Anal. Calcd. for $C_{30}H_{48}O_2$ (440.68): C, 81.76; H, 10.98. Found: C, 81.38; H, 10.95.

The material is resistant to sodium dichromate in acetic acid at 100°, and to boiling methanolic alkali.

Dihydro-desoxo-B.—Whereas desoxo-B is recovered essentially unchanged from the Huang-Minlon modification of the Kishner–Wolff reduction, treatment of 148 mg. with 1 g. of sodium in 12 ml. of ethanol and 1.25 ml. of hydrazine ("free base, 94%") for 16 hr. at 217–240° led to the isolation of a carbonyl-free *hydroxy compound.* The crude solid obtained by dilution of the contents of the bomb tube was recrystallized, yielding 112 mg. of needles (from chloroform–methanol, and from ethanol) that undergo a transformation below 260° and melt 288–290°, λ^{Chf} 2.80 (sharp), 2.95 (broad), 6.80, 7.22, 7.35, 7.70, 7.75, 7.85, 8.80 8.93, 9.12, 9.50, 10.00, 10.30, 10.60, 10.90, 11.05, 11.18, 11.45 μ.

Anal. Calcd. for $C_{30}H_{50}O_2$ (442.70): C, 81.39; H, 11.38. Found: C, 81.70; H, 11.52.

Dihydrodesoxo-B Acetate.—Acetylation of 56 mg. of dihydrodesoxo-B with 1.0 ml. of pyridine and 0.5 ml. of acetic anhydride for 7 minutes on the steam-bath led to only partial acetylation, but repetition of this treatment for 4 hours yielded 56 mg. of hydroxyl-free material that was recrystallized from absolute ethanol, yielding silky needles that begin to sublime near 236° and melt 241–242°, α_D −21.6° (*c* 2.03 Chf); λ^{Chf} 5.82, 6.83, 7.25, 7.35, 7.55, 7.70, 8.0 (broad), 8.65, 8.90, 9.12, 9.80, 10.10, 10.35, 10.60, 10.90, 11.35 μ.

Nor-B-dicarboxylic Acid and Anhydride.—A mixture of 528 mg. of B, 30 ml. of purified dioxane, 15 ml. of 0.1 *N* NaOH and 30 ml. of 0.618 *N* sodium hypochlorite solution was warmed to 64° (reflux temperature of methanol) for 1.5 hr. The solution was diluted with 100 ml. of distilled water and treated with ferrous sulfate solution until the potassium iodide–starch test became negative. The small quantity of ferric hydroxide formed was filtered and the filtrate acidified with phosphoric acid.

The precipitated organic acid was collected. The filtrate (240 ml.) was subjected to steam distillation in an apparatus with dephlegmator, the volume being maintained constant. Fifty-ml. portions of distillate were collected and examined acidimetrically for volatile acid. The results of these titrations were compared with those obtained in a blank run and it was found (a) that 0.04 ml. of acetic acid could be detected with certainty by the technique employed and (b) no volatile acid was formed in the oxidation of B.

The crude **diacid** on recrystallization from aqueous acetone gave 315 mg. of fine needles that change at 240–260° and melt 310–325° with decomposition. From the mother liquor 153 mg. of acid was isolated, which, by warming with 0.5 ml. of acetic acid and 1.0 ml. of acetic anhydride for 5 minutes, gave 96 mg. of the **anhydride**, which on recrystallization from benzene–petroleum ether gives needles and plates that at 290° change to hexagonal prisms of characteristic shape, melting 320–324°; λ^{Chf} 5.55, 5.67, 6.82, 7.20, 7.30, 7.65, 7.95, 9.00, 9.30, 9.45, 9.70, 9.90, 10.21, 10.55, 11.50 μ.

Anal. Calcd. for $C_{29}H_{44}O_4$ (456.64): C, 76.27; H, 9.71. Found: C, 76.32, 76.20; H, 9.64, 9.60.

