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DEGRADATIVE AND SYNTHETIC STUDIES ON COLCHICINE

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DEGRADATIVE AND SYNTHETIC STUDIES ON COLCHICINE

Donald Elwood Pack

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Degradative and Synthetic Studies on Colchicine

By

Donald Elwood Pack

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INTRODUCTION

Colchicine, the principal alkaloid of the Colchicum autumnale L., was first isolated in 1820 by Pelletier and Caventou (102). Since that time it has been the subject of extensive biological and chemical studies. Several reviews have been published on its chemistry (25,26,55,78,79,117). Also a recent book (38) has been published concerning colchicine, but it deals mainly with the biological aspects of the subject.

Initial pharmacological studies were directed towards investigation of the use of colchicine in gout therapy, but to this day the mechanism of its action is not understood (81). However, the most interesting and useful property of colchicine is its ability, discovered by Dustin and Lits (37), to bring cell division to an abrupt halt in the early metaphase. In the case of plant cells, tetra- or polyploidal forms may result and this effect is of great value in cytological, breeding and mutation studies.

Since faster growing cells are affected more than resting cells, it is possible that colchicine might be employed in the chemotherapy of cancer. Some early investigations (49) raised hopes that colchicine might be of value, but more detailed study revealed that its toxic effect is too great to expect any valuable curative effects. However, there is a possibility that the structural features responsible for anti-mytotic activity and for toxicity are separable, and, if so, the synthesis of less toxic anti-mytotics might be accomplished.

The mechanisms of action and of toxicity and the metabolism of colchicine is largely unknown. Radioactive colchicine could be valuable in elucidating these points, and therefore the development of methods for synthesizing isotopically labeled colchicine becomes important. At present

there are two such preparations in the literature (105, 136), both of which leave much to be desired. In one (136), uniformly labeled colchicine was prepared by growing colchicum plants in an atmosphere of carbon dioxide-C^{14}. However, the material is of low specific activity (27 μc per g.) and therefore is limited in its usefulness. In the second process (105), the labeling is in the very labile methoxyl (rings A and C) and acetyl (ring B) groups, again limiting its usefulness. The development of synthetic methods suitable for introducing the carbon isotope in high specific activity into one of the rings would overcome these limitations.

With these ends in mind, the elucidation of the structure of colchicine and the development of suitable methods for the synthesis of colchicine and related compounds (both radioactive and inactive) assumes great importance.

However, from a purely chemical viewpoint, colchicine offers many interesting and challenging aspects. Some of these, along with the above-mentioned goals, will be discussed in the following sections under (I) structure studies and (II) synthetic studies.

STRUCTURE STUDIES

Introduction — Zeisel (146) first isolated colchicine (I) in the crystalline state and assigned the empirical formula $C_{22}H_{25}O_6N$, which remains unchanged to this day. In 1910 Windaus (140) established the trimethoxyphenyl nature of ring A by the isolation of a trimethoxyphthalic acid as a product of the oxidation of colchicine with potassium permanganate. The position of the methoxy groups in relation to ring C was established by the work of Sachs (116).

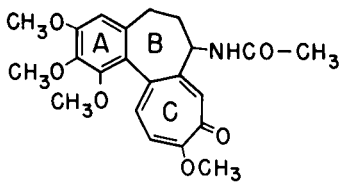
After forty years of conflicting postulations and misleading data obtained as a result of molecular rearrangements of the colchicine skeletons during structural investigations, the seven-membered structure of ring B and the location of the acetamido group thereon was established by Rapoport (112) and by Cook (23,24) through the synthesis of N-acetylcolchinol methyl ether (II), a degradation product of colchicine.

The only portion of the molecule for which a definitive proof of structure is lacking is ring C. Three postulated solutions have been offered: The six-membered methoxymethylene ketone structure (III) of Windaus (139), the pyran structure (IV) of Lettre (78), and the tropolone methyl ether structure (V) of Dewar (31). Most of the available evidence discounts the Windaus and Lettre structures and clearly indicates that ring C is tropoloid. This evidence includes spectral data (123), hydrogenation of colchicine (VI) to a glycol (5,6,32) which is cleaved by lead tetraacetate (32) and by periodic acid (5,6), and the parallelism in chemical properties between colchicine and known tropoloids (21,31,128). This similarity in properties, as has been pointed out (3), is necessary but insufficient to establish the tropoloid nature of ring C. Probably

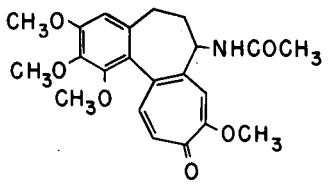
the most definitive evidence is X-ray diffraction data (69), which shows the correctness of the ring system as proposed by Dewar (31) and of the position of the oxygen functions in ring C of colchiceine (VI) as proposed by Cech and Santavy (20).

On the basis of mechanistic interpretations (20,34) of the rearrangements of colchiceine (VI) and the X-ray data (69), the two oxygen functions must be confined to the two positions shown in structure I instead of as in structure V as originally postulated by Dewar (31). Although colchicine is almost universally drawn as in Ia and isocolchicine as the alternate structure Ib, only one attempt (59) has been made to indicate the specific position of the carbonyl and methoxyl groups. From the similarity of infrared spectra (in the 7 μ region), ultraviolet spectra and optical rotations of colchiceine (VI), the mild hydrolysis product of colchicine and isocolchicine and several isocolchicine derivatives, it was suggested (59) that colchiceine was a single species belonging to the iso series, and this non-tautomeric nature of colchiceine could be due to hydrogen-bonding to the amide carbonyl. Thus the hydroxyl group of colchiceine and the methoxy of isocolchicine occupy the position more proximate to the acetamido group (as in structure VIa for colchicine), and this conclusion supports structure Ia for colchiceine. However, with the lack of analogies for such a system, it is also possible to rationalize to the reverse conclusion that colchicine is represented by structure Ib. Alas, it is difficult to see why, if the hydroxyl-hydrogen is hydrogen-bonded to the amide, methylation with diazomethane should give practically an equimolar mixture of the two isomers. It would appear, therefore, that valid evidence for the positions of these two oxygens is still lacking.

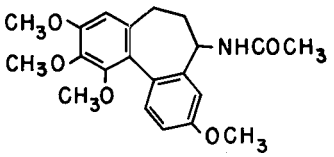
With the object in mind of providing degradative and synthetic data necessary to establish the seven-membered nature of ring C and to determine



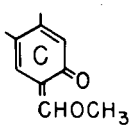
Ia



Ib

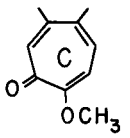


II

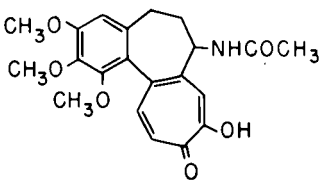


III

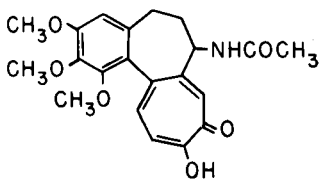
IV



V



VIa



VIb

the positions of the substituents thereon, Rapoport and co-workers (109,113, 114) degraded colchicine by the following rearrangement-free paths to products which presumably could be synthesized by unequivocal methods: Colchicine* was converted into N,N-dimethylaminocolchicide (VII) by reaction

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* For convenience, the structures in this degradative sequence will be shown as arising from structure Ia for colchicine. It must be kept in mind, however, that analogous structures arising from Ib are equally possible. This same pattern will be followed throughout this thesis, and unless otherwise stated or proven, all other possible isomeric structures (including the position of the double bond) are implied.

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with dimethylamine. VII was then hydrogenated to tetrahydrodemethoxycolchicine (VIII). VIII was then carried along two different paths to the final products. In the first, the carbonyl group was removed by conversion to the dimethylmercaptole and desulfurization by Raney nickel to yield hexahydrodemethoxydesoxycolchicine (IX). The acetamido group was then removed by reducing with lithium aluminum hydride, reacetylating, reducing again, Hofmann degradation and finally hydrogenation, yielding octahydrodemethoxydesoxydesacetamidocolchicine (X). The second path involved protecting the carbonyl group by means of the ethylene ketal, removing the acetamido group by the Hofmann path as before and finally hydrolysis to hexahydrodemethoxydesacetamidocolchicine (XI). The carbonyl group of XI was then removed as before, to yield the same octahydro compound (X). Synthesis of X would then prove the seven-membered nature of ring C, while synthesis of XI would also show the position of the original carbonyl group, thus fully establishing the structure of colchicine.

Rapoport and co-workers originally assigned the double bond, which remains resistant to hydrogenation, in all compounds from VIII on to the

7a-12a position* on the basis that this seemed like the most hindered

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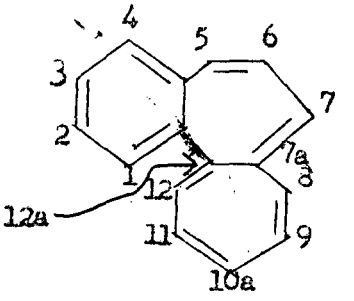
* In view of the strong evidence for the carbon skeleton of colchicine (I), the Chemical Abstracts numbering method of the parent ring system, benzo a heptalene (a) is used to locate substituents. However, the common names have been used for the degradation products since they are more lucid at present.



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position. However, as these workers realized, unsaturation between carbons 12 and 12a might also be subject to considerable resistance to hydrogenation, and no real decision could be made on the basis of the evidence available. It is even possible that the position of the double bond is not constant, but that it shifted during one of the reactions.** Either position is con-

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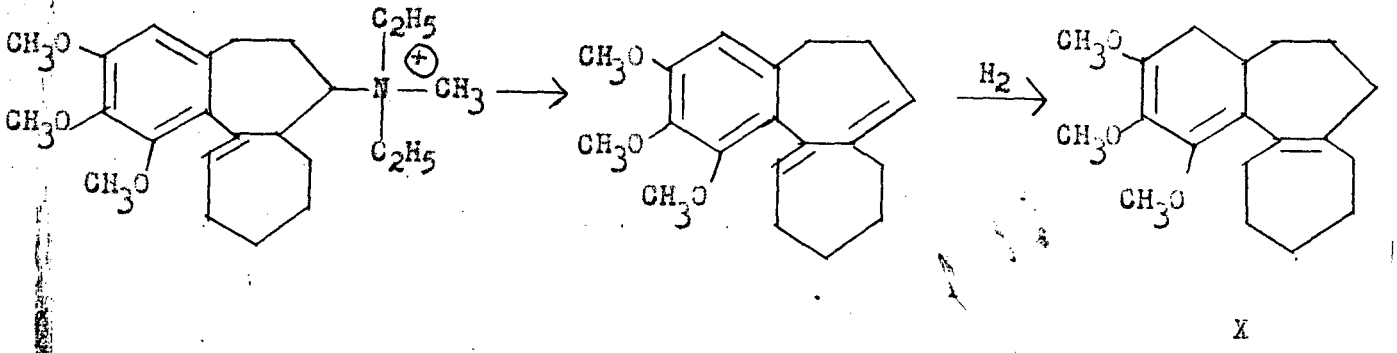
** A possible path that would account for a double bond shift is as follows:



X

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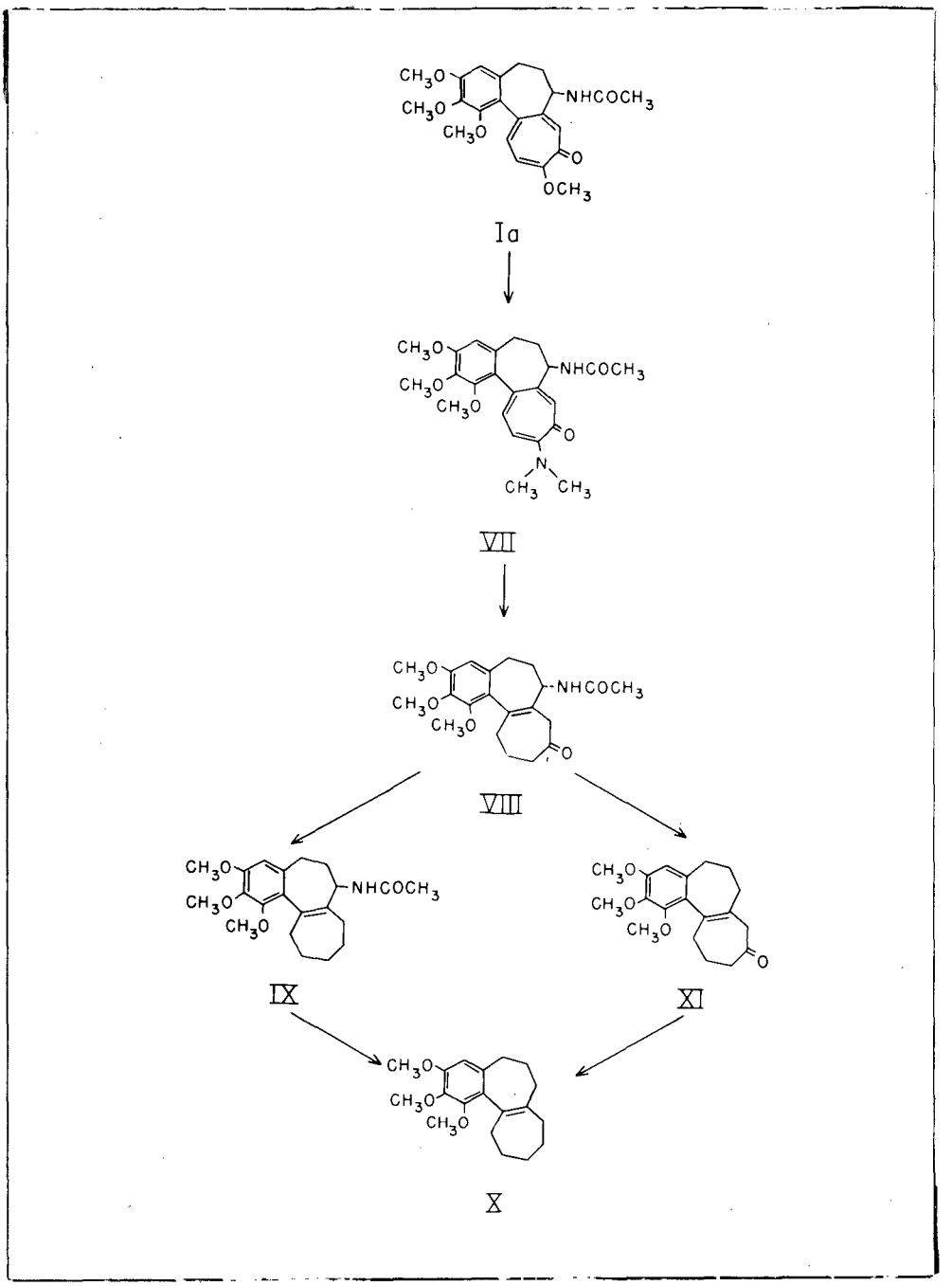
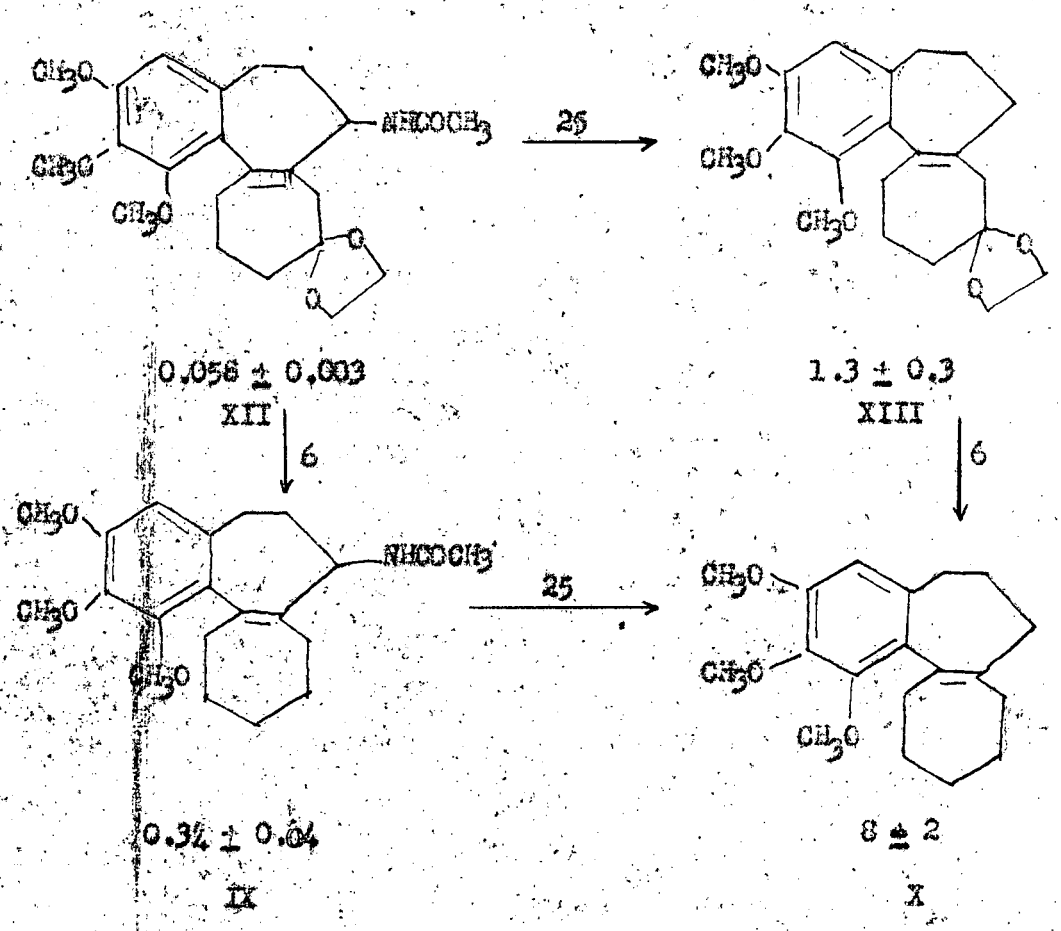
sistent with the ultraviolet absorption spectra, which indicate the presence of a styrene-like system, which is unconjugated with the carbonyl in those compounds containing that group.


CH3O
CH3O
CH3O
NHCOCH3
O
OCH3
Ia
CH3O
CH3O
CH3O
NHCOCH3
O
N
CH3
CH3
VII
CH3O
CH3O
CH3O
NHCOCH3
O
VIII
CH3O
CH3O
CH3O
NHCOCH3
IX
CH3O
CH3O
CH3O
O
XI
CH3O
CH3O
CH3O
X

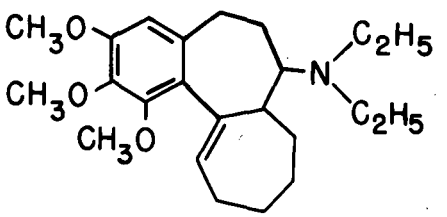
There are certain pieces of evidence, however, that, although not definitive and often negative, lead one to favor one structure over the other. The more conclusive of these will be discussed briefly: (1) The rate of epoxidation of four of the compounds is as follows (48), where the number under the formula is the rate constant and the number over the arrow is the ratio of the rates of the two compounds connected by that arrow:



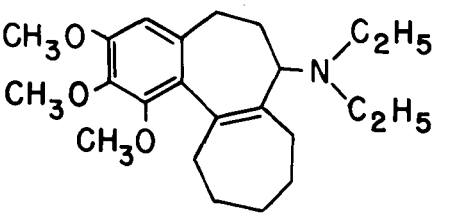
It is evident that the presence or absence of either the amide or the ethylene ketal group has a profound effect on the rate. Furthermore, the presence or absence of either group has no effect upon the value of the change in rate that accompanies removal of the other. This would imply that the ketal group, for example, bears the same relationship to the double bond

in XII as it does in XIII, i.e., the position of the double bond is constant, otherwise the effect on the rate of removing this group would not have been the same in the two cases. For a more detailed discussion of the effect of substituents on the rate of peroxidation, see (126). (2) Attempted von Braun degradations (17) of N,N-diethylhexahydrodemethoxydesoxydesacetylcolchicine (XIVa or XIV b) and N,N-diethyltetrahydrodemethoxydesoxydesacetylcolchicine (XVa or XVb) with cyanogen bromide resulted in recovery of approximately one-third of the starting material while the remainder was accounted for as a mixture of neutral products which contained no bromine. From the known ease of reaction of allylic tertiary amines (61) to give highly preferential introduction of bromine at the allylic position, this would constitute negative evidence for the non-allylic structures XIVa and XVa. (3) When the above two amines are treated with excess methyl iodide at 75°, the methiodides formed eliminate spontaneously to form the corresponding olefins (17,109,114). The facility with which this elimination occurs as compared with the forcing conditions usually necessary in the case of simple, aliphatic compounds (63) indicates some form of activation of the C-H bond that is broken. In the Δ^{12} compounds (XIVa and XVa) one of the β-hydrogens (7a) is allylic and therefore activated, while in the $\Delta^{7a(13a)}$ structures no such activated β-hydrogen is present, all β-hydrogens being of the paraffinic type.

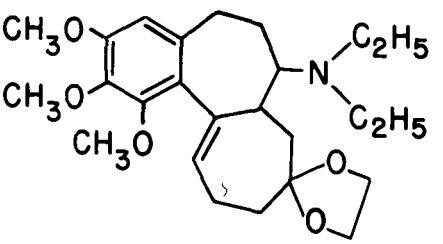
The above experimental data constitute evidence for the Δ^{12} double bond in all the degradation products. However, due to the uniqueness of the ring system (two fused seven-membered rings), any conclusions reached on the basis of analogies with much different systems must be regarded with caution. For example, in the case of the von Braun degradation, the compounds might have the allylic amine structure and yet unknown steric considerations might force the reaction to take an abnormal course.



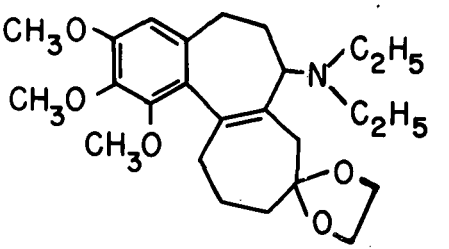
XIV a



XIV b



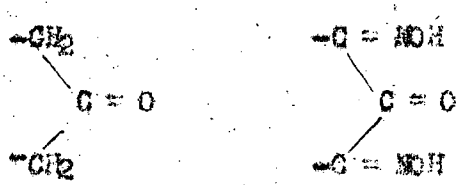
XV a



XV b

In order to obtain more definitive evidence for the structures of the various degradation products (and therefore of colchicine itself), various attacks were tried. These will be discussed in the following sections.

Relative Positions of the Double Bond and the Carbonyl Group — If the relationship between the positions of the double bond and the carbonyl in tetrahydrodemethoxycolchicine (VIII) could be established, then determination of the position of either one of them would establish the position of the other. One method of establishing this relationship would be to add an unsaturated group (e.g. oximino) to each of the carbon atoms alpha to the carbonyl:



Comparison of the ultraviolet absorption spectra of the two compounds should then show whether the double bond in VIII is β-γ or γ-δ to the carbonyl.*

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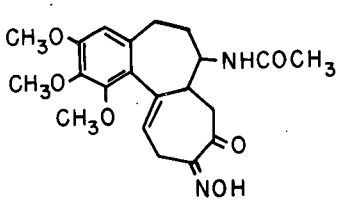
* These are the only two possibilities; α-β is ruled out (114) on the basis of the ultraviolet spectrum of VIII which shows a styrene-type of absorption.

As part of the synthetic sequence (see p. 72), VIII was converted to oximinotetrahydrodemethoxycolchicine (XVI or an isomeric form; λ_{max} 253 mμ, log ε_{max} 4.15) by the action of potassium t-butoxide and n-butyl nitrite. A single compound was isolated in a 95% yield of crude, base-soluble material (70% yield recrystallized).

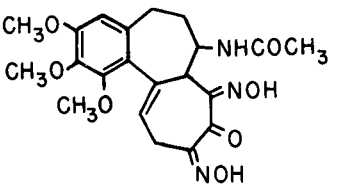
When an attempt was made to prepare dioximinotetrahydrodemethoxycolchicine (XVII or an isomeric structure) from VIII using 200 mole % of nitrite and 300 mole % of potassium, but otherwise following the same procedure as before, a crude, base-soluble product was isolted in an almost quantitative yield. Crystallization from benzene-chloroform gave a first crop of XVI in 40% yield. In view of the low solubility of XVII in chloroform and benzene, it seems probable that there was little, if any, present in the mother liquors.

