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Photochemistry of isocolchicine and related compounds

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PHOTOCHEMISTRY OF ISOCOLCHICINE AND RELATED COMPOUNDS

by

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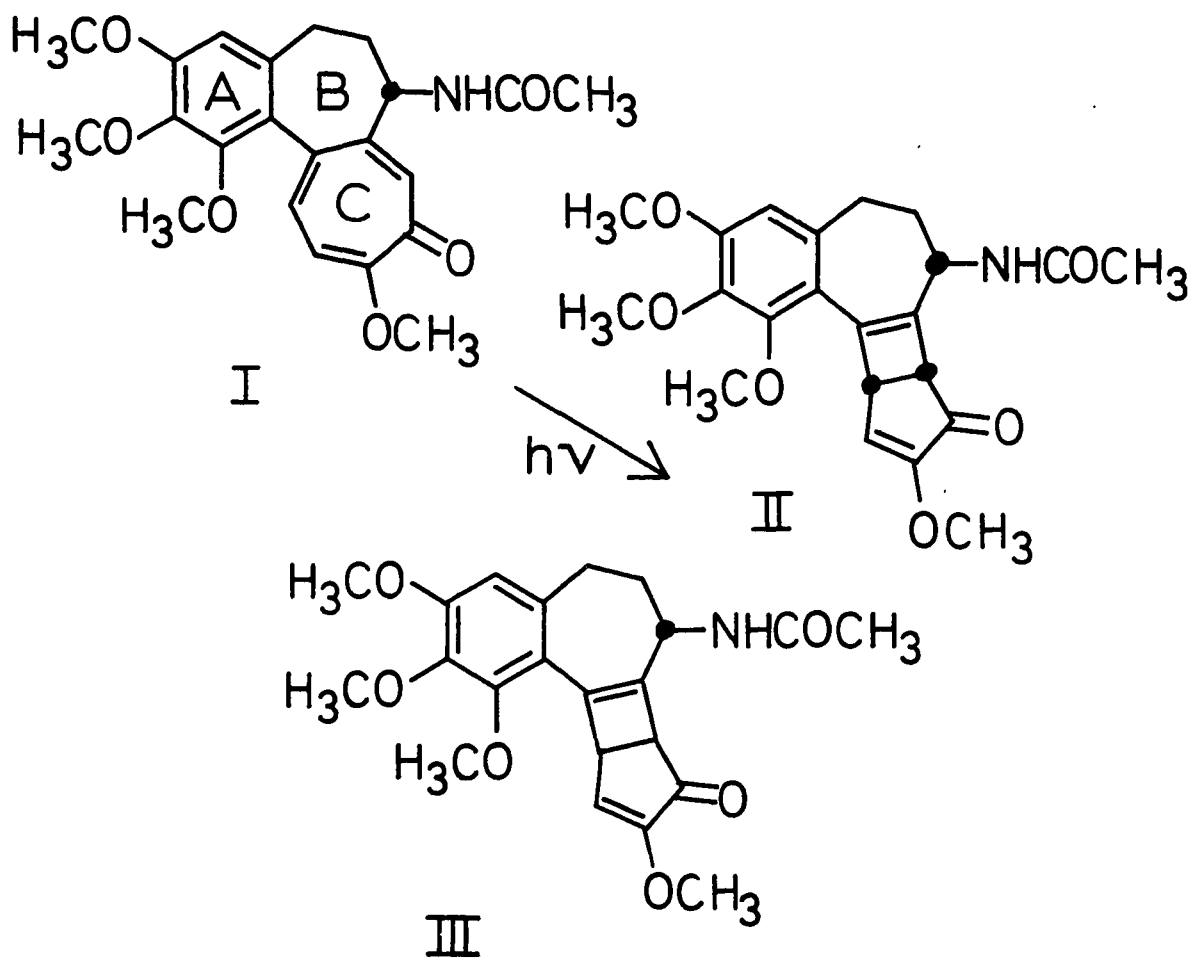
INTRODUCTION AND HISTORICAL

For nearly one hundred years, it has been known that colchicine (I) (1) and its solutions undergo change when exposed to sunlight (2). It was shown by Jacobi (3) that the photoproduct obtained was a product of photo-oxidation. Macht (4) showed that it was only the ultraviolet portion of the spectrum which caused the photochemical reactions.

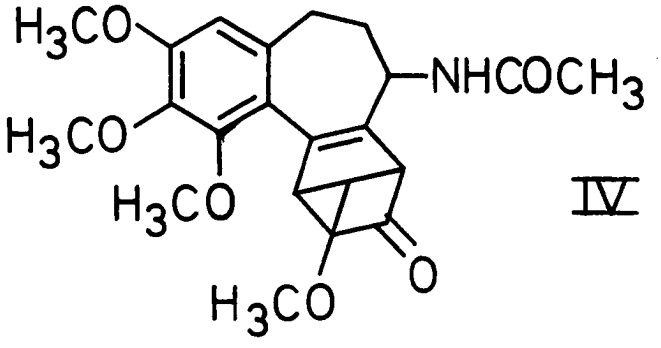
Using an ultraviolet light Grewe (5) was able to obtain small yields of a photoisomer of colchicine. When air was excluded from the irradiation vessels, sunlight was found to



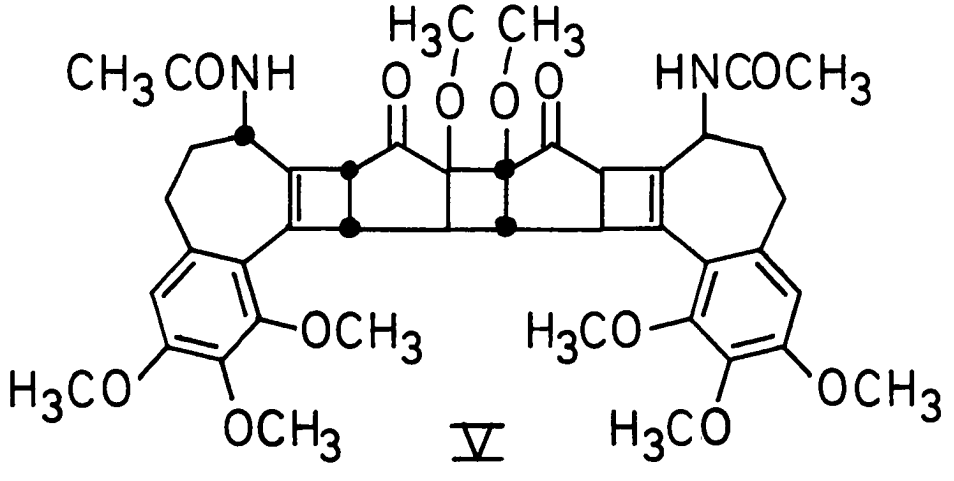
convert colchicine to its photoisomers in very high yield (6). Under these conditions Grewe (5) obtained three isomers which he designated as α-, β-, and γ-lumicolchicine. Lumicolchicines 1 and 2, obtained by Santavy (7), are identical to β- and γ-lumicolchicine.

In 1955, Forbes (8) suggested that β- and γ-lumicolchicine have gross structures II and III. Gardner (9) supported this work and suggested that β-lumicolchicine has the stereochemistry shown by structure II and γ-lumicolchicine has the stereochemistry shown by III. Chapman (10) has confirmed the structure and stereochemistry of β-lumicolchicine assigned by Gardner.

The erroneous structure (IV) of α-lumicolchicine



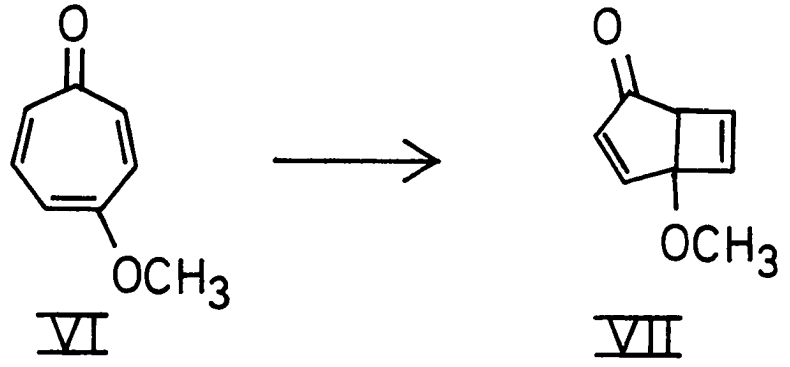
IV



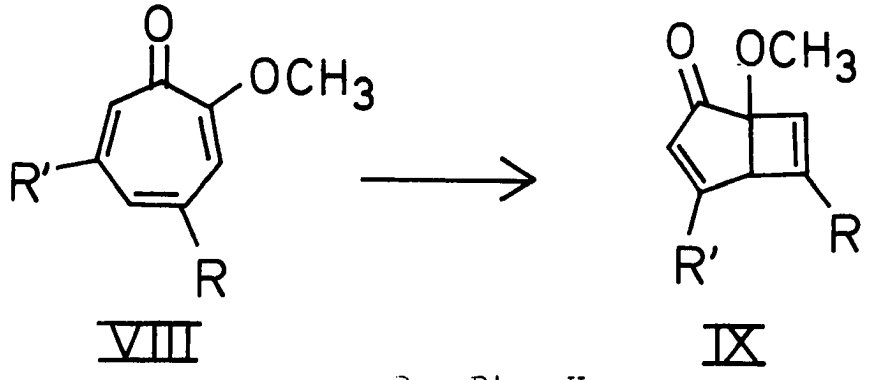
V

suggested by Schenck (11) has been corrected by Chapman (12). α-Lumicolchicine (V) is a head-to-head <u>trans</u> dimer of β-lumicolchicine.

Irradiation of simple tropolones such as γ-tropolone methyl ether (VI) (13) and α-tropolone methyl ethers (VIII a, b, and c) (14) gives good yields of bicyclic photoproducts VII and IX a, b, and c respectively.



VI VII



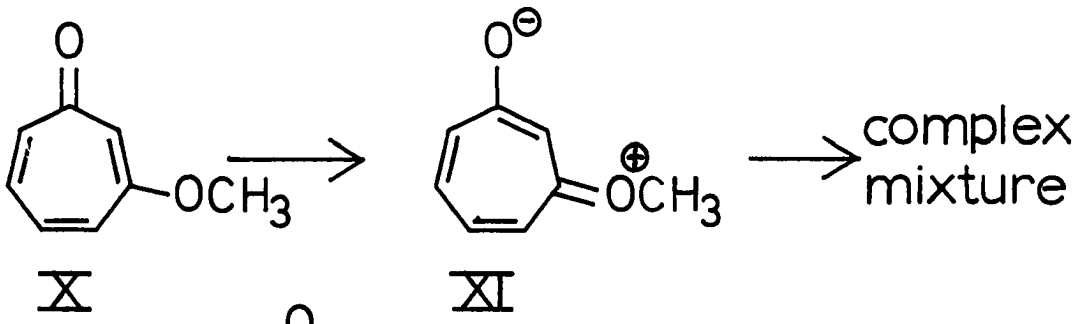
VIII IX

a; R = R' = H

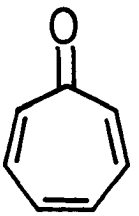
b; R = H, R' = CH_3

c; R = CH_3, R' = H

In direct contrast, β-tropolone methyl ether (X) (15) and tropone XII (16) give only complex mixtures of photoproducts.

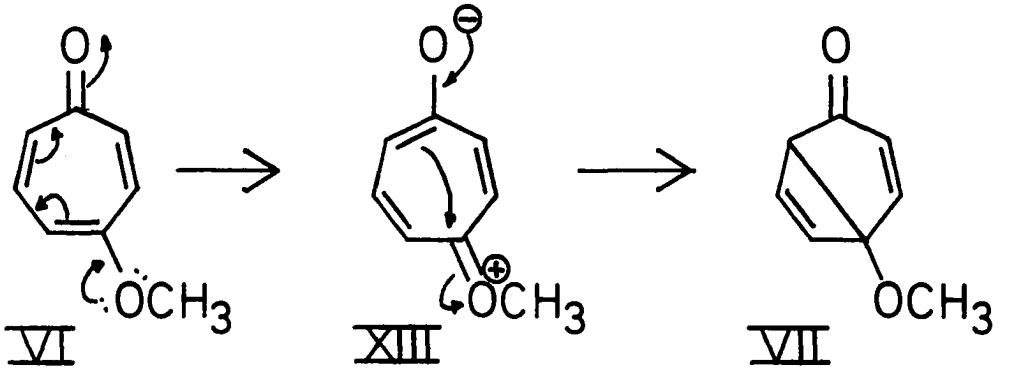


X XI

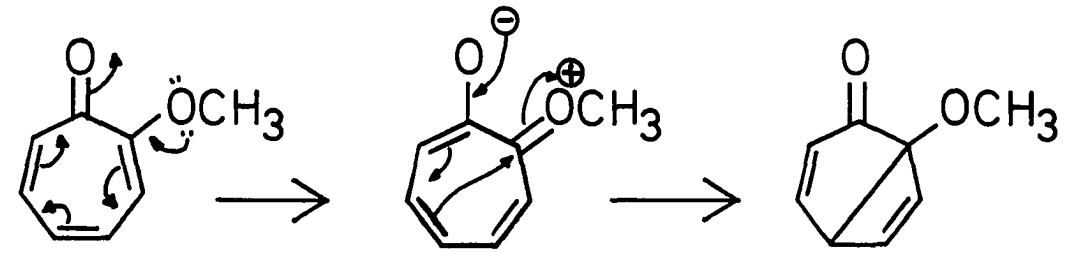


XII

It is evident that the methoxyl groups in the γ- and α-tropolone methyl ethers are involved <u>directly</u> in photo-excited states XIII and XIV as shown below. On the other



VI XIII VII

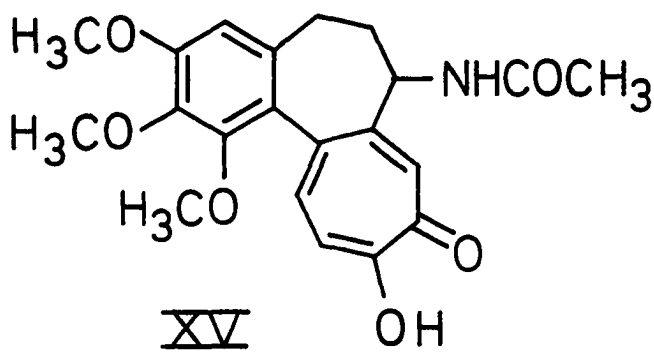


VIIIa XIV IXa

hand, the direct involvement of the methoxyl group in β-tropolone methyl ether (X ⟶ XI) does not lead to a stable photoproduct. Tropone (XII) has no methoxyl group to exert a directive effect.

It is interesting to note that, although colchicine is a substituted α-tropolone methyl ether, all three lumicolchicines do not originate from an intermediate which involves the methoxyl group in the same direct manner as in the simple tropolone ethers. It is thus apparent that retention of a trimethoxystyryl chromophore is more important in product formation than is the direct involvement of the methoxyl group.

It has been shown by Schenck (11) and Gardner (17) that colchiceine (XV) undergoes light-induced isomerization and

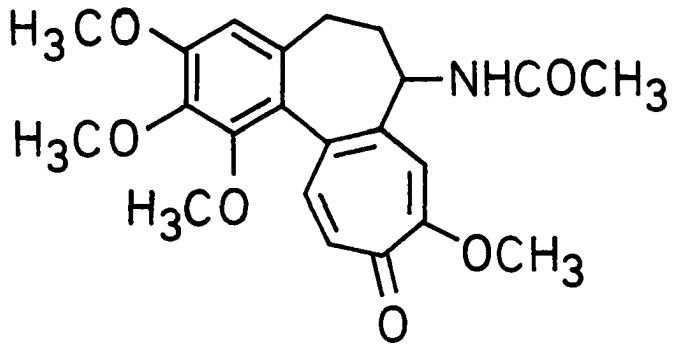


XV

dimerization reactions analogous to those of colchicine. These photoproducts have been converted to α-, β-, and γ-lumicolchicine. Once again the retention of the trimethoxystyryl chromophore is the factor governing the course of these photochemical reactions. It is apparent that the presence of a methoxyl group is not necessary in the photo-

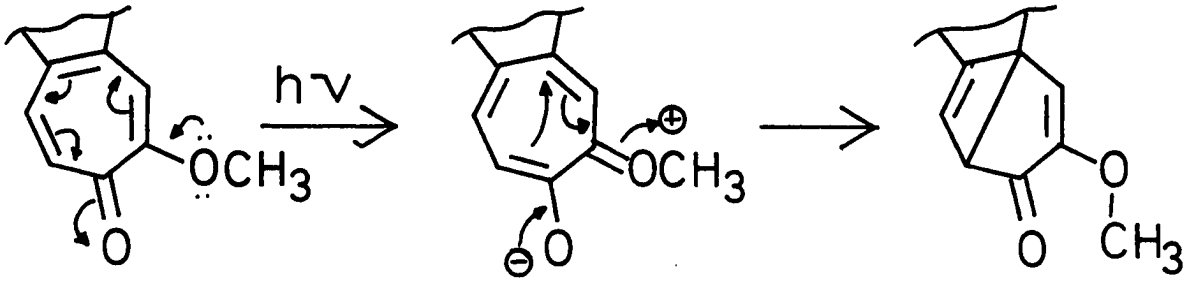
isomerization reactions. As a general rule, the preservation of the trimethoxystyryl chromophore is the dominant factor in the photoisomerization of colchicine and colchiceine.

Irradiation of isocolchicine (XVI) is an interesting test



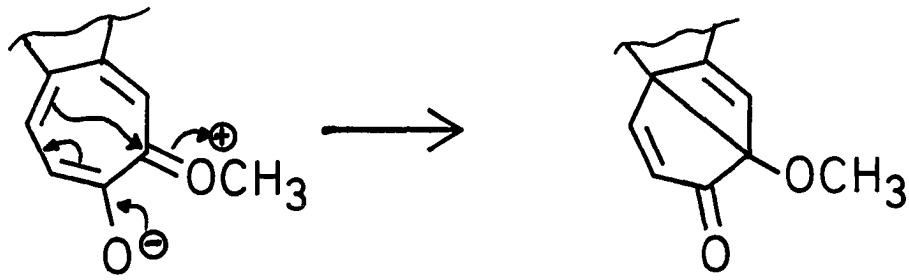
XVI

of the applicability of this generalization. If the generalization is valid, the photoexcited intermediate (XVII) should give XVIII as the predicted photoproduct. If the generaliza-



XVI XVII XVIII

tion is not valid, XVII should collapse to XIX, by a mechanism analogous to simple tropolone methyl ether collapse.



XVII XIX

Irradiation of aminodemethoxyisocolchicine (18) (XX)*

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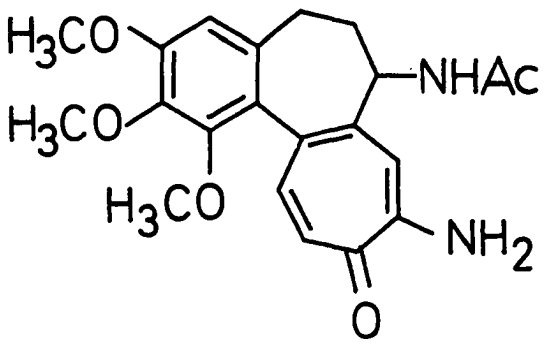
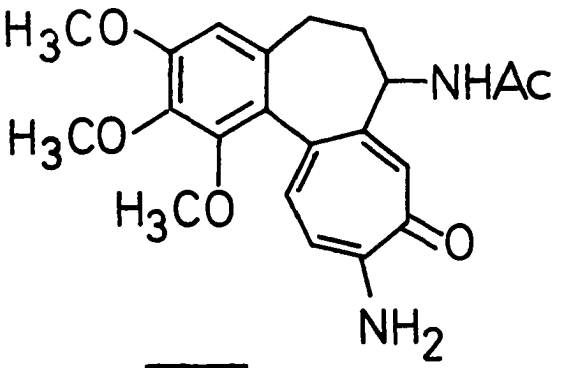
*"Some confusion exists in the nomenclature of this type of compound in which an amino group has replaced the methoxyl of colchicine. Zeisel (Monatsh. 9, 1 (1888)) who first prepared the amino compound, called it the amide of colchiceine, amide of acetyltrimethylcolchicinic acid, and colchicamide, and all three names have been used by various investigators. However, we find this nomenclature (which had its origin in the mistaken belief that colchicine was an ester and colchiceine a carboxylic acid) misleading, since the compounds are distinctly basic in aqueous media, a property not characteristic of amides. Furthermore, the term colchiceinamide" (18b, 18c) "leads to difficulty in naming the compound in which carbonyl and amino groups are interchanged. Isocolchiceinamide implies a non-existent compound, isocolchiceine, and the alternative, colchiceineisoamide implies an 'isoamide' as opposed to a normal amide, which is clearly not intended. For these reasons we have adopted the name aminocolchicide as being more suitable. The basic properties are clearly implied, and the root name colchicide may serve quite flexibly for any number of compounds in which the methoxyl is replaced by another group, colchicide itself serving for replacement by hydrogen. This nomenclature very conveniently accomodates the iso series also through isocolchicide." Quotation from footnote 13, p. 3694 of reference 18a.

The present author feels that the paragraph above states the difficulty of nomenclature clearly. However, it is felt that the introduction of the new term "colchicide" is an unfortunate and unnecessary addition to the problem of nomenclature. The term "demethoxycolchicine" implies the same structure as "colchicide" yet causes no confusion to the casual reader who is unfamiliar with the above quotation. So, at the risk of being damned (footnote continued on next page)

and aminodemethoxycolchicine (XXI) provides additional tests of the applicability of this generalization.

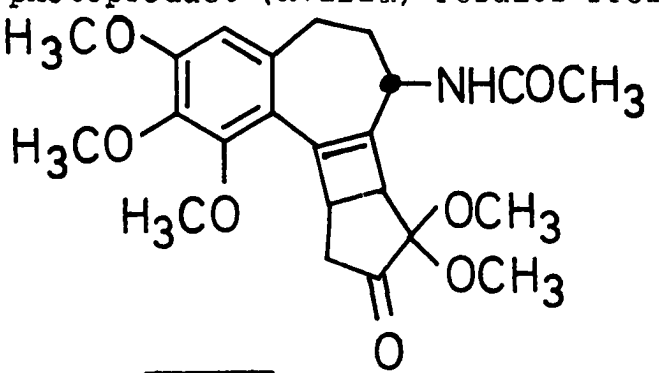


XX



XXI

After this work was completed, Dauben (19) published the structure of two photoproducts obtained by irradiation of isocolchicine in methanol. The major photoproduct, lumi-isocolchicine, has the same structure as photoisocolchicine (XVIII). The minor photoproduct (XVIIIa) results from addition of



XVIIIa

methanol to isocolchicine. Both photoproducts contain the trimethoxystyryl system.