Dimethyl Nor-B-dicarboxylate.—This ester is obtained by treatment of the C_{29}-acid with ethereal diazomethane and recrystallization from ethanol and from chloroform–methanol. The compound forms lozenge-shaped plates, m.p. 176.8–178.8° (cor.); λ^{Chf} 5.79, 6.80, 6.92, 7.20, 7.40, 7.65, 7.95, 8.70, 8.86, 9.10, 10.25, 10.60 μ.

Anal. Calcd. for $C_{31}H_{50}O_5$ (502.71): C, 74.06; H, 10.03; CH_3O, 12.36. Found: C, 73.68, 73.83; H, 10.12, 10.13; CH_3O, 12.25.

Unhindered Monomethyl Ester of Nor-B-dicarboxylic Acid.—A sample of crude nor-B-dicarboxylic acid (72 mg.) was treated with 1 ml. of methanol containing 0.05 ml. of concentrated sulfuric acid in a sealed tube for 8 hr. at 80°. A first crop of 45 mg. of needles was collected, and a second crop of 24 mg. Recrystallization from chloroform–methanol and from ethanol (under pressure) yielded well-formed needles, decomposing at 288–290° with gas evolution; the melt partially resolidifies and remelts near the m.p. of the anhydride. Treatment with diazomethane yields the diester, m.p. 178–179°, not depressed by admixture of authentic material. The monoester also results when the anhydride is boiled with 0.1 *N* sodium hydroxide in 90% methanol; the product was identified by its behavior on melting, by its spectrum and by conversion to the diester, m.p. 179°; λ^{Chf} 2.9–3.2 (broad shoulder), 5.82, 5.87, 6.85, 7.25, 7.35, 7.45, 7.50, 7.95, 8.70, 8.90, 9.10, 9.30, 10.0, 10.30, 10.60 μ.

Anal. Calcd. for $C_{30}H_{48}O_5$ (488.68): C, 73.73; H, 9.90; CH_3O, 6.35. Found: C, 73.86, 73.77; H, 10.08, 9.68; CH_3O, 5.72, 6.43.

Hindered Monomethyl Ester of Nor-B-dicarboxylic Acid.—A sample of dimethyl nor-B-dicarboxylate (11 mg.) was heated in a sealed vessel with 0.5 ml. of 20% methanolic potassium hydroxide on the steam-bath for 1.5 hr. The product (8 mg.) was precipitated by water and recrystallized from dilute methanol. The substance formed needles, m.p. 255–264°; no gas evolution was noted during rapid heating of a sample, and when mixed with the isomeric monomethyl ester described above the sample melted below 250°.

Anal. Calcd. for $C_{30}H_{48}O_5$ (488.68): CH_3O, 6.35. Found: CH_3O, 6.45.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING AND THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA]

The Degradation of Colchicine to Octahydrodemethoxydesoxydesacetamidocolchicine[1]

BY HENRY RAPOPORT, ARTHUR R. WILLIAMS,[2] JOHN E. CAMPION AND DONALD E. PACK

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Although a complex mixture of difficultly separable products is formed on hydrogenation of colchicine (I), replacement of the methoxyl of ring C by dimethylamino and hydrogenation of the thus formed N,N-dimethylaminocolchicide (II) results in a good yield of easily purified tetrahydrodemethoxycolchicine (III). By conversion to the mercaptole and desulfurization, the carbonyl group is transformed to methylene giving hexahydrodemethoxydesoxycolchicine (V). Removal of the acetamido group is accomplished by heating with phosphorus pentoxide in xylene and hydrogenation yields octahydrodemethoxydesoxydesacetamidocolchicine (VI). This degradation sequence very reasonably takes place without rearrangement in the colchicine carbon skeleton and thus affords a degradation product whose synthesis would establish the nature of ring C.

With the synthesis of colchinol methyl ether,[3,4] the only remaining portion of the colchicine molecule (I) for which a definitive proof of structure is lacking is ring C. The features yet to be established by decisive chemical evidence are the seven-membered, tropoloid nature of the ring[5] and the relative positions of the carbonyl and methoxyl groups.