This difficulty in adding two oximino groups is surprising in view of the ease with which some ketones (13,36,44,50,70,72,74,80,101,103, 110,142,144) add two moles of nitrite. In one case (144), it was even impossible to obtain the monoximino compound, even with excess ketone, the dioximino ketone and recovered starting material being the main products isolated. In another (74,110) the monoximino ketone was obtained in very low yield with equal moles of reactants, but the dioximino compound could easily be produced in good yield with an excess of nitrite. Steric hindrance of one of the alpha positions is probably involved here.

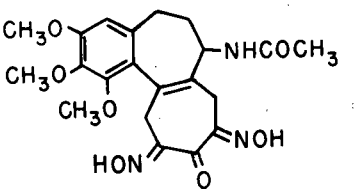
In view of the failure to produce the dioximino compound XVII from VIII directly, preparation of XVII was then attempted from the preformed monoximino compound XVI following the same procedure as with the monooximination of VIII. However, difficulty was experienced due to the low solubility of XVI in benzene and t-butyl alcohol. This difficulty was overcome by adding solid XVI to the t-butoxide solution, stirring until it had all dissolved and then adding the nitrite in benzene. A several-fold excess of potassium and butyl nitrite was used and the reaction mixture was worked up as before. The product was fairly insoluble in chloroform (even less so in benzene) and repeated hand extraction of the neutralized basic aqueous solution by chloroform removed only part of the material.



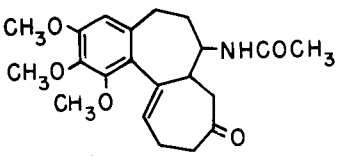
XVI



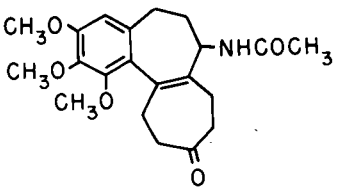
XVII a



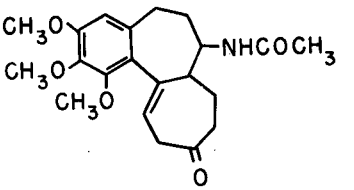
XVII b



XVIII a



XVIII b



XVIII c

However, continuous, liquid-liquid extraction with the same solvent removed most of the remaining product. The dioximino ketone was crystallized from isopropyl alcohol in fairly low overall yield and had λ_{max} 256 mμ with log ε_{max} 4.20.

There are four possible structures for tetrahydrodemethoxycolchicine (VIII, XVIIIa, XVIIIb and XVIIIc) and, therefore, there are eight possible oximinotetrahydrodemethoxycolchicine structures and four possible dioximinotetrahydrodemethoxycolchicine structures. However, a comparison of the ultraviolet absorption spectrum of VIII (λ_{max} 257; log ε_{max} 4.10) with the spectra of XVII shows essentially no shift in the position of the maxima and only a slight increase in the extinction coefficient. This indicates very strongly that the added oximino groups have not conjugated with the styrene system. The only structures consistent with these data are XVIIa and XVIIb for dioximinotetrahydrodemethoxycolchicine and XVIIIa and XVIIIb for tetrahydrodemethoxycolchicine.

If the position of the double bond could now be established, then the position of the carbonyl could be known and consequently the absolute position of the carbonyl (see p. 64) and the methoxyl of colchicine would be established.

Absolute Position of the Double Bond — With the determination of the relative position of the double bond and the carbonyl in tetrahydrodemethoxycolchicine completed, attention was then turned to establishing the absolute position of one or the other. On the basis of several considerations, it was felt that work on the double bond, rather than the carbonyl, would be more likely to succeed. Several experiments directed along this line were carried out and these will be discussed in the following sections.

Nuclear Magnetic Resonance — Meyer, Shika and Gutowsky (82) have shown that the shift in the position of the proton magnetic resonance of a proton-containing group is fairly constant for any one group, but varies with different groups. This "chemical shift" can thus be used as an indication of the type of proton bonds present in a molecule. Of the two possible structures for the compounds in the colchicine degradation sequence, only the one with the trisubstituted (Δ^{12}) double bond contains a vinylic hydrogen atom, and it might be possible to distinguish between those two possibilities by the use of proton magnetic resonance.

To this end, the high-resolution proton magnetic resonance spectra of tetrahydrodemethoxycolchicine (VIII), hexahydrodemethoxydesoxycolchicine (IX), N,N-diethyltetrahydrodemethoxydesacetylcolchicine ethylene ketal (XV), octahydrodemethoxydesoxydesacetamidocolchicine (X), and hexahydrodemethoxydesacetamidocolchicine ethylene ketal (XIII) were determined through the courtesy of Dr. James Shoolery, Varian Associates, Palo Alto, California. The samples employed were, in the case of the lower melting compounds, melted specimens somewhat diluted with carbon tetrachloride to reduce the viscosity; with the high melting materials, hot, saturated solutions in carbon tetrachloride were used. Spectra were obtained with both rotated and stationary samples.

Peaks were observed in all of the samples at shifts of -1.8,*

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* These figures are ten times the magnitude and of opposite sign as those used by Gutowsky (82), following the convention used by Varian Associates.

+0.8 and +2.9 parts per million relative to the reference (water), corresponding respectively to the aromatic, methoxyl and the sum of the cyclic methylene, allyl and benzyl proton common to those compounds. In addition,

-CO-NH-, C-H and O-CH_3 peaks at -2.5, +2.25 and +4.0 parts per million were obtained in the case of the compounds bearing these proton types. The result of a search for vinyl protons in the neighborhood of zero shift was negative in the case of each sample except VIII where a peak of the correct magnitude was visible at a shift of -0.3 parts per million, just at the base of the high-magnitude methoxyl peak. This result, then, implies that the styrene double bond occupies the trisubstituted position in VIII and the tetrasubstituted, or ring juncture, position in the rest of the compounds.

However, this assignment is to be viewed with some caution due to difficulties in interpretation of the presence or absence of vinyl proton bands in the spectra arising from: (1) the proximity of the large methoxyl peak which could very probably mask the less intense band, (2) limited solubility of some of the compounds in the solvent, lowering the traces into the noise level of the apparatus, and (3) high viscosities of the melts and concentrated solutions lowering the resolution obtainable and requiring further dilution.

Chemically, there is little reason to suspect a difference in double bond position between the various compounds. If the apparently unnatural division between the tri- and tetrasubstituted positions obtained experimentally is to be regarded as unreal, interpretation of the results obtained must follow one of two lines: either, as one extreme, the observed peak is regarded as spurious and unexplained and all the double bonds are assigned the ring juncture position, or, at the other extreme, the trisubstituted position exists in each compound, the small vinyl proton peak being obscured beneath the large methoxyl band in every case except that of VIII where, for some reason, it appears. Any intermediate

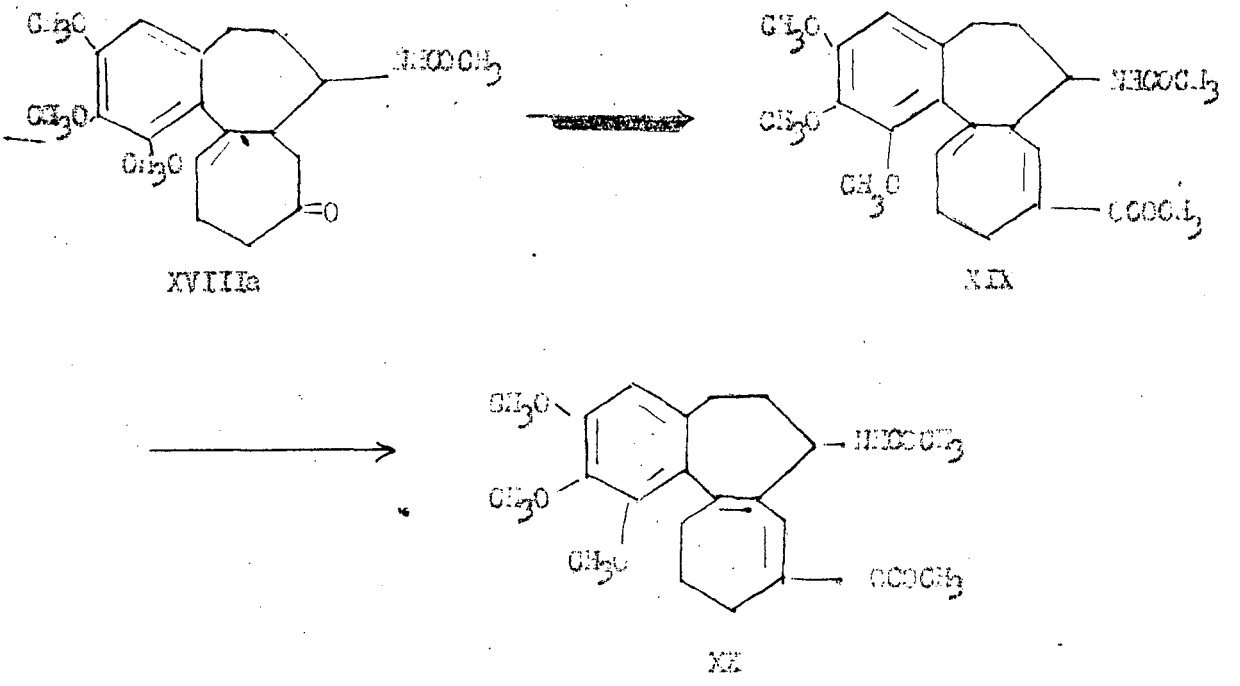
result is, of course, equally possible. The data furnish no motive for a choice between these possibilities.

In conclusion, the nuclear magnetic resonance spectra obtained are consistent with the main structural features of these compounds, but have assigned double bond positions which are open to question and will require independent verification.

Preparation of Double Bond Isomers — Although there are available several pieces of evidence indicating that the double bond is trisubstituted, this interpretation of the data, as was pointed out on page 10 is open to some doubt due to the lack of analogous compounds. However, if a double bond isomer of any one compound could be obtained, a direct comparison of the two compounds could be made without resorting to dubious analogies. This, then, would give the required definitive evidence and thus settle the question of the structure of colchicine.

The compound chosen for this isomerization work was hexahydrodemethoxydesoxycolchicine (IX), as it is the simplest structure in the series still containing the nitrogen function.

Enol Acetates — In connection with other work, an attempt was made to convert tetrahydrodemethoxycolchicine (VIII) into its enol acetate (XIX). An acetyl analysis of the product showed the addition of one acetyl group. The crude product, however, had an ultraviolet absorption maximum at 265 mμ, while the absorption maximum of XVII should differ only slightly from that of VIII (λ_{max} 257 mμ). This shift in the maximum indicates a rearrangement to give a different chromophoric system. One sequence of reactions that could explain this change is formation of the enol acetate (XIX) and then shift of the original double bond into conjugation with the enol double bond to give XX:

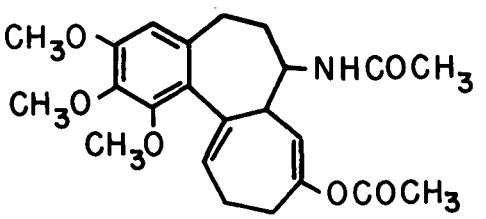


XVIIIa XIX XX

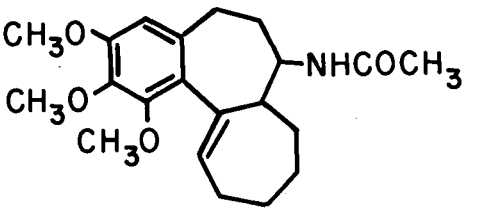
If this were true, then hydrolysis of XX should give an isomeric VIII which could then be reduced to the desired isodesacetyldesmethoxy-colchicine (XX).

The enol acetate was prepared from VIII by three methods: (1) isopropenyl acetate-p-toluene sulfonic acid; (2) acetic anhydride-sodium acetate; and (3) acetic anhydride-pyridine. In all three cases, the reaction mixture darkened rapidly on heating. However, only the products from the acetic anhydride-pyridine preparations were carried any further.

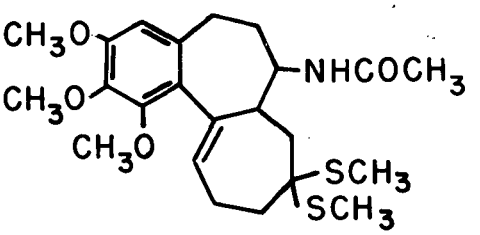
The crude material was obtained by refluxing the reaction mixture for several hours, evaporating the solvent under reduced pressure, and washing a benzene solution of the residue with sodium bicarbonate solution. The benzene was then removed by lyophilization to give a quantitative yield of a brown amorphous powder, which analysed for the addition of one acetyl group. All attempts to crystallize the product failed.



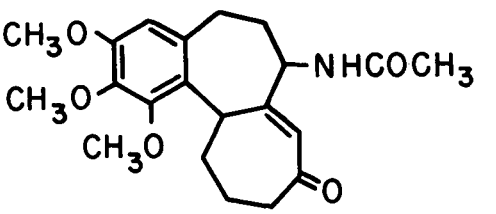
XIX



XXI



XXII



XXIII

An attempt was then made to purify this material by chromatography on alumina. One large peak (accounting for about 30% of the material) and several, rather ill-defined peaks were obtained. The weights and concentrations of the fractions, as well as the absorption maxima of the larger fractions, are given in Table 1. As can be seen from the absorption maxima, even the largest peak is not a single compound, and it is evident that the reaction gave several products, all in rather low yield. Although rechromatography of the larger peak might be expected to give purer components, this was not tried because of the results to be described next.

Although it would be desirable to isolate and characterize a pure enol acetate, this is not the desired final product, but rather isohexahydrodesoxydemethoxycolchicine (XII) through the isomeric tetrahydrodemethoxycolchicine. This isoketone would be produced by hydrolysis of the corresponding enol acetate. In view of the difficulty in isolating a pure enol acetate, it was decided to hydrolyze the crude acetate mixture, with the hope of being able to effect a purification of the ketone more easily. Formation of a bisulfite addition compound should be quite useful here.

The hydrolysis was first attempted by refluxing with dilute hydrochloric acid in aqueous ethanol. At the end of the reflux time, the 285 mμ absorption maximum had disappeared and a shoulder at 255-60 mμ had appeared. The solvent was then removed under reduced pressure and the residue was taken up in benzene, washed with aqueous sodium bicarbonate, dried, and evaporated to dryness. The residue was shaken overnight with 20% aqueous sodium bisulfite. (This treatment is sufficient to completely dissolve pure VIII.) The mixture was then filtered, basified with potassium carbonate to pH 9-10 and extracted with chloroform. Evaporation of the chloroform yielded a residue in about 30% recovery, based on starting VIII.

Table 1

Chromatography of "Enol Acetate" from 76 mg.

Tetrahydrodemethoxycolchicine (VIII)

Fraction No.	Eluent	Weight mg.	Conc. mg./100 ml.	λ_{max} mμ
0	benzene	3.6	1.3	
1	0.5% chloroform in benzene	0.6	0.7	
2		0.3	0.4	
3		0.6	0.9	
4		0.7	1.0	
5-6	1% chloroform in benzene	1.1	1.2	
7		0.8	1.2	
8		0.8	1.2	
9		0.5	0.7	
10-13		samples lost		
14-15	5% chloroform in benzene	0.8	0.9	
16-17	10% chloroform in benzene	0.1	0.1	
18	20% chloroform in benzene	2.2	2.7	281,365
19		1.8	2.1	290
20		2.0	3.6	290
21		2.9	3.7	250,265 (shoulders)
22		3.6	3.9	267
23		5.0	6.2	284
24		3.8	4.2	284
25-26	25% chloroform in benzene	8.0	10.8	282
27	50% chloroform in benzene	14.6	18.2	269
28		5.8	6.4	257
29		1.9	2.1	none
30		1.6	1.8	
32		1.4	1.6	
33		1.2	1.6	none
34	chloroform	5.5	9.2	329
35		7.2	6.9	327
36		2.7	6.7	none
37	ethanol	6.3	—	none
	Total	88.2 mg.		

The material insoluble in bisulfite was isolated with a 50% recovery. This material contained only about two-thirds as much acetyl as VIII, indicating that some hydrolysis of the N-acetyl had also occurred.

Another hydrolysis in more dilute acid and for a shorter reflux time gave similar results. In this run, the ketonic fraction was treated with methyl mercaptan to form the dimethylmercaptol. The only product isolated from this reaction was tetrahydrodemethoxycolchicine dimethyl mercaptol (XXII).

The low recovery of ketonic material from the acid hydrolysis might have been due to instability of isotetrahydrodemethoxydeoxycolchicine in the hydrolysis medium. With this in mind, attention was next turned to a mild, basic hydrolysis. Potassium bicarbonate has been successfully used before to hydrolyze enol acetates (115).

To this end, the crude enol acetate residue was refluxed for one hour with dilute potassium bicarbonate in aqueous methanol. This time, a ketonic fraction was isolated in approximately 40% yield and a non-ketonic fraction in about 25% yield. Again, the non-ketonic fraction had lost some of the N-acetyl of the original molecule. The other 35% of the material is unaccounted for.

It is possible, although unlikely, that hydrolysis of the conjugated enol acetate XX could give an α,β-unsaturated ketone XXIII. This ketone would then form a 1-4 addition compound with the bisulfite which would not be decomposed by the potassium carbonate used for decomposing the normal 1-2 addition product. To test this hypothesis, the bisulfite solutions from both the acid and basic hydrolyses were

basified with potassium carbonate and extracted with chloroform to obtain the normal ketonic products. The aqueous solutions were then basified with sodium hydroxide to pH>13 and extracted again with chloroform. This second extraction yielded very little additional material. Thus, if there were any of compound XXIII formed, it is present in only very low concentration.

The production of the non-ketonic material with less acetyl than tetrahydrodemethoxycolchicine is strange. Hydrolysis of the N-acetyl group usually requires rather vigorous conditions. Thus in colchicine (VI), hydrolysis of the N-acetyl group is usually accomplished by heating several hours in 3% hydrochloric acid (10%). Also the hydrolysis of hexahydrodesoxydemethoxycolchicine (IX) in alkali requires strong alkali, long periods and high temperatures. The much greater ease of hydrolysis in this case indicates a rearrangement (probably during formation of the enol acetate) to give a more labile N-acetyl group.

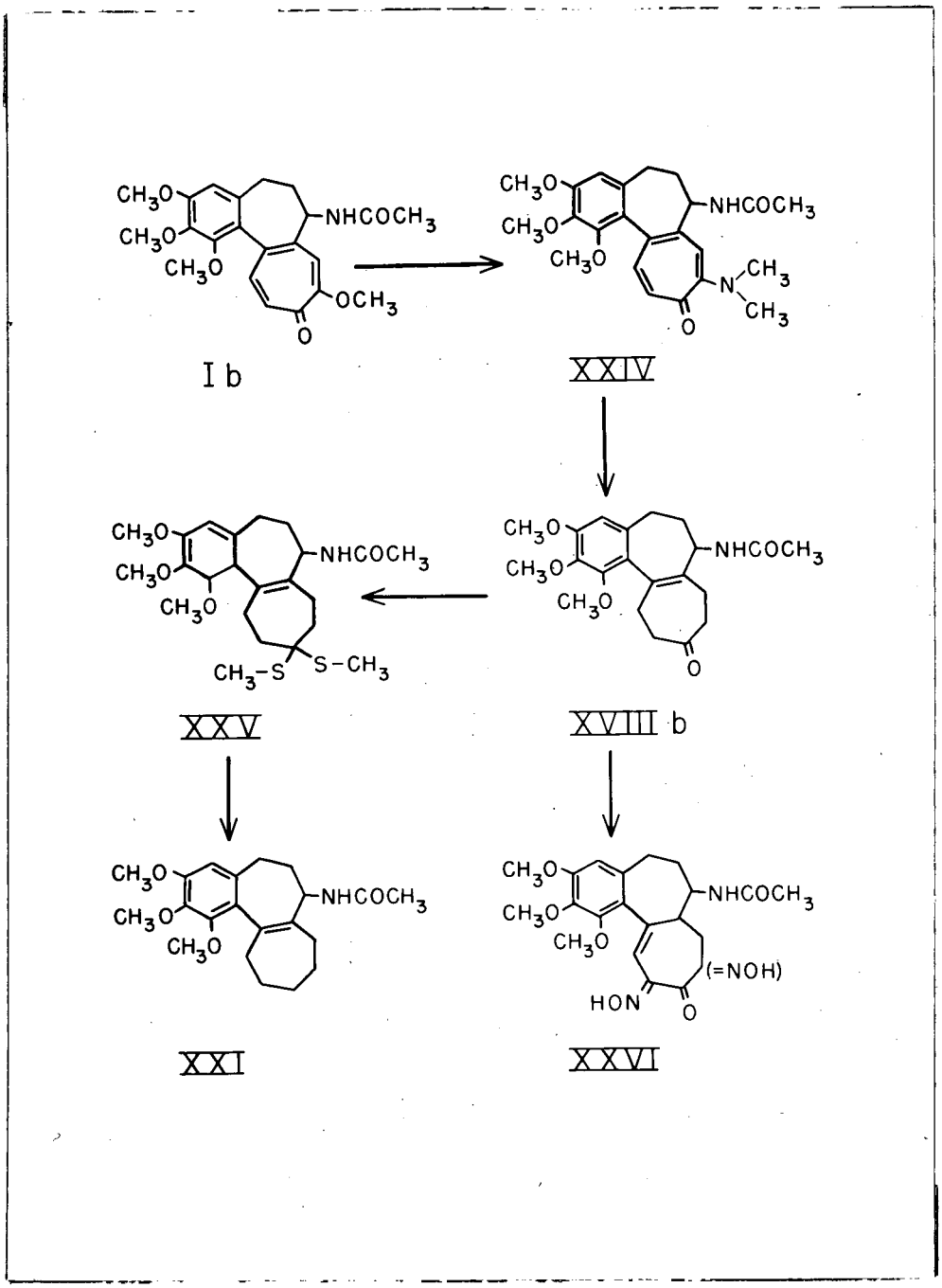
In view of the above results (especially the isolation of VIII as the only, or at least main, ketonic material), it does not seem likely that preparation of a double bond isomer is possible by this method.

Isocolchicine Series — After failure of the enol acetate method, preparation of isohexahydrodemethoxydesoxycolchicine (XXI) was then attempted starting with isocolchicine (Ib), through a sequence of reactions analogous to that used to produce hexahydrodemethoxydesoxycolchicine (IX) from colchicine (Ia). The hope was that hydrogenation of N,N-dimethylaminoisocolchicide (XXIV) would give a ketonic product (tetrahydrodemethoxyisocolchicine (XVIIIb)) with the double bond in a different position than it is in tetrahydrodemethoxycolchicine (XVIIIa).

Recent work by J. B. Lavigne (74) has made XXIV available in quantity sufficient for this study. Colchicine (I) is first hydrolyzed to colchiceine (VI), which is then remethylated with diazomethane to give an equimolar mixture of colchicine and isocolchicine. Previous workers (59, 124) had then separated this mixture by chromatography on alumina, a rather tedious process not easily adapted for the quantities needed here. Williams (138) was able to effect separation by partially hydrolyzing the mixture, colchicine being hydrolyzed faster than isocolchicine. Lavigne found, however, that pure isocolchicine could be obtained in 30% yield by crystallization from ethyl acetate, taking advantage of the faster rate of crystallization of isocolchicine and the faster rate of solution of colchicine. The mother liquors could be recycled to give an additional amount of isocolchicine. The isocolchicine is then reacted with dimethylamine to give an 85% yield of XXIV.

The hydrogenation of XXIV was first attempted using the same ratio of amine to catalyst as was used in the colchicine series. The rate of hydrogen uptake was greater than in the case of N,N-dimethylaminocolchicide (VII), but no definite break in the curve was observed, only a gradual leveling off at slightly less than four moles uptake of hydrogen. When the hydrogenation was stopped at slightly over a three mole pickup of hydrogen, and the reaction mixture worked up as in the normal series, only a 10% yield of neutral, ketonic material was obtained.

Since, in the normal series, using the Paar hydrogenation apparatus resulted in better yields of tetrahydrocolchicine (VIII) than when the atmospheric pressure apparatus was used, a


Ib
XXIV
XVIII b
XXV
XXI
XXVI
NHCOCH3
CH3O
OCH3
N(CH3)2
(=NOH)
HON
CH3-S
S-CH3

sample of XXIV was hydrogenated in the Paar apparatus. However, in this case only a 3% yield of ketonic material was obtained. This ketone was crystallized from ethyl acetate-butyl ether and has an ultraviolet absorption spectrum that is very similar to that of tetrahydrodemethoxycochiolone (XVIIIb), although the compounds are distinctly different, indicating the same or similar chromophoric groups.