(footnote continued from previous page) for adding fuel to the fire, the root demethoxycolchicine is introduced to replace colchicide. A prefix, such as amino, implies that the amino group has replaced the methoxyl on the colchicine ring. Also demethoxyisocolchicine causes no confusion in the isocolchicine series.

RESULTS AND DISCUSSION

Irradiation Apparatus

All the irradiations performed in this study utilized the same basic apparatus. The ultraviolet irradiation lamp is a quartz, high intensity mercury arc lamp (Type A, 550 watts) manufactured by Hanovia.*

The lamp (A) (Figure 1, p. 11), connected to the appropriate transformer (T), is inserted in a Pyrex well (B), fitted with cold water inlet (C) and outlet (D). The solution (E) of compound to be irradiated is enclosed in flask (F), which varies in capacity from 240 ml. to 2.0 liters. Flask (F) is equipped with two side arms (G and H) so that nitrogen can be bubbled into the solution continually during the irradiation. The nitrogen inlet (G) is fitted with a small serum cap through which a five-inch syringe needle (J) is inserted. A nitrogen atmosphere is maintained by bubbling a slow stream of nitrogen through the needle. Aliquots of the irradiation mixture are obtained by removing the nitrogen source from the needle, extracting the required amount with a syringe, and re-attaching the nitrogen source. The solution is stirred by a magnetic stirring motor (K) and Teflon stirring bar (L).

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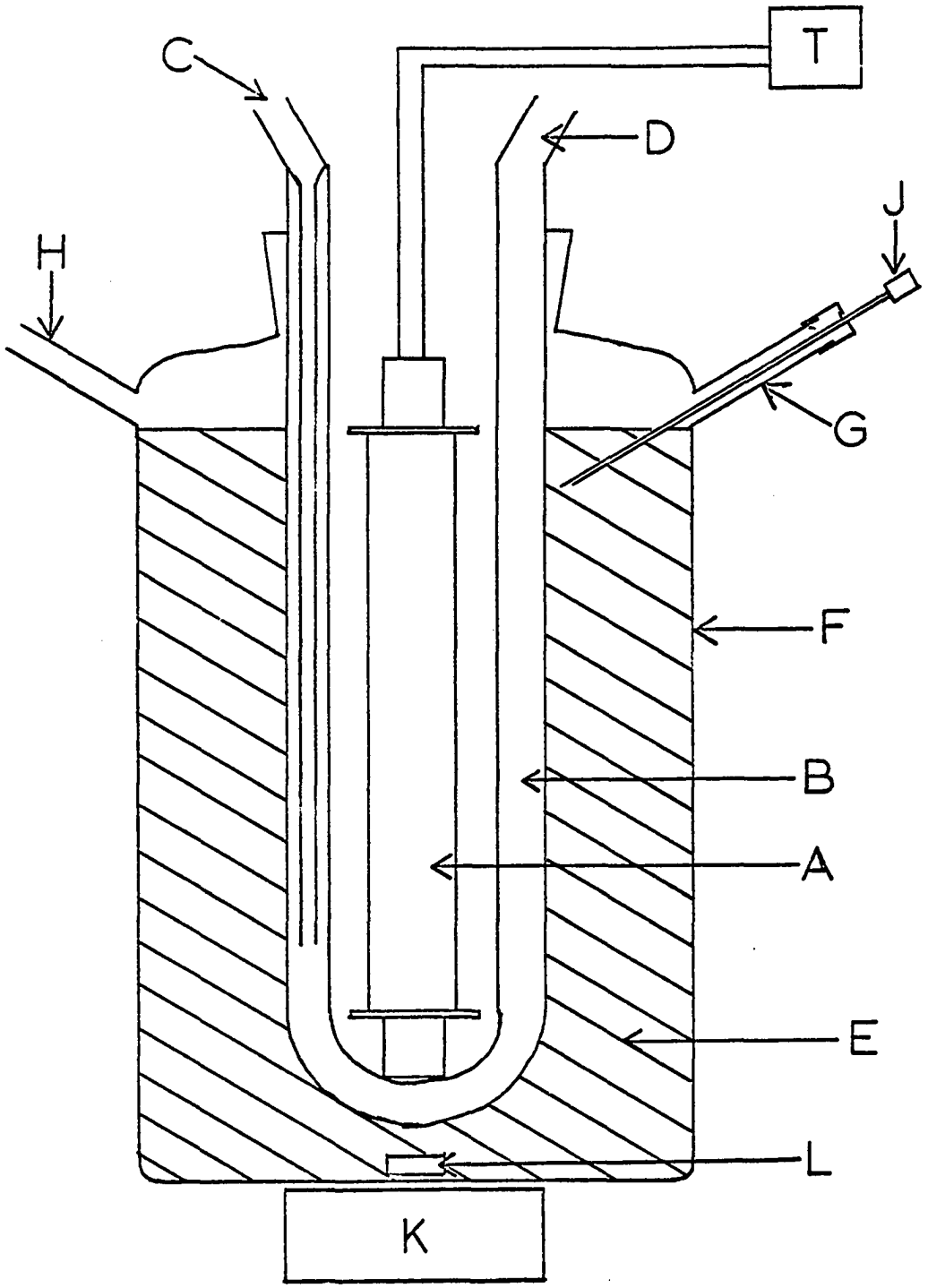
*Hanovia Lamp Division, Englehard Industries, 100 Chestnut Street, Newark 5, New Jersey.

Figure 1. Irradiation apparatus


T
C
D
J
H
G
F
B
A
E
L
K

Photoisocolchicine

The methoxyl group on the tropolone ring (ring C) of colchicine (I) is labile to mild acid hydrolysis (20, 21). Crystalline colchiceine (XV) is obtained by heating colchicine and 0.1 Normal hydrochloric acid on a steam bath for 1.5 hours. Treatment of the crude hydrolysis product with diazomethane in methylene chloride gives a mixture of colchicine and isocolchicine (XVI). The isomers can be separated by column chromatography as suggested by Sorkin (20c) giving isocolchicine in 35-40% yield (Figure 2, p. 25). Isocolchicine is almost completely insoluble in hot ethyl acetate, while colchicine is soluble. A method of obtaining pure isocolchicine was developed, based on this observation. The crude diazomethane reaction mixture is refluxed gently at first to remove and destroy excess diazomethane. After two hours the remaining methylene chloride is removed in vacuo. The red oil is twice dissolved in chloroform, evaporated in vacuo, dissolved in ethyl acetate, and boiled vigorously on a steam bath to remove the chloroform. Isocolchicine precipitates as a colorless powder in 25% yield when the mixture is scratched. The main feature of this preparation of isocolchicine is elimination of time consuming chromatography. This procedure has two disadvantages: a) the amount of isocolchicine obtained per run is lower and b) pure colchicine is not obtained. Neither of these objections is serious

since the noncrystalline material (a mixture of colchicine and isocolchicine) is recycled, treating it as pure starting colchicine. A series of four cycles can be run in two days giving 50-65% total yield of isocolchicine (based on starting colchicine) whereas two days are required to run one cycle utilizing column chromatography, when sizable amounts of material are involved.

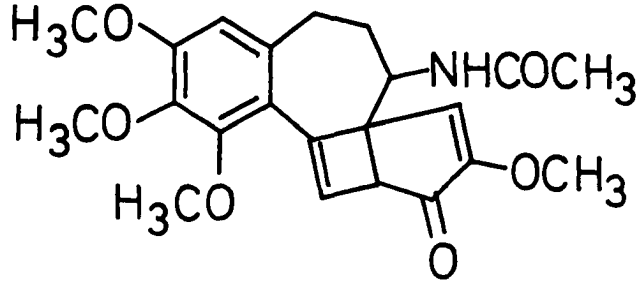
Three solvents were used for the irradiation of isocolchicine. Absolute methanol was used in the first experiment but no photoproduct was obtained after irradiation for 17.5 hours. Very dilute aqueous solutions also yielded no useful photoproduct. It was then discovered that deoxygenating the solution (no matter which solvent is used) is very critical in the formation of any photoproduct. Irradiation of aqueous solutions of isocolchicine (three grams in two liters) under nitrogen atmosphere gives 30-40% of a single photoisomer, based on unrecovered isocolchicine. The progress of the irradiation is followed by the decreasing intensity of the long wavelength maximum in the ultraviolet (342 mμ). When this reaction is about 40% complete (60-65 hours), the irradiation is stopped and the photoproduct isolated. Longer irradiation times do not increase the yield of photoisocolchicine and at the same time decrease the amount of isocolchicine which can be recovered for recycling. The third irradiation solvent was 95% ethanol. More concentrated (two

grams of isocolchicine per 240 milliliters) solutions were irradiated. Only four and three-quarters hours are required for 70% loss of the 342 mμ peak. At the same time the maximum at 245 mμ decreases and a shoulder develops at 260 mμ. If the reaction is allowed to go to 95% completion, the 342 mμ maximum disappears, maxima develop at 262 and 219 mμ, and a minimum appears at 250 mμ. There is no increase in total yield of photoproduct beyond 70% completion, and as above, less unreacted isocolchicine is recovered.

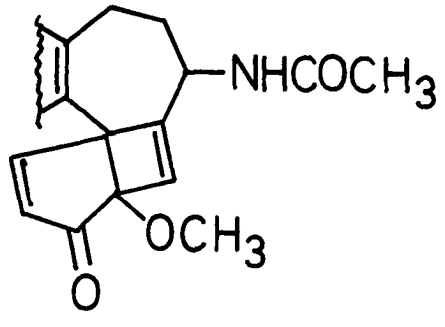
Photoisocolchicine is obtained from the aqueous medium by saturation with sodium chloride and extraction with chloroform. The chloroform extracts are concentrated and chromatographed on alumina. Unreacted isocolchicine can be removed by treatment with ethyl acetate before chromatography, as described above. This step is useful because less alumina and less time are required for the separation of the photoisomer. The ethanolic solutions are concentrated and chromatographed. It is important to remove all of the ethanol solvent from the crude irradiation mixture since traces of ethanol in the chromatography column allow the compounds to be eluted without good separation. Likewise, chloroform which contains ethanol as preservative should not be used. The photoproduct is easily eluted from neutral alumina using chloroform-benzene (1:1) solvent. Unreacted isocolchicine is recovered by elution with pure chloroform. No other

photoisomers are isolated.

Photoisocolchicine is obtained as a crystalline monohydrate from aqueous methanol or ethanol. The tightly-held water of hydration can be removed by high vacuum drying at 78° for at least two days. The monohydrate appears to be more stable to air oxidation. Photoisocolchicine has infrared maxima (Figure 3, p. 25) at 5.85, 6.01, 6.20, and 6.27 μ. The first maximum is characteristic of a cyclopentenone (22, 23). The acetamido carbonyl group appears at 6.01 μ. The two peaks at 6.20 and 6.27 μ are associated with the trimethoxybenzene ring. Two peaks in this region appear in only one other known compound in this series, β-lumicolchicine, all others have just one peak between 6.20 and 6.30 μ. Hypothetical structures XVIII and XIX both contain the required



XVIII



XIX

cyclopentenone moiety and must therefore be considered as possible structures for photoisocolchicine.

XIX does not contain the trimethoxystyryl chromophore while XVIII does. The ultraviolet spectrum of photoisocolchicine (Table 1) is useful in distinguishing between these

Table 1. Ultraviolet absorption maxima

Compound	Ultraviolet absorption spectra $\lambda_{max}^{95\% EtOH}$ mμ (ε)
Photoisocolchicine (XVIII)	218 (26,200), 260 (21,900)
XXII	212 (35,530), 270 (14,850)
XXIII	220 (25,000), 265 (15,200)
XXVI	207 (46,420)

two hypothetical structures. Photoisocolchicine has maxima at 260 (21,900) and 218 mμ (26,200). The first is characteristic of a trimethoxystyryl chromophore (8, 9). Structure XVIII is thus tentatively assigned as the structure of photoisocolchicine.

This conclusion is confirmed by the nuclear magnetic resonance spectrum (Figure 8, p. 29 and Table 2, p. 17). Three olefinic protons, in agreement with structure XVIII, appear as two singlets located at 3.48 and 3.65 τ (relative to internal tetramethylsilane) with area ratios of 1:2. The n.m.r. spectrum of photoisocolchicine in pyridine solution (Figure 9, p. 29) confirms that these three olefinic protons appear as three distinct singlets. It is unfortunate that pyridine is not more widely used in nuclear magnetic resonance spectroscopy. It has the ability to solvate specific sites on the molecule (24). Thus, protons which were previously equivalent in one solvent are rendered magnetically non-equivalent and appear separated in pyridine. The proton on nitrogen

Table 2. Nuclear magnetic resonance spectra[a]

Compound	Proton Aromatic	Cyclobutene	H(C=)OMe	N-H[b]	H(C)-N	O-CH_3	O=C-CH_3	Bridgehead	Methylene	H(C)O	Hydroxyl
XVIII[c] ($CDCl_3$)	3.48	3.65	3.65	3.74	5.58	6.14 6.18 6.35	8.08	6.54	7.12 7.90	--	--
XVIII (Pyridine)	3.45	3.34	3.36	--	5.10	6.18 6.22 6.36 6.65	8.02	5.96	7.06 7.68	--	--
XXII ($CDCl_3$)	3.52	3.71	5.24	4.54	5.78	6.17 6.27 6.40	8.07	6.67	7.22	5.32	7.70
XXIII ($CDCl_3$)	3.58	3.73	--	4.02	5.64	6.20 6.36	7.99	6.3 under OMe	7.23 8.0	5.64	6.2 under OMe
XXVI[d] ($CDCl_3$)	3.45	--	4.52	4.80	5.56	6.18 6.23 6.38 6.42	8.18	6.15 under OMe	7.13 8.0	--	--

[a]Resonance positions are given in τ -values relative to tetramethylsilane as internal standard.

[b]The exact position of this resonance depends strongly on concentration.

[c]Water of crystallization 7.50 τ. This resonance disappears when the solution is shaken for a few seconds with two drops of D_2O.

[d]Water of crystallization 7.58 τ. This resonance disappears when the solution is shaken for a few seconds with two drops of D_2O.

appears as a doublet centered at 3.74 τ (coupling constant J = 8 c.p.s.). The position of this peak is solvent dependent as shown in Table 3. The complex multiplet centered at 5.58 τ is assigned to the proton on carbon bearing nitrogen. The

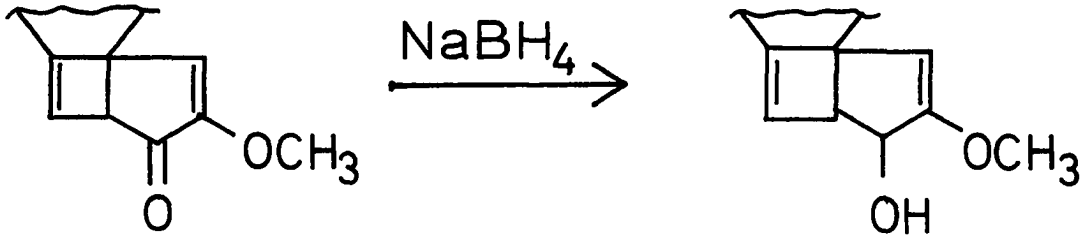
Table 3. Position of N-H doublet of photoisocolchicine at various concentrations in deuterochloroform

mg. of XIX/μl. $CDCl_3$	τ (relative to internal tetramethylsilane)
100/300	3.63
100/400	3.74
50/300	4.15
25/300	4.47
12.5/300	4.60
6.25/300	4.68
3.12/300	4.75

four methoxyl groups appear as three singlets located at 6.14, 6.18, and 6.35 τ, with area ratios of 3:3:6. These four methoxyls are separated in the spectrum obtained in pyridine solution (Figure 9, p. 29). The bridgehead proton appears as a slightly split (coupling constant J = 0.8 c.p.s.) doublet centered at 6.54 τ. The methylene protons occur as two multiplets centered at 7.12 and 7.90 τ. The acetamido methyl appears at 8.08 τ. The water of hydration at 7.50 τ is removed when the sample is shaken with deuterium oxide for a few seconds. The remaining assignments for the pyridine solution of photoisocolchicine (Figure 9, p. 29) are straight

forward and are found in Table 2, p. 17.

The structure of photoisocolchicine, XVIII, derived solely from spectral data, is confirmed by chemical transformations. Reduction of photoisocolchicine (XVIII) by sodium borohydride leads to a very acid-labile alcohol (XXII).



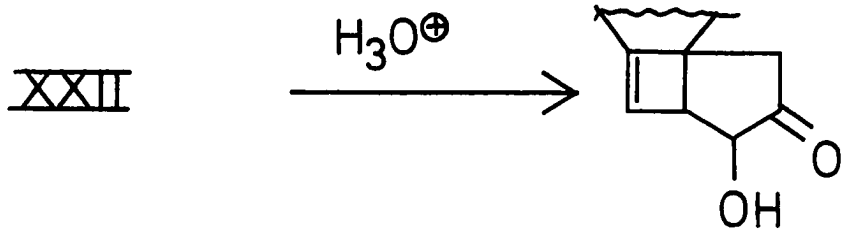
XVIII XXII

Photoisocolchicine alcohol (XXII) shows enol ether absorption in its infrared spectrum at 6.09 μ (Figure 4, p. 25). The ultraviolet absorption spectrum (Table 1, p. 16) shows the retention of the trimethoxystyryl chromophore ($\lambda_{max}^{95\% \text{ EtOH}}$ 212 (35,530) and 270 mμ (14,850)).

The n.m.r. spectrum of photoisocolchicine alcohol (XXII) (Figure 10, p. 31 and Table 2, p. 17) is very similar to the spectrum of XVIII. The obvious assignments are made in Table 2 and need no discussion. The aromatic and the cyclobutene protons remain at low field. The third olefinic proton, the enol ether vinyl proton, has shifted upfield to 5.27 τ, the normal position for the vinyl proton of an enol ether (25, p. 62). The proton on carbon bearing hydroxyl appears as a doublet, J = 9.35 c.p.s., centered at 5.35 τ. The bridgehead

proton is now a doublet, J = 9.35 c.p.s., centered at 6.68 τ. Consequently, the hydrogen which reduced the carbonyl group is attached to the carbon next to the bridgehead carbon. The hydroxyl proton is centered at 7.70 τ and is lost when the sample is treated with deuterium oxide for a few seconds. Slow scan of the two downfield olefinic protons (Figure 11, p. 31) shows that the proton at 3.71 τ is actually a slightly split doublet (J = 0.86 c.p.s.). Irradiation of the bridgehead proton in a double resonance experiment collapses this proton to a singlet (Figure 11, p. 31). The four-membered ring olefinic proton (3.71 τ) is on the carbon attached to the bridgehead carbon. Only structure XXII will accommodate these observations.

Mild acid hydrolysis of photoisocolchicine alcohol (XXII) gives photoisocolchicine ketol (XXIII). The ketol gives a



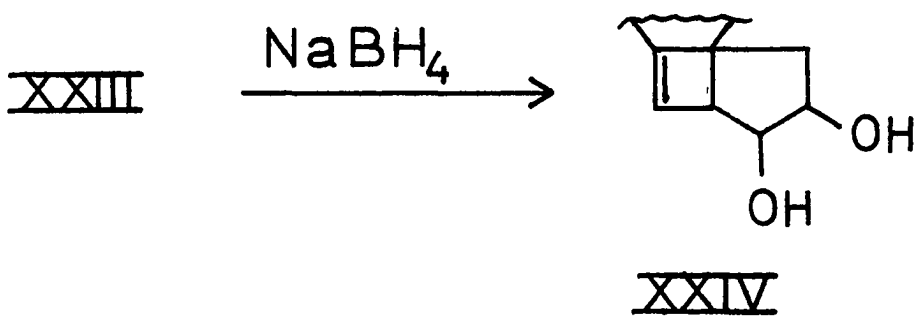
XXIII

positive periodic acid test showing that the carbonyl and hydroxyl functions are on adjacent carbons. The infrared spectrum of XXIII (Figure 5, p. 27) has a maximum at 5.70 μ, which confirms the presence of a cyclopentanone ring. The

ultraviolet absorption spectrum (Table 1, p. 16) shows the trimethoxystyryl chromophore.

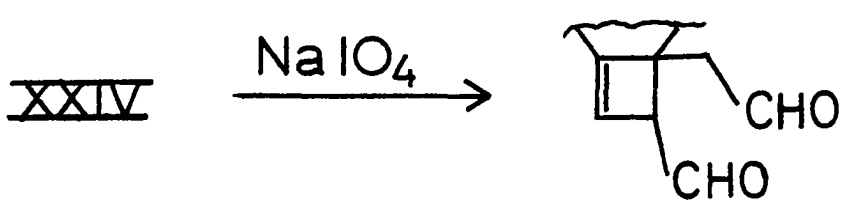
The nuclear magnetic resonance spectrum of XXIII (Figure 12, p. 33 and Table 2, p. 17) shows only three methoxyl groups located in two singlets (area ratio 6:3) at 6.20 and 6.36 τ. The enol ether vinyl proton of XXII has also been removed. The bridgehead proton and the hydroxyl proton also appear under the two methoxyl peaks.

Photoisocolchicine ketol XXIII gives a diol (XXIV) on



XXIV

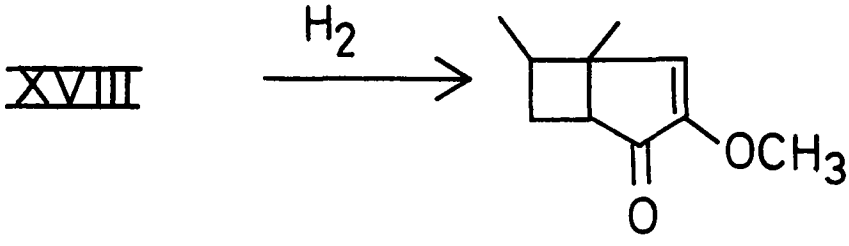
reduction with sodium borohydride. The amorphous diol is oxidized with sodium metaperiodate to the dialdehyde XXV which was characterized only by its infrared absorption.



XXV

The dialdehyde shows typical aldehyde absorption in the infrared at 5.84 μ (Figure 6, p. 27).