(1) Supported (in part) by Cancer Research Funds of the University of California and (in part) by the U. S. Atomic Energy Commission.

(2) American Cancer Society Postdoctoral Fellow, 1951.

(3) H. Rapoport, A. R. Williams and M. E. Cisney, THIS JOURNAL, **73**, 1414 (1951).

(4) J. W. Cook, J. Jack and J. D. Loudon, *J. Chem. Soc.*, 1397 (1951).

(5) First proposed by M. J. S. Dewar, *Nature*, **155**, 141, 479 (1945).

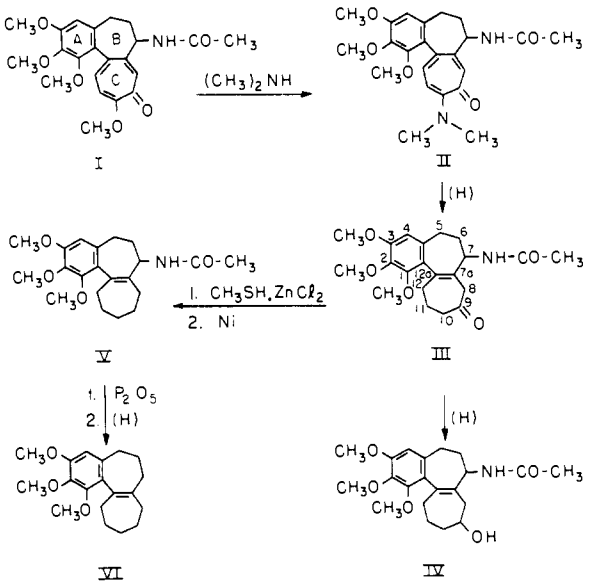
Much evidence[6] has accumulated supporting a tropolone methyl ether formulation as the most feasible structural representation for ring C. This includes spectral data,[6b] hydrogenation to a glycol,[6a,5] and the parallelism in properties between known tropoloid compounds and colchicine,[6c,d,e] albeit in several instances there are marked differences in degree. This similarity in properties, as has been pointed out,[6c] is necessary but insufficient to establish the tropoloid nature of ring C.

Although the carbonyl and methoxyl groups must be confined to the two positions shown in structure I on the basis of mechanistic interpretations[6c,7] of the rearrangements of colchicine, only one attempt[8] has been made to indicate the specific position of each group.[9] From the similarity of infrared spectra (in the 7 μ region) and optical rotations of colchiceine and several isocolchicine derivatives, it was suggested that colchiceine was a single species belonging to the iso series, and this non-tautomeric nature of colchiceine could be due to hydrogen-bonding to the amide carbonyl. Thus the hydroxyl group of colchiceine and the methoxyl of isocolchicine occupy the position more proximate to the acetamido group, and this conclusion supports structure I for colchicine.

We have sought direct chemical evidence for these structural features of ring C by unambiguous degradation to compounds, the synthesis of which would be feasible and would establish the structure in question. The present work reports the degradation to such a compound, octahydrodemethoxydesoxydesacetamidocolchicine (VI), containing the intact carbon skeleton of colchicine.[10]

A fruitful approach to the desired compound (VI) was suggested by the studies of Bursian[11] who reported the hydrogenation of colchicine and the isolation of hexahydrodemethoxycolchicine (IV) and a very small amount of what was apparently hexahydrodemethoxydesoxycolchicine (V). Since these compounds arise by mild catalytic hydrogenation, it is extremely probable that no rearrangement of the original, labile carbon skeleton of ring C is involved. In addition, they still retained a double bond, and since the most reasonable position for this resistant-to-hydrogenation double bond was at the fusion of rings B and C, the stereoisomer problem would be obviated. However, in a recent reexamination[12] of this work, the carbinol IV was isolated only in very small yield after a laborious separation procedure, and none of the desoxy compound V was detected among the hydrogenation products.