In an effort to increase the yield of XVIIIb, various modifications of the hydrogenation procedure were tried, such as varying the rate of stirring or the amine-to-catalyst ratio, or using platinum oxide or palladium on carbon alone. In all cases, except with palladium alone in which there was essentially no hydrogen pickup, the yield of ketonic material was only 5-11%. Also, the neutral fraction was only about one-half to three-fourths ketonic, the rest presumably being the corresponding carbinol. This is also in contrast to the normal series in which the neutral fraction is almost completely ketonic. Thus we must accept not only the low yield of hydrogenolysis products, but also the further hydrogenation of the desired ketone itself.

The bisulfite soluble fractions from several of the hydrogenation runs were combined and treated with methyl mercaptan and zinc chloride to produce the dimethylmercaptol (XXV). An attempt to crystallize the mercaptol from methanol-water gave only an oil. Chromatography on alumina gave only one peak which accounted for about 60% of the material. This material also could not be crystallized, but treatment with water gave a white powder which analyzed slightly low for sulfur. This powder was then rechromatographed. Only one peak was obtained which accounted for at least 97% of the material and was still an oil.

The oily mercaptol was desulfurized as such rather than risk losing the small amount of material available in further attempts at purification. The desulfurized product would show immediately whether or not the hydrogenation had given the desired double bond isomer. To this end, the oil was treated with Raney nickel and the product was chromatographed and crystallized. It was identical with authentic hexahydrodemethoxydesacetylcolchicine (III) as shown by mixed melting point and optical rotation. The ketone, therefore, is the carbonyl isomer, i.e. has the double bond isomer of tetrahydrodemethoxycolchicine (XVIIIa).

Thus, the hydrogenation of the amino compounds in both the normal and the iso series results in products with the double bond in the same position. It would appear that this is the most stable position for the double bond in this molecule, and that hydrogenation cannot be used to produce any double bond isomers.

One other use for the isocolchicine compounds suggests itself. In the discussion on diaminotetrahydrodemethoxycolchicine (XVII) (see p. 12), it was assumed that if the amino group were conjugated with the double bond, then this would be manifested by a large bathochromic shift in the ultraviolet absorption spectrum. However, there is a possibility, although remote, that steric factors causing non-coplanarity would prevent interaction between the two groups. Since the double bonds in tetrahydrodemethoxycolchicine (XVIIIa) and tetrahydrodemethoxyisocolchicine (XVIIIb) are in the same position while the two carbonyl groups are not, the double bond in one compound must be one carbon atom closer to the carbonyl than it is in the other. If an amino or diazonium compound (XVI) could be prepared from XVIIIb and this compound showed a bathochromic shift, then the relative

position of the double bond and the carbonyl group in the two ketones would be known for a certainty.

However, in three attempts to eximinate XVIIIb, no identifiable product was obtained. The yield of base-soluble material was considerably less than 50% (compare this with the almost quantitative yield obtained in the normal series). This material was dark brown and amorphous and could not be purified. The reason for these results is unknown, and no information regarding conjugation in the iso series could be obtained.

Reactions at C_7 — After exhaustion of methods of obtaining information regarding the position of the double bond by reactions involving functional groups on ring C, attention was next turned to reactions involving the nitrogen atom at C_7. In selecting suitable pathways, care must be taken to avoid reactions that might lead to rearrangements. Ideally, the reaction sequence should unequivocally leave the carbon skeleton intact and also the position of the double bond should remain the same or should shift in such a way that its new position would be known. Also the reagents used should not react with any other part of the molecule. Obviously, the above stipulations would immediately eliminate all reactions that might go through carbonium ion intermediates, free radical reactions, and reactions leading to carbon-carbon double bonds.

Dehydrodemethoxydesoxycolchicine (IX) was chosen as the starting point for this work as in it all functional groups in ring C have been removed. Inasmuch as the free amine, dehydrodemethoxydesoxydeacetylcolchicine (XXVII), rather than the amide IX, would be a common intermediate in practically all possible reaction sequences, its

preparation was first studied. The hydrolysis of IX proved to be more difficult than was anticipated. Saponification in refluxing methanolic or ethanolic alkali was quite slow, requiring several days for even a 25% yield of basic material.* When an attempt was made to increase

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* In all the hydrolyses, the reaction mixture was separated into acidic (including amphoteric), basic and neutral fractions by the appropriate acid and base extractions.

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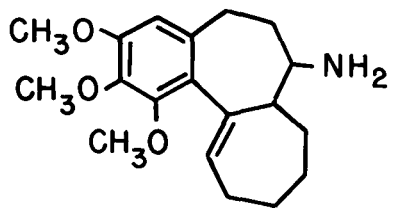
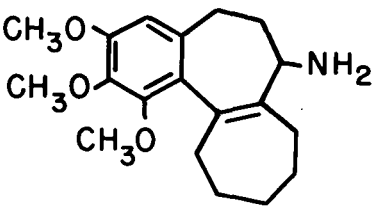
the rate of saponification by using ethylene glycol-water as a higher boiling solvent, the reaction mixture darkened and only about 25% of the material was recovered as neutral or basic. Treatment of IX with methanolic potassium hydroxide in a bomb tube in a steam bath for 12 days gave 40% basic material and 16% recovered neutral. When the bomb reaction was repeated at 150° for 16 hours, only 20% basic and 2% neutral material was obtained. The majority of the material, which was obtained as the acidic fraction, gave a positive ferric chloride test, and is probably phenolic. Apparently at elevated temperatures, ether cleavage is a competing reaction. In theory at least, the phenols could be remethylated, but it would be better if the ether cleavage could be avoided altogether.

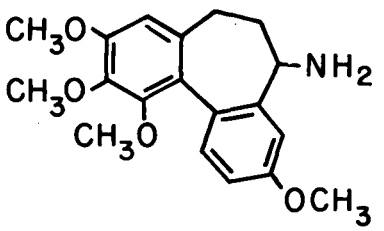
Although the hydrolysis of amides is usually more rapid in acid than in base, acidic conditions were originally avoided because of possible rearrangements the acid might cause. However, after the results obtained with the basic conditions, an acid hydrolysis was attempted. When IX was refluxed with sulfuric acid in aqueous acetic acid, an insoluble red oil, which accounted for 30% of the original material, separated. A 30% yield of basic material was obtained as a



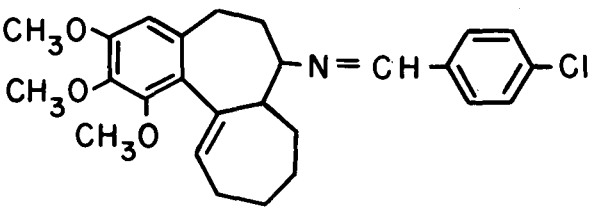
XXVII a



XXVII b



XXX



XXXI

brown oil. Even the neutral fraction (20% recovery) was colored, and contrary to the recovered IX from the basic hydrolysis, did not crystallize when the solvent was removed.

The best method for the hydrolysis, from the standpoint of material balance and purity of amine and recovered amide, is refluxing for several days in aqueous ethanolic potassium hydroxide. When the hydrolysis is conducted in a nitrogen atmosphere, the basic and neutral fractions are practically colorless and the recovered amide can be recycled to give a good overall yield of amine. The amine, however, could not be obtained in the pure, crystalline state, although in one run solid material was obtained from hexane, but this material did not analyse correctly, nor could the experiment be repeated. An attempt to form the picrate gave only an oil. The amine apparently is somewhat unstable on alumina, as the material obtained after chromatography was more colored than originally. Treatment with acetic anhydride gave back the amide IX and, therefore, the amine has the correct structure. The oily amine was used in the rest of the reactions without further attempts at purification.

Having available a supply of the amine, attention was next turned to reactions which could give the desired information regarding structure. Only a few methods for replacing a nitrogen with a hydrogen are known. Two of these (the Hofmann elimination and the von Braun cyanogen bromide reaction) have already been discussed (p. 10). Reductive cleavage (Emde reaction) probably would not work for the same reason that the von Braun reaction failed.

Mild oxidation of alkyl hydrazines yields the corresponding hydrocarbon (68). Although the amine XXVII could probably be converted to

the corresponding hydrazine (e.g. by reaction with hydroxylamine-o-sulfonic acid (27)), this oxidation has not been studied sufficiently to ensure that rearrangements would be unlikely. If, however, this reaction did give the same octahydrodemethoxydeoxydesacetamidocolchicine (X) as was obtained from the Hofmann elimination, then it would be established that the Hofmann did not shift the double bond. Also, in compounds with the Δ^{12} double bond, C_{7a} is asymmetric (as well as C_7 when the nitrogen is present) and presumably these compounds as isolated would represent single stereoisomers. Therefore, if the removal of the nitrogen by this mild oxidation gave an optically-active compound, then the position of the double bond would be established. There are several "ifs" in the above scheme and it was felt that this problem could better be attacked by other means.

A more direct way of obtaining information regarding the double bond would be replacement of the nitrogen with a carbonyl or other doubly bonded function. The ultraviolet absorption spectrum should then show whether or not the double bond is in conjugation with the oxygen and therefore would show its position. This ketone could then be converted, through reduction of the oxime, to an amine. If this amine is identical with XXVII (except for being racemic), then the position of the double bond in the colchicine degradation products will be established. If they are not identical, the double bond shifted to form the elusive double bond isomer. Here, again, the position of the double bond in the degradation products will be established.

Only a few methods are available for the replacement of a nitrogen with an oxygen. Nitrous acid on the amine is unsatisfactory

because of the rearrangements this reaction often gives. Treatment of primary amines with hypochlorous acid to yield the chloramine, followed by elimination of hydrogen chloride with sodium ethoxide and hydrolysis of the imine has yielded ketones, but hypochlorite would probabably also if not exclusively, attack some other part of the molecule. Primary amines have been oxidized with such agents as permonosulfuric acid to yield a small yield of oximes, along with further oxidation products. Here, again, the vigorous oxidant probably would attack other parts of the molecule. Phenyl azo alkanes on treatment with sodium ethoxide rearrange to the corresponding phenylhydrazones (28). However, no method is available for the preparation of this type of compound from alkyl amines.*

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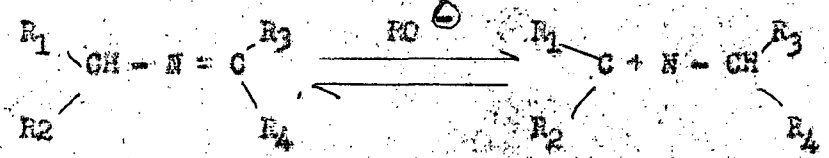
* Aromatic azo compounds can be prepared by reaction of nitroso benzene with an aromatic amine (58), but no report has appeared on the reaction of nitroso benzene with aliphatic amines. Exploratory experiments with cyclohexylamine were not promising, but more work along this line is needed.

A rearrangement similar to the azo-hydrazone reaction is the tautomeric change between azomethines of general structures XXVIII and XXIX (8,9,10,62,64,65,66,120,121,122).



XXVIII XXIX

XXVIIIa + XXIXa : $R_1 = C_6H_5$; $R_2 = R_3 = R_4 = H$

XXVIIIb + XXIXb : $R_1 = C_2H_5$; $R_2 = CH_3$; $R_3 = p\text{-}Cl\text{-}C_6H_4$; $R_4 = H$

XXVIIIc + XXIXc : $R_1 = C_6H_5CH_2$; $R_2 = CH_3$; $R_3 = p\text{-}Cl\text{-}C_6H_4$; $R_4 = H$

A study of this isomerization with a benzylidine of XXVII could give information concerning the position of the double bond in several ways: (1) The rate of isomerization would be a function of structure; (2) the ultraviolet absorption spectra would show whether the carbon-nitrogen double bond which had isomerized to C_7 is in conjugation with the carbon-carbon double bond in the ring; and (3) comparison of the amine obtained by hydrolysis of the isomerized product with the original amine (XXVII) would show whether the isomerization procedure had also shifted the carbon-carbon double bond. The last two would be sufficient to establish the structure of the colchicine degradation products.

The azomethines are easily prepared by condensation of the appropriate carbonyl and amine components. In most of the cases reported in the literature, the components were a benzyl amine and an aromatic aldehyde or ketone, and in all kinetic experiments this was the case. Only two groups (8,9,10,64) have reported work on azomethines from aliphatic amines. In one (8,9,10) various nuclear-substituted benzylidines of aliphatic amines was heated with aqueous glycerol or pyridine, and in some instances an aldehyde or ketone corresponding to the amine fragment was isolated, usually in low yield. In the second (64), methylene benzylamine (XXVIIIa) and benzylidine methylamine (XXIXa) were found to be stable to refluxing 3 N sodium ethoxide, conditions which would rapidly isomerize compounds in which both R_1 and R_2 are aromatic. When heated to 190° with sodium ethoxide and then hydrolyzed, XXVIIIa yielded a small amount of benzaldehyde, showing that the tautomerization can take place if the conditions are vigorous enough.

Several reports are available on the isomerization of azomethines in which both R_1 and R_3 are phenyl or substituted phenyl and R_2

and R_4 are hydrogen, alkyl or aryl (62,64,65,66,120,121,122). In all these cases sodium ethoxide in ethanol was the catalyst. It was found that in any one series, varying only the substituent on R_1 or R_3, the p-nitro compound isomerized the fastest, followed by m-chloro, p-chloro, and then others such as methyl, methoxyl, and dimethylamino. In all instances* the meta compound was slightly faster than the corresponding

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* No rate is available for the p-nitro compounds as these were unstable under the isomerization conditions (122).

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para isomer. The position of equilibrium, however, appears to be at random, but in all cases the equilibrium constant is between 0.3 and 3.

In the above cases, the rate of isomerization and the position of equilibrium were determined by treating the azomethine, after varying lengths of time in the isomerization solution, with p-nitrophenylhydrazine, forming the hydrazone in quantitative yield. The composition of this hydrazone mixture was then determined by comparison of its melting point with the melting points of mixtures of known composition. In those cases where there was a significant difference between the two possibilities, the composition was checked by elemental analysis. However, the rate of isomerization has been shown to be equal to the rate of racemization in those compounds in which the nitrogen is on an asymmetric carbon atom (62,65,66,97,120). Measurement of the change in optical rotation, then, should suffice to determine the rate of isomerization in other compounds that are studied. The mathematical analysis is also simpler when using optical activity, as the reverse reaction forms only racemic compound and does not interfere.

Of the two possibilities for hexahydrodemethoxydeoxydesacetylcolchicine (XXVII), XXVIIb has a cinnamyl amine structure (a vinylagous benzylamine), while XXVIIa, in which C_7 is insulated from the unsaturation by a saturated carbon atom (C_{7a}), is more analogous to β-phenylethylamine. The optically-active amines chosen as analogies were d-α-phenylethylamine, d-amphetamine (d-1-phenyl-2-aminopropane), d-2-aminobutane and colchinol methyl ether (XXX). With the exception of the colchinol methyl ether, the amines are the simplest possible compounds containing the necessary features. All contain the nitrogen on the asymmetric carbon atom. α-Phenylethylamine has the benzylamine structure, while amphetamine is a β-phenylethylamine with the phenyl group insulated from the nitrogen-containing carbon atom. The aminobutane was used to determine the effectiveness of the insulation. Colchinol methyl ether was used because, of the available compounds of known structure, it most closely resembles XXVII and probably is the best point of reference available to link the complex ring system of XXVII with the simple aliphatic systems of the other amines.

In theory, at least, the choice of the aldehyde should not matter, as long as the same one is used throughout. p-Chlorobenzaldehyde was chosen because of availability, stability of the benzilidines, a convenient rate* of racemization, and the fact that the chlorine would

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* Although p-nitrobenzilidines gave the fastest rate, undesirable side reactions have been reported (121).

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serve as a useful analytical label.

The benzylidines were prepared by heating the amine and the aldehyde together, either in methanol or without solvent, the reaction apparently be-

ing quantitative. With the exception of N-(p-chlorobenzylidine)-hexahydrodemethoxydeoxydesacetylcolchicine (XXXI), the benzylidines were obtained crystalline or were fractionally distilled. Several attempts to crystallize XXXI gave only oils. Chromatography also failed, the only products isolated being the amine and aldehyde components. The racemization studies on XXXI were done on the crude oil.

Although in the case of the aliphatic compounds, sufficient material was available for large scale runs, the rate runs were all run on a small scale (approximately 100 mg.) in anticipation of the experiments with XXVII and XXX in which only a limited amount of material would be available. It was found that these benzylidines were rapidly oxidized by air in the alkoxide solutions used for the racemizations, even at room temperature, and therefore the experiments were run in evacuated tubes. Aliquots were taken at various times and the rotation was either measured on this solution directly, first diluted with ethanol, or, in those cases in which the solution was too dark for measurement of the optical rotation, the benzylidine was isolated and the specific activity measured.

Previous work (62,64,65,66,120,121,122) has shown that the racemization is first order with respect to the benzylidine. The first order rate constant was calculated as follows: The rate of a first order reaction is given by

$$\frac{dc}{dt} = -kc \qquad (1)$$

where c is the concentration of the reactant at time = t. Integration of (1) yields

$$\log c = 0.434kt + \log c_0 \qquad (2)$$

where c_0 is the concentration at t = 0. Since the concentration is

proportional to the optical rotation (α) when the final rotation is zero, equation (2) can be rewritten

$$\log \alpha = -0.434\, kt + \log \alpha_0 \qquad (3)$$

A plot of log α versus t should give a straight line of slope $-0.434\, k$, and this was the case in those runs in which sufficiently accurate data were obtained.

The results of the rate runs are summarized in Table 2 which gives the first order rate constants at 75°C. For comparison, the constants have been corrected to unit base concentrations, assuming first order dependence. First order dependence on the base concentration follows from the mechanism proposed (62,65,66,120,122) for the isomerization and was found to be approximately the case of N-(p-chlorobenzylidine)-d-2-aminobutane.

Table 2

First Order Rate Constants for the Base-catalysed Racemization of N- p-Chlorobenzylidines at 75°C and Unit Base Concentration

Amine	k	
	Catalyst NaOEt	Catalyst KO-t-Am
d-α-Phenylethylamine	0.8	*
Colchicol methyl ether (XXX)	ca. 0.5	—
d-Amphetamine	<0.0001**	0.2
d-2-Aminobutane	—	0.04
Hexahydrodemethoxydeoxydesacetyl-colchicine (XXVII)	0.006	4

* Too rapid to measure, even at room temperature
** Unchanged after 24 hrs.

It can be seen that, except for LXXIII, the benzylidines fall into two distinct classes: (1) Benzyl amines which are racemized with sodium ethoxide and (2) non-benzyl amines that are unchanged in sodium ethoxide and require potasium t-amylate.* XXXI, although inter-

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* See p. 51 for a discussion of methods for racemizing amines.

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mediate between the two groups, apparently is closer to category (2).

It is difficult to see why, if structure XXVIIb prevailed for the amine, the rate should be two powers of ten slower than it is with colchinol methyl ether and phenylethylamine, while the rates of the last two are nearly the same. Sterically, colchinol methyl ether should be more like XXVIIIb than to phenylethylamine. XXVIIb is a cis-cinnamyl amine, but this should increase the rate over a benzyl amine, rather than decrease it.**

** Compare the dissociation constants of the following acids: Acrylic acid, pK 4.26; benzoic acid, pK 4.17; trans-cinnamic acid, pK 4.44; cis-cinnamic acid, pK 3.96.

On the other hand, it is reasonable that structure XXVIIawould racemize faster than does amphetamine, the experimentally determined factor of 20 between the rates being within reason. XXVIIa contains two groups which, by virtue of their inductive effects, would increase the acidity of the hydrogen on C_7 (compare the factor of 5 between amphetamine and 2-aminobutane). In addition, the inductive effect of the phenyl ring can be transmitted both through C_5-C_6 and the bridge head atoms (C_{12a} - C_{7a}). Also the inductivity of an α-styrenyl system is unknown and could be greater than the sum of its parts. The methoxyl in

ring A should decrease the inductive effect over that of phenyl (121, 122), but this effect is only minor.

The results of the racemization experiments, therefore, indicate that the double bond is in the trisubstituted position. However, this conclusion was drawn from analogies with simple systems which do not necessarily apply to the complicated ring system of the degradation products. Although practically all of the evidence available indicates that this is the position of the double bond, it is all drawn from the same type of analogies and the Δ^{12}-structure for the degradation products, although now on firmer ground, must still be regarded as tentative.

With the aim of obtaining more definitive evidence for the position of the double bond, further reactions were carried out on some of the racemized benzylidines. No further work was done on the benzylidines of phenylethyl amine and colchinol methyl ether, as there is ample work reported in the literature (62,65,65,66,120,121,122) on these types of compounds. Hydrolysis of the equilibrated benzylidines yielded the two carbonyl compounds and the two amines that would be expected from structures XXVIII and XXIX.

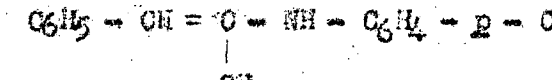
In the case of the racemate of N-(p-chlorobenzylidine)-2-aminobutane, two structures are theoretically possible: The original benzylidine XXVIIIb and the isomeric XXIXb. However, the imine double bond in XXVIIIb is conjugated with the phenyl ring, whereas in XXIXb this is not so. For this reason, the equilibrium mixture would be expected to be almost exclusively XXVIIIb.

To check this postulate, a large scale racemization of the benzylidine was run for about 15 half lives. Only about 30% of the material distilled, the remainder being dark, non-volatile pot residue and is probably polymeric. The distillate had no measurable rotation and the infrared spectrum was very similar to that of the original

benzylidine, although there were significant differences. Also, the ultraviolet spectra were similar. However, the elemental analysis was not correct, the carbon being too high and the chlorine too low. The material was then fractionated and a low boiling fraction was isolated which accounted for about 5% of the material, the remainder (3 fractions) distilling over a 1.2° range and at the proper temperature for N-(p-chlorobenzylidine)-2-aminobutane. The three major fractions analyzed correctly and had the same infrared and ultraviolet spectra as the original benzylidine. The low boiling fraction, however, contained less than half as much chlorine as the benzylidine and more carbon and hydrogen. The infrared spectra were quite different, strong bands in the low boiling fraction appearing at 6.9, 13.2 and 14.4 μ. This material was not investigated any further.

Since XXIXb is not conjugated, the ultraviolet extinction would be much less than that of XXVIIIb, and if any XXIXb were present in the racemate, the extinction would then be less than that of pure XXVIIIb, but this was not the case. Also the conjugated imine peak in the infrared is at 6.10 μ, whereas a non-conjugated imine absorbs at about 6.05 μ. If appreciable amounts of XXIXb were present, then a peak or at least a shoulder at about 6.05 μ should have been observed, but none was observed in any of the fractions. Therefore, the equilibrium mixture is composed of at least 95% and probably over 99% XXVIIIb.