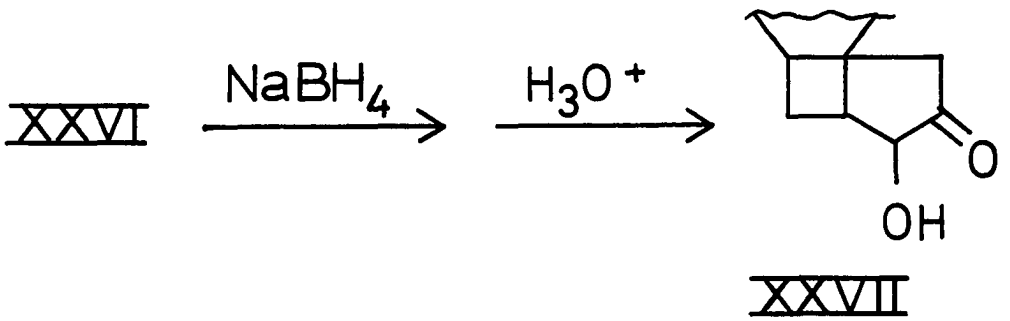
Photoisocolchicine (XVIII) can be selectively reduced with one mole of hydrogen over Adams catalyst. Dihydrophotoisocolchicine (XXVI) retains the cyclopentenone chromophore as shown by a 5.84 μ maximum in the infrared spectrum (Figure 7, p. 27). The ultraviolet spectrum (Table 1, p. 16) shows only a single maximum at 207 mμ. This shows that the trimethoxystyryl chromophore has been replaced by a trimethoxy-



XXVI

benzene chromophore. The nuclear magnetic resonance spectrum of XXVI (Figure 13, p. 33 and Table 2, p. 17) confirms the loss of the trimethoxystyryl chromophore. Only two low field protons are present, the aromatic proton at 3.45 τ and the cyclopentenone vinyl proton at 4.52 τ. The latter value is in the normal range for this proton, although it previously appeared in the range 3.3-3.7 τ (Table 2, p. 17). The previous low position of this proton is due to paramagnetic shielding of the vinyl enol ether proton by the trimethoxystyryl double bond.

Sodium borohydride reduction of XXVI followed by acid hydrolysis gives dihydrophotoisocolchicine ketol (XXVII) which gives a positive periodic acid test.



XXVII

The structure of photoisocolchicine is shown conclusively to be XVIII. This structure is proved by use of instrumental analysis on photoisocolchicine and reduction products of both the four- and five-membered rings. At the present time, no satisfactory method has been devised for proof of the stereochemistry of photoisocolchicine.

From this study it appears that the retention of a trimethoxystyryl chromophore in photoisomers derived from colchicine and isocolchicine is more important than direct interaction of the α-methoxyl group. Irradiation of amino substituted colchicines and isocolchicines, as shown in the following sections, substantiate this generalization.

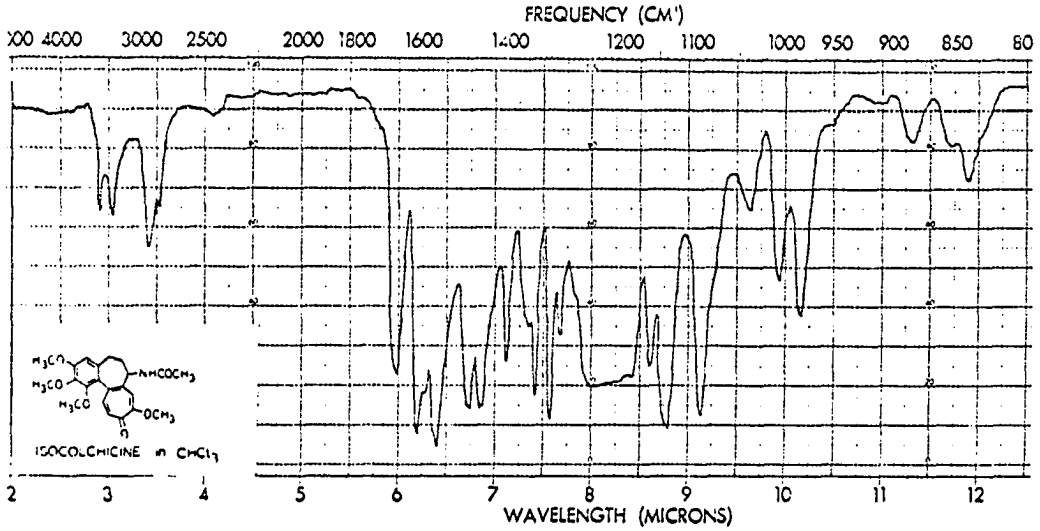
Photoaminodemethoxyisocolchicine

Preparation of aminodemethoxyisocolchicine (XX) requires only sufficient supplies of isocolchicine. The procedure of

Figure 2. Infrared spectrum of isocolchicine (XVI)

Figure 3. Infrared spectrum of photoisocolchicine (XVIII)

Figure 4. Infrared spectrum of photoisocolchicine alcohol (XXII)


FREQUENCY (CM')
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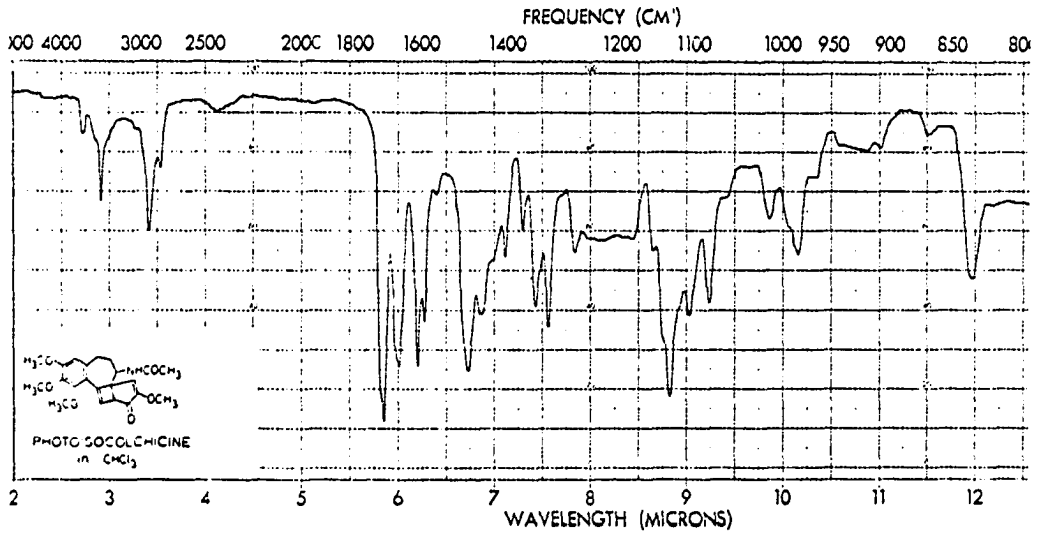
ISOCOLCHICINE in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)


FREQUENCY (CM')
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PHOTOISOCOLCHICINE in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)

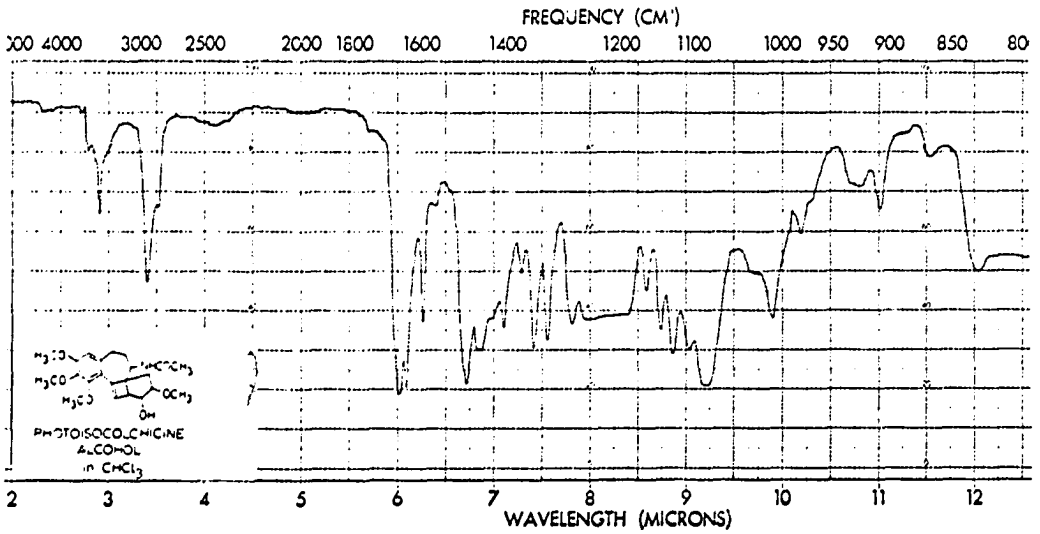
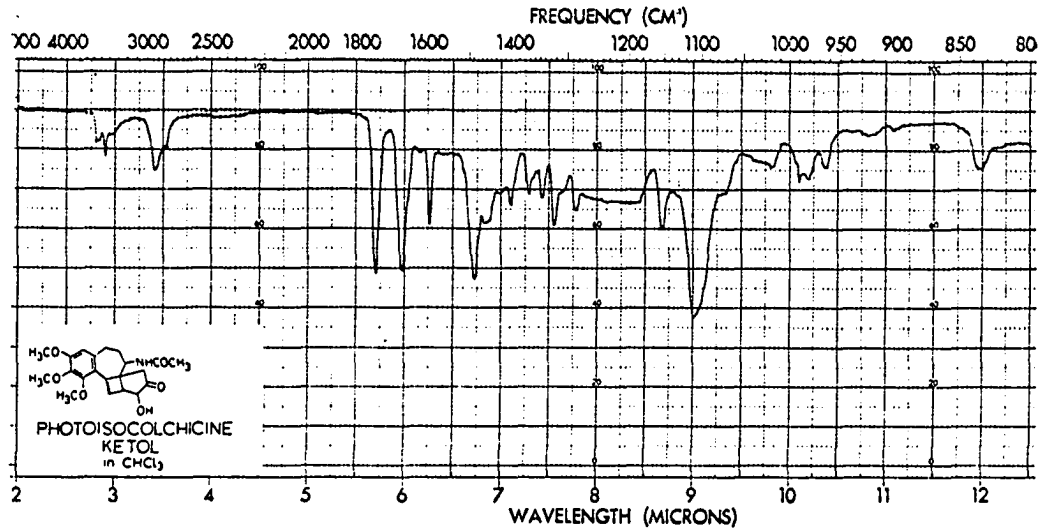

FREQUENCY (CM')
3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850
PHOTOISOCOLCHICINE ALCOHOL in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)

Figure 5. Infrared spectrum of photoisocolchicine ketol (XXIII)

Figure 6. Infrared spectrum of photoisocolchicine dialdehyde (XXV)

Figure 7. Infrared spectrum of dihydrophotoisocolchicine (XXVI)


FREQUENCY (CM⁻¹)
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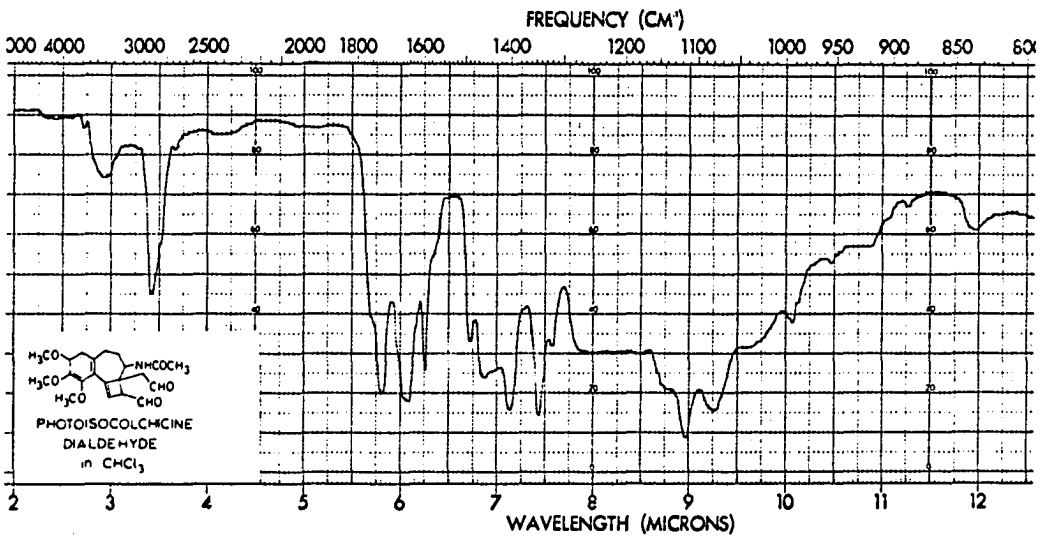
PHOTOISOCOLCHICINE
KETOL
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)


FREQUENCY (CM⁻¹)
000 4000 3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850 60
PHOTOISOCOLCHICINE
DIALDEHYDE
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)

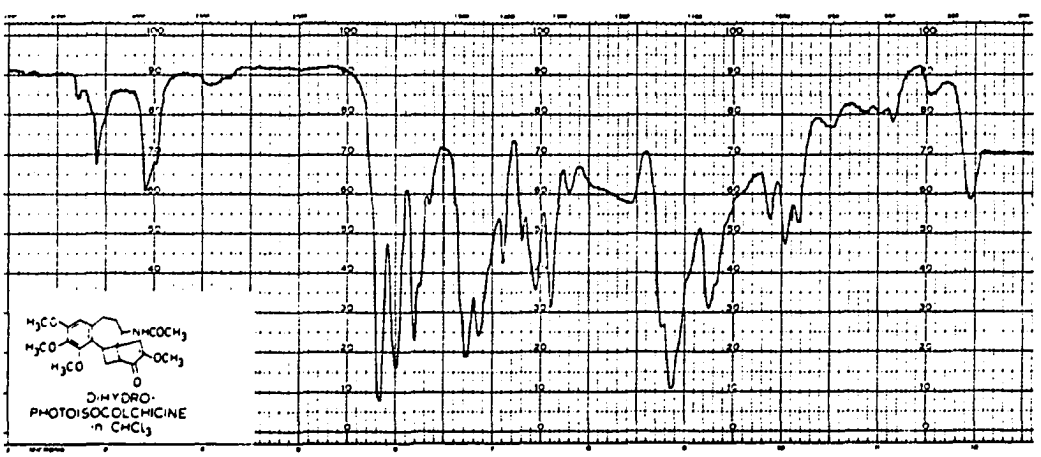
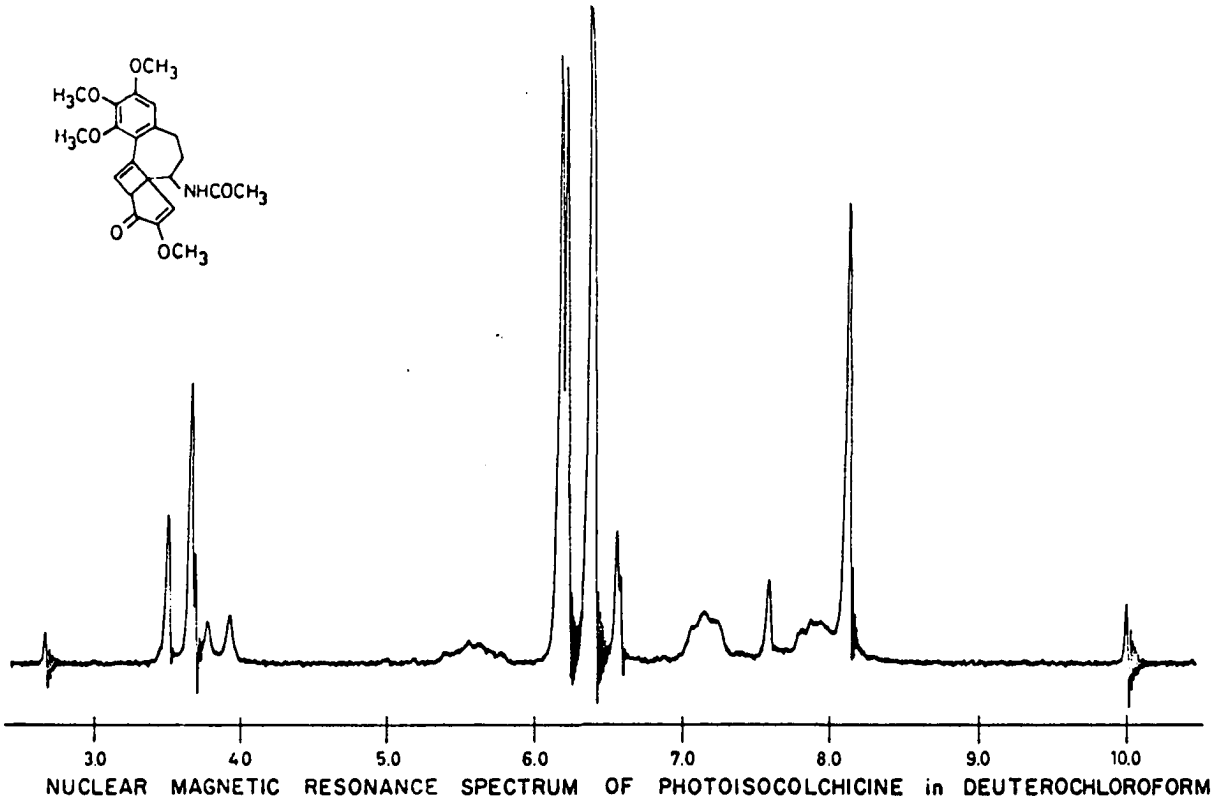
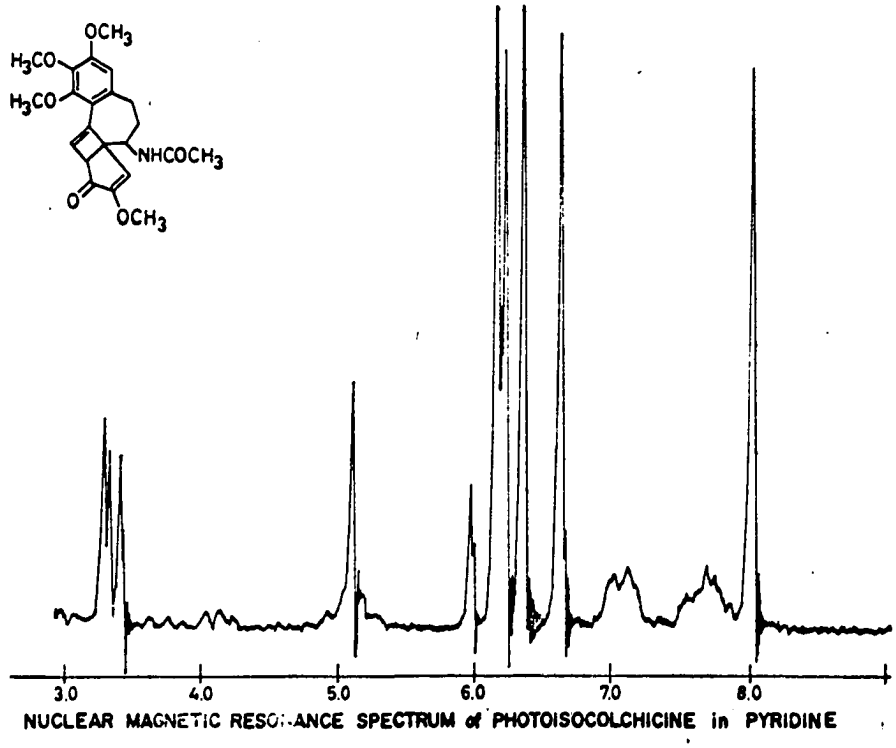

DIHYDRO-
PHOTOISOCOLCHICINE
in CHCl3

Figure 8. Nuclear magnetic resonance spectrum of photoisocolchicine (XVIII) in deuterochloroform

Figure 9. Nuclear magnetic resonance spectrum of photoisocolchicine (XVIII) in pyridine



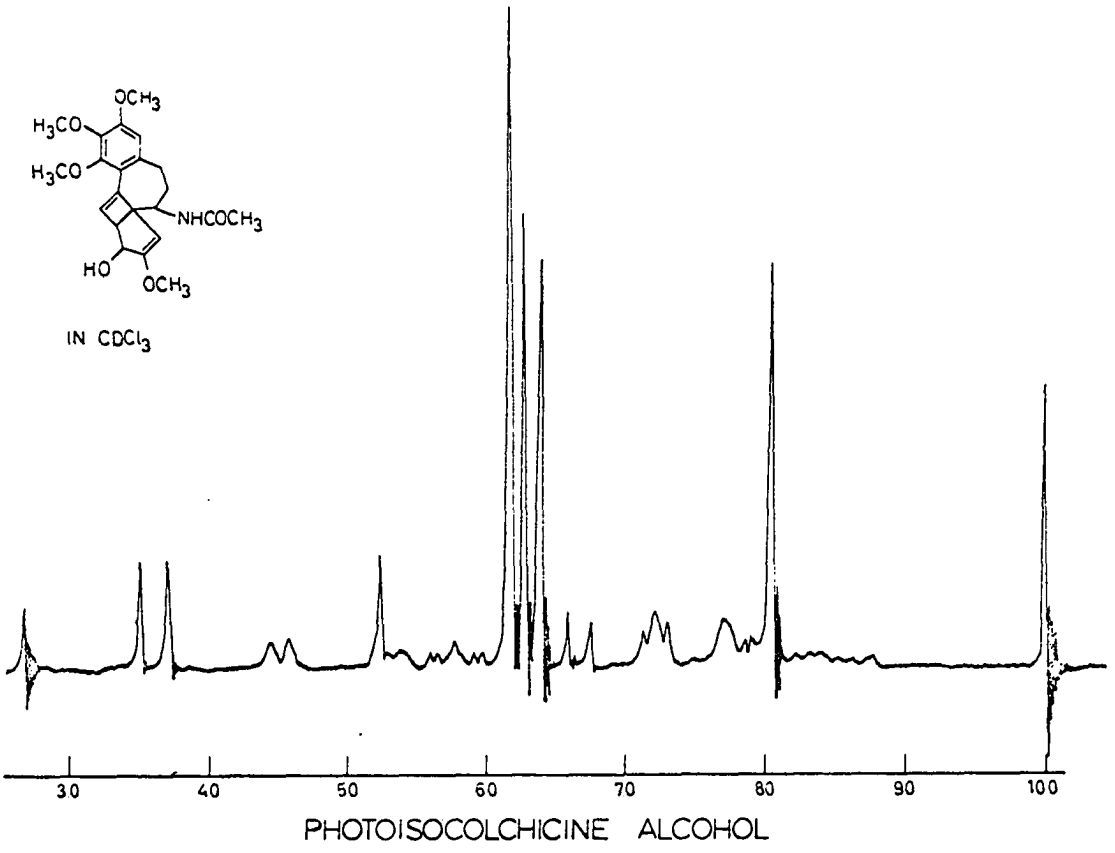
NUCLEAR MAGNETIC RESONANCE SPECTRUM OF PHOTOISOCOLCHICINE in DEUTEROCHLOROFORM



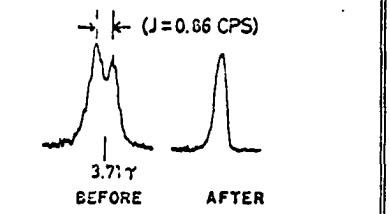
NUCLEAR MAGNETIC RESONANCE SPECTRUM of PHOTOISOCOLCHICINE in PYRIDINE

Figure 10. Nuclear magnetic resonance spectrum of photoisocolchicine alcohol (XXII) in deuterochloroform

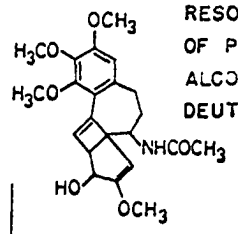
Figure 11. Double resonance experiment on photoisocolchicine alcohol (XXII) in deuterochloroform



PHOTOISOCOLCHICINE ALCOHOL



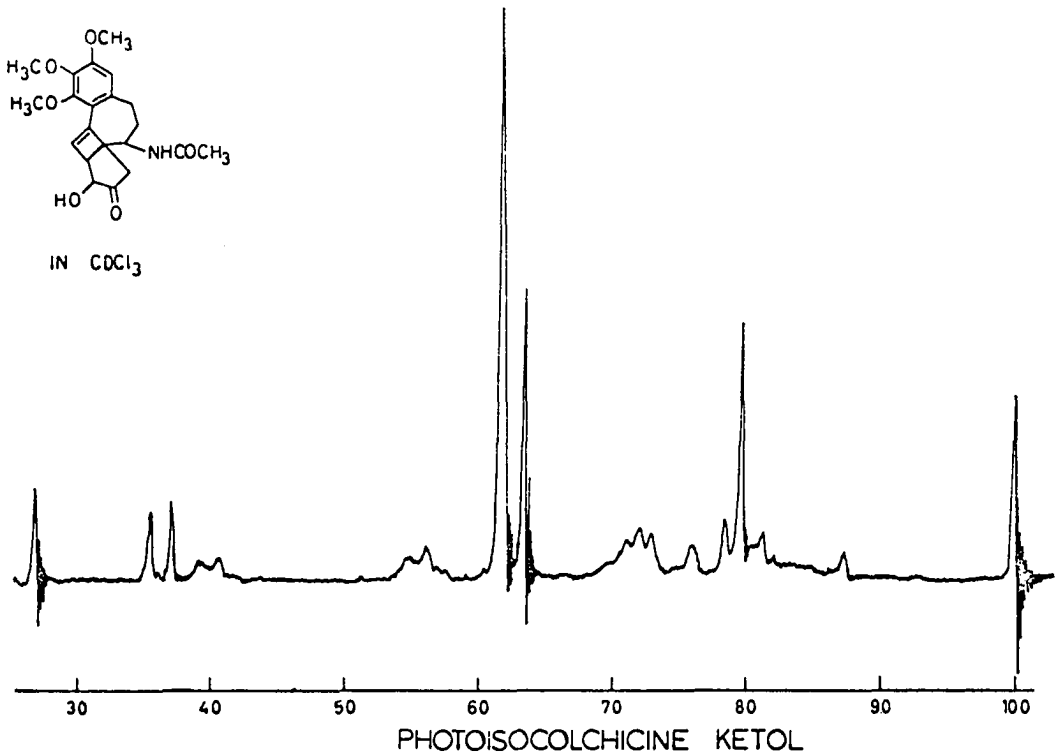
EXPANDED VIEW OF CYCLO-BUTENE PROTON BEFORE AND AFTER SATURATION OF BRIDGEHEAD PROTON.