An attractive alternative which might markedly facilitate separation of the hydrogenation products was to hydrogenate the derivative in which methoxyl had been replaced by amino. Non-hydrogenolyzed material now might be easily separable by virtue of its basic properties. The derivative chosen for this purpose was N,N-dimethylaminocolchicide (II),[13] formed by heating colchicine with dimethylamine.



N,N-Dimethylaminocolchicide has been reported several times in the literature[15–17] with a m.p. varying from 203 to 207°. Although we have prepared this compound numerous times, following as closely as the published details permit the prescribed conditions of the other investigators and also using some variations of our own, our purified material always melted at 178–179°. That this difference in m.p. is not due to an impurity[18] in our product

(6) (a) H. R. V. Arnstein, D. S. Tarbell, G. P. Scott and H. T. Huang, THIS JOURNAL, **71**, 2448 (1949); (b) G. P. Scott and D. S. Tarbell, *ibid.*, **72**, 240 (1950); (c) W. E. Doering and L. H. Knox, *ibid.*, **73**, 828 (1951); (d) J. W. Cook, A. R. Gibb, R. A. Raphael and A. R. Somerville, *J. Chem. Soc.*, 503 (1951); (e) D. S. Tarbell and J. C. Bill, THIS JOURNAL, **74**, 1234 (1952).

(7) J. Čech and F. Šantavý, *Collection Czechoslav. Chem. Communs.*, **14**, 532 (1949).

(8) R. M. Horowitz and G. E. Ullyot, THIS JOURNAL, **74**, 587 (1952).

(9) Also, an X-ray diffraction study by M. V. King, J. L. deVries, and R. Pepinsky, *Acta Cryst.*, **5**, 437 (1952), indicated the substituents are in positions corresponding to those suggested by Čech and Šantavý[7] for colchiceine.

(10) A preliminary report of this work appeared as a Communication to the Editor, H. Rapoport and A. R. Williams, THIS JOURNAL, **73**, 1896 (1951).

(11) K. Bursian, *Ber.*, **71**, 245 (1938).

(12) A. D. Kemp and D. S. Tarbell, THIS JOURNAL, **72**, 243 (1950).

(13) 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 has 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 certainly not characteristic of amides. Furthermore, the term colchiceinamide,[8,14,15] 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 the replacement by hydrogen. This nomenclature very conveniently accommodates the iso series also through isocolchicide.

(14) A. Uffer, *Helv. Chim. Acta*, **35**, 2135 (1952).

(15) J. L. Hartwell, M. V. Nadkarni and J. Leiter, THIS JOURNAL, **74**, 3180 (1952).

(16) A. J. Ewins, J. N. Ashley and J. O. Harris, British Patent 577,606 (1945).

(17) F. Šantavý, *Chem. Listy*, **46**, 280 (1952).

(18) A possible but highly improbable prospect that our compound was N-methylaminocolchicide for which m.p.'s of 173-174°,[16] 176-178°,[17] and 230–232°[15] have been recorded was eliminated by the marked depression exhibited in a mixed m.p. determination with authentic N-methylaminocolchicide (m.p. 172-174°).

is demonstrated by the fact that it displayed constant properties on crystallization from any of six different solvents, chromatography on alumina or sublimation. Also, a paper chromatogram on alumina-impregnated paper[19] showed only one spot under ultraviolet light. Fortunately, an optical rotation has been reported,[17] and our value is in good agreement.[20] On the basis of the above data, we have concluded our compound is undoubtedly a dimorphic form; and its characteristic ultraviolet absorption spectrum is given in Fig. 1.