The case of amphetamine is somewhat more complicated. In addition to the two imine forms XXVIIIc (conjucated) and XXIXc (unconjugated), a third form is also possible, namely, the enamine

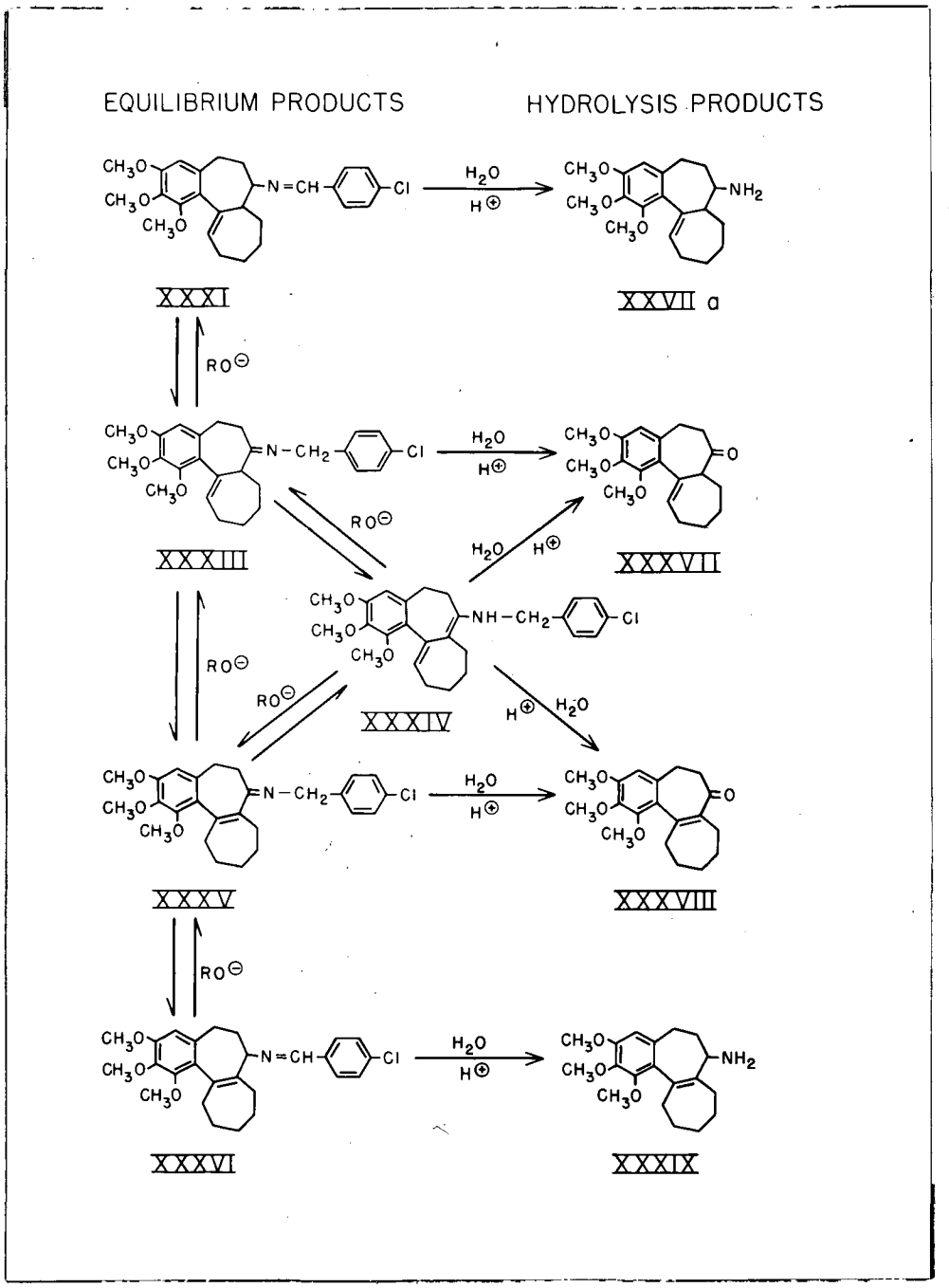


XXXII

due to the acidity of the benzylic hydrogen and the fact that the double bond in this form is also conjugated. What was mentioned above concerning the position of equilibrium between XXVIIIb and XXIXb would also apply to XXVIIIc and XXIXc, but nothing can be said about the equilibrium between XVIIIc - XXIXc and XXXII, except that an appreciable quantity of the latter could be present.

When the total recovered material from a racemization run for ten half lives on N-(p-chlorobenzylidine)-amphetamine was hydrolyzed with acid, 80% of theory of basic material was recovered. This amine fraction was converted to the benzoyl derivative, which was found to contain 2% chlorine. The amide was then twice recrystallized, but still it contained 2% chlorine and the melting point was lower than that for pure N-benzoyl-dl-amphetamine. A carbon, hydrogen, nitrogen and chlorine analysis checked well with that calculated for a mixture of 14% N-benzoyl-p-chlorobenzyl amine and 86% N-benzoylamphetamine. Since hydrolysis of XXXII, as well as XXIXc, would give rise to the p-chlorobenzyl amine, and since very little of XXIXc should be present, the equilibrium mixture probably contains about 14% XXXII, the remainder being XXVIIIc.

N-(p-Chlorobenzylidine)-hexahydrodemethoxydesoxydesacetylcolchicine (XXXI) is even more complex. The five possible structures in the equilibrium are shown on the left hand side of the following page; the products resulting from the hydrolysis of each of these are shown on the right. Structure XXXIII, having the unconjugated imine double bond can be eliminated from consideration as a major constituent. None of the other forms, however, can be discarded this easily, and the equilibrium mixture could be a combination of all four.


EQUILIBRIUM PRODUCTS
HYDROLYSIS PRODUCTS
XXXI
XXVII a
XXXIII
XXXVII
XXXIV
XXXV
XXXVIII
XXXVI
XXXIX

The infrared spectra of XXXI and the racemate are similar, although there are significant differences, such as changes in the relative intensity and position of several bands. Both the conjugated imine peak at 6.10 μ and the conjugated aromatic peak at 6.36 μ were reduced in intensity relative to the intensity of the aromatic peak at 6.26 μ, which indicates a decrease in the amount of conjugation in the molecule. The ultraviolet absorption also indicates such a decrease, the extinction coefficient at the maximum (257 mμ for XXXI and 246 mμ for the racemate) decreasing from 33,000 to 11,000.

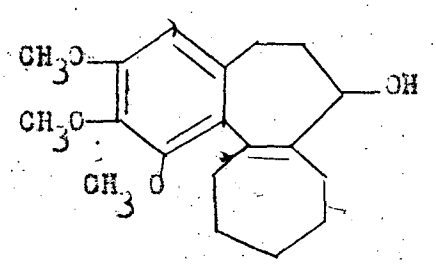
When the racemate was hydrolyzed, over 80% of the non-volatile product isolated was neutral, the remainder being basic. The basic fraction was converted to the amide, which was found to be identical with hexahydrodemethoxydesoxycolchicine (IX), except that it was optically inactive, as determined by infrared and ultraviolet spectra and the rate of reaction with perbenzoic acid.

The neutral fraction yielded a crystalline product which analyzed for a ketone of structure XXXVII or XXXVIII. The infrared showed a peak at 6.02 μ, which is characteristic of a conjugated ketone. It also had ultraviolet absorption maxima at 240 and 302 mμ with extinction coefficients of 13,700 and 3460, respectively. These data show that XXXVIII, in which the double bond is $\Delta^{7a(12a)}$, is the structure. The extinction at 302 mμ is much lower in intensity than is normally observed with such a chromophore, but it can be explained on the basis of a large amount of non-coplanarity between the aromatic ring, the double bond and the carbonyl group. The absorption at 240 mμ is probably due to an α-β unsaturated ketone system with the benzene ring out of the plane (later evidence will

show that the styrene system with the double bond in this position absorbs around 255 mμ). This means that the majority of the material in the racemate has structure XXXV (much of XXXIV is precluded as it does not contain an imine double bond, which is required according to the infrared spectrum). The remainder is either XXXI or XXXVI. A choice between these two still cannot be made, although on the basis of the evidence already discussed, XXXI is preferred.

Now that the ketone XXXVIII is available, with the position of the double bond known, conversion of this compound by rearrangement-free paths into any/several of the colchicine degradation products or an isomer thereof would then settle the question of the position of the double bond. One path would be conversion of the ketone group into an acetamido group to form hexahydrodemethoxydesoxycolchicine (IX), and a second would be formation of octahydrodemethoxydesoxydesacetamidocolchicine (X) by reduction of the carbonyl group to a methylene group.

When the ketone XXXVIII was hydrogenated in a micro-hydrogenation apparatus with palladium-on-carbon in ethanol, one mole of hydrogen was absorbed and a highly crystalline alcohol (XL) was obtained. XL shows the



XL

typical ultraviolet absorption maximum at 256 mμ with log ε_{max} 4.07.

Since the oxygen functions in the ketone and alcohol are allylic, treatment with hydrogen under the proper conditions might be expected to give hydrogenolysis of the carbon-oxygen bond to form $\Delta^{7a(12a)}$-octahydrodemethoxydemoxydesacetamidocolchicine (X). When the ketone was hydrogenated in a micro-hydrogenation apparatus with palladium on carbon in ethanol with a drop of perchloric acid added, hydrogen absorption ceased at 1.4-1.5 moles. However, no unreacted ketone was observed and less than 10% of the alcohol was obtained. When the alcohol was treated under the same conditions, hydrogen was initially absorbed, but later on the volume of the gas began to increase. The reason for the reversal in the system is unknown.

The product from a large scale run was then chromatographed on alumina (elution being accomplished with a solvent that would not remove the alcohol), sublimed and then crystallized from hexane. This product, however, analyzed for 74.4% carbon and 9.1% hydrogen, whereas the calculated values for the expected hydrogenolysis product are 75.5% and 8.7% respectively. The product from another run was chromatographed, sublimed and then carefully fractionally rechromatographed. Only one peak was obtained and the main fraction (8 mg. out of 32) analyzed for 72.5% carbon and 9.2% hydrogen.

Assuming the remainder to be oxygen and that the molecule still contains 19 carbon atoms, the analysis indicates about 3.5 atoms of oxygen per molecule. Formation of a dimer is ruled out on the basis of the ease of sublimation. The only obvious explanations for the analysis are either that the product is a mixture or that the molecule lost some carbon atoms.

Neither product showed any hydroxyl or carbonyl bands in the infrared spectra and the latter product has λ_{max} 281 mμ and log ε_{max} 3.15. This absorption is typical of substituted benzenes, rather than of styrenes. Obviously, the desired product was not formed, and this line of attack was abandoned.

An attempt to form the p-toluene sulfonate (tosylate) of the alcohol

was made. The plan was to then accomplish hydrogenolysis with lithium aluminum hydride. However, no tosylate was obtained when the reaction was carried out at 0°, the alcohol being recovered. No attempt was made to carry out the reaction at higher temperatures for fear that displacement of the allylic tosylate with chloride would occur.

A Wolff-Kishner reduction of the ketone was next attempted, following the mild conditions described by Anderson and Wade (4). As ether cleavage occurs during the reaction, the product was remethylated during the workup. A product was isolated in about 30% yield after chromatography. This material was then sublimed to give a crystalline product which analyzed correctly for octahydrodemethoxydesoxydesacetamidocolchicine (X), but had $\lambda_{max.}$ 256 mμ with log $\varepsilon_{max.}$ 3.80. The infrared absorption spectrum was significantly different from that of the octahydrodemethoxydesoxydesacetamidocolchicine produced via the Hofmann path.

Assuming that the Wolff-Kishner product is the pure $\Delta^{7a(12a)}$ compound, then the low extinction coefficient (6300 as compared with the 12,600 usually observed for the compounds in the degradation sequence) is somewhat surprising. If this is true, then the Hofmann product is the Δ^{12} product. On the other hand, the product could be a mixture of $\Delta^{7a(12a)}$ and Δ^{7} compounds.* If 12,000 is taken as about the correct extinction coefficient for the $\Delta^{7a(12a)}$

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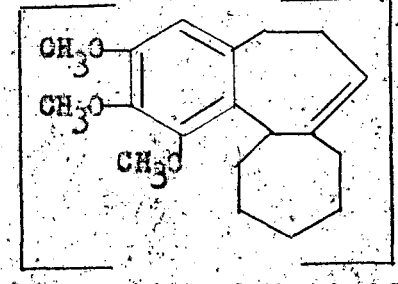
* The Δ^{7} compound



Would be produced by a shift of the double bond of a type that is well known in the Wolff-Kishner reduction of α-β-unsaturated carbonyl compounds.

compounds (compare the 7-hydroxy-$\Delta^{7a(12a)}$ compound (XL) above), then the mixture would contain about 50% of this compound, the remainder being the Δ^{7} product, which would contribute little to the absorption spectrum.

Of the two possibilities, the product being a mixture is the more probable. The ultraviolet extinction coefficient is lower than would be expected. Although the extinction coefficient of a $\Delta^{7a(12a)}$ compound without substitution at C_7 is unknown, one would not expect a hydroxyl group at C_7 to double the extinction coefficient without changing the absorption maximum, especially since the absorption spectrum of the 7-hydroxy compound is so similar to those of the compounds in the Hofmann degradation sequence. The infrared spectrum also indicates that the Wolff-Kishner product is a mixture, as the peaks are broader than they are in the spectrum of the Hofmann product. Also in all of the degradation products (including the 7-hydroxy product (XL)), two sharp peaks are present at about 6.25 and 6.40 μ which are probably due to the conjugated aromatic ring. In the Wolff-Kishner product, however, the 6.40 μ peak is missing, a shoulder appearing in its place. On the other hand, the product crystallized readily and melted over about a two degree range, behavior which is not usually observed with 1:1 mixtures, especially in the colchicine degradation compounds, many of which do not crystallize or do so only with difficulty unless quite pure. However, the Δ^{7} and $\Delta^{7a(12a)}$ compounds could be similar in shape and thus crystallize well together. Therefore, no decision can be made as to whether the Wolff-Kishner product represents a single pure compound or a mixture, and no comparison between this product and the Hofmann product can be made as yet.

There are several other methods that may resolve the problem. If the Wolff-Kishner product represents a mixture of the Δ^{7} and $\Delta^{7a(12a)}$ compounds, then the two components probably would have considerably different

rates of reaction with perbenzoic acid, the tetra-substituted compound ($\Delta^{7a(12a)}$) being the faster. Determination of the rate curve would then show whether or not this is the case, and if so, then the reaction could be stopped when only the one compound had reacted. The epoxide could then be isolated and compared with the epoxide obtained from the Hofmann product.

Another path to remove the oxygen would be conversion of the ketone into a dimethyl mercaptol followed by desulfurization with Raney nickel. One attempt was made to prepare the mercaptol from the 7-keto compound. An oil was obtained, which apparently was the desired product as the carbonyl band at 6.02 μ was no longer present in the infrared spectrum. No further work was done with this material, although this path seems quite promising and should be pursued further.

Another series of reactions that seems promising, on paper, at least, is conversion of the ketone (XXVIII) into the corresponding acetamide through a path that presumably would not effect the double bond. One method of accomplishing this would be formation of the oxime in a basic medium (e.g., pyridine) followed by reduction to the amine (again under basic conditions, such as sodium in alcohol) and then acetylation with acetic anhydride in pyridine. This process would have the advantage that the amide would be more crystalline and less soluble than the desacetamido compound (X) and therefore easier to purify. This amide could then be compared with the racemic amide (dl-IX) produced from the racemization experiment. Identity or nonidentity of the two compounds would then establish the position of the double bond.

In conclusion, the work presented in this thesis, while not as conclusive and definitive as would be desired, has placed the position of the double bond at Δ^{12} in the colchicine degradation products and the structure

of colchicine as in Ia (the keto group at C_9 and the methoxyl at C_{10}) on firmer ground. More work is necessary, though (reactions with 7-keto-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine (XXXVIII) will probably be the most fruitful approach) before the question of structure will be unequivocally settled.

Racemization of aliphatic amines. The racemization of primary, aliphatic amines in which the nitrogen is connected to the asymmetric carbon atom is a subject that is conspicuous by the paucity of information in the literature. This same statement would also hold true for all types of amines, but this discussion is limited to primary amines. Also, the discussion is limited to those cases in which there is no neighboring-group interaction with other parts of the molecule which would make the racemization especially facile. A well-known example of this interaction is the racemization of α-amino acids (84) under the influence of aqueous acids, which apparently involves protonation of the carboxyl group.

The only method to appear in the literature which claims generality is a patent (7, 125) in which the amine is treated with a nickel catalyst at 100-300° for several hours. Although good yields of recovered, partially racemized amines are reported, these conditions are quite vigorous and might, in fact, be too vigorous for molecules that are more sensitive than the ones reported, which contained only carbon, hydrogen and the amine group.

Williams (137) attempted to racemize colchinol methyl ether (XXX) by heating it with an imine in the presence of a strong base. The hope was that a reversible hydrogen transfer between the amine and the imine would take place, much as it does between an alcohol and a ketone in the presence of base. Any colchinol methyl ether that had been converted to the imine

and then back to the amine form would, of course, be racemic. Apparently the hydrogen transfer did not take place, as only the unracemized amine was recovered. Williams was able to effect the racemisation, however, by treating the benzylidine of the amine with base and then hydrolysing to obtain the racemic amine (112).

Several workers (62, 65, 66, 97, 130) have racemized amines by treating the benzylidines with sodium ethoxide. In these cases, though, the object was to study the mechanism of the equilibration of the benzylidines, rather than to obtain racemic amines. Also, their work was done with benzylic amines, and therefore, is not necessarily applicable to other types of amines. However, work reported in this thesis (p. 39) has shown that if the base is strong enough, benzylidines of nonbenzylic amines can easily be racemized.

However, the recovery of racemized amines can be quite low (e.g., 2-aminobutane) and the product can be contaminated with considerable amounts of difficult-to-remove impurities (e.g., amphetamine). The low recovery is probably due to the formation of polymers and possibly the recovery can be improved by the proper choice of conditions, such as solvent, base and temperature. Changing the carbonyl component to one that would increase the rate of racemization (e.g., p-nitrobenzaldehyde or benzophenone) and thereby reducing the reaction time, might result in less polymerization. The main impurity is the amine formed from the carbonyl moiety. The proper choice of the carbonyl compound would give an amine that could be easily separated from the desired product. For example, in the case of amphetamine, the p-chlorobenzyl amine impurity is very difficult to remove, but if benzophenone had been used, then the benhydryl amine would have been easy to remove from the amphetamine by a simple distillation.

No other methods or racemizing amines were found in the literature, and even these methods will not work if the asymmetric carbon atom is tertiary. Of course, any series of reactions that gives a carbonyl (or similar doubly bonded carbon atom) which can then be converted back into the amine will, in effect, accomplish a racemization. However, such methods are usually rather indirect and are beyond the scope of this discussion.

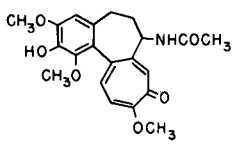
SYNTHETIC STUDIES

Introduction -- Literature survey of approaches to the problem of the synthesis of colchicine. Work on the synthesis of colchicine can be divided into two categories: (1) Conversion of closely related degradation products back to colchicine; and (2) synthesis of the colchicine ring system from simpler, monocyclic systems.

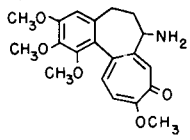
To date, the only published work dealing with the synthesis of colchicine from degradation products has been the remethylation and reacetylation of the corresponding demethyl and deacetyl compounds. Colchicine (VI) when treated with diazomethane yields a mixture of colchicine (Ia) and isocolchicine (Ib), which were first separated by Sorkin (124). Raffault, et al., (105) applied this reaction (using diazomethane-C^{14}) in the synthesis of colchicine-10-methoxyl-C^{14} and isocolchicine-9-methoxyl-C^{14}. They also prepared colchicine-2-methoxyl-C^{14} by treatment of 2-desmethylcolchicine (XLI) with diazomethane-C^{14}, colchicine-acetyl-1-C^{14} from trimethylcolchicinic acid methyl ether (XLII) and acetyl chloride-1-C^{14}, and trimethylcolchicinic acid methyl-C^{14}-ether and the isomeric 9-methyl compound (XLIII) from diazomethane-C^{14} and trimethylcolchicinic acid (XLIV). These reactions, although perhaps necessary for the eventual total synthesis of colchicine, are not very exciting or productive as far as the synthetic problem is concerned.

Work directed towards the formation of the colchicine ring system itself can be characterized first by the order in which the three rings are attached or formed. In almost all attempts, as might be expected, the aromatic A ring was the starting point, although in many of the model experiments this ring contained no methoxyl substituents.

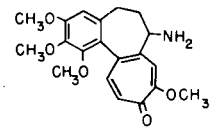
One method of attack was to attach substituents to a benzosuberone and



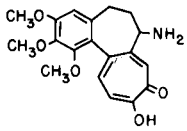
XLI



XLII

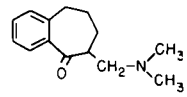


XLIII

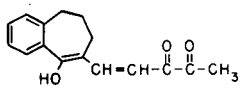


XLIV

XLV



XLVI



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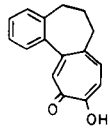

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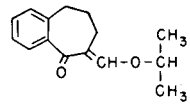

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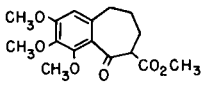
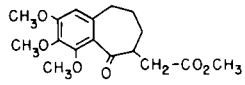


L



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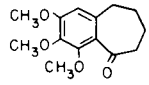

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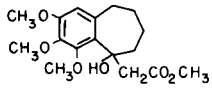

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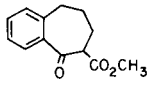

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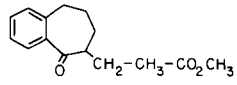


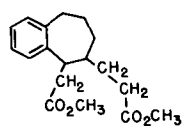
LV

then close the C ring. Tarbell and co-workers (93, 130) reacted benzosuberone (XLV) with dimethyl amine and formaldehyde to form 6-N,N-dimethylaminomethylbenzosuberone (XLVI). XLVI was then reacted in low yield with biacetyl monoketal to form XLVII, the plan being to then cyclicize XLVII to the substituted tropolone, XLVIII. An alternate path to XLVII which gave better yields was to react XLV with formaldehyde and then isopropyl iodide to form 6-isopropoxymethylenebenzosuberone (XLIX), which was then reacted with biacetyl monoketal to give XLVII. All attempts at cyclization of XLVII failed, however.

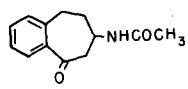
Gardner and Horton (43) reacted methyl 2,3,4-trimethoxybenzosuberone-6-carboxylate (L) with methyl bromoacetate to give methyl 2,3,4-trimethoxybenzosuberone-6-acetate (LI). Attempted Reformatsky reactions between LI and methyl bromoacetate or methyl-γ-bromocrotonate or a Darzens reaction with methyl chloroacetate failed. This is in sharp contradistinction with the reaction between 2,3,4-trimethoxybenzosuberone (LII) and methyl bromoacetate (43), which easily gives the expected product (LIII).

Anderson and Greef (3) were able to affect the Reformatsky reaction with a substituted compound similar to LI, but which lacked the methoxyl groups. Methyl benzosuberone-6-carboxylate (LIV) was condensed with methyl β-bromopropionate to yield methyl benzosuberone-6-propionate (LV). LV was then reacted with methyl bromoacetate to give, after dehydration and hydrogenation, a low yield of LVI. Although ring closure by an acyloin condensation would lead to the colchicine ring system, no experiments along this line were reported by the authors. In view of Gardner and Horton's experience with the methoxylated compounds, this line of approach probably will not be very fruitful, anyway.

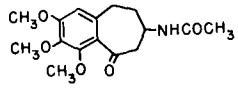
Horton and Thompson (60) have synthesized 7-acetamidobenzosuberone (LVII), which contains the amide group as it is in colchicine. They con-



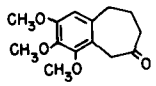
LVI



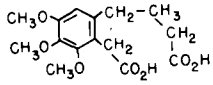
LVII



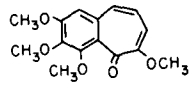
LVIII



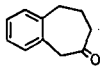
LIX



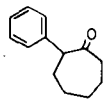
LX



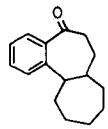
LXI



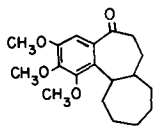
LXII



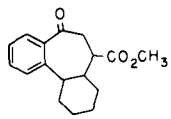
LXIII



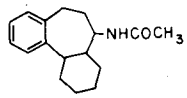
LXIV



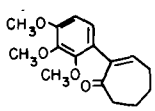
LXV



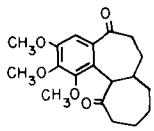
LXVI



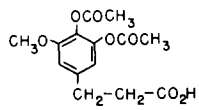
LXVII



LXVIII



LXIX



LXX

densed 2-phenyl-1-bromoethane with diethyl acetamidomalonate. The product was then hydrolyzed and decarboxylated to give γ-phenyl-α-aminobutyric acid. After protection of the amine group as the phthalimide, the chain was extended with an Arndt-Eistert reaction. Then the imide was hydrolyzed, the amine acetylated and the product ring closed to give LVII. However, when an identical series of reactions was tried with 2-(2,3,4-trimethoxyphenyl)-2-bromoethane, none of the corresponding aminovaleric acid could be obtained from the Arndt-Eistert reaction. Even if conditions could be worked out to get the desired 2,3,4-trimethoxy-7-acetamidobenzosuberone (LVIII), on the basis of the work of Gardner and Horton (43), this compound would probably be of little value as a precursor to colchicine compounds.

Rapoport and Campion (103), in an effort to circumvent the reduced activity of the carbonyl in the substituted benzosuberones, have prepared 2,3,4-trimethoxybenzocycloheptene-6-one (LIX). They started with 3,4,5-trimethoxybenzaldehyde and after several steps prepared γ-(2-carboxymethyl-3,4,5-trimethoxyphenyl)-butyric acid (LX). This compound, as the dimethyl ester, was then cyclized by the Dieckmann procedure to give LIX. LIX has also been prepared (41) by the reduction of purpurogallin tetramethyl ether (LXI). Also, the corresponding unmethoxylated compound benzocycloheptene-6-one (LXII) has been prepared (99), presumably as a model compound, starting with the peracetic acid ring-opening oxidation of β-naphthol and going through intermediates similar to those used by Rapoport and Campion (103). However, no further work on these compounds has been reported, except by Campion (17) who treated the sodium enolate of LIX with γ-bromobutyryl nitrile, but did no further work on the product.