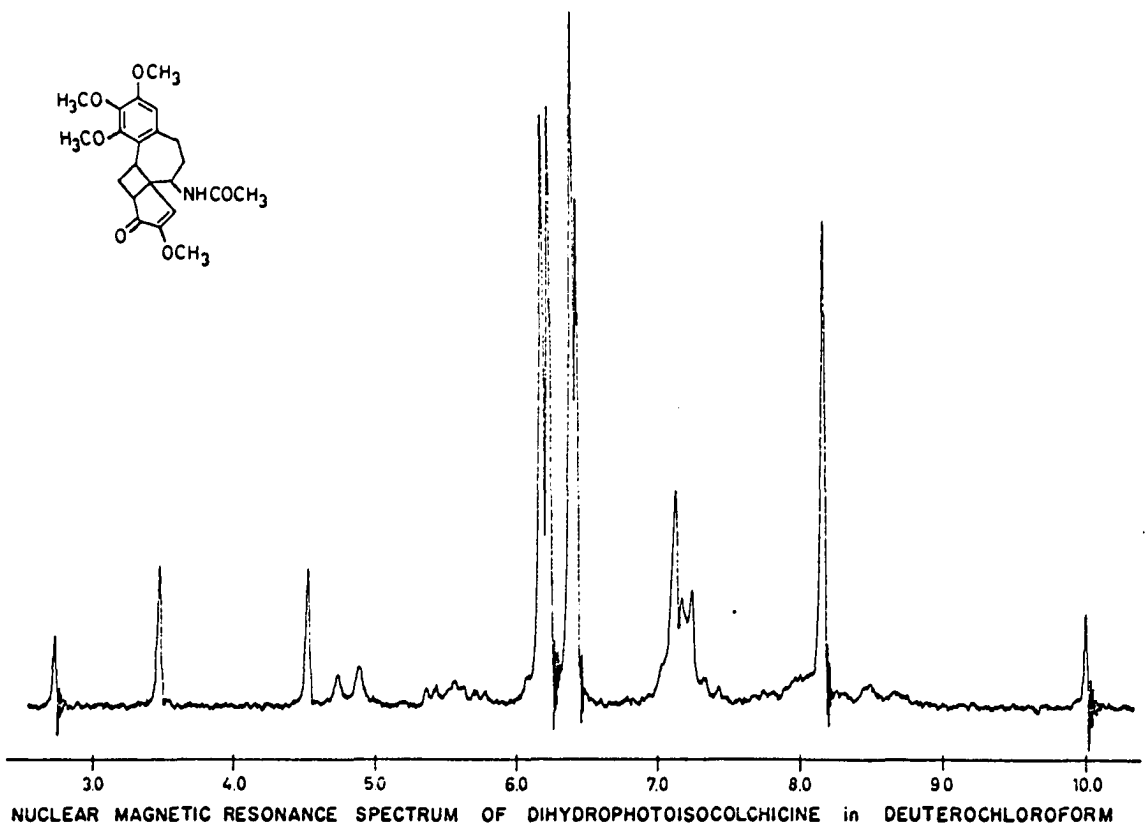
NUCLEAR MAGNETIC RESONANCE SPECTRUM OF PHOTOISOCOLCHICINE ALCOHOL IN DEUTEROCHLOROFORM

Figure 12. Nuclear magnetic resonance spectrum of photoisocolchicine ketol (XXIII) in deuterochloroform

Figure 13. Nuclear magnetic resonance spectrum of dihydrophotoisocolchicine (XXVI) in deuterochloroform



PHOTOISOCOLCHICINE KETOL



NUCLEAR MAGNETIC RESONANCE SPECTRUM OF DIHYDROPHOTOISOCOLCHICINE in DEUTEROCHLOROFORM

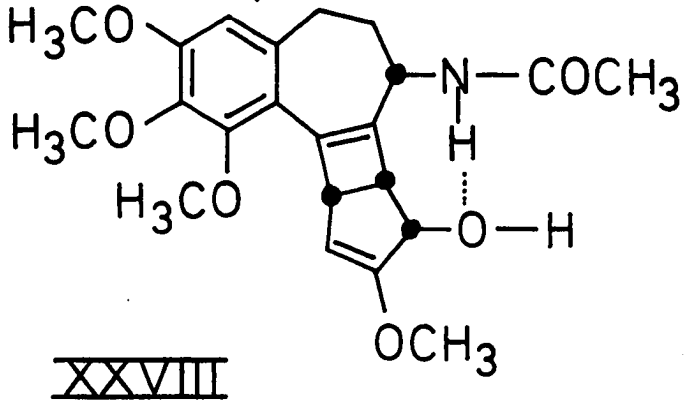
Horowitz and Ullyot (26) gives good yields of XX.

Aminodemethoxyisocolchicine (XX) is dissolved in 95% ethanol (3.7 grams in 240 milliliters of solution) and nitrogen is bubbled through the solution for 1.5 hours. The irradiation is followed by the disappearance of the 353 mμ band in the ultraviolet (Figure 14, p. 44). The four isobestic points which appear during this irradiation are located at 308, 269, 232, and 211 mμ. After the irradiation is 80% complete (7.25 hours) the bright red solution is concentrated and chromatographed on Woelm Neutral Alumina (Activity Grade III). It is surprising that no product is obtained from the column until it is eluted with a 99:1 mixture of chloroform-methanol. The black-green residue (1.82 grams) eluted with this solvent is crystallized from methanol-water (1:1) giving 1.25 grams of lustrous, colorless platelets.

Photoaminodemethoxyisocolchicine has infrared maxima at 5.70, 6.04, and 6.26μ (Figure 15, p. 46). The first maximum is too low for a cyclopentenone carbonyl group but could be a cyclopentanone carbonyl group. The maximum at 6.04μ corresponds to the acetamido group, and the trimethoxybenzene group is assigned to the 6.26μ maximum. The photoproduct has maxima at 276 (12,700) and 225 mμ (14,320) in the ultraviolet showing that the trimethoxystyryl chromophore is retained.

The n.m.r. spectrum (Figure 18, p. 48 and Table 4, p. 68)

of photoaminodemethoxyisocolchicine is unlike any other n.m.r. in the colchicine series. The acetamido nitrogen proton is located at 0.97 τ. This is a very dramatic downfield shift for this proton (from 3.7-4.7 τ in the photoisocolchicine series for instance). Moreover, this proton has a much smaller coupling constant (J = 3.5 c.p.s.) than previously observed (J = 8 c.p.s. for photoisocolchicine). The proton on carbon bearing acetamido appears at 5.74 τ. This multiplet is not as broad as the corresponding multiplets in the colchicine and isocolchicine series. The dramatic downfield shift of the acetamido -NH is similar to the downfield shift observed by Chapman (10) in β-lumicolchicine alcohol (XXVIII).



XXVIII

Chapman attributes this shift to a hydrogen bond as shown. The downfield shift of the acetamido -NH in photoaminodemethoxyisocolchicine must be due to similar hydrogen bonding. The only low field proton present (at 3.56 τ) is the aromatic proton. This implies that photoaminodemethoxyisocolchicine has undergone further reactions after simple tropolone ring collapse. One way to remove the olefinic protons

is simple dimerization analogous to the dimerization of β-lumicolchicine to α-lumicolchicine. There are three bridgehead protons, a triplet (slightly split) at 6.36 τ, a doublet (J = 3.5 c.p.s.) centered at 6.81 τ, and a sharp singlet at 7.25 τ. The three methoxyl groups appear at 6.17 τ, and the acetamido methyl appears at 8.03 τ. The peak at 7.50 τ and the underlying multiplet account for seven protons. Shaking the sample with deuterium oxide for a few seconds removes three protons from this multiplet leaving a multiplet containing four protons as a diminished peak at 7.50 τ (Figure 19, p. 48), assigned to the two methylene groups.

Osmometric determination of the molecular weight of photoaminodemethoxyisocolchicine confirms that the photoproduct is a dimer. Elemental analysis shows that the photodimer contains only two nitrogen atoms. The ring C amino group has been replaced by an oxygen function. Exhaustive drying to constant weight of the analytical sample by the analysts* at 78° and 0.04 mm. pressure resulted in a weight loss of 4.27%. This corresponds to one mole of water of hydration per monomer unit or two moles in the dimer.

One part of the puzzle of the n.m.r. spectrum is thus solved. Treatment with deuterium oxide removes the water of hydration and the hydroxyl proton in the multiplet at

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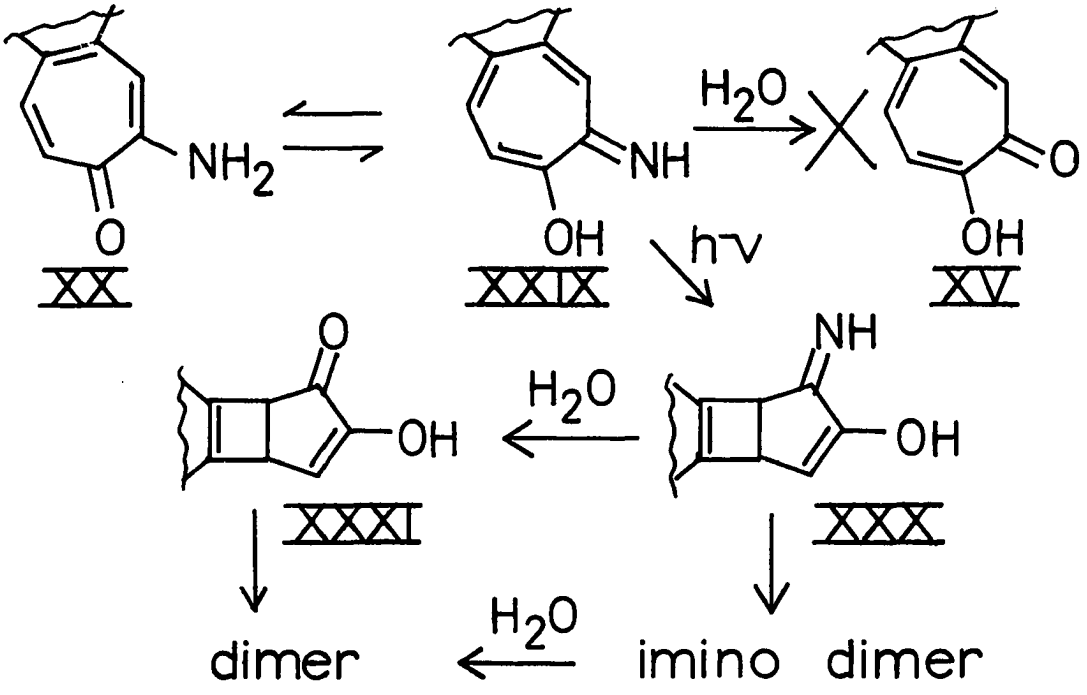
*Schwarzkopf Microanalytical Laboratory, 56-19 37th Ave., Woodside 77, New York.

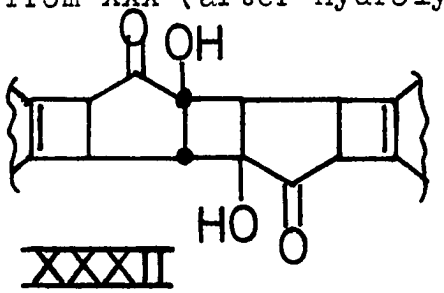
7.50 τ. The two methylene groups remain in this multiplet.

The formation of photoaminodemethoxyisocolchicine (dimer) can be rationalized by the following reaction sequence. XX is tautomerized to XXIX, which in turn collapses to XXX.

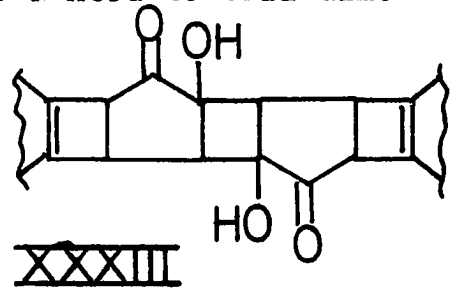


There is no way of telling whether the hydrolysis occurs before or after formation of the dimer. In a control experiment, XX was refluxed in 95% ethanol for 7.5 hours. Work up gave only unchanged aminodemethoxyisocolchicine (XX). Since XX is recovered unchanged, the hydrolysis of the imino group must occur after XXX is formed. Similarly, light-catalyzed hydrolysis of XXIX to colchiceine (XV) does not occur, since none of the photoproducts of XV (11, 17) are obtained. Consequently, hydrolysis must occur after XXX has been formed.

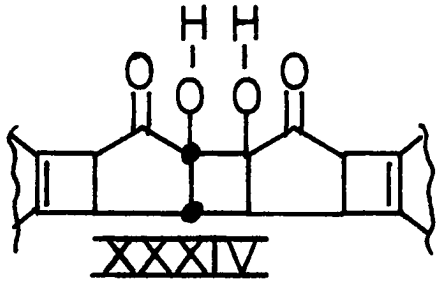
There are four possible dimers which can be obtained from XXX (after hydrolysis). XXXII is a head-to-tail dimer



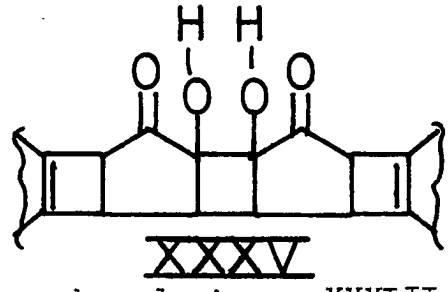
XXXII



XXXIII



XXXIV



XXXV

with trans-fusion of the central four-membered ring. XXXIII is the head-to-tail dimer with cis-fusion of the four-membered ring. XXXIV and XXXV are head-to-head dimers with trans- and cis-ring fusion respectively. These four possible dimers are drawn on the assumption that there is only one bicyclic monomer present. If two such monomers are present, another set of four homogeneous dimers plus an additional set of eight mixed dimers are possible.

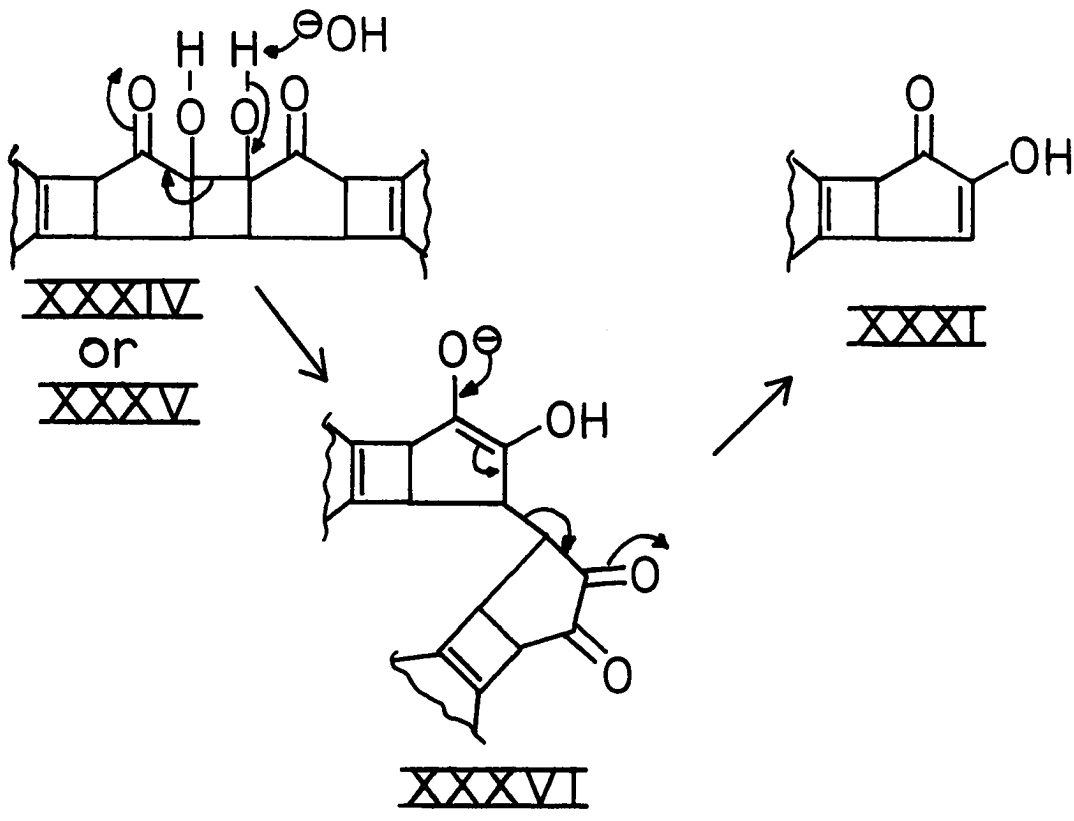
The infrared carbonyl absorption at 5.70μ is consistent with the cyclopentanone carbonyl of the dimer. Although this value is slightly low compared with cyclopentanone itself (5.75μ), it is consistent with a similar α-hydroxycyclopentanone group (photoisocolchicine ketol, XXIII, Figure 5, p. 27, at 5.70μ). The ultraviolet absorption spectrum of the photodimer is consistent with the ultraviolet spectrum

of colchiceine photodimer obtained by Schenck (11) (λ_{max}^{MeOH} 276, 227 mμ). The two photodimers, however, show different melting points. Schenck reports that his "lumicolchiceine A" gives α-lumicolchicine on treatment with diazomethane. He was not aware of the dimeric nature of α-lumicolchicine and thus was not aware that "lumicolchiceine A" was also dimeric. Gardner (17) reports that his lumicolchiceine dimer is methylated by treatment with methyl iodide and potassium carbonate.

It is reasonable to assume that the dimer obtained from aminodemethoxyisocolchicine can be methylated. Treatment with diazomethane or methyl iodide-potassium carbonate, however, gives only unreacted dimer. Even treatment with diazomethane using fluoroboric acid catalyst (27) gives only unreacted dimer.

It was hopefully assumed that the dimer is a head-to-head dimer. Treatment by base could split a head-to-head dimer as demonstrated by the following mechanism. Mild treatment by base does indeed cleave the dimer. The products of the hydrolytic cleavage are not satisfactorily purified on an alumina or a Unisil column. Treatment of the crude hydrolysis products with diazomethane converts XXXI to the enol ethers which are easily purified on an alumina column.

The first product obtained is β-lumicolchicine (II) (15% yield). Its melting point and infrared absorption

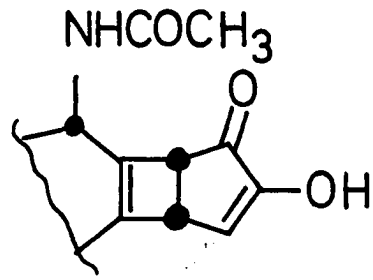


spectrum are identical with an authentic sample (see Figure 16, p. 46 for the infrared spectra for comparison). γ-Lumicolchicine (III) (9% yield), identified by its infrared spectrum, is eluted next although no crystalline material is obtained for melting point comparison (Figure 17, p. 46).