Another approach to the problem is attaching a preformed C ring to ring A and then building up and closing ring B. Gutsche (51) treated phenyldiazomethane with cyclohexanone to give 2-phenylcycloheptanone (LXIII).

This ketone was then reacted with bromoacetate in a Reformatsky reaction, dehydrated, and hydrogenated to give a substituted acetic acid. This acid was then chain-extended and ring closed to give 5-keto-5,6,7,7a,8,9,10,11, 12,12a-decahydrobenzo[a]heptalene (LXIV). This represents the first recorded synthesis of the colchicine ring system. Gutsche and Flemming (52) synthesized the corresponding trimethoxy compound (LXV), starting with 2,3,4-trimethoxyphenyl magnesium iodide and cycloheptanone. The alcohol formed was dehydrated, the olefin reacted with perbenzoic acid and the glycol rearranged with acid to give a ketone corresponding to LXIII. This ketone was then converted to XLV through a sequence of reactions similar to that used for LXIV. This last sequence then gives the colchicine ring system containing the three methyoxyls in ring A, which, although still far removed from the goal, is the closest anyone has come as yet to the synthesis of colchicine.

Gutsche and Seligman (53) have synthesized ring B containing the acetamido group as present in colchicine. A Stolbe condensation between 2-phenylhexanone and dimethyl succinate gave a substituted succinic half ester, which was ring closed to the keto ester, LXVI. Reduction of the keto group, Curtius degradation, and acetylation of the resulting amine then gives LXVII, which contains the complete B ring. Although the authors did not mention anything about attempting the Stolbe condensation with phenyl-cycloheptanone or with methoxylated compounds, there does not seem to be any a priori reason why these also would not work to give the methoxylated A ring and the seven-membered C ring at the same time the acetylated B ring is formed.

Ginsberg and Pappo (47) condensed phenyl lithium and 2,3,4-trimethoxy-phenyl lithium with cyclohexanone and cycloheptanone and then eliminated water from the alcohols formed to give phenyl- and trimethoxyphenyl-substituted cyclic olefins. The olefins were then treated with nitrosyl chloride and then

with pyridine to eliminate hydrochloric acid. The oximino group was then hydrolyzed to give substituted cyclic enones (e.g., LXVIII). These last three steps worked for all compounds, except in the one case that would lead to the colchicine A and C rings, trimethoxyphenylcycloheptene. Another attempt (46) to prepare LXVIII was the reaction of 2,3,4-trimethoxyphenyl magnesium bromide with cycloheptan-1,2-dione and then elimination of the hydroxyketone formed. However, the elimination step did not go, although it did if the methoxyl groups were absent. LXVIII was prepared in small yield by the selium dioxide oxidation of 1-(2,3,4-trimethoxyphenyl)-cycloheptene. A Michael condensation of LXVIII with diethyl malonate, followed by hydrolysis, decarboxylation and ring closure gave the diketone, LXIX.

Boekelheide and Pennington (12) connected rings A and C using the von Pechmann coumarin condensation. Ethyl cyclohexanone-2-carboxylate was condensed with the pyrogallol derivative LXX to give the substituted coumarin LXXI. Opening of the coumarin ring with dimethyl sulfate and esterification of the resulting dibasic acid, followed by Dieckmann ring closure gave the ketone LXXII. The plan was to then convert the carbonyl group into an acetamido function. However, when the above sequence of reactions was tried with ethyl cycloheptanone-2-carboxylate, the coumarin condensation, which should have produced LXXIV, would not take place. An alternate route to LXXIV was to condense ethyl cycloheptanone-2-carboxylate with pyrogallol to give LXXIV in good yield and then to add the propionic acid residue. However, using the cyclohexanone analog of LXXIV was a model compound, the authors found that the compound would not substitute, except in the case of the Claisen vinyl ether rearrangement which gave LXXV. However, this olefin could not be converted into the desired acid.

In all of the above references, ring C, when formed, was not tropoloid.*

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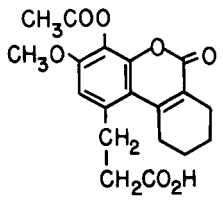
* In the case of Ott and Tarbell (95, 130), the proposed sequence would have produced a tropoloid C ring, had it worked.

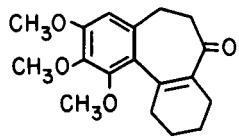
Two groups (90, 92, 96, 129) started with a tropolone, hoping to add the rest of the colchicine molecule to it. Tarbell (129) condensed o-nitrobenzaldehyde with o-carboxy-4-carbomethyltropolone (LXXVI) to give the styryl tropolone, LXXVII. Reduction of the nitro group followed by treatment with nitrous acid and copper gave no ring closure, but only resulted in tars. This failure of the ring closure is attributed to the two rings being in a trans arrangement, a fact which is supported by the infrared spectrum. In addition to doing the above condensation, Nozoe (90, 92, 96) condensed phenylacetaldehyde with LXXVI to get, after hydrolysis of the lactone which is formed and elimination of water, a substituted propylene (LXXVIII). No further work has been reported along these lines.

From the above discussion several observations might be made. Replacing a cyclohexane ring with a cycloheptane ring often unpredictably alters or even stops a reaction; replacing a phenyl with a trimethoxyphenyl also often does the same. In only three isolated instances (12, 53, 60) is any attempt made to introduce the acetamido group as present in colchicine. In all other cases, it is not immediately evident as to just how this might be accomplished. Only two groups (90, 92, 96, 129) have made any attempt at all at making ring C tropoloid, although presumably the other plans (3, 17, 41, 43, 60, 99, 103) which would form ring C last would leave an oxygen function at C_9 or C_{10}. In no case, however, has any attempt been made to include both the acetamido group and the ring C oxygen function, which would be necessary for a synthesis of colchicine.

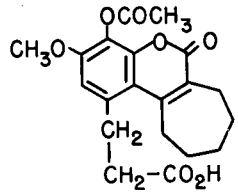
In view of the large number of reactions which gave good results with model compounds but failed to work when applied to the trimethoxyphenyl and cycloheptane systems, the use of "model compounds" is of limited, and even doubtful, usefulness. Even in cases where the colchicine ring system is synthesized (including the methoxyl groups), but where no provision is



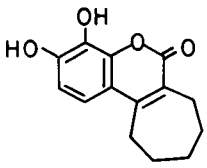
LXXI



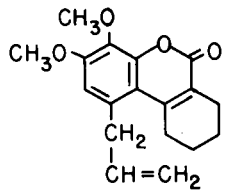
LXXII



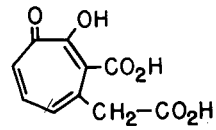
LXXIII



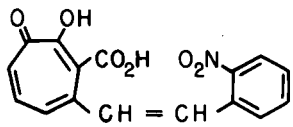
LXXIV



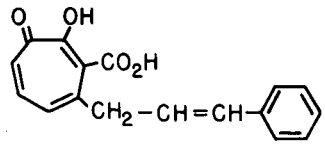
LXXV



LXXVI



LXXVII



LXXVIII

made to include the acetamido group or the ring C oxygens, the inclusion of these groups could, and in all probability would, again alter the course of the reactions to such an extent that one would be faced with a new problem.

In short, one might say that the synthesis of colchicine, and even of colchicine degradation products which still contain the colchicine ring system, is a difficult problem that is still far from being solved. Much work has been expended on model compounds and probably much more will be done before enough is known about this unique ring system to be able to actually synthesize colchicine. Until this is done, though, much useful and necessary work can still be done towards solving the problem by starting with degradation products which still contain the necessary amide and oxygen functions.

Until the work of Rapoport and co-workers (109, 113, 114), no suitable derivative was available in sufficient quantity for such a study. As a result of their work, tetrahydrodemethoxydesoxycolchicine (VIII) is available in quantities limited only by the amount of colchicine available (30-40% yield from colchicine) and the attempted synthesis of colchicine from this compound is the subject of the rest of this report.

Structure of tetrahydrodemethoxycolchicine (VIII) relative to that of colchicine (Ia). Before any synthetic studies can be initiated on tetrahydrodemethoxycolchicine (VIII), it must first be shown that the structural features present in VII (such as ring size and position of the carbonyl) represent the corresponding features in colchicine. The intermediate product in the degradation of colchicine to VIII is N,N-dimethylaminocolchicide (VII), which is formed by the action of dimethylamine on colchicine. VIII is then produced from VII by a hydrogenation and hydrogenolysis using platinum oxide and palladium on carbon as catalysts. Because of discrepancies between the work at this laboratory and other work reported in the literature (42, 54, 118), this intermediate has been reexamined.

Ewins, et al., (42) reported a melting point for VII of 204-206°, but no other physical constants or analysis were given. Santaby (118) reported a melting point of 205-207° and $[\alpha]_D^{21}$ +510° (c. 0.79, chloroform), but no analytical data were given. Hartwell, et al., (54) reported a melting point of 203-205° (foams at 145°), and an analysis for nitrogen and methoxyl was in good agreement with the calculated values. Repoport and Williams (113), however, reported from this laboratory a melting point of 174-176° (later value 178-9°) and $[\alpha]_D^{25}$ +69.4° (c. 1.03, ethanol) which was unchanged after recrystallization from various solvents, sublimation or chromatography on alumina. They also reported an analysis for carbon, hydrogen, nitrogen and methoxyl which was in good agreement with the calculated values. This compound formed a picrate which also analyzed correctly. Three other workers (109, 114) have since repeated Williams work with the same results. Also Lavigne (111) in this laboratory has prepared an identical product by the reaction of dimethylamine with methylthiocolchicide (LXXIX).

The only obvious differences in the procedures used by the various workers were the source of the dimethylamine, the molar ratio of amine to colchicine, the use of an acid and base extraction in the workup by us, and the recrystallization solvent. In order that our procedure would be more nearly the same as those of the other workers, these differences were eliminated as much as possible.

The original source of the dimethyl amine was cylinder dimethylamine supplied by the Matheson Company and claimed by the manufacturer to be 98% dimethylamine (neither the method of determining this value nor the nature of the other 2% was stated). In order to get a purer sample of the amine, Eastman crystalline dimethylamine hydrochloride was recrystallized twice from absolute ethanol with approximately a 50% recovery each time. The free amine was then liberated with potassium hydroxide and absorbed in methanol. The use of this solution yielded a compound which had a melting point of 179-180°, but in about the same yield as when the cylinder amine was used. The main effect of using the purified amine was to give a higher yield of material melting above 178° in the first crystallization than when the cylinder amine was used, but the total yield (75%) remained the same. However, in the last run made, the yield of crystalline product was 85%, but whether this increase was due merely to the increased experience of the operator is not known.

The original molar ratio of amine to colchicine used was 3 to 1. Reduction of this ratio to 1.25 to 1 resulted in no change in the product.

It is conceivable, though unlikely, that the treatment at room temperature with acid and base could have changed the product. In order to test this, the procedure of Ewins (42), which used no acid or base extraction, was followed as closely as possible. Again no difference in the

product was observed. In Ewins' procedure, the crystallizing solvent was ethyl ether-light petroleum, but our product was only sparingly soluble in ether and even less so in a mixture of ether and pentane.

Recrystallization from ethyl ether, acetone, methyl isobutyl ketone, xylene, ethanol, or ethyl acetate gave identical products with no change in the melting point. It seems very unlikely that an impurity would co-crystallize with our compound in a constant amount from each of the widely different solvents, so as to cause a depression of the melting point from 205° to 179° each time. As N-methyl aminocolchicide could be an impurity,* a mixed melting point determination of methylaminocolchicide (m.p. 172-174°, reported mp. 173-174° (43), 176-178° (118), 230-232° (54)) and VII was made; a depression of over 20° was observed.

From the fact that the melting point did not change after recrystallization from widely different solvents, chromatography or sublimation, it seems highly probable that our material is of high purity. Also chromatography on filter paper impregnated with alumina (29, 30) using benzene-ethanol as the developing solvent gave only one spot detectable by ultraviolet absorption, indicating that only one compound was present.

In order to determine the purity in some way other than recrystallization from various solvents to constant melting point, a solubility analysis (57, 73, 75, 86, 145) was attempted. When accurate data is obtained, this method is considered to be one of the most reliable methods for the determination of purity. In this case, however, accurate data was not obtained, the experimental points scattering more than would have been predicted from the accuracy of the volume and weight measurements. This is probably due

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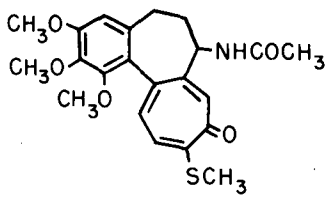
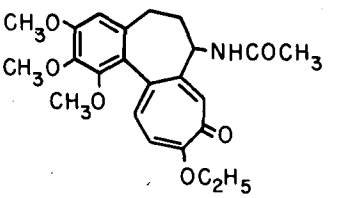
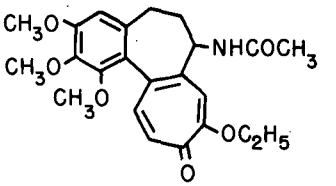
* Unpublished work of J. Lavigne has shown that methylamine is present in the cylinder dimethylamine by the isolation of N-methylaminoisocolchicide from the reaction mixture of isocolchicine and cylinder dimethylamine.



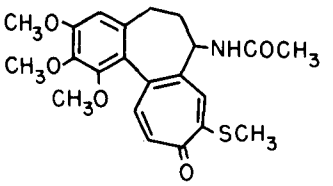
LXXIX



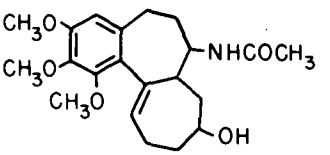
LXXX



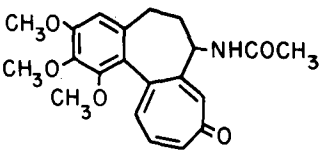
LXXXI



LXXXII



LXXXIII



LXXXIV

to the failure of the system to reach equilibrium in the time allowed (3 days) due to the extreme slowness with which this compound dissolves in and crystallizes out of various solvents. For this reason no conclusions regarding the purity can be drawn from this attempt. In order to get more accurate data it would be necessary to allow the solutions to equilibrate for much longer periods of time before analyzing them. It is felt that the information gained from more determinations of this nature would not be worth the amount of time spent in acquiring the data.

During the course of these investigations, the following observations were made on the optical rotation of various samples of dimethylaminocolchicide:

Table III

Sample		95% Ethanol			Chloroform			Remarks
No.	m.p.	$[\alpha]_D$	Temp.	c.	$[\alpha]_D$	Temp.	c.	
12-1	176-7°	+ 114°	--	1.02	+ 452°	--	1.03	Produced from cylinder amine and dried at 40° at aspirator pressure.
12-1a	176-7	+ 64.5	23°	1.01	+ --	--	--	12-1 dried at 100° at less than 1 mm. pressure for 18 hrs.
20-2	177	+ 105	24.5	1.01	+ 311	24.5	1.00	Similar to 12-1 but a different run.
24-2	177-8	+ 69	25	1.00	+ 380	25	.79	Produced from purified amine and dried at 100° and than 1 mm. pressure.
26-12	179	--	--	--	+ 483	--	1.00	Similar to 24-2 but different run and 2 months old.
29-12	179	+71.5	25	1.01	+ 463	24	1.00	26-12 redried at 100° and less than 1 mm. pressure.
29-24	179	--	--	--	+ 481	23	1.06	29-12 recrystallized from ethyl acetate and air dried at room Temp.
29-29	179	--	--	--	+ 508 + 483 + 465	17 23 27.5	1.04 1.03 1.00	29-12 recrystallized from ethyl acetate and dried at 100° and 1 mm. pressure.

Taking into account the large temperature coefficient (-4°/°C.), the values in the last row of the table (which are believed to be the best values) are in good agreement with the value reported by Santavy (118) $[\alpha]_D^{21}$ + 510 ± 10°). The reason for the large variations in the values is unknown, but it is evident that the optical rotation is very sensitive to factors which are as yet unknown.

Our product (VII) can be hydrolyzed to colchiceine (IV) (114) in 60% yield, thus showing that the carbon skeleton has remained intact and that the positions of substitution are the same as it is in colchicine, except that the carbonyl could now be at C_{10} and the amino group at C_9 (the iso-colchicide structure, XXIV).

There are two possible paths by which a reagent can react with a tropolone methyl ether (excluding ring contraction to benzenoid compounds): (1) Direct displacement of the methoxyl group by the substituting reagent and (2) attack at C_1 (i.e., the carbonyl group), the carbonyl oxygen being displaced to C_2 which then displaces the methoxyl. The products from the two paths are isomeric, as only path (2) involves a rearrangement (i.e., an interchange of the two groups). Most examples (see (100) for references) follow path (1), and only one case has been reported (1) in which path (2) appears to operate. In two other examples (59, 134, 135) both isomers were isolated. The ethanolysis of colchicine with p-toluene sulfonic acid monohydrate (59) yields, besides the expected ethoxy colchicide (LXIV), a small amount (5%) of ethoxyisocolchicide (LXV). Treatment of colchicine with methyl mercaptan and p-toluene sulfonic acid monohydrate not only gives methylthiocolchicide (LXXIX), but also about 5% of the isomeric methyl-thioisocolchicide (LXXII) (135,136). In both these cases water and acid were present, and the iso compound could have been formed by first an hydrolysis to colchiceine (IV) rather than by operation of mechanism 2.

Even in these cases, though, the major product was the one produced by direct displacement.

All substitutions by ammonia, amines, hydrazine, mercaptans (anhydrous), etc., apparently follow the direct displacement path. This is shown (1,2, 95, 111) by the demonstration that when the same compound can be obtained by one, two or three successive replacements, the same product results in every case. For example, VII is prepared either directly from colchicine or by a two-step process *via* methylthiocolchicide (LXXIX). This result cannot be explained if the substituent changes place with the the carbonyl oxygen in any of the steps.

Colchicine, being a tropolone methyl ether, should then also follow the direct displacement mechanism when treated with amines. Further evidence (54, 59, 74, 111, 133, 134, 135) is available from a comparison of some of the physical properties of the various derivatives in both series. The products from the reactions (including VII) fall into two groups; all those obtained from colchicine have, like the parent compound, lower negative specific rotation, slightly higher main maxima in the ultraviolet and higher potency in biological tests than isocolchicine and its derivatives, a regularity suggestive of the configurational correspondence expected from the direct replacement mechanism. Also, compounds in the iso-series mutarotate (74, 104, 133, 134), while those in the normal series do not. Perhaps the most direct evidence is the fact that hydrogenation of VII* (and also of VIII gives the same compound, hexahydrodemethoxycolchicine (LXXIII), as does hydrogenation of colchicine (16, 128) and colchicide (LXXIV) (111).

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* It has been shown (2) that hydrogenation of a dimethylaminotropone gives hydrogenolysis of the dimethylamino group without rearrangement of the carbonyl function.

It thus seems safe to say that both the purity and identity of our product has been determined, and that the position of the carbonyl in N,F-dimethylaminocolchicine (VII), and therefore also of tetrahydrodemethoxycolchicine (VIII), is the same as it is in colchicine (Ia). The difference in the melting point of our product (VII) and that of the products of the other workers (42, 54, 113) is probably due to dimorphism.

Synthetic Experiments. Several methods for the synthesis of tropolones are available. Inasmuch as an excellent and complete review on tropoloids (including synthetic methods) has been published recently (100), no effort will be made in this report to cover the literature on this subject, except where directly applicable to the present problem.

The starting material for this study, tetrahydrodemethoxycolchicine (VIII), is a substituted, unconjugated cycloheptenone, and methods that have been used to convert cycloheptenones to tropolones should be applicable here. It must be remembered, however, that because of the complexity of the rest of the molecule, VIII certainly is not a typical cycloheptenone and any extrapolations made from the simpler monocyclic systems must be made with reservations.

Perhaps the simplest and best method available for making tropolone is the treatment of cycloheptanone with bromine to give mainly 2,4,7-tribromotropone (39). This material is then transformed into a mixture of dibromotropolones with potassium acetate. With a final hydrogenolysis of the remaining bromine atoms, this process gives up to a 45% yield of tropolone from cycloheptanone.

The bromination of VIII was attempted following Monroe's procedure (91,93), which consists of adding bromine to the compound in acetic acid in the cold, and then heating on the steam bath to eliminate hydrobromic acid. VIII reacted immediately with bromine to give a light orange-red solution. When the solution was heated, however, it soon turned quite dark red and a black precipitate was deposited. This precipitate contained 25% bromine. As bromo tropones are colorless or yellowish, it is evident that this precipitate is not the desired product. Probably what happened was that the liberated hydrobromic acid reacted with the methoxyl

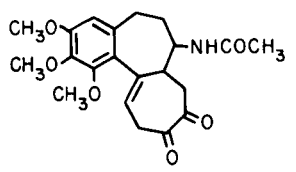
group liberating phenolic material which then oxidized or polymerized to give the insoluble material. This route therefore is not satisfactory.

Several groups (see (100) for references) used a 1,2-cycloheptanedione as an intermediate, which was converted to the tropolone with bromine, N-bromosuccinimide or palladium. One of the most used methods of converting a ketone into a diketone is reaction with selenium dioxide. This is the only reagent that has been used in the monocyclic tropolone series and has also been used with moderate success for the synthesis of benztropolones (18, 19, 27). Selenium dioxide, however, is often not a specific reagent for carbonyl groups (see (107) for a review of this reagent), as it will also oxidize allylic and benzilic positions and in some cases (39, 127) introduces a double bond rather than an oxygen.

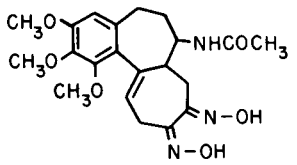
A more indirect, though more specific, route would be oximination of the ketone to an oximinoketone (XVI) followed by hydrolysis to the diketone (LXXV). This procedure has been applied in the benztropolone series (11, 18, 19), but has not yet been applied to other cases, presumably because selenium dioxide, which gives excellent yields, is easier and more direct.

Several methods are available for the oximination of ketones, and these have recently been reviewed (132). Although acid catalysis is by far more common in the literature than basic catalysis, it was felt that acidic conditions probably would lead to undesirable side reactions (e.g., nitrosation of the amide group), and that basic conditions would be more likely to succeed.

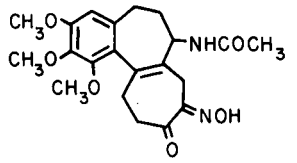
When VIII was reacted with an equal molar amount of n-butyl nitrite and a slight excess of potassium t-butoxide in t-butyl alcohol, a crude base soluble fraction was obtained in 95% yield. Recrystallization of



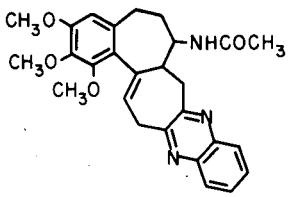
LXXV



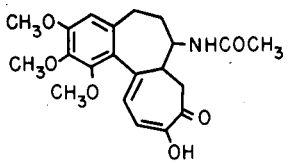
LXXVI



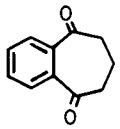
LXXVII



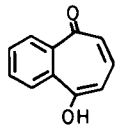
LXXVIII



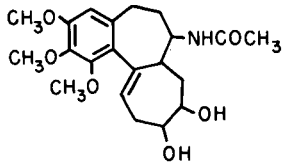
LXXIX



LXXXI



LXXXII



LXXXIII

this material gave a single compound (XVI) in 70% yield. XVI gave a dioxime (LXXVI) on reaction with hydroxylamine.

It might be well to stop at this point and speculate on the structure of XVI. The structure of tetrahydrodemethoxycolchicine (VIII), although not completely known, is probably either XVIIIa or XVIIIb (see page 12). The fact that only one oximinoketone is isolated and in high yield plus the fact that a second oximino group is much more difficult to introduce (p.13) indicates strongly that the two positions alpha to the carbonyl differ markedly in reactivity (possibly due to steric effects). If structure XVIIIa prevails for the ketone, than C_{10} is certainly less hindered than C_8 and the structure of the oximino ketone would be expected to be XVI. If structure XVIIIb is correct, then a choice between C_9 and C_{11} is more difficult to make, although it appears that C_9 would be more favored. If reaction did occur at C_9, then the oximinoketone would be represented by LXXVII. In either case, if our ideas are right concerning the position of reaction, the two functional groups in ring C are on the same carbon atoms as are the oxygen functions in colchicine, a condition which is certainly necessary if the synthesis is to succeed.