It is apparent that the dimer has the gross structure shown by XXXIV or XXXV and that the basic cleavage proceeds according to the scheme shown XXXIV or XXXV ⟶ XXXVI ⟶ XXXI. It is unlikely that the dimer is a mixed dimer of β- and γ-lumicolchiceine because of the simplicity of the n.m.r. spectrum. The formation of γ-lumicolchicine from XXV is probably an artifact of the methylation, the work-up, or the chromatography. This conclusion is based on work

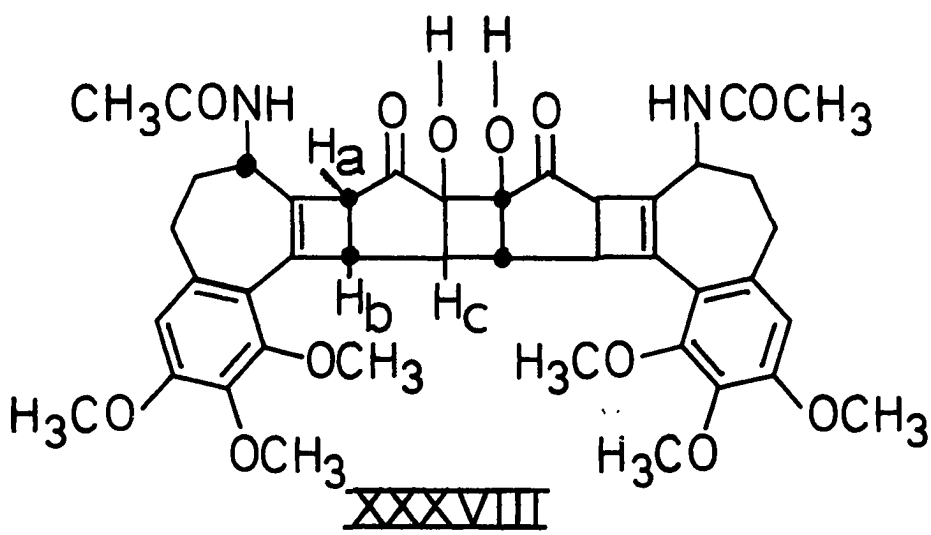
described later in the thesis.

Since β-lumicolchicine can come only from XXXI with the same stereochemistry, the basic hydrolysis product must have the stereochemistry shown in XXXVII. The basic hydrolysis



XXXVII

shows that the dimer is a head-to-head dimer. The stereochemical requirements of a head-to-head dimer make the formation of XXXV (cis-fused center ring) impossible. Moreover, a more complicated n.m.r. spectrum should be expected from a cis-fused dimer, since a cis-fused dimer would not be symmetrical. XXXVIII is probably the structure of the dimer obtained by irradiating aminodemethoxyisocolchicine. This structure accommodates all that is known about the dimer.



XXXVIII

The acetamido -NH proton on the back side of the left half of this molecule is in good position for hydrogen bonding with the left hydroxyl on the center ring, as required by the n.m.r. spectrum. The slightly split triplet at 6.38 τ is H_b, the doublet at 6.81 τ is H_a, and the singlet at 7.25 τ is H_c (with a coupling constant which is fortuitously zero). The basic cleavage requires the head-to-head dimer and the stereochemistry requires the trans-ring fusion. If this dimer could be methylated, it would give α-lumicolchicine (V) and the structure (including stereochemistry) would be solved. Until the methylation conditions are published in detail by Schenck (11) or Gardner (17) nothing more can be said about the structure of the dimer formed by irradiating aminodemethoxyisocolchicine.

Photoaminodemethoxycolchicine

Irradiation of aminodemethoxycolchicine (XXI) was conceived as an experiment designed to answer two questions. 1) Is the retention of the trimethoxystyryl chromophore applicable in this case also? 2) Is amino group hydrolysis the exception or the rule?

Aminodemethoxycolchicine (XXI) is prepared by the procedure of Horowitz and Ullyot in good yield. Irradiation of XXI in 95% ethanol is similar to the irradiation of XX. The irradiation time is directly proportional to the amount of

Figure 14. Irradiation of aminodemethoxyisocolchicine (XXI) in 95% ethanol

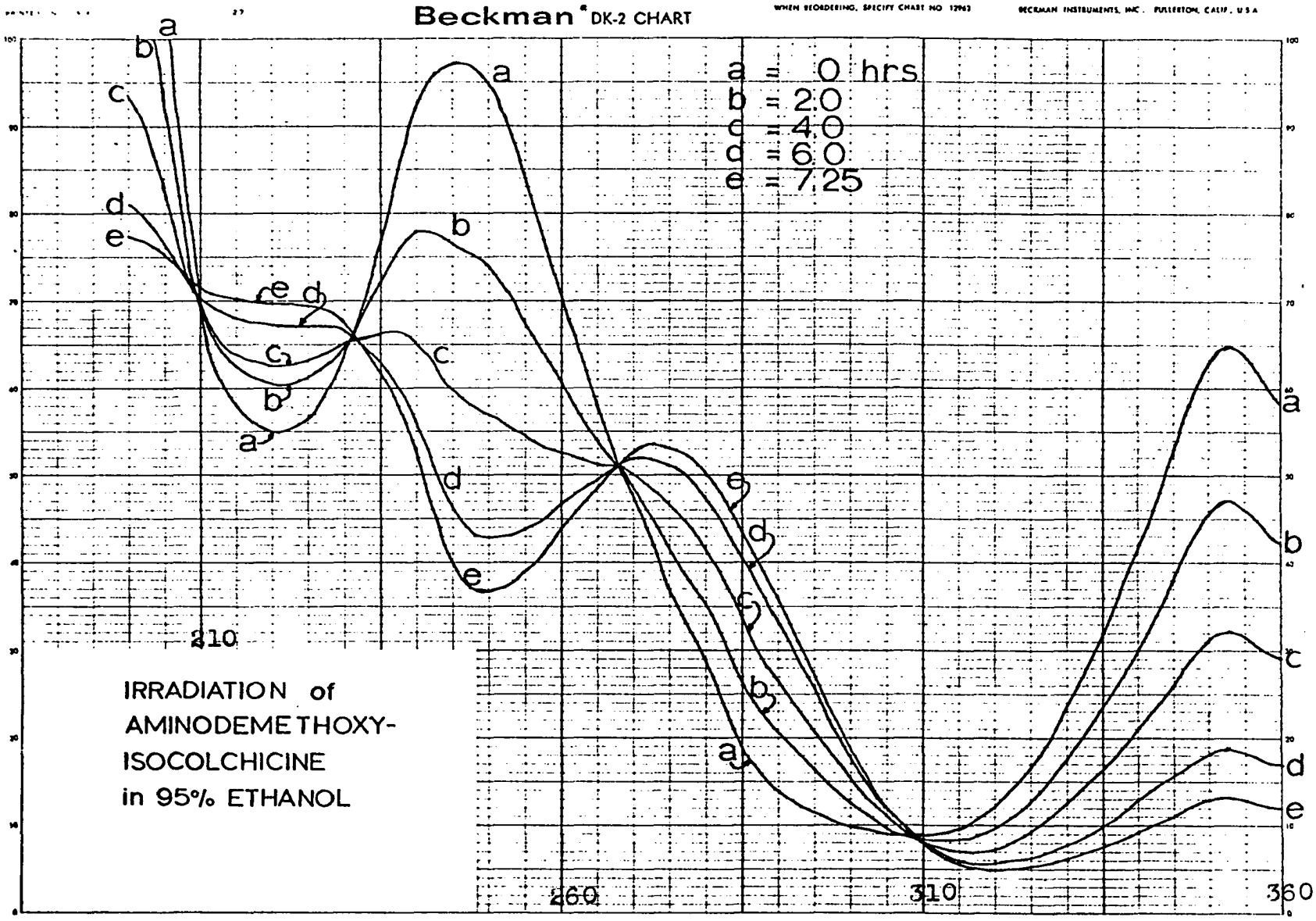

Beckman DK-2 CHART
a = 0 hrs
b = 2.0
c = 4.0
d = 6.0
e = 7.25
210
260
310
360
IRRADIATION of
AMINODEMETHOXY-
ISOCOLCHICINE
in 95% ETHANOL

Figure 15. Infrared spectrum of photoaminodemethoxy-isocolchicine (XXXVIII)

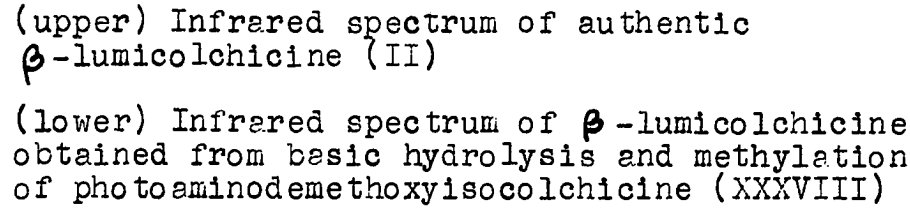


Figure 16. (upper) Infrared spectrum of authentic β-lumicolchicine (II)

(lower) Infrared spectrum of β-lumicolchicine obtained from basic hydrolysis and methylation of photoaminodemethoxyisocolchicine (XXXVIII)

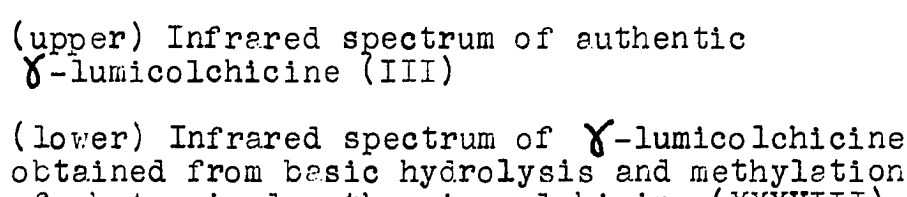
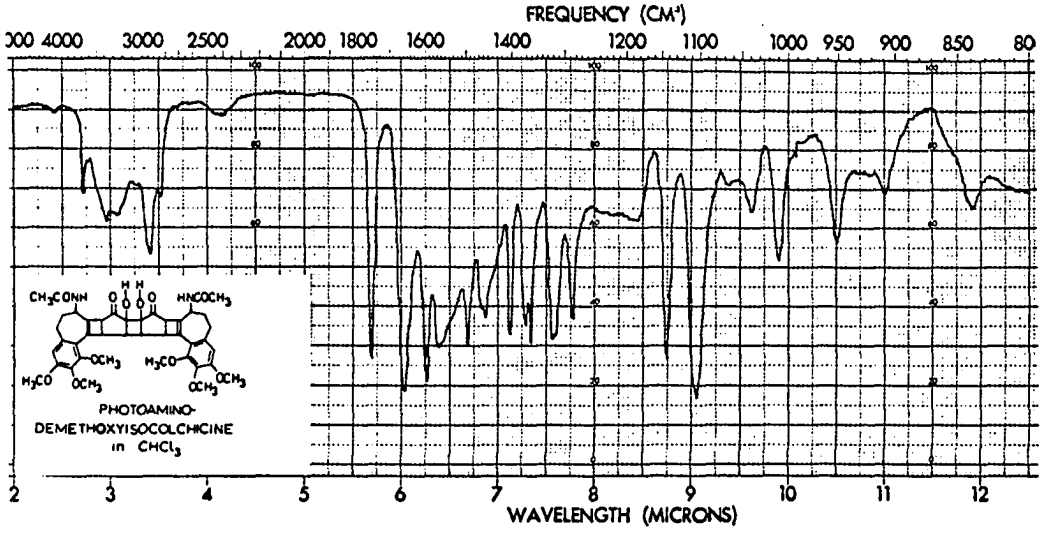
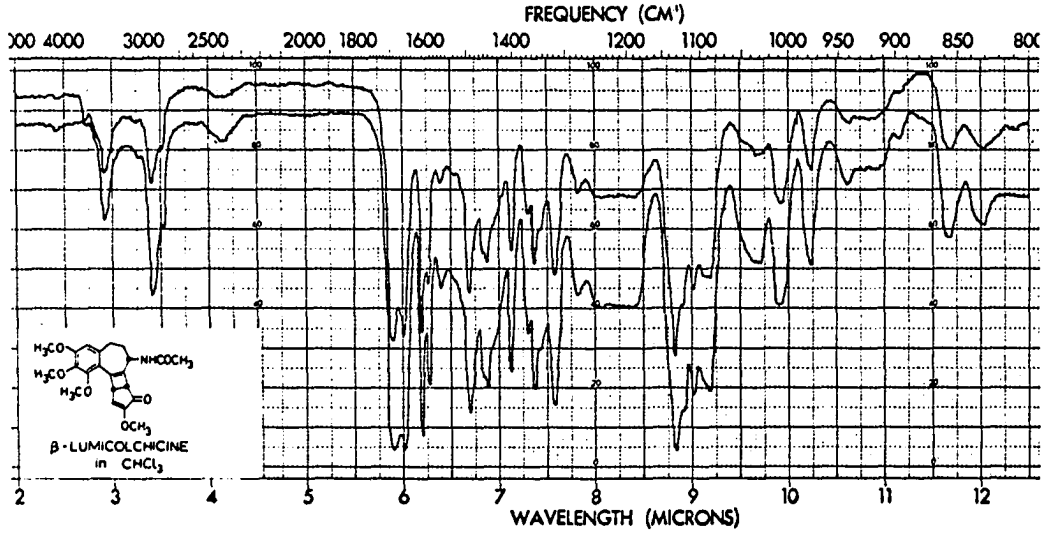


Figure 17. (upper) Infrared spectrum of authentic γ-lumicolchicine (III)

(lower) Infrared spectrum of γ-lumicolchicine obtained from basic hydrolysis and methylation of photoaminodemethoxyisocolchicine (XXXVIII)


FREQUENCY (CM⁻¹)
3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850
PHOTOAMINO-
DEMETHOXYISOCOLCHICINE
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)


FREQUENCY (CM⁻¹)
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β-LUMICOLCHICINE
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)

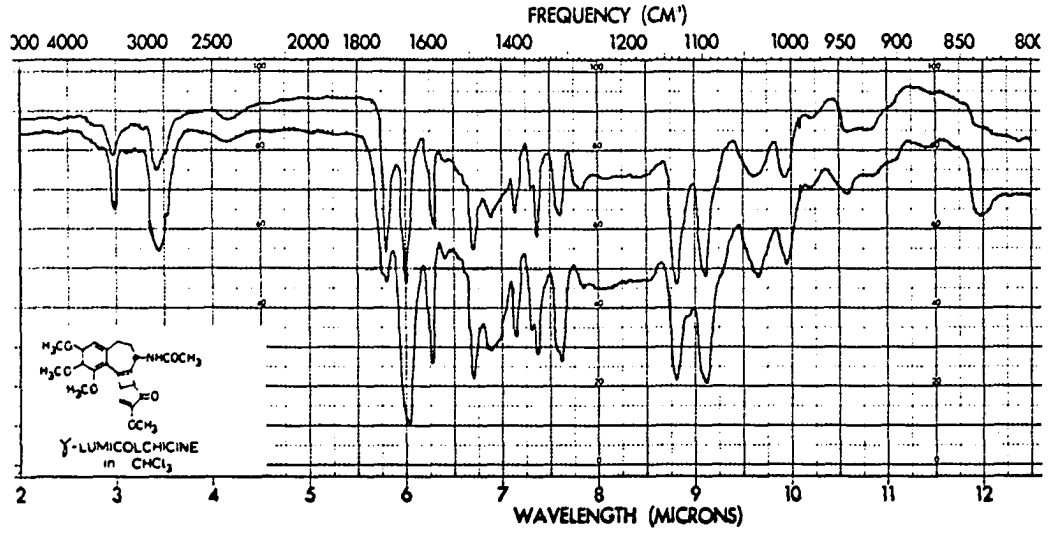
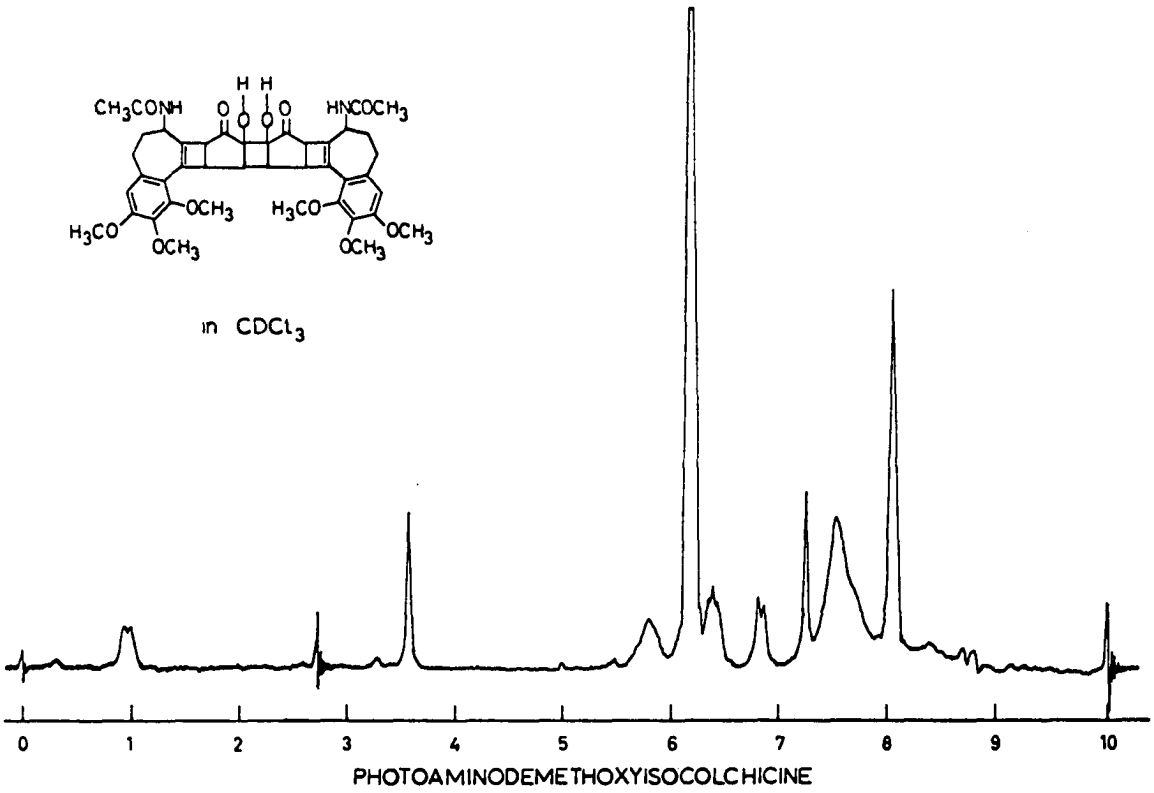
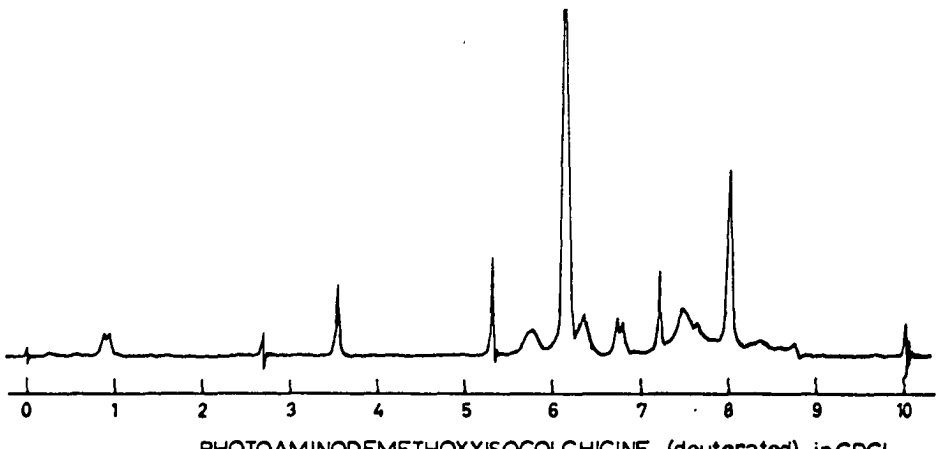

FREQUENCY (CM⁻¹)
3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850
γ-LUMICOLCHICINE
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)

Figure 18. Nuclear magnetic resonance spectrum of photoaminodemethoxyisocolchicine (XXXVIII) in deuterochloroform

Figure 19. Nuclear magnetic resonance spectrum of photoaminodemethoxyisocolchicine (XXXVIII) in deuterochloroform after treatment with deuterium oxide



PHOTOAMINODEMETHOXYISOCOLCHICINE



PHOTOAMINODEMETHOXYISOCOLCHICINE (deuterated) in $CDCl_3$

XXI in solution. The progress of the irradiation is followed by the decreasing intensity of the maximum at 353 mμ. (The progress of a typical irradiation is shown as Figure 20, p. 58.) A solution of 6.0 grams of XXI in 240 milliliters of ethanol requires 4.75 hours for 80% loss of the 353 mμ band. During the reaction, maxima develop at 274 and 217 mμ with a minimum at 252 mμ replacing the 246 mμ maximum of XXI. Isobestic points appear at 316, 266, 299, and 208 mμ.

The irradiation mixture is concentrated and treated with chloroform, as described above, to completely remove ethanol. Chromatography of 9.0 grams of crude irradiation mixture on Woelm Neutral Alumina (Activity Grade III) affords 2.6 grams of a single crystalline photoisomer (from methanol-water) by elution with benzene-chloroform (1:1). Continued elution with chloroform-methanol (9:1) gives unreacted starting material (0.5 grams).

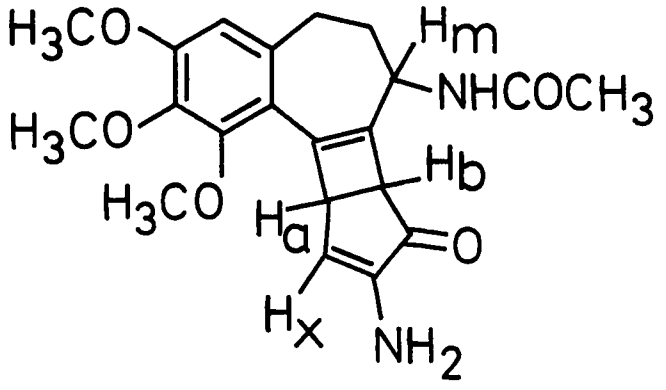
Molecular weight determination shows that the photoproduct is a monomer. Combustion analysis shows two nitrogens present, indicating that the amino group is retained.

The infrared absorption spectrum (Figure 21, p. 60) of photoaminodemethoxycolchicine has a very intense maximum at 5.98-6.01μ. The acetamido carbonyl group is part of this maximum but, in previous examples, the acetamido carbonyl group is only slightly more intense than the benzene maxima at 6.28μ. Consequently, another chromophore must also be

absorbing in this range. The maximum at 6.13 μ is assigned to an enamine.

The ultraviolet absorption spectrum has maxima at 274 (21,100) and 219 mμ (21,600) showing the presence of the trimethoxystyryl chromophore.