Having acquired a sufficient quantity of the oximino ketone, XVI, the next step is the conversion of XVI to the diketone LXXV. Oximinoketones have been hydrolyzed to the diketone with hydrochloric acid and formaldehyde (11, 13), but these strong acid conditions appeared to be rather drastic. Of the various methods of hydrolyzing carbonyl derivatives, an oxime interchange with pyruvic acid in aqueous acetic acid (56, 141) seemed to be the mildest.

Accordingly, oximinotetrahydrodemethoxycolchicine (XVI) was refluxed in 50 volume-percent aqueous acetic acid containing 200 mole percent of pyruvic acid. In the exploratory, small scale runs, aliquots were taken at various times for determination of the ultraviolet spectra. The original solution showed a maximum at 252 mμ. As the reaction proceeded, this peak diminished in intensity and a peak at about 300 mμ appeared. This new peak reached its maximum intensity after about 8 hours, and then remained constant after 25 hours. As the hydrolysis is apparently complete in 8 hours, all further runs were stopped after 8 hours of refluxing.

After the reflux time was over, the hydrolysis solution was evaporated, the residue taken up in benzene and extracted with acid and alkali. The benzene was then lyophilized to give a yellow, amorphous powder. A carbon, hydrogen, nitrogen and methoxyl analysis agreed well with the calculated values for dihydrocolchicine (LXXV). This material could not be crystallized or chromatographed on alumina. Treatment with hydroxylamine gave the same dioxime (LXXVI) as was obtained from oximinotetrahydrodemethoxycolchicine (XVI). Reaction of LXXV with o-phenylenediamine (a reaction that is characteristic of 1,2-diketones) gave the quinoxaline LXXVII. High purity of LXXV is indicated by the fact that chromatography of a complete reaction mixture between LXXV and o-phenylenediamine gave only one peak (the quinoxaline) in over 80% yield.

Dihydrocolchicine (LXXV) differs from colchicine (VI) by only two hydrogen atoms. However, the removal of these two hydrogens proved to be more difficult than was originally anticipated. Several methods are available for converting diketones directly to the corresponding tropolones. All these were tried including a couple that have not been used for tropolone synthesis, but in none of the cases could any colchicine be isolated.

Palladium on carbon in boiling trichlorobenzene gave good results in the benztropolone series (11, 22, 27), but gave only tars in an attempted tropolone synthesis (88). Application of this method to LXIV resulted in most of the dihydrocolchiceine being recovered. When colchiceine was treated under the same conditions, 50% was recovered. If any colchiceine had been formed, some of it should have been detected. Any attempt to increase the rate of dehydrogenation by raising the temperature would probably result in even more destruction of any colchiceine that might be formed.

Treatment of 2,4-cycloheptadienone (127) and 2-phenyl-2,4-cycloheptadienone (39) with selenium dioxide gave the corresponding tropone directly, the selenium dioxide acting purely as a dehydrogenation agent. The enol form of dihydrocolchiceine (LXXV) is a hydroxycycloheptadienone and selenium dioxide might be expected to effect a dehydrogenation of this compound. However, when LXXV was heated with selenium dioxide, no selenium precipitated and no colchiceine was detectable. Colchiceine itself is slowly destroyed by selenium dioxide, but the reaction is slow enough (half life approximately 18 hours) so that any colchiceine produced would have been observed.

Two reagents (mercuric acetate (119) and silver carbonate (111a)) which have not been used before for tropolone synthesis and one (chloranil (28)) which was previously unsuccessful were also tried. When LXXV was refluxed with mercuric acetate in dioxane-acetic acid (119), the ultraviolet absorption due to LXXV decreased, but no increase in extinction appeared in the region of 350 mμ. When LXXV was refluxed with silver carbonate in benzene (111a), no change in the appearance of the silver carbonate occurred (in the oxidation of codeine to codeinone (111a), the

greenish silver carbonate turns black), and no material could be obtained with an absorption around 350 mμ. LXXV was refluxed with chloranil in xylene, but the trace of base soluble material did not give a color with ferric chloride.

In one case in the literature (28), a substituted cycloheptanedione was dehydrogenated with iodine in boiling nitrobenzene. When LXXV was treated under these conditions, the solution rapidly turned black. A light yellow, base soluble product was isolated in 12-22% yield. This material contained no iodine and gave a green color with ferric chloride and a chloroform-soluble complex with cupric ion, so it probably is a tropolone. However, it had an absorption maximum at 360 mμ and therefore is not colchiceine (λ_{max} 349 mμ).

The most widely applicable method for converting a diketone to a tropolone is bromination (with either bromine or N-bromosuccinimide) followed by dehydrobromination (see (100) for references). LXXV, however, contains several positions besides the desired ones in ring C which should be reactive to bromination. It was hoped, of course, that the rate of bromination would be greatest at the positions alpha to the carbonyl groups. This apparently was not the case, as the following discussion will indicate.

Bromination was first attempted with one mole of N-bromosuccinimide in carbon tetrachloride. After bromination, the product was dehydrobrominated with lutidine. A base soluble noncrystalline product, which gave positive tests to ferric and cupric salts, was isolated in low yield. It had an absorption maximum at 380 mμ, though, and contained bromine. The bromine content, however, was only about one-third of that calculated for bromocolchicoine. The bromination was then repeated, adding a trace

of benzoyl peroxide, with similar results (15% yield of base soluble fraction). A carbon, hydrogen and bromine analysis agreed well with that calculated for a mixture of 67% colchiceine and 33% bromocolchiceine. Sixty-seven percent colchiceine should easily have been detected in the spectrum, but there is no indication of even an inflection in the curve around 349 mμ, so if any colchiceine were produced, it was certainly in only a very small quantity.

The bromination was then attempted using 2 moles of N-bromosuccinimide. The product, isolated in about 30% yield, contained twice the amount of bromine as the one-mole product, but the spectra of the two products, although not identical, were very similar. An attempt to remove the bromine by hydrogenolysis using a palladium on carbon catalyst, resulted in an uptake (after making the catalyst correction) of 1.6 times the calculated amount of hydrogen. The product however still contained half the original bromine and the ultraviolet spectra before and after hydrogenation were almost identical. Apparently the bromine is in such a position in the molecule that it has very little effect on the absorption, and therefore cannot be on the tropolone ring.#

Bromination of LXIV with one mole of bromine in carbon tetrachloride in the presence of potassium carbonate gave an immediate reaction, even in an ice bath, and a brown solid precipitated. Reaction with two moles of bromine in chloroform in the presence of sodium bicarbonate also was rapid. In both cases, though, very little base soluble material was iso-

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The substitution of bromine into a tropolone nucleus ring shifts the maximum to appreciably longer wave lengths (132a).

lated. Bromination with one mole of bromine in glacial acetic acid, however, gave a 45% yield of base soluble material, but the absorption spectrum of this material was similar to those obtained from N-bromosuccinimide.

It is quite possible that the failure to obtain colchiceine is due to the high reactivity of the rest of the molecule. If so, then more specific methods must be found for this conversion. For example, treatment of the diketone, LXXX, with bromine yielded only tars, and treatment with N-bromosuccinimide followed by aqueous trimethylamine gave a non-acidic product (15). However, when the dienol acetate was reacted with N-bromosuccinimide followed by base, 7-hydroxybenzocycloheptatriene-3-one (LXXXI) was obtained.

Conversion of dihydrocolchiceine into an enol acetate might activate the C-ring sufficiently so that colchiceine could be prepared. If the rest of the molecule is still too reactive, then perhaps the enol acetate could be reacted with N-iodosuccinimide to form an α-iodoketone. Djerassi and Lenk (33,34) have shown that this reagent reacts specifically with enol acetates to form α-iodo ketones, while double bonds and other groups that normally react with halogens do not react. Hydrogen iodide would then be eliminated from the iodoketone to form colchiceine.

It is also possible that the oxygen function that was added <u>via</u> the oximino group is not on the same carbon atom as it is in colchicine. The product obtained, then, would not be colchiceine, but would be an isomer of it and could be called pseudo-colchiceine. This compound in itself would be an extremely interesting one, but would be of no value as far as the synthetic problem is concerned. The position of the oxygen could be checked by hydrogenation of LXXV to a diol (or diols) and com-

perison of its properties with those of the diols (LXXXII) formed by direct hydrogenation of colchicoine (32, 128).

If it is found that LXXV does not have the structure corresponding to a dihydrocolchicoine, then some other method of preparing dihydrocolchicoine must be found. Hexahydrocolchicoine (LXXXII), in which the oxygens are in the correct position, could be a source for dihydrocolchicoine, if a suitable method for specifically oxidizing the glycol group to a ketol or a diketone could be found. No such specific method could be found in the literature.

In conclusion, it may be said that the synthesis of colchicine, even from closely related degradation products, is a problem yet to be solved, and much more work must still be expended before the synthesis can be accomplished. This work has shown, though, that extrapolation of methods that are satisfactory for simple systems to the colchicine problem must be done with great care, and often a "good" method will be of no value in the more complex situation.

EXPERIMENTAL*

Attempted preparation of dioximinotetrahydrodemethoxycolchicine (XVII). Dry (over sodium) benzene was added to 1.92 g. n-butyl nitrite (87)** to a total volume of 25 ml. and 3.8 ml. (2.8 mM) of this solution was then added to a solution of 500 mg. tetrahydrodemethoxycolchicine (VIII) in 10-15 ml. dry benzene. This solution was then added dropwise at room temperature to a stirred solution of 147 mg. (3.8 mM) potassium in t-butyl alcohol (distilled from sodium). After standing overnight, the solution was evaporated under reduced pressure to a brown oil. The oil was dissolved in water and extracted with 3 portions of benzene, each benzene layer in turn being washed twice with distilled water. The combined benzene fractions were dried with anhydrous sodium sulfate and evaporated to yield 10 mg. residue (approximately 2%).

The combined aqueous phases were treated with solid carbon dioxide until saturated (pH approximately 5). The solution was then extracted three times with chloroform, the chloroform layers being washed once with water. The combined chloroform layers were dried with sodium sulfate and evaporated to yield 540 mg. residue. (Theoretical yield 540 mg. for the addition of one oximino group and 580 mg. for the addition of two such groups.) The residue was crystallized from benzene-chloroform to yield

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* All melting points above 200° were taken in evacuated capillaries; all liquid-liquid extractions were done in a countercurrent manner; microanalyses were performed by the Microchemical Laboratory, University of California; ultraviolet absorption spectra were taken on a Cary recording spectrophotometer Model 11 and in 95% ethanol unless otherwise stated; infrared absorption spectra were taken on a Baird Recording infrared spectrophotometer.

** The butyl nitrite was stored in a refrigerator and distilled *in vacuo* at room temperature into a dry ice trap just prior to use. Any water in the nitrite was frozen out at the temperature of dry ice and aliquots taken while the material was still cooled in the dry ice were sufficiently dry for these reactions.

210 mg. (40%) of a light yellow solid.

Anal. Calculated for $C_{21}H_{26}N_2O_6$: C, 62.67; H, 6.51; N, 6.96

$C_{21}H_{26}N_3O_7$: C, 58.46; H, 5.84; N, 9.74

Found: C, 60.36; H, 6.51; N, 7.12

The product had an ultraviolet absorption spectrum identical with that of oximinotetrahydrodemethoxycolchicine (XVI).

Dioximinotetrahydrodemethoxycolchicine (XVII). Potassium (145 mg.) was dissolved in t-butyl alcohol which had been distilled from sodium. Oximinotetrahydrodemethoxycolchicine (XVI) (500 mg.) was then added and stirred until the solid dissolved. n-Butyl nitrite (2.15 g.) was added to dry benzene to a total volume of 25 ml. and 7.0 ml. (5.8 mM) of this solution was added to the oximinotetrahydrodemethoxycolchicine solution dropwise with stirring at room temperature over a period of one hour. After standing overnight, the brown solution was evaporated under reduced pressure to a brown glass. The residue was taken up in water and extracted with benzene. The benzene fractions were combined, dried and evaporated to yield 15 mg. residue which was discarded.

The aqueous solution was saturated with carbon dioxide and a yellow solid precipitated. The solution was extracted five times with chloroform (most of the solid dissolved), the chloroform layers being washed with water. The chloroform was then dried and evaporated to yield 140 mg. residue, which was crystallized first from benzene-chloroform and then from isopropyl alcohol to yield 9 mg. of a yellow solid, m.p. 247° dec. (after drying overnight at 115° and less than 1 mm. pressure).

Anal. Calcd. for $C_{21}H_{25}N_3O_7$: N, 9.74

Found: N, 10.02.

The aqueous layers were again saturated with carbon dioxide and extracted continuously with chloroform. As the extraction proceeded, the chloroform became yellow and yellow crystals were deposited. After several hours, the chloroform extract was cooled and the solid collected by filtration and dried to yield 110 mg. solid, m.p. 243° dec.

Anal. Found: N, 8.69.

The product was recrystallized from isopropyl alcohol and dried overnight at 115° and less than 1 mm. pressure to give 63 mg. of a yellow crystalline solid, m.p. 248° dec., λ_{max} 256 mμ, log ε_{max} 4.20; $[\alpha]_D^{23}$ -417° (c, 0.836, 95% EtOH).

Anal. Calcd. for $C_{21}H_{25}N_3O_7$: C, 58.46; H, 5.84; N, 9.74; OCH_3, 21.58

Found: C, 58.78; H, 5.85; N, 9.44; OCH_3, 21.61.

Reaction of tetrahydrodemethoxycolchicine (VIII) with acetic anhydride-pyridine. A typical example is as follows: A mixture of 76 mg. VIII, 20 ml. acetic anhydride and 5 ml. pyridine (dried over potassium hydroxide) was refluxed for one hour. The solvent was then evaporated under reduced pressure to a brown glass. This residue was dissolved in benzene and washed with sodium bicarbonate solution and then water, dried and evaporated. The residue was then taken up in a small amount of benzene, filtered and lyophilized to yield a brown, amorphous powder, λ_{max} 284 mμ, log ε_{max} 4.04; λ_{min} 255, log ε_{min} 3.91. All attempts to crystallize this material failed.

Anal. Calcd. for $C_{23}H_{29}NO_8$: CH_3CO, 20.7

Found: CH_3CO, 20.9.

Hydrolysis of "Enol acetate." A. Acid hydrolysis. Tetrahydrodemethoxycolchicine (VIII) (503 mg.) was heated under reflux for 2-1/2 hours

with 40 ml. acetic anhydride and 10 ml. pyridine and worked up as above. The residue was dissolved in 30 ml. of 0.1 N hydrochloric acid in 70% aqueous ethanol and heated under reflux for 1/2 hour. After cooling, excess solid sodium bicarbonate was added and the solution was evaporated under reduced pressure and at room temperature to a small volume. The mixture was then extracted with benzene and the benzene extract was treated with anhydrous sodium sulfate and filtered to remove insoluble matter.

The benzene solution (approximately 30 ml.) was extracted with 3 x 50 ml. of 20% aqueous sodium bisulfite, stirring each extraction vigorously for 20 minutes. Each aqueous phase was washed with a small amount of benzene. The aqueous phases were combined, basified with solid sodium carbonate and extracted with chloroform. Drying and evaporation of the chloroform yielded 100 mg. of a straw colored oil (sample A). The aqueous phases from the chloroform extraction were basified with potassium hydroxide to pH >13 and extracted again with chloroform to yield an additional 20-30 mg. of material.

The benzene phases from the above sodium bisulfite extractions were dried and evaporated. The residue was dissolved in a small amount of methanol and 150 ml. of 20% sodium bisulfite solution was rapidly added, at which time a precipitate formed. After shaking the mixture overnight, it was filtered. The residue was then dissolved in methanol and shaken for 2 days with 150 ml. of sodium bisulfite solution. Filtering yielded 120 mg. residue. The two aqueous solutions from above were separately basified with sodium carbonate and extracted with chloroform to yield 50 and 40 mg. residue respectively. These residues were combined (sample B).

Conversion to the dimethyl mercaptol. Samples A and B above were separately treated with 100 mg. zinc chloride (fused in vacuo), 300 mg.

anhydrous sodium sulfate and 10-20 ml. methyl mercaptan in a bomb tube. After standing 2 days at room temperature, the bombs were opened and the methyl mercaptan allowed to evaporate. The residue was dissolved in benzene and water. The benzene phase was washed with normal sodium hydroxide and then water. The benzene was dried with sodium sulfate and evaporated to yield 80 mg. from A and 50 mg. from B. The mercaptols were crystallized from methanol-water and dried at 100° and less than 1 mm. pressure.

A: yield 52 mg. white crystals, m.p. 192.5-193°, mixed m.p. with tetrahydrodemethoxycolchicine dimethyl mercaptol (XXII) 191.5-192.5°.

B: yield 24 mg. tan crystals, m.p., 178-180°. Recrystallization gave 20 mg., m.p. 185-187°, mixed m.p. with XXII, 187-189°.

In an earlier hydrolysis which was heated for 2 hours in 0.2 N hydrochloric acid in 70% ethanol, the nonketonic material analyzed for 7.7% acetyl, while VIII calculated for 11.5% acetyl.

B. Determination of the stability of VIII under the hydrolysis conditions. VIII (99 mg.) was heated under reflux for 1/2 hour in 25 ml. 0.1 N hydrochloric acid in 70% ethanol. The solution was then cooled, solid sodium bicarbonate was added and the solvent evaporated almost to dryness under reduced pressure and at room temperature. The residue was taken up in benzene and washed with water. Drying and evaporation of the benzene yielded 90-100 mg. residue, which was shaken overnight with 50 ml. 20% sodium bisulfite solution.

The bisulfite solution was filtered, basified with sodium carbonate and extracted with chloroform. The chloroform was dried (sodium sulfate) and evaporated and the residue was lyophilized from benzene to give 33 mg. (85% recovery), $[\alpha]_D^{21}$ -171° (c. 1.01, 95% ethanol). $[\alpha]_D^{25}$ for pure VIII is -174° in ethanol.

C. Basic hydrolysis. An "Enol acetate" product from 122 mg. VIII was dissolved in 15 ml. methanol. Three ml. water and 2 ml. 1 N potassium bicarbonate solution was added and the solution was heated under reflux for 1 hour. The solvent was removed under reduced pressure and at room temperature. The residue was taken up in 25 ml. benzene and washed with a small amount of water. The benzene solution was then extracted with 5 x 50 ml. of 20% aqueous sodium bisulfite solution, stirring vigorously for 20 minutes each time. Each aqueous extract was washed with benzene, adding the benzene wash to the original benzene solution after every second extraction. The benzene was finally washed once with water. The benzene solution was dried (sodium sulfate) and evaporated to yield 27 mg. residue.

Anal. Calcd. for $C_{21}H_{27}NO_5$: CH_3CO, 11.5

Found: CH_3CO, 5.7.

Solid potassium carbonate was added to the aqueous phases until basic (pH approximately 10) and the solution was extracted with chloroform, which was dried and evaporated to yield 43 mg. residue. The aqueous solution (approximately 500 ml.) was then basified to pH >13 with sodium hydroxide and extracted with 3 x 100 ml. chloroform by continuously shaking for 3 hours for the first two extractions and overnight for the last. This extraction yield only an additional 9 mg.

N,N-Dimethylaminoisocolchicide (XXIV). Two to 2.5 g. isocolchicine (Ib) (m.p. 221.5-222.5°, prepared by the method of Lavigne (74)) was reacted with 12 ml. of 2.35 M dimethylamine (prepared from recrystallized dimethylamine hydrochloride) in methanol in a sealed tube at 170°, following the general directions given by Lavigne (74). After working the reaction mixture up as directed, the maximum yield of recrystallized product was 85%, m.p. 138-139°. This represents a 10% increase in yield

over that reported by Lavigne and is probably due to the larger scale in which these runs were made.

Tetrahydrodemethoxyisocolchicine (XVIIIc). XXIV and the catalyst were stirred together in glacial acetic acid in an atmospheric pressure hydrogenation apparatus until 3 or slightly more moles of hydrogen had been absorbed. The mixture, which had lost its yellow color, was then filtered through a mat of super-cel and evaporated to dryness under reduced pressure. The residue was taken up in benzene, washed with potassium carbonate solution, water, 2 N hydrochloric acid and again with water. The acid solutions were basified and extracted with chloroform to yield the major portion of the material as the basic fraction, but no further work was attempted with this material.

The benzene, containing the neutral fraction, was dried with sodium sulfate and evaporated. The residue was then shaken with 25-50 ml. 20% sodium bisulfite solution overnight. The mixture was then filtered and the filtrate was basified with solid potassium carbonate and extracted with chloroform to yield the ketonic fraction. The following table gives the weights of XXIV and catalysts and the volume of the acetic acid used and the yields of the neutral and ketonic fractions (see Table 4).

Table 4

Weight XXIV mg.	Weight PtO_2 mg.	Weight 5% Pd/C mg.	Volume HOAc ml.	Yield neutral %	Yield ketonic %
206	13	25	10	21(?)	10(?)
2060*	124	250	50	6	3
501	25	50	25	11	9
500	26	50	25	12	5
501	50	101	25	14	5
200	0	50	10	no H_2 uptake	
200	10	0	10	11	7
206	13	25	10	8	5
206	13	25	10	11	6
207	12	25	10	14	6
500	30	60	25	13	9
500**	30	60	25	16	11

* This run was done in a Paar hydrogenation apparatus.
** This row represents an average of six runs of 500 mg. each.

The combined ketonic fractions (334 mg.) from one series of runs was crystallized from ethyl acetate-dibutyl ether to yield 244 mg. of light tan crystals, m.p. 182-184°; λ_{max} 219, 255 mμ, log ε_{max} 4.45, 4.03; λ_{min} 240 mμ, log ε_{min} 3.95; $[\alpha]_D^{20}$ -143° (c. 1.16, ethanol).

Anal. Calcd. for $C_{21}H_{27}NO_5$: C, 67.6; H, 7.3

Found: C, 67.5; H, 7.0.

Tetrahydrodemethoxyisocolchicine dimethyl mercaptol (XXV).

XVIIb (143 mg.), the combined bisulfite-soluble fractions from several hydrogenation runs, 150 mg. fused zinc chloride and 300 mg. anhydrous sodium sulfate were sealed in a bomb tube with methyl mercaptan. After standing at room temperature overnight, the bomb was opened and the mercaptan allowed to evaporate. The residue was taken up in benzene and water. The benzene layer was washed twice with 1 N sodium hydroxide and three times with water. The benzene was then dried with sodium sulfate and evaporated to yield 150 mg. of an oily residue. An attempt to crystallize this material from methanol-water gave only an oil. The material was then chromatographed on alumina. The material was put on the column in benzene and eluted with 0.5% absolute ethanol in benzene. The main fractions were combined to yield 119 mg.

This material still could not be crystallized. However, when water was added to a solution of the oil in a small volume of methanol, an oil separated which hardened and became solid and fluffy on being rubbed. Filtration, washing with water and drying at 55° and less than 1 mm. pressure overnight yielded 100 mg. of a white powder.

Anal. Calcd. for $C_{23}H_{33}NO_4S_2$: S, 14.2

Found: S, 13.4.

Rechromatography of 63 mg. of this powder gave 66 mg. (97%) as a single peak.

Desulfurization of tetrahydrodemethoxyisocolchicine dimethyl mercaptol (XXV). XXV (66 mg.), 2.5 ml. settled Raney nickel (33) (stored under absolute ethanol) and 25 ml. 90% ethanol were refluxed under a nitrogen atmosphere with stirring for approximately 24 hours. The mixture was then cooled and filtered through a mat of super-cel. The nickel was extracted twice by refluxing with benzene for 1/2 hour each time.

The combined filtrates were evaporated to dryness to yield 64 mg. of a white, partially crystalline residue. This material was then chromatographed on alumina, eluting with 0.25% absolute ethanol in benzene. The main fractions were combined and crystallized from methanol-water to yield 25 mg. white crystals, m.p. 182-183°, mixed m.p. with hexahydrodemethoxydesoxycolchicine (IX) 182-183°, $[\alpha]_D^{21}$ -152° (c. 1.04, 95% ethanol). The constants for authentic IX are m.p. 183-184° and $[\alpha]_D^{21}$ -158° (c. 1.03, 95% ethanol).

Hexahydrodemethoxydesoxydesacetylcolchicine (XXVII). Hexahydrodemethoxydesoxycolchicine (IX) was prepared from colchicine following the directions given by Rapoport (114), m.p. 183-184°, $[\alpha]_D^{25}$ -158° (c. 1.08, ethanol); reported (114) m.p. 183.5-184°, $[\alpha]_D^{25}$ -162° (c. 1.10, ethanol). A typical hydrolysis is as follows: 2.0 g. of IX was dissolved in 30 ml. ethanol, then 30 ml. water and 10 g. potassium hydroxide was added. The clear solution was then heated under reflux in a nitrogen atmosphere for 8 days. At the end of this time the mixture had separated into two layers. The mixture was then poured into 100 ml. water and extracted with benzene. The benzene layers were then extracted with 100 ml. 1 N hydrochloric acid. Drying (potassium carbonate and sodium sulfate) and evaporation of the benzene gave 1.2 g. recovered crystalline IX, which was recycled. The hydrochloric acid solution was basified with potassium carbonate and extracted with chloroform. Drying (sodium sulfate) and evaporation of the chloroform gave 0.8 g. of XXVII as a colorless oil, which could not be crystallized.

Hexahydrodemethoxydesoxycolchicine (IX) from hexahydrodemethoxydesoxydesacetylcolchicine (XXVII). XXVII (47.4 mg.) in 3 ml. pyridine and 3 ml. acetic anhydride were heated on the steam bath for 15 minutes and then allowed to stand at room temperature overnight. The solution was then evaporated to dryness under reduced pressure. The residue was

taken up in benzene and chromatographed on alumina, eluting with 0.3% absolute ethanol in benzene to give 39 mg. white crystalline residue. Recrystallization from methanol-water gave 33 mg., m.p. 183.5-184.5°.

N-(p-chlorobenzylidine)-d-α-phenylethyl amine. d-α-Phenylethyl amine (5.0 g.) $[\alpha]_D^{}$ +39°, obtained from Dr. Rapoport), and 6.6 g. (47 mM) p-chlorobenzaldehyde (recrystallized from hexane m.p. 46°) in 50 ml. methanol was heated gently on a steam bath for one-half hour. The solution was then removed from the steam bath and allowed to stand for 1-1/2 hours. The mixture was then cooled in ice and crystals appeared. Filtration, washing with methanol-water (1:1) and drying overnight and at the water pump gave 8.3 g. of white flakes, m.p. 77-77.5°, $[\alpha]_D^{21}$ -90° (c. 1.0% ethanol). The mother liquors yielded an additional 1.3 g. of slightly yellow material for a total yield of 9.6 g. (95% of theory).

Anal. Calcd. for $C_{15}H_{14}NCl$: C, 73.91; H, 5.79

Found: C, 74.22; H, 6.03.

N-(p-chlorobenzylidine)-colchinol methyl ether. Colchinol methyl ether (XXX) was prepared by the method of Rapoport, Williams and Gigney (112), but was not crystallized. Only XXX (420 mg.) and 300 mg. p-chlorobenzaldehyde in 10 ml. of methanol was heated under reflux for 10 minutes. The methanol was then evaporated under reduced pressure and the excess aldehyde was removed by sublimation at 60° bath temperature and less than 1 mm. pressure. The residue was dissolved in hexane and filtered to remove a small amount of colored insoluble matter. After obtaining only oils in attempting to crystallize the benzylidine from several solvents, the oil was allowed to stand free of solvent for 2 days, when it had partially crystallized. Partial solution in hot hexane and cooling gave 320 mg. (after air drying) of tan crystals m.p. 85-87° (softens 81°);

$[\alpha]_D^{25}$ +43° (c. 1.32, absolute ethanol). The analytical sample was prepared by decolorising a hot solution of the above crystals in hexane with norite to yield a colorless solution which deposited white fluffy needles on cooling, m.p. 38-39.5°.

Anal. Calcd. for $C_{26}H_{26}NClO_4$: C, 69.09; H, 5.80; Cl, 7.85

Found: C, 69.26; H, 5.91; Cl, 7.60.

N-(p-chlorobenzylidine)-d-amphetamine. d-Amphetamine sulfate (obtained from the Stayner Corporation, Berkeley, California) was treated with sodium hydroxide solution and the liberated amine was extracted with methylene chloride. Distillation yielded d-amphetamine, b.p. 201-203°. d-Amphetamine (5 g.) and 5.2 g. p-chlorobenzaldehyde in 40 ml. methanol were heated on the steam bath for 1/2 hour. A small amount of insoluble matter was filtered off and the solution was allowed to cool. The methanol was removed under reduced pressure, toluene was then added and the mixture was distilled *in vacuo* to remove the water. Fractional distillation yielded 7.6 g., b.p. 162-162.5° at 2 mm. pressure; m.p. 38-40°; $[\alpha]_D^{22}$ +263° (c. 1.19 ethanol).

Anal. Calcd. for $C_{16}H_{16}NCl$: C, 74.55; H, 6.26

Found: C, 74.44; H, 6.47.

Resolution of 2-aminobutane.* d-Tartaric acid (312 g.) was dissolved in 1 l. water and 150 g. dl-2-aminobutane was cautiously added with cooling. After standing overnight no crystals had appeared. The solution was then concentrated to approximately one third of the original volume on a steam bath with an air stream. Cooling gave a solid mass

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* The resolution of 2-aminobutane with tartaric acid has been reported several times in the literature (76, 77, 131). However, as experimental details are all but lacking, detailed directions are given here.

which was broken up with a heavy spatula, filtered without rinsing and pressed dry with a rubber dam to yield 224 g. moist 2-aminobutane bitartrate monohydrate, m.p. (after drying overnight at 80° and less than 1 mm.) 136-138°; reported m.p. 146-147° for the pure l-amine salt (76). The moist salt was then dissolved in 200 ml. water and again the solution was below saturation. Concentration as before and cooling gave 82 g. moist salt, m.p. (after drying) 139-141°. Seventy-six g. of this salt was then placed in a distillation flask, 40 ml. 19 N sodium hydroxide was added and the liberated amine was distilled. Drying with potassium hydroxide and potassium carbonate, decantation and fractional distillation gave 21.0 g. (92% recovery) of d-2-aminobutane, b.p. 62.5-63°; $[\alpha]_D^{25}$ +4.63° (neat); reported $[\alpha]_D^{15}$ +7.30° (76), $[\alpha]_D^{20}$ +7.44° (131), $[\alpha]_D^{20}$ +5.38° (134). The amine is therefore about 90% resolved, which is sufficient for the racemization experiment.

N-(p-chlorobenzylidine)-d-2-aminobutane. d-2-Aminobutane (19.6 g.) and 37.0 g. p-chlorobenzaldehyde were mixed, heat was evolved, the aldehyde dissolved and a second liquid phase (water) appeared. The reaction was completed by heating for a short period on the steam bath and then allowing it to stand overnight. Pentane was then added, the mixture was dried with magnesium sulfate, filtered and fractionally distilled. The fraction boiling at 125-125.5° at 14 mm. weighed 42.9 g (89%); $[\alpha]_D^{25}$ +56.2° (c. 1.12 ethanol); n_D^{25} 1.5347; d^{25} 1.029; λ_{max} 253, log ϵ_{max} 4.34.

Anal. Calcd. for $C_{11}H_{14}NCl$: C, 67.51; H, 7.21; N, 7.16; Cl, 18.12

Found: C, 67.95; H, 7.27; N, 6.76; Cl, 18.13.

N-(p-chlorobenzylidine)-hexahydrodemethoxydesoxydesacetylcolchicine (XXXI). A solution of hexahydrodemethoxydesoxydesacetylcolchicine (XXVII) and 2-3 molar equivalents of p-chlorobenzaldehyde in methanol was heated under reflux for 15-30 minutes. The methanol was then removed under reduced pressure and the excess aldehyde was removed by sublimation for several hours at less than 1 mm. and 70-80° to leave a straw colored oil in quantitative yield.* The oil was then dissolved in hexane and decolorized with Norite. Evaporation of the hexane yielded a colorless oil which could not be crystallized; $[\alpha]_D^{25}$ -53.0° (c. 1.60, benzene); λ_{max} 257 mμ, log ϵ_{max} 4.52. This material, as well as the other benzylidines, showed a conjugated imine peak in the infrared region at 6.10 μ.

Racemization rate experiments. The experimental conditions common to all the rate runs are as follows: All glassware was dried several hours at 110° before use. Sodium ethoxide solution was prepared by dissolving under a nitrogen atmosphere the appropriate amount of sodium in absolute ethanol which had been heated under reflux several hours with sodium and diethyl phthalate and then distilled. Potassium t-amylate was prepared in a similar manner from potassium and t-amyl alcohol which

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* One experiment was run to check the completeness of the reaction: 102 mg. XXVII was dissolved in 10 ml. methanol; this solution had α_D^{25} - 2.12°. Forty-one mg. (1 molar equivalent) p-chlorobenzaldehyde was then added to 3.4 ml. of this solution and an aliquot removed for determination of the rotation, which was found to be slowly changing toward zero. The remainder of the solution was then heated under reflux for 1/2 hour. Two ml. was then removed, 47 mg. (approximately 2 molar equivalents) more aldehyde was added and the solution was heated for another 1/2 hour. The rotations of the mixture after the 1st and 2nd heatings were -0.13° and -0.16°, respectively. These rotations probably are the same within experimental error, but even if the differences are real, this represents essentially complete reaction even with one mole of the aldehyde.

had been heated under reflux several hours with sodium and then distilled. The base concentration was determined by titrating an aliquot with standard acid. A portion of the base solution was then added to a sample of the benzylidine (in some cases dissolved in a small amount of the dry alcohol) and the mixture shaken until any solid had dissolved. The solution was then transferred to a tube fitted with a ground glass joint and an aliquot was removed for determination of the optical rotation at zero time. The tube was then closed with a stopper containing a stopcock, immersed in a dry ice bath and evacuated to less than 1 mm. The stopcock was then closed and the tube was placed in the vapor of boiling methyl chloroform (b.p. 75°). After the desired length of time, the tube was again cooled in dry ice and then brought to room temperature. Dry air was admitted and an aliquot was removed. The tube was then again sealed, cooled, evacuated and replaced in the heating bath.

As the method of following the racemization varied with the different compounds, each will be described separately. The numerical data are given in table 5.

N-(p-chlorobenzylidine)-d-α-phenylethyl amine. (1) Sodium ethoxide: The benzylidine (100 mg.) was dissolved in 1 ml. of absolute ethanol and this solution was then added to 9.5 ml. 1.2 N sodium ethoxide solution in the reaction tube. The rate of racemization was determined by measuring the rotation of the solution directly in the polarimeter tube. At the end of the racemization experiment (2 hours), the remainder (7 ml.) of the solution was poured into water and extracted with ether. The ether was dried with sodium sulfate and evaporated and the residue was sublimed to give a light yellow semisolid mass, $[\alpha]_D^{25}$ -0.203° (c. 1.15 ethanol). The calculated value for the rotation of the original benzyli-

dine at this concentration is -1.06° and, therefore, the compound is 19% unracemized.

(2) Potassium t-amylate: The benzylidine (60 mg.) was dissolved in 6 ml. of 1.4 N potassium t-amylate solution. After 1/2 hour at room temperature, the solution had a rotation of -0.025°, whereas, if no racemization had occurred, the rotation would have been approximately -1°.

N-(p-chlorobenzylidine)-colchinol methyl ether. The benzylidine (101 mg.) was added to 10 ml. 0.95 N sodium ethoxide solution. Only part of the solid dissolved and the supernatant was used for the rate run. The solution was read directly in the polarimeter tube, except for the last sample (12 hours), which was too darkly colored. This sample was poured into water and extracted with hexane. The residue (24.9 mg.) left after evaporation of the hexane was dissolved in 2 ml. absolute ethanol; $[\alpha]_D^{25}$ -0.007°.

N-(p-chlorobenzylidine)-d-amphetamine. (1) Sodium ethoxide: The benzylidine (99.8 mg.) was dissolved in 10 ml. of 1.79 N sodium ethoxide solution. The optical rotations were determined directly on this solution.

(2) Potassium t-amylate: The benzylidine (277 mg.)was added to approximately 13 ml. of 1.33 N potassium t-amylate solution. For determination of the optical rotation, 2 ml. aliquots were diluted to 5.2 ml. with absolute ethanol.

N-(p-chlorobenzylidine)-2-eminobutane. The benzylidine (10.3 ml.) was added to about 10 ml. of 1.34 N potassium t-amylate solution. For determination of the optical rotation, 2 ml. aliquots were diluted to 5.2 ml. with absolute ethanol.

N-(p-chlorobenzylidine)-hexahydrodemethoxydesoxydesacetylcolchicine (XXXI). (1) Sodium ethoxide: The benzylidine (242 mg.) was added to

10 ml. 1.6 N sodium ethoxide solution, warming slightly to hasten solution. Only part of the material dissolved and the supernatant was removed for the rate determination. The solution darkened rapidly on heating and the rotation could not be measured directly. With a five-fold dilution, the rotation could be measured, but the actual rotation was too small and the reading errors too great to be of any value. The rotations were determined by pouring the aliquots into water, extracting with chloroform, and determining the specific rotations in benzene of the residues left after evaporation of the chloroform.

(2) Potassium t-amylate: Approximately 55 mg. of the benzylidine was dissolved in 1 ml. of absolute t-amyl alcohol and 5 ml. 1.29 N potassium t-amylate solution was added. For determination of the rotations, aliquots were poured into water and extracted with hexane. The residue left after evaporation of the hexane was taken up in benzene and the specific rotation determined.

Table 5

Racemization Rate Data on p-Chlorobenzylidines of Some Amines at 75°.

Amine	Catalyst	Time hours	α^{25}_D	% Unrac.	k at unit base conc.
Phenylethyl amine	NaOEt	0	-1.056	100	.9
		1	-0.399	37.9	
		2	-0.159	15.1	
Colchinol methyl ether	NaOEt	0	+0.203	100	.5
		1/2	+0.143	70	
		1-1/4	+0.134	66	
		12	0	0	
Amphetamine	NaOEt	0	+2.63	100	0
		1	+2.61	100	
		6	+2.63	100	
		24	+2.58	100	
	KOt-Am	0	+1.773	100	.2
		1	+1.331	75.1	
		3	+0.767	43.2	
		23	+0.008	0	
2-Aminobutane	KOt-Am	0	+0.657	100	.05
		5	+0.499	76.2	
		12	+0.336	51.2	
LXXIII	NaOEt	0	-43.5*	100	.006
		21	-34.4*	81	
	KOt-Am	0	-53.0*	100	4
		5 min	-38.0*	71.7	
		10 min	-26.1*	49.6	

* Specific rotation $[\alpha]^{25}_D$.

Racemization of N-(p-chlorobenzylidine)-d-2-aminobutane with product isolation. The benzylidine (40.6 g.) was added to approximately 30 ml. of a 1.3 M potassium t-amylate solution and the mixture was heated under reflux in a nitrogen atmosphere for 45 hours. The solvent was then partially removed under reduced pressure and the residue was taken up in methylene chloride and water. The organic phase was then dried with magnesium sulfate and distilled under reduced pressure to remove the methylene chloride and residual amyl alcohol. The residue (33 g.) was then distilled through a short-path distilling head at 1-2 mm. pressure and at a bath temperature of 100-120° to yield 12.8 g. of a colorless liquid; $[\alpha]_D^{25}$ 0.0° (c. 1.95, ethanol); λ_{max} 253 mμ, log ε_{max} 4.33. The infrared absorption spectrum of this material was similar, but not identical, to that of the original benzylidine.

Anal. Calcd. for $C_{11}H_{14}NCl$: C, 67.5; H, 7.2; N, 7.2; Cl, 18.1

Found: C, 68.8; H, 7.3; N, 6.8; Cl, 17.3.

The distillate was then distilled through a fractionating column at 14 mm. pressure. The following fractions were obtained:

Table 6

Fraction Number	b.p. °C.	Wt. g.	λ_{max} mμ	log ε_{max}	Anal. C	H	N	Cl
1	90-126	0.59	257	4.29	75.7	8.2	7.3	7.3
2	126-127.2	2.79	253	4.34	68.0	7.3	7.1	17.6
3	127.2	6.44	253	4.33	67.8	7.3	7.1	18.0
4	127.2	1.12	253	4.32	68.3	7.9	6.2	17.8

Racemization of N-(p-chlorobenzylidine)-d-amphetamine with product isolation. The benzylidine (1.00 g.) was added to 30 ml. of a 1.2 N solution of potassium t-butoxide in t-butyl alcohol and the mixture was heated under reflux in a nitrogen atmosphere for 24 hours. The reaction mixture was then cooled, poured into water and extracted with chloroform. Drying (sodium sulfate) and evaporation of the chloroform gave a straw colored liquid residue which was shaken with 30 ml. of 1 N hydrochloric acid for 19 hours.* The solution was then extracted with chloroform and the aqueous phase was treated as described in the following paragraph. The organic phase was dried and evaporated. The residue was dissolved in 30 ml. methanol, 17 ml. 2 N hydrochloric acid added and the mixture was heated on the steam bath for 1/2 hour. After standing overnight at room temperature, the solution was extracted with chloroform, basified and extracted again with chloroform. The second chloroform extract was evaporated and the residue dissolved in water. Titration with 0.100 N hydrochloric acid required less than 0.1 ml. and therefore the original hydrolysis was complete.

The original acid solution was basified and extracted with chloroform. The chloroform was dried with sodium sulfate and evaporated. The residue was dissolved in water and titrated with 0.100 N hydrochloric acid, using brom cresol green as the indicator. The recovered amine accounted for 77% of the starting material. The solution from the titration was then basified and extracted with chloroform. The chloroform was dried and evaporated. The residue was taken up in 10 ml. of

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* An experiment with the pure benzylidien showed that hydrolysis in 1 N hydrochloric acid at room temperature was quite rapid, a precipitate of p-chlorobenzaldehyde appearing within 5 minutes, and that the recovery of the amine was essentially quantitative.

pyridine, 0.5 ml. benzoyl chloride was added, and the mixture was heated on the steam bath for 1/2 hour. The solvents were removed in an air stream on the steam bath. The residue was taken up in benzene, washed with 1 N hydrochloric acid, 1 N sodium hydroxide and finally with water. The benzene layer was dried with sodium sulfate and evaporated to yield 750 mg. of the amide.

Anal. Found: Cl, 2.36.

The amide was crystallized from methanol-water to yield 530 mg., m.p. 115-125° (N-benzoyl-dl-amphetamine melts at 132-133°). Recrystallization yielded 430 mg., m.p. 115-125°; $[\alpha]_D^{25}$ 0.0° (c. 2.96, ethanol).

Anal. Calcd. for $C_{16}H_{17}NO$ (A): C, 80.3; H, 7.2; N, 5.9; Cl, 0

Calcd. for $C_{14}H_{12}NOCl$ (B): C, 68.4; H, 4.9; N, 5.7; Cl, 14.4.

Calcd. for 86% A and 14% B: C, 78.9; H, 7.0; N, 6.0; Cl, 2.0.

Found: C, 78.5; H, 6.8; N, 5.8; Cl, 2.0.

Racemization of N-(p-chlorobenzylidine)-hexahydrodemethoxydesoxydesacetylcolchicine (XXXI) with product isolation. The benzylidine prepared from 630 mg. hexahydrodemethoxydesoxydesacetylcolchicine (XXVII) was dissolved in 9 ml. absolute t-amyl alcohol and this solution was added to 25 ml. of a 1.3 N potassium t-amylate solution. The mixture was placed in an oil bath at 75° for 3 hours under a nitrogen atmosphere.* The solution was then cooled, poured into water and extracted with hexane (part of the solution was lost at this point). The yellowish hexane

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* A later run was made by heating under reflux a solution of the benzylidine and potassium t-butoxide in t-butyl alcohol for 3 hours with essentially the same results.

solution was dried with sodium sulfate, decolorized with Norite and evaporated to yield 600 mg. of a yellow oil; λ_{max} 248 mμ, log ε_{max} 4.04.

The racemate was then heated on the steam bath for 1/2 hour with 1 N hydrochloric acid.* The hydrolysis mixture was then extracted with benzene, the aqueous layer basified with potassium carbonate and extracted with chloroform. Drying and evaporation of the benzene yielded 443 mg. neutral material and drying and evaporation of the chloroform gave 90 mg. of basic material.

dl-Hexahydrodemethoxydesoxycolchicine (dl-IX). The basic fraction from the above hydrolysis was treated with 10 ml. pyridine and 10 ml. acetic anhydride by warming on the steam bath and then allowing the reaction mixture to stand overnight. The solvents were evaporated under reduced pressure and the residue, which partially crystallized, was chromatographed on alumina. The main band (eluted with 0.2-0.5% absolute ethanol in benzene) weighed 66 mg. and was crystallized from benzene-hexane to yield 33 mg. white crystals, m.p. 134°; $[\alpha]_D^{25}$ +4.5° (c. 0.40, ethanol)(the actual rotation was +0.018°, which may be zero within the experimental error); λ_{max} 219, 256 mμ, log ε_{max} 4.37, 4.07; λ_{min} 240 mμ, log ε_{min} 3.91. (Hexahydrodemethoxydesoxycolchicine (IX) has λ_{max} 219, 256 mμ), log ε_{max} 4.39, 4.10 and λ_{min} 240 mμ, log ε_{min} 3.92.) The infrared spectra of IX and dl-IX were identical.

Anal. Calcd. for $C_{21}H_{29}NO_4$: C, 70.17, H, 8.13

Found: C, 70.15, H, 8.03.

Rate of reaction of perbenzoic acid with hexahydrodemethoxydesoxycolchicine (IX) and dl-hexahydrodemethoxydesoxycolchicine (dl-IX). A stock solution of perbenzoic acid in chloroform was prepared following

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* Experiments with the original benzylidine indicated that XXXI was not hydrolyzed as rapidly at room temperature as were the aliphatic benzylidines, and for this reason the more vigorous conditions were used. The recovery of the amine under the above conditions is essentially quantitative.

the directions given by Braun (14) as modified by Kolthoff (71) and stored in the refrigerator. For the rate run, a portion of the stock solution was diluted with chloroform to approximately 0.04 M and placed in an ice bath. The concentration was determined by adding a 200 λ aliquot to 2 ml. chloroform, adding 2 ml. 0.1 N acetic acid and 2 ml. 0.5% potassium iodide, shaking vigorously, and titrating to the starch end point with standard sodium thiosulfate. IX or dl-IX (3.32 mg.) were placed in a 2 ml. volumetric flask, the flask then being placed in the ice bath. After the flasks and solution had equilibrated with the bath, the perbenzoic solution was added to the amide to the 2.0 ml. mark and the contents were mixed. 200 λ aliquots were removed at various times and the concentration of perbenzoic acid was determined as before. The concentration of the perbenzoic acid in the remainder of the original solution was also checked at the end of the run and was found to have remained constant. Table 7 on the following page gives the results that w were obtained.

The second order rate constant (k) in Table 7 was calculated from the following equation:

$$k = \frac{2.303}{t(a-b)} \left[\log \frac{a-x}{b-x} - \log \frac{a}{b} \right] \qquad (1)$$

where a is the initial concentration of perbenzoic acid, b is the

Table 7

IX (0.01157 M)			dl-IX (0.01157 M)		
t-min.	conc. of perbenzoic	k_{IX} $l\text{-}min^{-1}\text{-}moles^{-1}$	t-min.	conc. of perbenzoic	$k_{dl\text{-}IX}$ $l\text{-}min^{-1}\text{-}moles^{-1}$
0	0.04130	---	0	0.04206	---
5	0.03981	0.927	10	0.03868	0.853
10	0.3898	0.693	20	0.03737	0.563
15	0.03860	0.538	30	0.03688	0.504
20	0.03743	0.401	40	0.03632	0.442
30	0.03743	0.401	50	0.03582	0.402
45	0.03631	0.369	60	0.03545	0.369
60	0.03527	0.365	70	0.03471	0.382
75	0.03486	0.324	80	0.03440	0.362
90	0.03336	0.349	90	0.03387	0.369

initial concentration of IX or dl-IX, and x is the amount of perbenzoic acid consummed at time t. It is obvious that the k's are not constant, but in both runs, k seems to be approaching similar limiting values. If we let $d = \frac{2.303}{a-b}\left[\log \frac{a-x}{b-x} - \log \frac{a}{b}\right]$

then

$$t = \frac{d}{k} \qquad (2)$$

Then, according to equation (2), a plot of t vs. d should give a straight line of slope $\frac{1}{k}$ going through the origin.* A plot of the above data gives two parallel straight lines, but both intercept the t-axis below the origin.