Photoaminodemethoxycolchicine is assigned structure XXXIX because of the similarity of its n.m.r. spectrum (Figure 24, p. 62 and Table 4, p. 68) to those of β-lumicolchicine and γ-lumicolchicine. The two low field protons are located



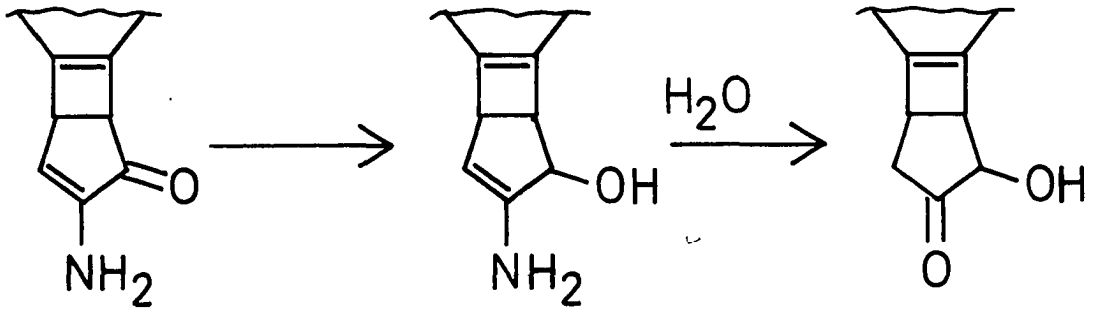
XXXIX

at 3.45 τ (a doublet, J = 3.6 c.p.s.) assigned to H_X and at 3.54 τ (the aromatic proton). The N-H (of the acetamido) appears as a doublet (J = 7.8 c.p.s.) centered at 3.80 τ. The proton on carbon bearing acetamido is the characteristic multiplet centered at 5.24 τ. One bridgehead proton is a multiplet centered at 5.97 τ, while the other bridgehead proton (a quartet) is centered at 6.47 τ. The three methoxyl groups appear as two singlets (area ratio 3:6) at 6.08 and 6.21 τ. The $-NH_2$ group is a broad singlet at 6.35 τ. One

methylene is centered at 7.34 τ while the other methylene is a multiplet under the acetamido methyl at 7.97 τ. Water of hydration appears at 7.75 τ and is removed when the sample is shaken with deuterium oxide for a few seconds. The $-NH_2$ protons at 6.35 τ also disappear during the deuterium oxide treatment.

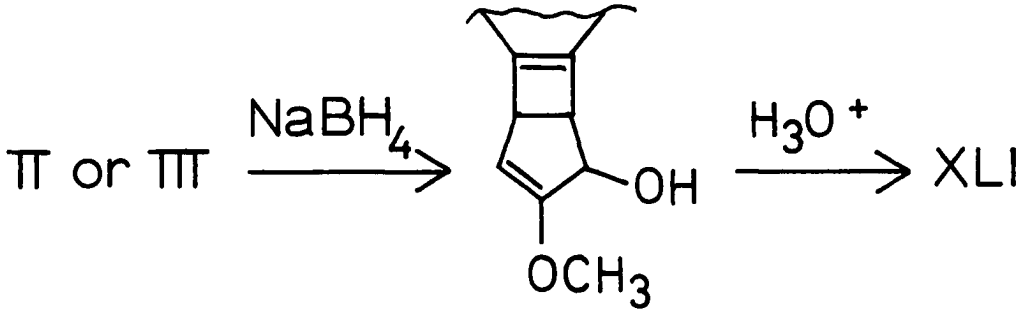
A series of double resonance experiments was performed on XXXIX (Figure 25, p. 62). Irradiation of the bridgehead proton at 5.97 τ caused the olefinic doublet at 3.45 τ to collapse to a singlet, thus locating H_a as the multiplet at 5.97 τ. Coupling between H_a and H_x was also established by irradiation of H_x. H_x changed from a multiplet to a triplet while H_b remained a quartet. J_{ab} must be nearly the same as J_{ax} to observe the triplet when a quartet is expected. Long range coupling was established between H_a--H_m and H_b--H_m by irradiation of the multiplet at 5.24 τ and observing the collapse of H_a to a triplet and of H_b to a doublet. Of course, H_a and H_b split one another.

Since XXXIX is a photoisomer similar to β- or γ-lumicolchicine, various attempts were made to convert it to intermediates derived from either of the lumicolchicines. Reduction with sodium borohydride should give XL. Since XL is an enamine it should be easily hydrolyzed to XLI. Both stereoisomers of XLI are available from the lumicolchicines by a



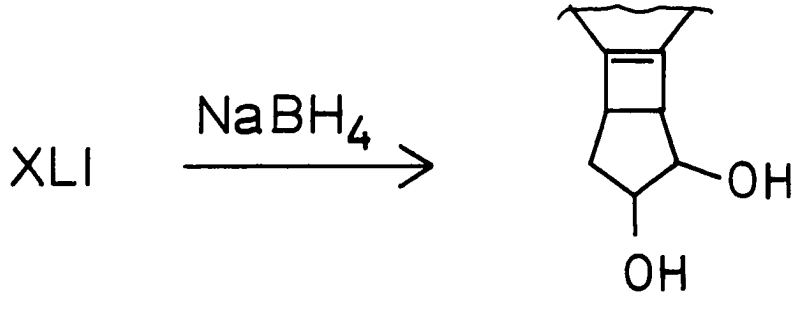
XXXIX XL XLI

similar route as shown below (II or III ⟶ XLII ⟶ XLI).



XLII

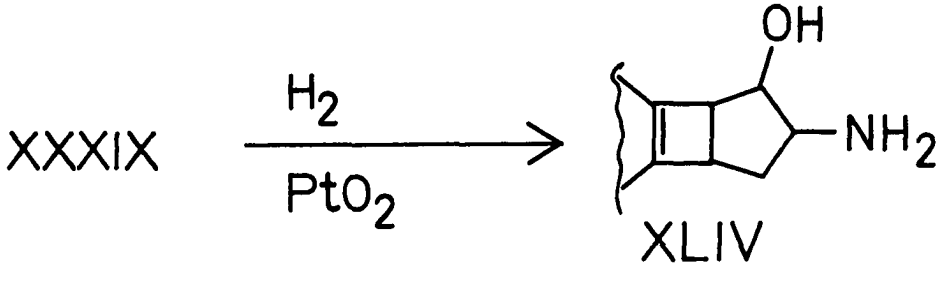
Unfortunately, reduction of XXXIX by sodium borohydride in tetrahydrofuran-water solution gives a further reduction product of XLI, the diol XLIII. This diol was not characterized.



XLIII

Formation of the diol shows that, after the first reduction has occurred, the aqueous medium must hydrolyze XL to XLI, which is further reduced to XLIII. A second attempt was made to isolate either XL or XLI by reduction in dry tetrahydrofuran, freshly distilled from lithium aluminum hydride. The excess reducing agent was complexed by addition of dry acetone. Then the complexes were destroyed by addition of dilute acid. Even with these precautions, only the amorphous diol was obtained.

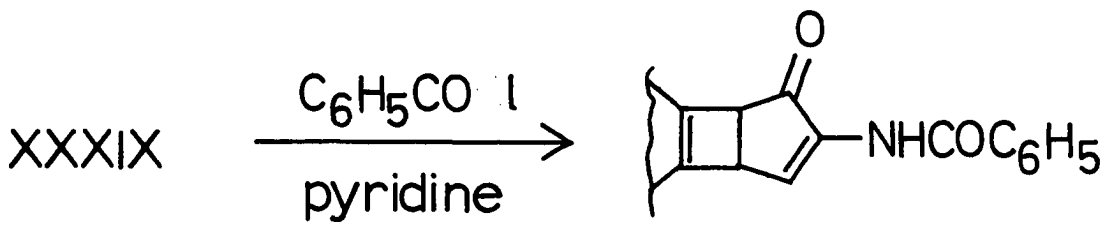
Reduction of the enamine double bond by hydrogen in 95% ethanol is the second method for degrading the molecule. There is no decrease in rate of hydrogenation after one mole is absorbed. Exhaustive hydrogenation consumes two moles of hydrogen. The product retains the trimethoxystyryl chromophore, showing that the α-aminocyclopentenone has been reduced to an α-amino alcohol (XLIV). In addition, there is



no evidence for a cyclopentanone in the infrared spectrum. Since the enamine is so easily hydrolyzed, it is possible that if carbonyl hydrogenation occurs first, the diol XLIII

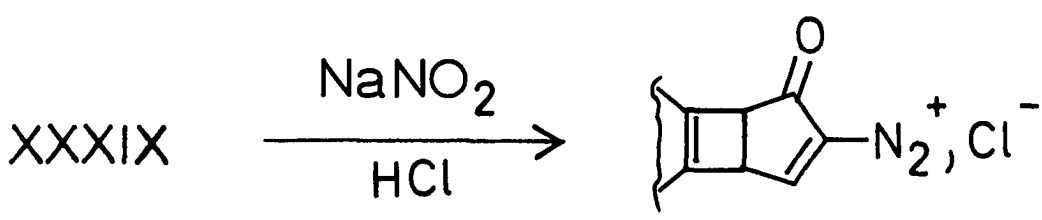
might be the product of hydrogenation. Oxidation of the hydrogenation product (either XLIII or XLIV) by sodium meta-periodate does not give a characterizable product.

An attempt was made to convert the amino group to a less reactive group so that sodium borohydride reduction might be stopped at an intermediate. Attempted benzoylation of XXXIX to XLV failed.



XLV

Treatment of XXXIX with hydrochloric acid-sodium nitrite in an attempt to make a diazonium compound (XLVI) also failed,

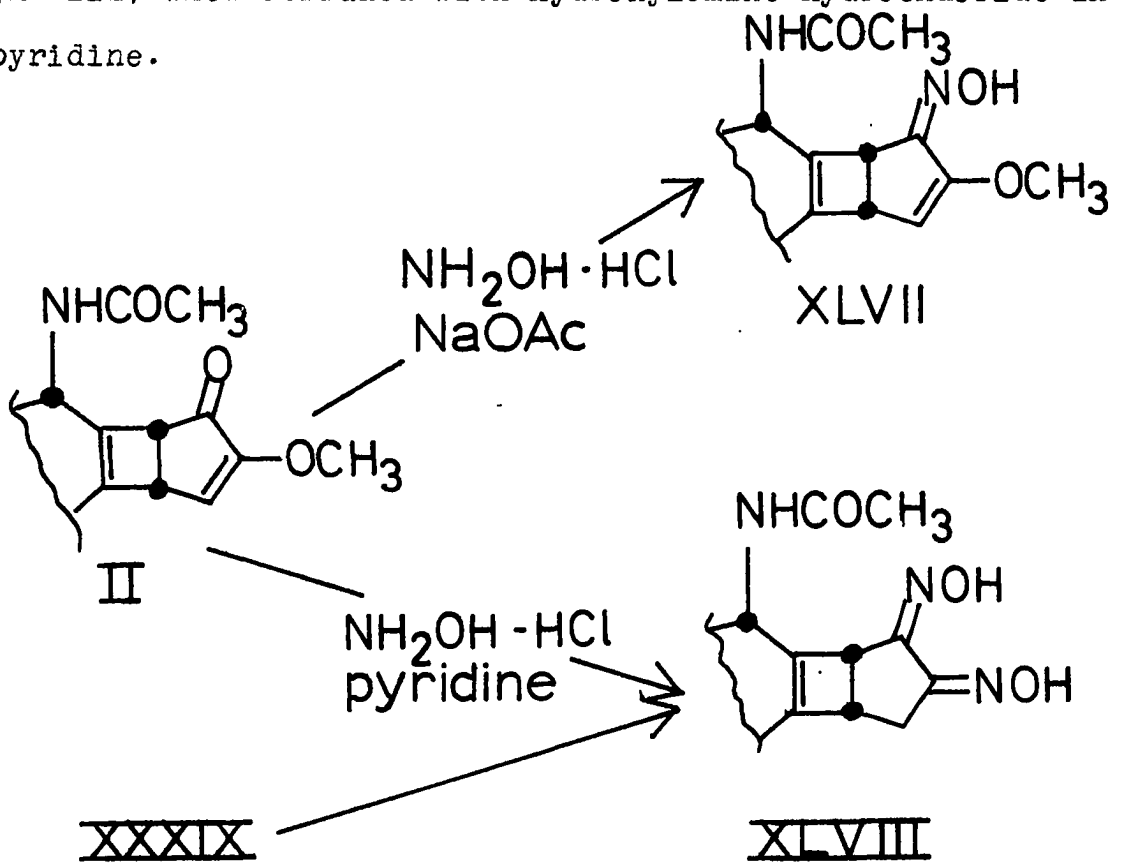


XLVI

because a salt precipitated on addition of acid. This salt does not melt below 350°C. and resists all attempts to reconvert it to the parent amine. The salt is insoluble in

every solvent that would give a useful n.m.r. spectrum. Its infrared spectrum in potassium bromide shows many of the maxima of XXXIX.

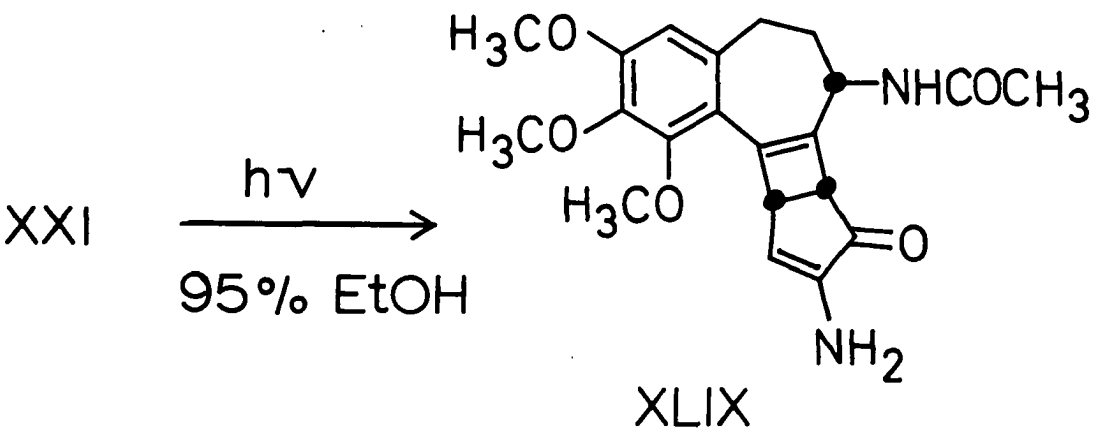
Forbes reported that β-lumicolchicine (II) (8) gives an oxime (XLVII) on treatment of II with hydroxylamine hydrochloride and sodium acetate. Moreover, II forms a dioxime (XLVIII) when refluxed with hydroxylamine hydrochloride in pyridine.



Dioximes prepared from both XXXIX and β-lumicolchicine have identical melting points (although lower than reported by Forbes) and superimposable infrared spectra (see Figure 22, p. 60 for comparison).

Since the melting points were slightly low, another derivative was desirable for absolute proof of structure of XXXIX. The 2,4-dinitrophenylosazones of both XXXIX and β-lumicolchicine were prepared and are identical in melting point (and in agreement with the reported melting point) (8), and the infrared spectra are superimposable (see Figure 23, p. 60 for comparison).

This firmly establishes the structure of the photoisomer of aminodemethoxycolchicine (XXI) as XLIX, including the appropriate stereochemistry.



XLIX

It is interesting that the formation of both the dioxime and osazone from XLIX proceeds much more rapidly (5 minutes from XLIX, 1.5 hours from β-lumicolchicine for the osazones) and in much higher yields. This confirms that the enamine is much more reactive than the enol ether, a fact which is anticipated from the borohydride and hydrogenation experiments.

Figure 20. Irradiation of aminodemethoxycolchicine (XXI) in 95% ethanol

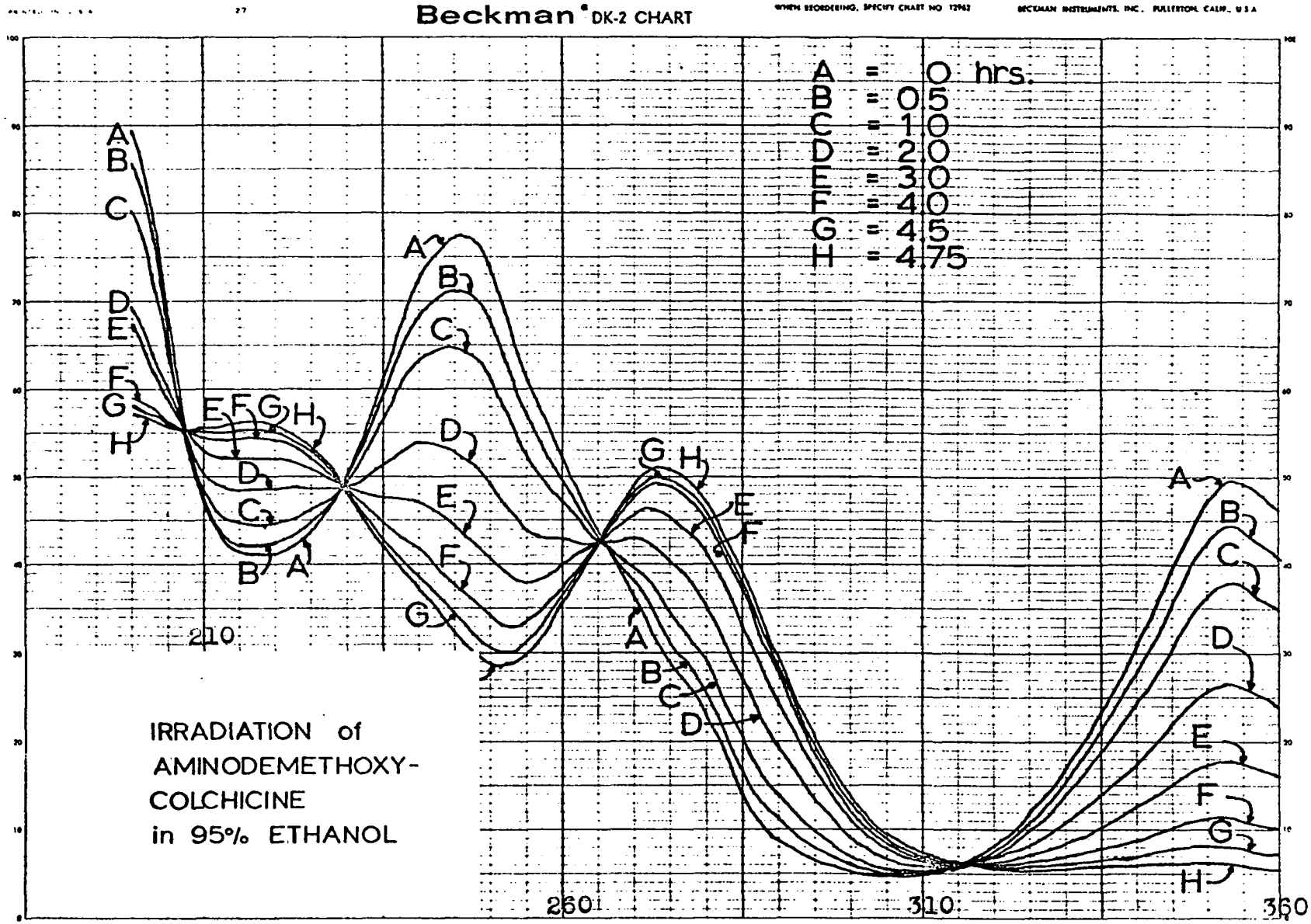

Beckman DK-2 CHART
A = 0 hrs.
B = 0.5
C = 1.0
D = 2.0
E = 3.0
F = 4.0
G = 4.5
H = 4.75
210
260
310
360
IRRADIATION of
AMINODEMETHOXY-
COLCHICINE
in 95% ETHANOL

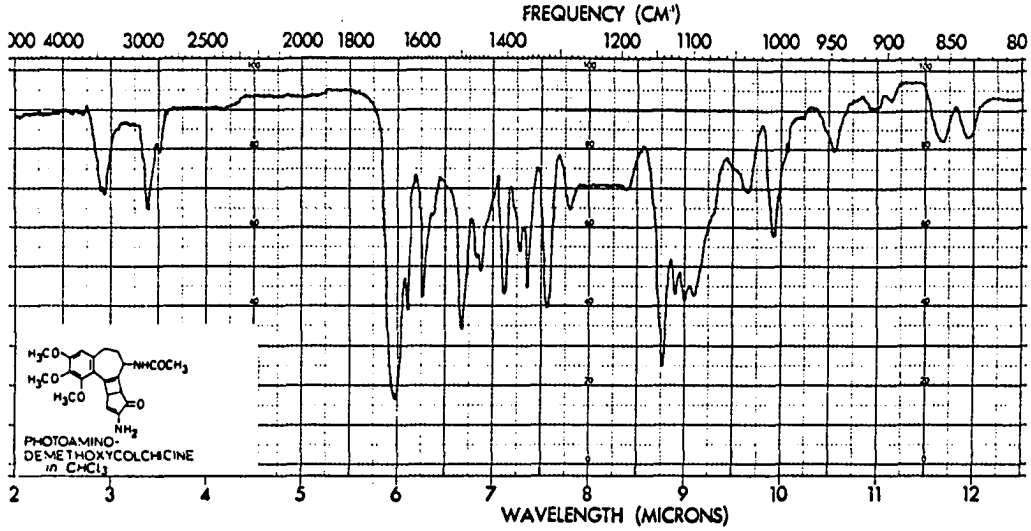
Figure 21. Infrared spectrum of photoaminodemethoxy-colchicine (XXXIX)

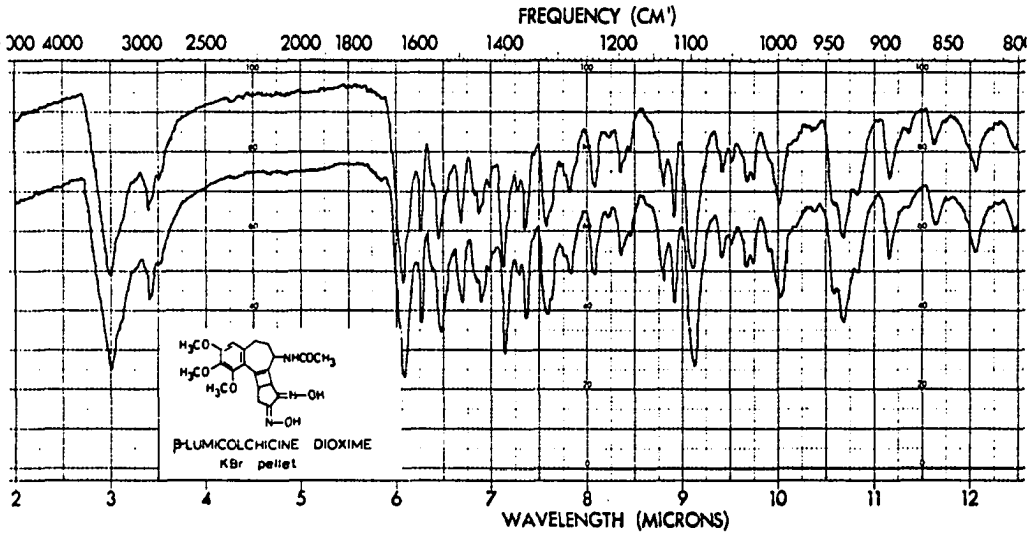
Figure 22. (upper) Infrared spectrum of photoamino demethoxycolchicine dioxime (XLVIII)

(lower) Infrared spectrum of β -lumicolchicine dioxime (XLVIII)

Figure 23. (upper) Infrared spectrum of photoamino-demethoxycolchicine-2,4-dinitrophenylosazone (XLIX)

(lower) Infrared spectrum of β-lumicol-chicine-2,4-dinitrophenylosazone (XLIX)


FREQUENCY (CM')
PHOTOAMINO-
DEMETHOXYCOLCHICINE
in CHCl3
WAVELENGTH (MICRONS)


FREQUENCY (CM')
β-LUMICOLCHICINE DIOXIME
KBr pellet
WAVELENGTH (MICRONS)

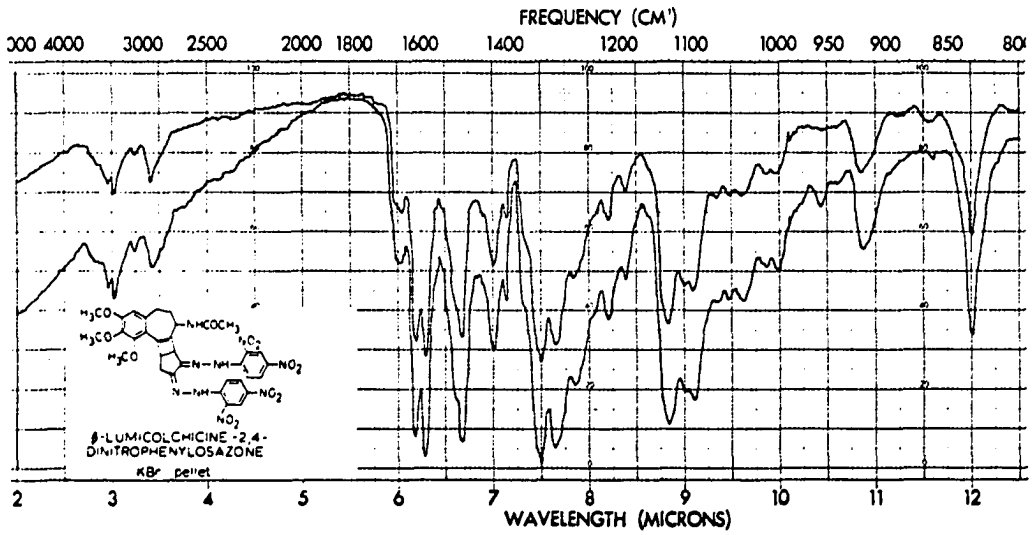

FREQUENCY (CM')
β-LUMICOLCHICINE -2,4-
DINITROPHENYLOSAZONE
KBr pellet
WAVELENGTH (MICRONS)

Figure 24. Nuclear magnetic resonance spectrum of photoaminodemethoxycolchicine (XXXIX) in deuterochloroform

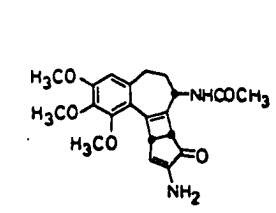
Figure 25. Double resonance experiments on photoaminodemethoxycolchicine (XXXIX) in deuterochloroform

(upper left) Experiment 1, saturate 5.97τ, scan 3.45τ

(upper center) Experiment 2, saturate 5.24τ, scan 6.47τ

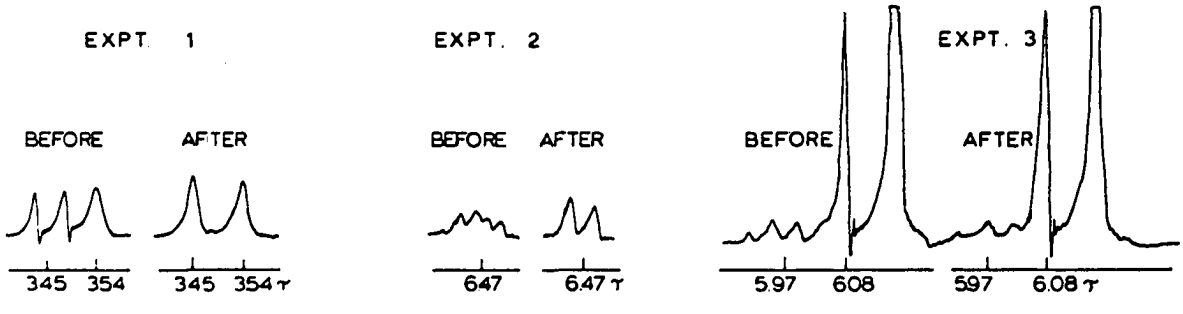
(upper right) Experiment 3, saturate 5.24τ, scan 5.97τ

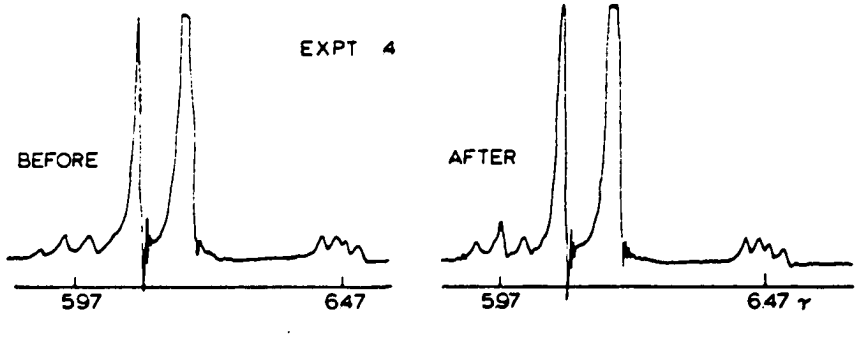
(lower) Experiment 4, saturate 3.45τ, scan 5.97 and 6.47τ



IN $CDCl_3$

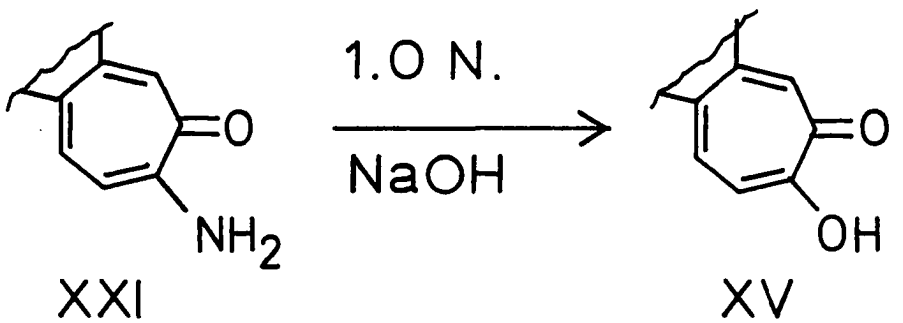
PHOTOAMINODEMETHOXYCOLCHICINE





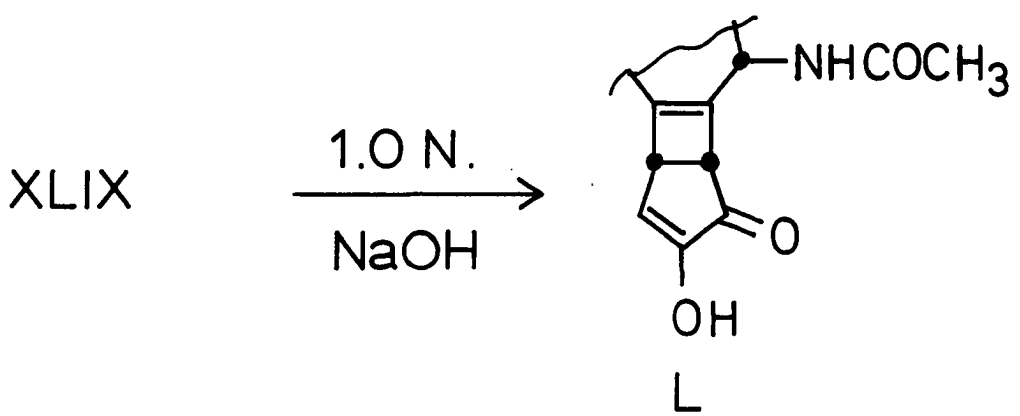
DOUBLE RESONANCE EXPERIMENTS on PHOTOAMINODEMETHOXYCOLCHICINE

Horowitz and Ullyot (26) generate colchiceine (XV) by gentle basic hydrolysis of their aminodemethoxycolchicine (XXI ⟶ XV). Similar treatment of XLIX gives a lumi-



XXI XV

colchiceine (L) which crystallizes from the neutralized reaction mixture. At first it was believed that no reaction



L

had occurred, since the infrared spectrum (Figure 26, p. 67) and melting point of L are very similar to XLIX. The melting point of L is similar to that of β-lumicolchiceine prepared by Gardner (17).

The n.m.r. of L (Figure 27, p. 67) shows that it is a pure compound and that it is very similar to XLIX. The assignments for L are the same as for XLIX and are found in Table 4 (p. 68). The amino group and water of hydration of

XLIX are missing, and the enol hydroxyl proton of L is located under the aromatic proton at 3.50 τ .

Methylation of this pure isomer gives a crude oil. The infrared spectrum of this oil is superimposable on the spectrum obtained from basic hydrolysis of photoaminodemethoxyisocolchicine (dimer) followed by methylation. Recall that the crude oil of the dimer gave β-lumicolchicine and apparently γ-lumicolchicine. This means that the γ-lumicolchicine is probably formed during the methylation, the work up, or the chromatography.

L was prepared a second time and methylated by a slightly different procedure. The infrared spectrum of the crude oil from this preparation showed the oil to be richer in β-lumicolchicine than the previous crude oils. Chromatography of the second crude oil gives good yields of pure β-lumicolchicine (Figure 28, p. 67) with no evidence of γ-lumicolchicine, although traces of other products appear.

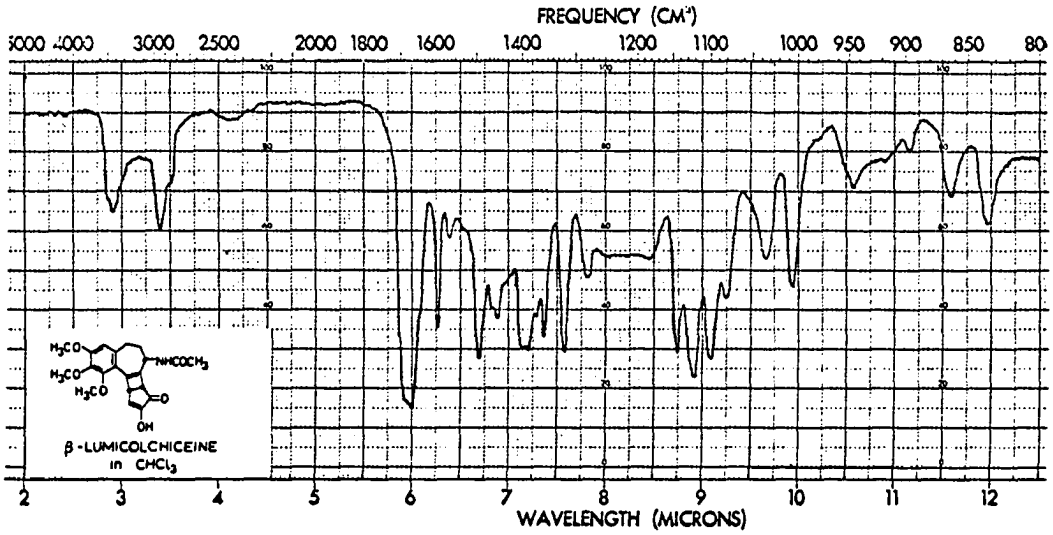
The trimethoxystyryl chromophore is once again present in photoproduct XLIX. This study has produced three new photoproducts of colchicine derivatives and all three retain this chromophore. In addition, there are at least five, and possibly as many as eight, other photoproducts from colchicine and colchiceine which contain this chromophore. Since there is no report of a photoproduct which does not contain this chromophore, it must be concluded that the preservation

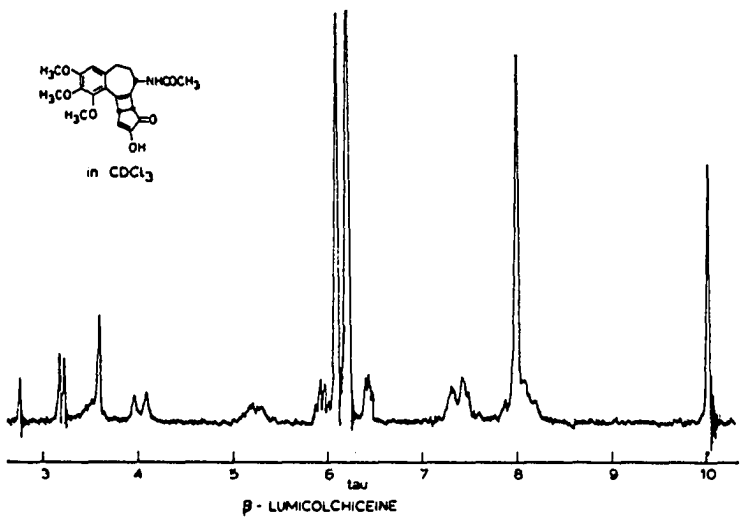
of the trimethoxystyryl chromophore is the main factor in the formation of photoproducts from colchicine and its derivatives.

Figure 26. Infrared absorption spectrum of β-lumicolchiceine (L)

Figure 27. Nuclear magnetic resonance spectrum of β-lumicolchiceine (L) in deuterochloroform

Figure 28. Infrared spectrum of β-lumicolchicine (II) obtained from methylation of β-lumicolchiceine (L)


FREQUENCY (CM⁻¹)
5000 4000 3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850 800
β-LUMICOLCHICEINE
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)


in CDCl3
3 4 5 6 7 8 9 10
tau
β - LUMICOLCHICEINE

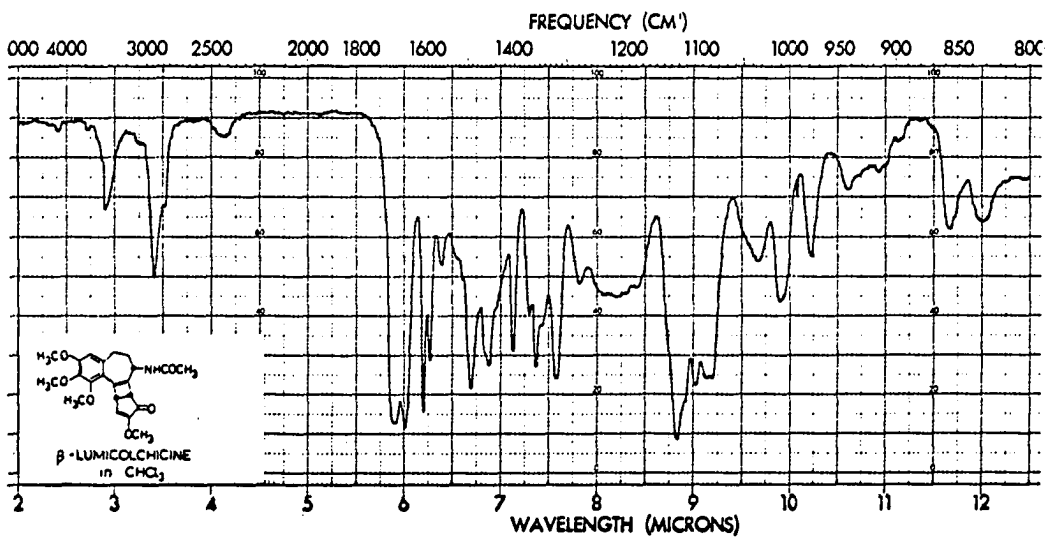

FREQUENCY (CM⁻¹)
000 4000 3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850 800
β-LUMICOLCHICINE
in CHCl3
2 3 4 5 6 7 8 9 10 11 12
WAVELENGTH (MICRONS)

Table 4. Nuclear magnetic resonance spectra[a]

Compound (Proton)	Aromatic	Cyclopentene	-N-H[b]	OH	NH_2	H -C-N	OCH_3	Bridgehead	Methylene	-C-OH	O -C-CH_3
XXXVIII[c] ($CDCl_3$)	3.56	--	0.97	--	--	5.78	6.17	6.38 6.81 7.25	7.50 8.00	7.50	8.03
XXXIX[d] ($CDCl_3$)	3.54	3.45	3.80	--	6.35	5.24	6.08 6.21	5.97 6.47	7.33 7.97	--	7.97
L ($CDCl_3$)	3.56	3.18	4.00	3.56	--	5.24	6.07 6.18	5.94 6.44	7.41 7.97	--	7.97

[a]Resonance positions are given in τ values relative to internal tetramethylsilane.

[b]The exact position of this resonance depends strongly on concentration.

[c]The water of crystallization at 7.50 τ disappears when the solution is shaken with deuterium oxide for a few seconds.

[d]The water of crystallization at 7.75 τ disappears when the solution is shaken with deuterium oxide for a few seconds.

EXPERIMENTAL

Photoisocolchicine

Colchiceine (XV)

A solution of colchicine (40.0 g., 0.10 mol.) in water (438 ml.) was treated with concentrated hydrochloric acid (7.0 ml.) and heated to 93° on a steam bath with stirring for 1.5 hr. (20). (If the orange-yellow oil which forms does not crystallize after 1 hr. a few seed crystals should be introduced.) After cooling to 8° with stirring the slurry was filtered, washed with ice-cold water (2 x 100 ml.), and dried giving 29.9 g. colchiceine (77.7%, m.p. 175-178°; lit. 178-179° (20). A second crop of colchiceine (2.7 g.) was obtained by heating the mother liquors as above for an additional 1.5 hr. Total yield: 32.6 g., 84.7%. Colchiceine can be purified by recrystallization from aqueous ethanol (m.p. 178-179°).

Isocolchicine (XVI)

Diazomethane (29) was prepared by distillation at 40° from a mixture of N-nitroso-N-methylurea (30, p. 843, Method 2) (30 g.) and 40% potassium hydroxide (60 ml.) in methylene chloride (500 ml.).

Colchiceine (XV) (41.2 g., 0.107 mol.), dissolved in methylene chloride (50 ml.), was slowly added to the stirred

(Teflon covered stirring bar) ice-cold solution of diazomethane (250 ml.) described above. The reaction mixture was slowly warmed to 40° with stirring over a period of 2 hr. (to remove the excess diazomethane) then evaporated to dryness *in vacuo*. The red viscous oil was twice dissolved in chloroform (100 ml.) and evaporated to dryness *in vacuo*.

The red viscous oil was then dissolved in ethyl acetate (200 ml.) and boiled vigorously on a steam bath for 0.5 hr. (to remove chloroform). The hot solution (approx. 125 ml.) was vigorously scratched with a glass rod. The isocolchicine which precipitated was filtered (after cooling the solution to 0°) and washed with cold ethyl acetate (2 x 25 ml.). If the isocolchicine (11.2 g., 0.0281 mol., 26.2%, m.p. 218-222°; lit. 225-226° (19c)) was not colorless, it was boiled with ethyl acetate (25 ml.) for a few minutes, and the slurry was filtered while hot (m.p. 223-224°, lit. 225-226° (20c)), $\lambda_{max}^{CHCl_3}$ 5.98, 6.20, 6.27 (sh), 6.40 μ ; Figure 2, p. 25.

Irradiation of isocolchicine in water

Isocolchicine (XVI) (3.0 g., 0.0075 mol.) was dissolved in water (2.0:1.0) and flushed with nitrogen for 1 hr. The solution was stirred with a Teflon coated stirring bar, and a slow stream of nitrogen was bubbled into the solution for the duration of the irradiation. A mercury arc lamp (Hanovia Type A, 550 watts) was inserted in a Pyrex immersion well

cooled with water (12°). The progress of the irradiation was followed by disappearance of the 343 mμ band in the ultraviolet. Maximum yields were obtained after irradiation for 60-65 hr. (40% complete).

The aqueous solution was saturated with sodium chloride and extracted with chloroform (4 x 250 ml.). The combined organic extracts were dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* giving 3.0 g. of an orange viscous mass. This mass was dissolved in ethyl acetate (20 ml.) and boiled vigorously in a beaker, precipitating unreacted isocolchicine (1.2-1.5 g., 40-50% recovery). The mother liquors were evaporated to dryness *in vacuo*, dissolved in chloroform (25 ml.), evaporated to dryness, and chromatographed as described below.

Irradiation of isocolchicine (XVI) in 95% ethanol

Isocolchicine (2.0 g., 0.00501 mol.) was dissolved in 95% ethanol (240 ml.) and purged with nitrogen for 1 hr. The solution was irradiated as described above for 4.5 hr. (70% complete). The progress of the reaction was followed as above.

The red reaction mixture was evaporated to dryness *in vacuo* giving 2.0 g. of red oil which was chromatographed as follows.

Chromatography of irradiation mixtures

Neutral alumina (25 g., 25 g. per g. of irradiation mixture) was packed in benzene. The irradiation mixture (1.0 g.) was dissolved in benzene-chloroform (3:1, 20 ml.), placed on the column and eluted with benzene-chloroform (3:1, 150 ml.) giving 0.02 g. of an unidentified colorless oil. Continued elution with benzene-chloroform (1:1, 600 ml.) gave crude photoisocolchicine (XVIII) (0.54 g.) as a yellow glass which crystallized from methanol-water (1:1, 5 ml.) as colorless crystals (0.30 g.; m.p. 119.5-120.5°, resolidified from 130-145°, remelted 196-199°; $\lambda_{max}^{CHCl_3}$ 5.85, 6.02μ (Figure 3, p.25); $\lambda_{max}^{95\%\ EtOH}$ 218 (26,200), 260 mμ (21,900). The nuclear magnetic resonance spectrum (Figures 8 and 9, p. 29) showed one water of hydration at 7.50τ which was removed by shaking with deuterium oxide for a few seconds.

Anal. Calcd. for $C_{22}H_{25}O_6N \cdot H_2O$: C, 63.30; H, 6.52; N, 3.36.

Found: C, 63.56; H, 6.63; N, 3.09.

Subsequent elution of the column with chloroform (300 ml.) gave a yellow glass (0.35 g.) which crystallized when boiled in ethyl acetate (5 ml.) giving unreacted isocolchicine (XVI). Final elution with methanol gave a yellow glass (0.1 g.) which crystallized as unreacted isocolchicine (XVI) as above.

Photoisocolchicine alcohol (XXII)

Sodium borohydride (0.2 g.) in water (2 ml.) was added to a stirred solution of photoisocolchicine (0.150 g., 0.38 mmol.) in tetrahydrofuran (20 ml.). The stirred mixture was refluxed for two hours and cooled to room temperature. Water (12 ml.) was added, and the reaction mixture was concentrated *in vacuo* to 10 ml. The milky solution (pH 11) was extracted with chloroform (4 x 3 ml.), and the combined organic extracts were dried over magnesium sulfate-potassium carbonate, filtered, and evaporated *in vacuo* giving a colorless, viscous oil (0.15 g.). Trituration with benzene (3 ml.) gave photoisocolchicine alcohol (XXII) as a yellow powder which was recrystallized from acetone-methanol giving colorless, diamond-shaped crystals (0.070 g., 47%, m.p. 206-209°(d.); $\lambda_{max}^{CHCl_3}$ 6.02, 6.09μ (Figure 4, p. 25); $\lambda_{max}^{95\%\ EtOH}$ 212 (35,530), 270 mμ (14,850); nuclear magnetic resonance spectra (Figures 10 and 11, p. 31).