There are several systematic errors in the experimental determinations that are due to the small scale in which the rate runs were made. One of these is the zero time, for when the perbenzoic acid solution is transferred to the reaction flask, it warms up, and when it comes in contact with the amide, it is considerably warmer than 0°. The reaction, therefore, will be more rapid at that time than it should be. Also, when removing aliquots, the sample will rapidly warm to room temperature and again the reaction will speed up.** This increase in the rate of the reaction would mean that the concentration of perbenzoic acid as measured actually corresponds to a time greater than t, i.e., t + c. Assuming that the error in taking samples is constant for any one run (an assumption that is implied by the fact that the above mentioned plots approximate straight lines), then c will be a constant and equation (2) can be modified to include c

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* This reaction run on a larger scale actually does obey this equation and the value of k_{IX} was found to be 0.34 ± .04 $l\text{-}min^{-1}\text{-}moles^{-1}$. (J. Gordon, private communication.)

** These errors could probably be eliminated by working in a cold room so that the glassware would be near 0°.

$$t + c = \frac{d}{k} \qquad (3)$$

Here again a plot of t vs. d will give a straight line of slope $\frac{1}{k}$; but in this case the t-intercept will be -c. Solving equation (3) by means of the method of least squares gives $k_{II} = 0.30$ l-min^{-1}-moles^{-1} and $k_{dl\text{-}II} = 0.29$ l-min^{-1}-moles^{-1}, which are the same within the experimental error.

7-Keto-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine (XXXVIII). The neutral fraction (400 mg.) from the hydrolysis of the racemized benzylidine (XXXI) was chromatographed on alumina eluting with benzene-hexane (1:1) and then benzene. The main fractions (136 mg.) were combined and twice crystallized from hexane and dried at 80° and less than 1 mm. overnight to yield 64 mg. light tan crystals, m.p. 106.5-107.5°; λ_{max} 214, 240, 302 mμ, log ε_{max} 4.54, 4.14, 3.62; λ_{min} 231, 271 mμ, log ε_{min} 4.11, 3.49. A strong absorption in the infrared occurred at 6.02 μ.

Anal. Calcd. for $C_{19}H_{24}O_4$: C, 72.1; H, 7.7
Found: C, 71.7; H, 7.7.

7-Hydroxy-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine (XL). 7-Keto-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine (XXXVIII) (99 mg.) in 10 ml. absolute ethanol was hydrogenated in the presence of 97 mg. 5% palladium on carbon for 26 hours. The mixture was filtered through super-cel and evaporated to dryness in an air stream on the steam bath to yield a colorless oil which slowly crystallized. Crystallization from hexane yielded, after drying overnight at 80° and less than 1 mm., 71 mg. white fluffy crystals, m.p. 153-153.5°; λ_{max} 219, 256 mμ, log ε_{max} 4.37, 4.07; λ_{min} 240 mμ, log ε_{min} 3.91.

Anal. Calcd. for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23
Found: C, 71.53; H, 8.33.

Attempted hydrogenolysis of 7-hydroxy-$\Delta^{7a(12a)}$-octahydrodemethoxy-desoxydesacetamidocolchicine (XL). A mixture of 44 mg. XL, 45.7 mg. 5% palladium on carbon, 10 ml. absolute ethanol and two drops perchloric acid was hydrogenated in an atmospheric pressure apparatus for 21 hours. The reaction mixture was then filtered into 5% sodium bicarbonate solution and extracted first with hexane and then with chloroform. The hexane yielded 29 mg. residue and the chloroform 11.5 mg. The hexane residue was chromatographed on alumina, eluting with hexane and then with benzene-hexane (1:3) to yield 25 mg. This product was sublimed at 70° bath temperature and 0.02 mm. pressure for 3 hours to yield an oil which slowly crystallized. The sublimate was dissolved in hexane and transferred to a micro centrifuge filter tube and slowly cooled to -60° in a methanol-dry ice bath. The tube was then centrifuged and the crystalline product was dried at 40° and 0.5 mm. pressure for 2 hours and then overnight at room temperature and 0.5 mm. pressure to yield 10.5 mg. white crystals, m.p. 70-72°.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.7; OCH_3, 30.6.

Found: C, 73.4; H, 9.1; OCH_3, 29.3.

In another run 57.7 mg. XL was hydrogenated as above with 56 mg. palladium on carbon for 23 hours and worked up as before. The sublimate, however, instead of being crystallized was fractionally rechromatographed on fresh alumina, eluting with benzene-hexane (1:3). Only one peak was obtained and the main fraction (8 mg., crystalline) had λ_{max} 281 mμ and $\log \varepsilon_{max}$ 3.15.

Anal. Found: C, 72.5; H, 9.2.

Attempted preparation of the p-toluene sulfonate of 7-hydroxy-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine (XI). A solution of 15.3 mg. XL in 2 ml. pyridine (distilled from potassium hydroxide) in a

tube protected with a drying tube was cooled in ice. A solution of 10.7 mg. p-toluene sulfonyl chloride in 0.6 ml. pyridine was added in 3-4 portions with shaking. The sulfonyl chloride was rinsed in with an additional 0.6 mg. pyridine. The reaction mixture was then allowed to stand in the ice bath for 9 hours. The reaction mixture was then poured into ice water and extracted with benzene. The benzene was then washed with 3% acetic acid, water, 5% sodium bicarbonate and finally with water. The benzene was then dried with sodium sulfate and evaporated at 40° bath temperature under reduced pressure. Hexane was then added to the partially crystalline residue and the entire product crystallized. The hexane was then evaporated to yield 13 mg. crystalline residue, m.p. 143°. The infrared spectrum showed a moderately strong hydroxyl band and was very similar to that of the starting material.

Wolff-Kishner reduction of 7-keto-$\Delta^{7a(12a)}$-octahydrodemethoxy-desoxydesacetamidocolchicine (XXXVIII). A mixture of 90 mg. XXXVIII, 12.5 mg. 85% hydrazine hydrate and 9 ml. absolute ethanol was heated under reflux for 1 hour. The condenser was then removed and the solution was boiled until slightly cloudy. Diethylene glycol (12 ml.) was then added and the solution was boiled until the internal temperature reached 185°. The solution was then cooled, 1.6 g. sodium hydroxide was added and the solution was again boiled until the temperature reached 180°. The mixture was then heated under reflux in a nitrogen atmosphere for 4-1/2 hours. The reaction mixture was then cooled, poured into water, acidified with hydrochloric acid and extracted with benzene. Drying and evaporation of the benzene yielded a residue of 84 mg. The residue was then shaken with 25 ml. of 3 N sodium hydroxide and 2.5 ml. of methyl sulfate for 2 hours. An additional 2.5 ml. of methyl sulfate was then added and the mixture was shaken for another 2 hours. The

mixture was then extracted with benzene. Drying and evaporation of the benzene yielded 51 mg. which was chromatographed on alumina, eluting with benzene-hexane (1:3), to yield 32 mg. This material was then sublimed at 60-70° bath temperature and 0.02 mm. pressure to yield a crystalline sublimate, m.p. 58-60°; λ_{max} 256 mμ, log ε_{max} 3.80.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.7

Found: C, 75.3; H, 9.0.

7-Keto-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine dimethyl mercaptol. Zinc chloride (100 mg.) was fused under vacuum in a bomb tube. After cooling, dry air was admitted and 103 mg. of 7-keto-$\Delta^{7a(12a)}$-octahydrodemethoxydesoxydesacetamidocolchicine (XXXVIII) was added. The tube was then placed in a dry ice bath and methyl mercaptan was condensed into the tube, which was then sealed and allowed to stand at room temperature overnight. The bomb was then opened, the red contents were poured into a flask and the methyl mercaptan was allowed to evaporate. The residue was then taken up in benzene and water. The benzene layer was then washed with 1 N sodium hydroxide and then water. Drying and evaporation of the benzene yielded 105 mg. residue, which had no selective absorption at 6 μ in the infrared spectrum.

Preparation of Purified dimethylamine: Eastman-Kodak crystalline dimethylamine hydrochloride was recrystallized twice from absolute ethanol, each time with an approximately 50% recovery. The final product was dried at 100° under reduced pressure. The calculated amount of the hydrochloride was placed in a flask equipped with a dropping funnel, distillation head and condenser which dipped below the surface of some methanol in the receiver. A nearly saturated solution of potassium hydroxide in methanol was added to the flask and the liberated amine distilled into the receiver. An aliquot of this solution was titrated with standard acid to determine the concentration of amine. A solution 2-3 N

in dimethylamine was generally used.

Preparation of N,N-dimethylaminocolchicide (VII). The general method described by Campion (17) was used with the exceptions that the amine solution used was the purified solution described above and that chromatography on alumina (using ethyl acetate, 1% absolute ethanol in benzene, or benzene-chloroform (1:1) as eluent) was used as the last step prior to crystallization. The general procedure is as follows: A mixture of colchicine (Ia) and the dimethyl amine solution (molar ratio 1:3) was heated in a sealed tube at 105° for 18 hours. The solvent was evaporated, the residue taken up in benzene and extracted into aqueous acid. The acid layer was then basified and the product extracted with benzene. Chromatography on alumina using one of the above mentioned eluents removed a small amount of highly colored material. The product was then crystallized from ethyl acetate.

Preparation of N,N-dimethylaminocolchicide (VII) without using acid extraction. A mixture of colchicine (Ia) and dimethylamine solution in methanol was heated at 105° for 18 hours. The liquid was evaporated and the residue dissolved in benzene. This solution was then passed through a column of alumina, elution being accomplished with benzene-chloroform (1:1). The solvent was removed and the product was crystallized from ethyl ether, yielding yellow crystals melting at 177-178°.

Paper chromatography. Whatman No. 1 filter paper strips were dipped into a solution of aluminum sulfate (65 g/l) and drained. The paper was then soaked in 2 N aqueous ammonia and washed under a running tap for 5 hours. It was then dried at 110° and stored over phosphorus pentoxide.

A quantity of the solution to be chromatographed containing approximately 25-50 micrograms of material was then placed about 2 inches from one end of the paper and allowed to dry. The paper was then

developed (descending) using a suitable solvent, e.g., 10% absolute ethanol in benzene. The paper was then examined under ultraviolet radiation to determine the position of the compounds.

Solubility analysis: Accurately weighed samples (30-60 mg.) were placed in 10 ml. volumetric flasks, a glass bead added to aid mixing, and 5 ml. of sodium-dried xylene added. The flasks were stoppered, the stoppers being lightly greased with Dow-Corning silicone lubricant and held in place with rubber bands. The flasks were then placed in a water bath at 25.0° (held constant to ± .05°) and rotated. At the end of 3 days filtered aliquots were taken and transferred to tared weighing bottles. The solvent was evaporated and the samples dried to constant weight at 100° and 1 mm. pressure.

Bromination of tetrahydrodemethoxycolchicine (VIII). VIII (500 mg.) was dissolved in 5 ml. 100% acetic acid in a flask fitted with a stirrer, dropping funnel and reflux condenser, all outlets being protected with drying tubes. Bromine (1.07 g.) in 15 ml. acetic acid was added to the cooled and stirred solution over a period of about one hour. The mixture was then allowed to stand at room temperature overnight. The orange-red solution was then heated on a steam bath. Hydrogen bromide was liberated and the solution turned dark red. After being heated overnight, the solution was a very dark red-brown and a black solid had precipitated. This precipitate was soluble in sodium carbonate solution and was re-precipitated upon acidification.

Anal. Found: C, 44.2; H, 3.17; Br, 24.3; residue, 2.0.

Oximinotetrahydrodemethoxycolchicine (XVI). t-Butyl alcohol was refluxed over sodium for several hours and then distilled into the reaction flask* equipped with a reflux condenser, sealed stirrer, and dropping

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* All equipment was dried in an oven at 110° just prior to being used.

funnel. Potassium (1.3-1.7 moles per mole VIII) was then added and the alcohol was refluxed until all of the potassium had dissolved. The solution was then cooled to room temperature and a solution of VIII and an equivalent amount of freshly distilled n-butyl nitrite in benzene (dried over sodium) was added with stirring over a period of about one hour. After standing overnight, the reaction mixture was then taken to dryness *in vacuo*. The residue was taken up in water and extracted with benzene. Drying (sodium sulfate) and evaporation of the benzene yielded a dark glass which accounted for about 5% of the starting material. In one 10 g. run, 0.56 g. of base-insoluble material was obtained. This residue was then extracted with hot 20% sodium bisulfite solution, but only 0.05 g. was dissolved, the remainder apparently being nonketonic.

The basic aqueous phase from the first extraction was saturated with carbon dioxide and extracted with chloroform. Drying (sodium sulfate) and evaporation of the chloroform gave a brownish residue in about 95% yield, which was crystallized from benzene-chloroform to give a 70% yield of crystalline material. The analytical sample was recrystallized twice more to yield white, very feathery needles, m.p. 214° dec.*; $[\alpha]_D^{23}$ -395° (c. 1.00, ethanol); λ_{max} 253 mμ, log ε_{max} 4.15; λ_{min} 241 mμ, log ε_{min} 4.11.

Anal. Calcd. for $C_{21}H_{26}N_2O_6$: C, 62.67; H, 6.51; N, 6.96; OCH_3, 23.13

Found: C, 62.92; H, 6.77; N, 6.96; OCH_3, 23.02.

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* This is the maximum value obtained. The melting point is quite dependent upon the rate of heating, and values below 200° have been obtained with slower rates of heating.

Dihydrocolchiceine (LXXV). A mixture of 5.1 g. XIV (12.2 mM), 4 ml. of a 7.4 N pyruvic acid solution* and 300 ml. of 50 volume % acetic acid was heated under reflux for 8 hours. The solution was then evaporated to a syrup under reduced pressure and less than 50°. The yellow residue was then taken up in benzene and extracted with potassium carbonate solution, 0.2 N sodium hydroxide (with added sodium chloride to salt out any LXIX that might go into the aqueous phase), 2 N hydrochloric acid, and finally washed with water. Acidification or basification of the aqueous phases gave 0.29 g. as acid soluble and 0.6 g. as base soluble materials. The benzene solution containing the neutral fraction was dried and evaporated. The final drying was done by lyophilization to yield 3.62 g. (72%) of amorphous yellow powder, which could not be crystallized or chromatographed; $\lambda_{max}^{CHCl_3}$ 340 mμ, log ε_{max} 3.76; $\lambda_{min}^{CHCl_3}$ 290 mμ, log ε_{min} 3.54; λ_{max}^{EtOH} 305 mμ, log ε_{max} 3.69; λ_{min}^{EtOH} 280 mμ, log ε_{min} 3.63.

Anal. Calcd. for $C_{21}H_{25}NO_6$: C, 65.10; H, 6.51; N, 3.62; OCH_3, 24.03.

Found: C, 65.28; H, 6.61; N, 3.82; OCH_3, 24.74.

Dihydrocolchiceine dioxime (LXXVI). A. From oximinotetrahydrodemethoxycolchicine (XVI). XVI (100 mg.) and 100 mg. hydroxylamine hydrochloride were heated under reflux in 5 ml. pyridine and 5 ml. absolute ethanol for 3 hours. The solvents were then evaporated under an air stream and the residue was dissolved in chloroform and extracted into 0.2 N

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* The pyruvic acid solution was prepared by adding about an equal weight of water to freshly distilled pyruvic acid and was stored in a refrigerator. The acid concentration was determined by titration with standard alkali.

sodium hydroxide. The aqueous layer was acidified with hydrochloric acid and extracted with chloroform, which was then dried (sodium sulfate) and evaporated to yield 110 mg. Crystallization from benzene-chloroform yielded 50 mg. white crystals, m.p. 232-233° dec.

Anal. Calcd. for $C_{21}H_{27}N_3O_6$: C, 60.42; H, 6.52; N, 10.06.
Found: C, 60.32; H, 6.75; N, 9.79.

B. From dihydrocolchiceine (LXXV). In a similar manner 49 mg. LXXV was reacted with 85 mg. hydroxylamine hydrochloride to give 20 mg. crystalline product, m.p. 226-7° dec.; mixed m.p. of the two dioximes from A and B 226-7° dec.

Anal. Calcd. for $C_{21}H_{27}N_3O_6$: C, 60.42; H, 6.52; N, 10.06
Found: C, 60.17; H, 6.12; N, 9.83.

o-Phenylene diamine derivative (LXXVIII) of dihydrocolchiceine (LXXV). LXXV (34 mg.) and 10 mg. o-phenylene diamine (recrystallized from ethanol-water) were heated under reflux for 1-1/2 hours in absolute ethanol. The solvent was evaporated in an air stream and the residue was crystallized twice from ethanol-water to yield 27 mg. white crystals, m.p. 110-115° and 190-190.5° (on the Koefler micro hot stage the crystals melted at 110-115° and then recrystallized to the higher melting form as the temperature increased); λ_{max} 243, 282, 335 mμ; log ϵ_{max} 4.34, 4.35, 4.07; λ_{min} 234, 251, 293 mμ; log ϵ_{min} 4.32, 4.32, 3.78.

Anal. Calcd. for $C_{27}H_{29}N_3O_4$: C, 70.26; H, 6.77; N, 9.10.
Found: C, 70.20; H, 6.40; N, 9.14.

Attempted dehydrogenation of dihydrocolchiceine (LXXV) with palladium. LXXV (10.7 mg.) and 34.4 mg. 5% palladium on carbon were heated under reflux overnight in 10 ml. of 1,2,4-trichloro-benzene (b.p. 205-210°, purified by washing with concentrated sulfuric acid) in a nitrogen sweep. The mixture was then filtered through a pad of

super-cel, rinsing with chloroform. The filtrate was then extracted with 0.1 N sodium hydroxide. Acidification of the aqueous layer and extraction with chloroform yielded a trace of residue, λ_{max} 248, 309, 354 mμ.

When 10.1 mg. colchiceine (VI) was treated the same way, the ultraviolet absorption spectrum of the base soluble was similar to that for colchiceine, although the extinction at 349 mμ showed that only 51% of the original colchiceine remained.

Attempted dehydrogenation with selenium dioxide. LXXV (10.9 mg.) and 1.55 mg. selenium dioxide in 2.5 ml. dioxane were heated under reflux for 5 hours. No selenium precipitated. The absorption spectrum became flatter and lower in optical density, but no peak appeared in the region where colchiceine or any other tropolone would absorb. Colchiceine treated in a like manner showed a gradual reduction in the extinction with a half-life of about 18 hours.

Attempted dehydrogenation with mercuric acetate. LXXV (7.3 mg.) was heated under reflux in 1 ml. dioxane and 1 ml. acetic acid containing 9.6 mg. mercuric acetate. Aliquots were taken for determination of the absorption spectra after varying periods of time up to 6 hours, but no absorption appeared in the region of 390 mμ. Colchiceine treated the same way showed a decrease in the absorption maximum with a half-life of about 6 hours.

Attempted dehydrogenation with silver carbonate. LXXV was heated under reflux with excess silver carbonate in benzene for several hours. The silver carbonate, which had not changed appreciably in appearance, was removed by filtration and a base soluble fraction was isolated by extraction of the benzene with sodium hydroxide solution. The ultra-

violet spectrum of this material showed only a maximum at 207 mμ, which is probably due to LXIX.

Attempted dehydrogenation with chloranil. LXXV (100 mg.) and 64 mg. chloranil were heated under reflux in 15 ml. xylene for 5 hours. The solvent was then removed in vacuo and the residue was treated with a 1:1 mixture of pyridine and acetic anhydride for 1/2 hour. The solvents were removed under reduced pressure and the residue was taken up in benzene. The benzene was then extracted with 2 N hydrochloric acid and 10% potassium carbonate. The carbonate solution was acidified and extracted with chloroform to yield a small amount of a yellow residue. This residue, however, gave no color with ferric chloride.

Reaction of dihydrocolchiceine (LXIX) and iodine. LXXV (25.3 mg.) and a crystal of iodine were heated under reflux in nitrobenzene for 2-1/2 hours. The mixture turned black as soon as heating had started. The solvent was then evaporated under an air stream. The residue was taken up in benzene, filtered to remove the black insoluble material, and extracted with potassium carbonate solution. The aqueous phase was then acidified and extracted with chloroform to yield 3 mg. (approximately 12%) residue. This material gave a green color with ferric chloride and a yellow-green chloroform soluble complex with cupric ammono sulfate and had absorption maxima at 247, 311, 381 mμ. A second run with 100.5 mg. LXXV refluxed 1 hour have 22.5 mg. (22%) base soluble.

Anal. Found: I, 0.0.

Reaction of dihydrocolchiceine (LXXV) with N-bromosuccinimide. LXXV, N-bromosuccinimide (recrystallized from carbon tetrachloride) and a trace of benzoyl peroxide (except in the first two runs) were refluxed 4-7 hours in carbon tetrachloride. The solvent was then evaporated and the residue was heated under reflux 2-4 hours in 2,6-lutidine. The

lutidine was then evaporated and the residue was taken up in benzene, extracted with hydrochloric acid and then sodium hydroxide. Acidification of the hydroxide solution and extraction with chloroform yielded the acidic fraction. This material gave a green color with ferric chloride and a yellow solution in chloroform with cupric ammono sulfate. The individual runs are tabulated in Table 8:

Table 8

Run No.	Molar ratio*	% Yield base sol.	% Br in base sol.	λ_{max} mμ
1	1	**	**	307, 365, 380***
2	1	13	5.0	365, 380***
3	1	15	5.8	230, 247, 304, 361
4	2	35	12.6	306, 380
4a	-	-	5.3	306, 380
5	1	22	**	**
6	3	26	**	**

* Ratio of N-bromosuccinimide to dihydrocolchiceine.
** Not determined.
*** Absorption maxima at lower wavelengths not determined.

Anal. of No. 3: Calcd. for $C_{21}H_{23}NO_5$(A): C, 65.4; H, 6.0; Br, 0.0
Calcd. for $C_{21}H_{22}NO_5Br$(B): C, 54.3; H, 4.8; Br, 17.2
Calcd. for 67% A and 33% B: C, 61.8; H, 5.6; Br, 5.8
Found: C, 62.2; H, 5.9; Br, 5.8.

Fifty-four mg. from run No. 4, 28 mg. sodium acetate and 12 mg. 5% palladium on carbon in 10 ml. ethanol was hydrogenated in an atmospheric pressure apparatus. Uptake of hydrogen ceased after 1 hour and was 1.6 moles hydrogen per mole bromine. The residue from the

hydrogenation constitutes run No. 4a.

Reaction of dihydrocolchiceine (LXXV) with bromine. A. LXXV (100 mg.) was dissolved in 20 ml. carbon tetrachloride, 200 mg. potassium carbonate was added and the mixture was cooled in an ice bath. A solution of 41 mg. (0.262 mM) bromine in 10 ml. carbon tetrachloride was then added with stirring over a period of 1-1/2 hours. During the addition a yellow solid appeared. After 1/2 hour more, the mixture was filtered and washed with chloroform, which dissolved the yellow precipitate. The filtrate was then evaporated and the residue was taken up in benzene and lyophilized to yield 75 mg. of a yellow powder.

Anal. Found: Br, 10.9.

The above powder (54.4 mg.) was heated under reflux for 1 hour in 25 ml. 2,6-lutidine. The solvent was then evaporated and the residue was taken up in benzene, extracted with 2 N hydrochloric acid and 10% potassium carbonate. Acidification of the carbonate solution gave only 4 mg. of base soluble material. Drying and evaporation of the benzene phase yielded 31 mg. neutral material.

Anal. Found: Br, 6.1.

B. LXXV (100.5 mg.) was dissolved in 25 ml. chloroform, 200 mg. sodium bicarbonate was added and the mixturewas cooled in ice. A solution of 83 mg. (0.513 mM) bromine in 10 ml. carbon tetrachloride and 10 ml. chloroform was then added with stirring over a period of 3 hours. After the reaction mixture had risen to room temperature over 1 hour, it was filtered.* The filtrate was evaporated and the residue was heated

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* The residue contained 38.2 mg. bromide ion.

under reflux 1 hour in 25 ml. 2,6-lutidine. The lutidine was then removed *in vacuo* and the residue was taken up in benzene and extracted with potassium carbonate solution. The benzene layer gave 100 mg. on evaporation and the carbonate solution yielded 10 mg. on acidification and extraction with chloroform.

C. Bromine (20.7 mg.) in 10 ml. glacial acetic acid was added at room temperature with stirring to a solution of 50 mg. (0.129 mM) LXXV in 25 ml. acetic acid over a period of 4 hours. After standing overnight, the reaction mixture was heated on a steam bath for 1 hour, during which time the red-orange solution turned redder and darker. The acetic acid was then removed under reduced pressure at about 50°. The residue was then taken up in benzene, filtered to remove a red insoluble oil, extracted with water (removed a red color), and 10% potassium carbonate. Evaporation of the benzene gave 36 mg. of a dark brown residue. The carbonate phase was acidified and extracted with chloroform to yield 22.4 mg. (45%) of a brown residue, which gave a green color with ferric chloride; λ_{max} 247, 303, 382 mμ.

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