Anal. Calcd. for $C_{22}H_{27}O_6N$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.83; H, 7.00; N, 3.70.

Photoisocolchicine ketol (XXIII)

A solution of photoisocolchicine alcohol (XXII) (0.075 g., 0.19 mmol.), ethanol (95%, 1 ml.), water (4 ml.), and sulfuric acid (1.0 N, 1.0 ml.) was heated in a test tube on a steam bath for five minutes. The reaction mixture was

cooled, then extracted with chloroform (3 x 1.5 ml.). The combined extracts were dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo to a colorless viscous oil which was dissolved in ethanol (5 ml.) and evaporated again (to remove chloroform). The crude product gave a positive periodic acid test. Colorless platelets of photoisocolchicine ketol (0.050 g.) were obtained by crystallization from aqueous ethanol or aqueous acetone, m.p. 229-231°; $\lambda_{max}^{CHCl_3}$ 5.71, 6.00μ (Figure 5, p. 27); $\lambda_{max}^{95\%\ EtOH}$ 220 (25,000), 265 mμ (15,200); nuclear magnetic resonance spectrum (Figure 12, p. 33).

Anal. Calcd. for $C_{21}H_{25}O_6N$: C, 65.10; H, 6.50; N, 3.62.

Found: C, 65.40; H, 6.73; N, 3.47.

The ketol was reduced with sodium borohydride as above giving a diol (XXIV) ($\lambda_{max}^{CHCl_3}$ 6.01μ) which was cleaved to the dialdehyde (XXV) ($\lambda_{max}^{CHCl_3}$ 5.84, 6.01μ ; Figure 6, p. 27) when treated with sodium metaperiodate.

Dihydrophotoisocolchicine (XXVI)

A solution of photoisocolchicine (XVIII) (1.01 g., 0.253 mmol.) in ethanol (95%, 70 ml.) was reduced with hydrogen over Adams catalyst (52 mg.). After 1.05 moles of hydrogen were absorbed, the catalyst was filtered, and the filtrate was evaporated to dryness in vacuo. The light yellow oil was

crystallized from ethanol-water (1:3, 20 ml. total) giving colorless needles (0.69 g., 0.172 mmol., 68%; m.p. 113.5-115.5°, resolidified between 140-170°, remelted 219-225°; $\lambda_{max}^{CHCl_3}$ 5.84, 6.01 μ (Figure 7, p. 27); $\lambda_{max}^{95\% EtOH}$ 208 mμ (46,420); nuclear magnetic resonance spectrum (Figure 13, p. 33).

Anal. Calcd. for $C_{22}H_{27}O_6N \cdot H_2O$: C, 62.99; H, 6.97; N, 3.34.

Found: C, 62.89; H, 6.95; N, 3.64.

Conversion of XXVI to XXVII

Reduction of (XXVI) (0.125 g.) with sodium borohydride (0.125 g.) as above gave a colorless oil ($\lambda_{max}^{CHCl_3}$ 6.03, 6.11 μ) which was hydrolyzed with sulfuric acid (1.0 ml., 1.0 N) in water (4 ml.) and ethanol (4 ml.). The reaction mixture was extracted with chloroform (3 x 4 ml.), dried over anhydrous magnesium sulfate, filtered, and concentrated to dryness giving (XXVII) as a colorless powder (m.p. 140-148°) which could be crystallized from ethyl acetate-ethanol (m.p. 146-149°); $\lambda_{max}^{CHCl_3}$ 5.72, 6.00 μ. The product gave a positive periodic acid test.

Photoaminodemethoxyisocolchicine

Aminodemethoxyisocolchicine (XX)

Aminodemethoxyisocolchicine was prepared as described by Horowitz and Ullyot (26).

Irradiation of aminodemethoxyisocolchicine (XX)

A solution of aminodemethoxyisocolchicine (3.70 g., 0.0097 mol.) in ethanol (95%, 240 ml.) was purged with nitrogen for 1.5 hr. The irradiation lamp (described above) was placed in a Pyrex immersion well with internal cooling (12°). The progress of the irradiation (Figure 14, p. 44) was followed by watching the decrease of the 353 and 246 mμ maxima and the subsequent formation of maxima at 274 and 220 mμ and minima at 321 and 250 mμ. After 7.25 hr. the irradiation was stopped (70% complete). The color of the solution changed from straw yellow to deep red during the irradiation.

The irradiation mixture was concentrated *in vacuo* giving a red oil which was twice dissolved in chloroform (50 ml.) and evaporated to dryness *in vacuo* (3.7 g.). The red residue was dissolved in benzene-chloroform (1:1, 25 ml.) and chromatographed on Woelm Neutral Alumina (Activity Grade III, 245 g., column length 200 mm.). Elution with 100% chloroform (2000 ml.) gave 0.17 g. of unidentified glass. Elution with chloroform-methanol (99:1, 1000 ml.) gave 1.82 g. of green-

black glass which could be crystallized by scratching a vigorously boiled (to remove chloroform) solution of the eluate in methanol-water (1:1, 50 ml. total) giving colorless, lustrous platelets (1.25 g. (34%)), m.p. 198-199.5° (color disappears in polarizer at 190°). Photoaminodemethoxyisocolchicine gave a slow (1.5 min.) positive periodic acid test. Photoaminodemethoxyisocolchicine showed $\lambda_{max}^{CHCl_3}$ 5.70, 6.04 and 6.28μ (Figure 15, p. 46); $\lambda_{max}^{95\% EtOH}$ 276 (12,700), 225 mμ (14,320); nuclear magnetic resonance spectra (Figures 18 and 19, p. 48).

Drying of the sample (80°, 0.04 mm., 24 hr.) resulted in loss of 4.27% by weight. This corresponds to a loss of two moles of water per mole of dimer (calcd. 4.54%).

Anal. Calcd. for $C_{42}H_{46}O_{12}N_2$: C, 65.44; H, 6.02; N, 3.64.

Found: C, 65.28; H, 6.30; N, 3.84.

The molecular weight was determined in chloroform solution by the osmometric method. Colchicine dissolved in chloroform was used as the standard.

Three determinations gave molecular weights of 632, 975, and 810, average 803. The molecular weight calculated for a dimer is 768.75.

A similar molecular weight determination on photoaminodemethoxycolchicine gave a molecular weight of 362 (calculated 384.37).

Control experiment on irradiation conditions

Aminodemethoxyisocolchicine (XX, 0.380 g.) was dissolved in ethanol (95%, 25 ml.) and refluxed vigorously for 7.5 hr. The solution was evaporated to dryness *in vacuo* and twice dissolved in methanol (10 ml.) and evaporated *in vacuo*. The yellow powder was crystallized from methanol-water (1:1, 20 ml.) giving 0.35 g. of unreacted starting material, identified by melting point and comparison of infrared spectra.

Attempted hydrogenation of photoamino-demethoxyisocolchicine (XXXVIII)

(XXXVIII) (50 mg.) was dissolved in ethanol (95%, 15 ml.) and added to a prereduced slurry of Adams catalyst (26 mg.) in ethanol (95%, 25 ml.). After 40 minutes only 25% of the theoretical amount of hydrogen had been absorbed. The reaction mixture was filtered and evaporated to dryness *in vacuo* giving a colorless oil (50 mg.) which was crystallized from methanol-water (1:1, 10 ml.). The colorless platelets obtained (45 mg.) proved to be starting material as shown by melting point, mixed melting point, and superimposable infrared spectra.

Attempted methylation of photoamino-demethoxyisocolchicine (XXXVIII)

(XXXVIII) (100 mg.) was added to a stirred slurry of anhydrous acetone (25 ml., dried over magnesium sulfate),

and fused, powdered potassium carbonate (250 mg.) in a three-necked flask fitted with nitrogen atmosphere inlet, condenser, and magnetic stirrer. After addition of methyl iodide (1.0 ml.) the reaction mixture was refluxed gently for 40 hr.

Water (20 ml.) was added to the cooled reaction mixture, and the solution was extracted with chloroform (4 x 20 ml.). The combined organic extracts were washed with water (2 x 20 ml.), dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo. Addition of methanol (1 ml.) to the light yellow oil caused a precipitate to separate which, after filtration, was identified as starting material (60 mg.) as shown by melting point and superimposable infrared spectra. Addition of water (1 ml.) to the mother liquors gave unreacted starting material (20 mg.).

Attempted reaction of photoaminodemethoxy-isocolchicine with diazomethane (29)

A. Preparation of fluoroboric acid (HBF_4) (31) Fused boric acid (3.63 g.) was added slowly with swirling to hydrofluoric acid (47%, 10 ml.) in a polyethylene bottle at zero degrees. All of the necessary precautions were taken, especially having available a slurry of magnesium oxide in alcohol for hydroflouric acid burns. Some of the boric acid crystals did not dissolve, even when the solution was warmed to room temperature.

B. Preparation of fluoroboric acid catalyst (27) Although the reference called for concentration of the acid, it was used here as prepared above. The acid (0.5 ml.) was added to diethyl ether (38 ml.) in a 50 ml. volumetric flask, and the solution was diluted to the mark with methylene chloride.

C. Preparation of diazomethane (29) Diazomethane was prepared by distillation from a methylene chloride (100 ml.) slurry of N-nitroso-N-methylurea (4.0 g.) treated with ice-cold potassium hydroxide solution (40%, 10 ml.). Benzoic acid (0.030 g.) required 0.77 ml. of the diazomethane solution, showing it to be 0.319 Molar.

D. Reaction of photoaminodemethoxyisocolchicine, diazomethane, and fluorboric acid Photoaminodemethoxyisocolchicine (0.280 g.) was dissolved in methylene chloride (5 ml.) and treated with the fluoroboric acid catalyst solution (0.20 ml.). This solution was slowly added to the diazomethane solution (10 ml., 4-fold excess). After stirring at room temperature for 1 hr., water (10 ml.) was added to the reaction mixture, followed by sodium bicarbonate (1.0 g.). The organic layer was separated, washed with water (2 x 20 ml.), dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo, giving a tan powder (0.27 g.).

Chromatography of this powder on Woelm Neutral Alumina (Activity Grade III, 28 g.) gave thirteen fractions. Five

fractions were obtained by elution with chloroform-benzene (3:1, 250 ml., net 0.05 g.), four from chloroform (250 ml., 0.05 g.), and four from chloroform-methanol (99:1, 250 ml., 0.18 g.). All these fractions had similar infrared spectra and were crystallized from methanol-water giving 0.22 g. of unreacted starting material.

Reaction of photoaminodemethoxyisocolchicine with base

Photoaminodemethoxyisocolchicine (1.0 g., 0.00130 mol.) was dissolved in ethanol (95%, 45 ml.) and heated with stirring to 55°. Sodium hydroxide (10%, 5 ml.) was added to the stirred solution. The progress of the reaction was followed by the slight shift of the 276 mμ band to 273 mμ. After two hours of heating, the bright red reaction mixture was cooled in an ice bath, diluted with water (20 ml.), and brought to pH 2 by addition of dilute hydrochloric acid (2%, 24 ml.). The acid solution was extracted with chloroform (3 x 50 ml.), and the combined organic extracts were dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo. The orange-red powder showed no 5.70 μ peak in its infrared spectrum. The powder could not be induced to crystallize from a variety of solvents and could not be purified by column chromatography on Woelm Neutral Alumina (Activity Grade III) or Unisil.

Reaction of the basic hydrolysis product with diazomethane

The crude orange-red powder obtained above was dissolved in methylene chloride (20 ml.) and added slowly to an ice-cold, stirred solution of diazomethane in methylene chloride (75 ml.), generated as described previously from N-nitroso-N-methylurea (4.0 g.) and cold potassium hydroxide solution (40%, ≈5 ml.). The reaction mixture was warmed slowly with stirring to 40° to decompose the excess diazomethane. Chloroform (30 ml.) was added to the remaining solution (35-50 ml.), and the solution was boiled vigorously for a few minutes then evaporated to dryness in vacuo. The orange residue was dissolved in benzene (20 ml.) and chromatographed on a 200 mm. column of Woelm Neutral Alumina (Activity Grade III, 54 g.). Elution with benzene (300 ml.) and benzene-chloroform (4:1, 300 ml.) gave 0.01 g. each of unidentified oil. Elution with benzene-chloroform (3:2, 150 ml.) gave 0.11 g. of unidentified yellow glass. Continued elution with the same solvent (≈50 ml.) gave 0.14 g. of a light yellow glass which had an infrared spectrum identical to the infrared spectrum of authentic β-lumicolchicine (Figure 16, p. 46). The glass was crystallized from ethanol-water (1:2, 2 ml.) giving 0.06 g. of light yellow needles, m.p. 177-184°; lit. (8) 180° from ethyl acetate-ethanol or 205° from aqueous ethanol. Repeated crystallizations from aqueous ethanol gave colorless needles,

m.p. 179.5-183°. The infrared spectrum of these crystals (Figure 16, p. 46) is superimposable on the infrared of authentic β-lumicolchicine recrystallized from ethanol-water (m.p. 182-184°). Continued elution with chloroform-benzene (1:1, ≈50 ml.) gave a yellow glass (0.09) g. whose infrared spectrum (Figure 17, p. 46) is superimposable upon the infrared spectrum of γ-lumicolchicine (Figure 17, p. 46). This oil could not be crystallized.

Photoaminodemethoxycolchicine

Aminodemethoxycolchicine (XXI)

Aminodemethoxycolchicine was prepared as described by Horowitz and Ullyot (26).

Photoaminodemethoxycolchicine (XXXIX)

A solution of aminodemethoxycolchicine (6.0 g., 0.0156 mol.) in ethanol (95%, ≈40 ml.) was purged with nitrogen for 1 hr. The solution was irradiated with a mercury arc lamp (described above) using a water-cooled internal Pyrex well. During the irradiation, the color of the solution changed from straw yellow to deep red. The progress of the irradiation (Figure 20, p. 58) was followed by the disappearance of the 353 and ≈246 mμ maxima with concommitant generation of maxima at ≈274 and ≈217 mμ. Total time required was 4.75 hr.

(Subsequent irradiations on 6.0 and 1.85 g. of aminodemethoxycolchicine required 4.75 and 2.6 hr. respectively.)

The irradiation mixture was evaporated to dryness *in vacuo*, dissolved in chloroform, and again evaporated to dryness (to remove ethanol). The red viscous oil (6.0 g.) was combined with the residue (3.0 g.) from a second irradiation (of 3.0 g.) and chromatographed on Woelm Neutral Alumina (Activity Grade III, 345 g.) packed in benzene. The red oil was placed on the column in chloroform-benzene (1:3, 15 ml.) and eluted with the same solvent mixture (1000 ml.) affording 0.01 g. of unidentified oil. Elution with chloroform-benzene (1:1, 500 ml.) gave 0.03 g. of crude photoproduct. Continued elution with the same solvent system (3500 ml.) gave 5.3 g. of crude photoproduct. The combined fractions were crystallized from methanol, chilled, and filtered cold, giving 3.15 g. of yellow-brown crystals. Subsequent recrystallization from water-methanol (3:2, 100 ml.) gave 2.60 g. (29%) of fluffy colorless needles. M.p. 208.5-209.5°, resolidified above 210°, and slowly remelted with decomposition above 220°, $\lambda_{max}^{CHCl_3}$ 5.95, 6.12, 6.26 μ (Figure 21, p. 60); $\lambda_{max}^{95\% \text{ EtOH}}$ 274 (21,100), 219 mμ (21,600); nuclear magnetic resonance spectrum (Figure 24, p. 62).

The analytical sample was dried for 24 hr. at 78° and 0.4 mm. Subsequent drying by the analysts resulted in no loss of weight.

Anal. Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.51; H, 6.29; N, 7.29.

Found: C, 65.62; H, 6.44; N, 7.24.

Hydrogenation of photoaminodemethoxycolchicine (XXXIX)

Photoaminodemethoxycolchicine (XXXIX) (200 mg.) was reduced with hydrogen in ethanol (95%, 45 ml.) utilizing prereduced Adams catalyst (45 mg.). There was no discernable change in rate of hydrogen absorption after 1 mole of hydrogen had been consumed. Exhaustive hydrogenation consumed 2.04 moles of hydrogen. The catalyst was filtered, and the filtrate was evaporated to dryness in vacuo. Chromatography of the crude straw yellow powder was unsuccessful. The crude reaction product shows the loss of the enamine absorption at 6.13 μ in the infrared spectrum and gives a slow periodic acid test. The reaction product was not further characterized.

In a similar experiment, the hydrogenation was stopped after 1 mole of hydrogen had been absorbed. Chromatography of the reaction products gave some starting material (20-30%) in addition to other oils which were not further characterized.

Oxidation of tetrahydrophotoamino-demethoxycolchicine (XLIII or XLIV)

A solution of sodium metaperiodate (110 mg., 1 mol.) in water (4 ml.) was added to a solution of the crude tetrahydrophotoproduct above (200 mg.) in methanol (4 ml.). The white precipitate (sodium iodate), which was formed after stirring at room temperature for 3 hr., was filtered and the mother liquors were evaporated to dryness *in vacuo*. The infrared spectrum of the crude reaction product showed that no reaction (aldehyde formation) occurred.

Photoaminodemethoxycolchicine dioxime (XLVIII)

The dioxime was prepared by refluxing a solution of the photoproduct (XXXIX) (0.2 g.), hydroxylamine hydrochloride (0.1 g.), ethanol (95%, 1.5 ml.), and pyridine (2.0 ml.) on a steam bath for 1.5 hr. The white precipitate, which formed almost immediately, was soluble in water and gave a positive silver nitrate test. The filtrate was concentrated *in vacuo* giving a light yellow oil which crystallized when triturated with methanol (2.5 ml.). The colorless platelets (0.18 g., 83%) were recrystallized from methanol-ethanol giving colorless platelets (0.14 g.); m.p. 175-178°; lit. (8) m.p. 184-186°; m.p. of dioxime prepared from β-lumicolchicine by the method of Forbes (8) 175-177°. The infrared spectra of both dioximes in potassium bromide were superimposable (Figure 22,

60).

Photoaminodemethoxycolchicine-2,4-dinitrophenylosazone

(XXXIX) (30 mg.) was dissolved in methanol (2 ml.) and added to a solution of 2,4-dinitrophenylhydrazine (30 mg.) in methanol (3 ml.) to which one drop of concentrated hydrochloric acid had been added. The reaction mixture was warmed gently on a hot plate causing a voluminous scarlet precipitate to appear in less than five minutes. The cooled reaction mixture was filtered, and the precipitate was washed with cold methanol (5 ml.). The dried precipitate (30 mg.) melted at 209-212°, lit. (8) 212°.

The 2,4-dinitrophenylosazone of β-lumicolchicine was prepared as described by Forbes (8). The scarlet precipitate required at least 1.5 hr. to form. Melting point of the dried material (10 mg.) was 211-213°. The infrared spectra of the two 2,4-dinitrophenylosazones in potassium bromide were superimposable (Figure 23, p. 60).

Attempted reduction of photoaminodemethoxycolchicine

(XXXIX) (0.500 g.) was placed in a flame dried three-necked flask and dissolved in anhydrous tetrahydrofuran (20 ml., freshly distilled from lithium aluminum hydride). Sodium borohydride (0.100 g.) was added, and the reaction mixture

was stirred for 10 hr. at room temperature. Anhydrous acetone (2.0 ml.) was added to complex with the excess borohydride. After stirring for one half hour, water was added (10 ml.), and the reaction mixture was concentrated in vacuo to 8 ml. The milky solution was extracted with chloroform (3 x 5 ml.), and the combined, dried organic extracts were evaporated to dryness giving a yellow oil which had an infrared spectrum identical to the product of hydrogenation (2 moles of hydrogen) and to the product obtained from a similar borohydride reduction which also had 10 ml. of water present in the reaction mixture. No further attempt at purification was made.

Unsuccessful experiments

An attempt to form an N-benzoyl derivative of photoaminodemethoxycolchicine in pyridine, in order to lower the reactivity of the enamine, failed.

Any reaction attempted (such as a diazotization) which requires an acidic medium results in precipitation of a salt, which does not melt below 350°. This salt is slightly soluble in chloroform, but not soluble enough to give a meaningful n.m.r. spectrum. It is insoluble in pyridine, dimethylsulfoxide, water, ethanol, benzene, methanol, and ether. Neutralization of an aqueous slurry with sodium hydroxide (10%) results in recovery of salt, λ_{max}^{KBr} 5.85, 6.10, 6.17, 6.28 μ.

Basic hydrolysis of XLIX

XLIX (0.25 g.) was dissolved in a mixture of ethanol (5.0 ml., 95%) and normal sodium hydroxide (12.5 ml.), and refluxed on a steam bath for 5 hr. The cooled solution was neutralized with normal sulfuric acid (14.0 ml.) to pH 2. The neutralized solution deposited tan needles of L on standing overnight in a refrigerator (0.165 g., 66%, m.p. 209-218°; $\lambda_{max}^{CHCl_3}$ 5.95 (sh), 6.00, 6.26μ (Figure 26, p. 67); $\lambda_{max}^{95\%\ EtOH}$ 223 (33,900), 268 mμ (30,580); nuclear magnetic resonance (Figure 27, p. 67).

Methylation of L

Diazomethane was generated as above from N-nitroso-N-methylurea (2.0 g.) and cold potassium hydroxide (20 ml., 40%) in methylene chloride (20 ml.) A solution of XLIX (0 09 g.) in methylene chloride (4 ml.) was added dropwise to the ice-cold, stirred solution of diazomethane in methylene chloride (20 ml.). The reaction mixture was slowly warmed to decompose excess diazomethane then evaporated nearly to dryness. No chloroform was used in this workup. Ethanol (5 ml., 95%) was added, and the reaction was boiled gently for a few minutes then evaporated to dryness *in vacuo*. The crude yellow oil showed characteristic β-lumicolchicine absorption in the infrared. Crystallization from methanol-water failed. The aqueous solution was extracted with chloroform (4 x 3.0 ml.),

and the combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Chromatography of the light yellow oil (0.08 g.) on Woelm Neutral Alumina (Activity Grade III, 18 g.) afforded β-lumicolchicine as a colorless oil (0.050 g., 55%) on elution with benzene-chloroform (3:2, 250 ml.). β-Lumicolchicine obtained by crystallization from methanol-water (1:3, 1.6 ml., 0.030 g.) was identical with an authentic sample in melting point and infrared spectrum (Figure 28, p. 67 for β-lumicolchicine obtained in this experiment, Figure 17, p. 46, for authentic β-lumicolchicine).

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