Since N,N-dimethylaminocolchicide (II) was a key compound in the degradation scheme, its structure was of considerable importance and was established by the fact that (a) hydrolysis gave colchiceine, and (b) hexahydrodemethoxycolchicine (IV) could be obtained by hydrogenation. The hydrolysis to colchiceine indicates the same two positions of ring C that were occupied by methoxyl and carbonyl in colchicine were now occupied by dimethylamino and carbonyl; and the hydrogenation to IV, the same compound obtained from the hydrogenation of colchicine,[11,12] indicates the carbonyl group has retained its initial position. Therefore, N,N-dimethylaminocolchicide (II) has resulted through replacement of the methoxyl of colchicine by dimethylamino.[21]

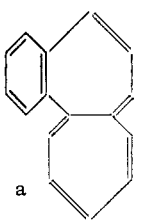
As was expected, N,N-dimethylaminocolchicide was quite reactive toward hydrogen. After an initial, rapid absorption of three moles of hydrogen, absorption continued at a slower but still appreciable rate and ceased at a final value of five moles. Both three- and five-mole reaction mixtures were examined and each found to consist of a neutral and basic portion. In neither case could any crystalline material be isolated from the basic fraction. The neutral fraction, however, afforded hexahydrodemethoxycolchicine (IV) from the five-mole reaction and tetrahydrodemethoxycolchicine (III) from the three-mole reaction. Since the ketone appeared more promising for conversion to the desoxy compound (V), the three-mole hydrogenation was investigated in detail and conditions established which led to a 53% yield of tetrahydrodemethoxycolchicine (III),[22] isolated *via* its bisulfite addition product.

(19) S. P. Datta and B. G. Overell, *Biochem. J.*, **44**, xliii (1949); S. P. Datta, B. G. Overell and M. Stack-Dunne, *Nature*, **164**, 673 (1949).

(20) In this regard, it should be noted that the optical rotation is quite variable with temperature, a not uncommon behavior for colchicine derivatives. For N,N-dimethylaminocolchicide in chloroform, the specific rotation varies inversely with the temperature to the extent of 4.1°/°C.

(21) A comparison of the specific rotation of N,N-dimethylaminocolchicide [$[\alpha]^{27.5}D$ +465° (chloroform)] and N,N-dimethylaminisocolchicide [$[\alpha]^{27}D$ −520° (chloroform), unpublished work by J. B. Lavigne] is also consistent with this structural assignment according to the generalizations of Horowitz and Ullyot[8] (see also Uffer, ref. 14) who find the iso compound of a given pair to have the more negative rotation.

(22) In view of the strong evidence for the carbon skeleton of colchicine (I) and therefore for that of tetrahydrodemethoxycolchicine (III), we have employed the *C. A.* numbering method of the parent ring system, benzo[a]heptalene (a), to locate substituents. However, the common names as derived from colchicine have been used for the degradation products since they are more lucid at present.



a

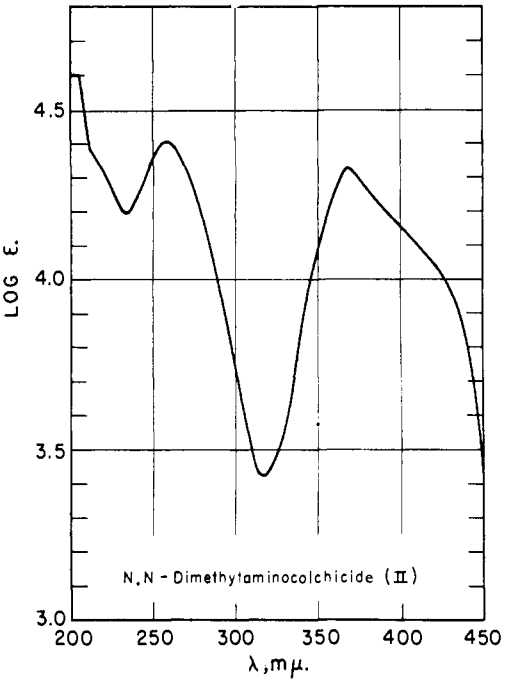


Fig. 1.—Ultraviolet absorption spectrum of N,N-dimethylaminocolchicide in 95% ethanol.

The carbonyl group of III is undoubtedly at the same position as that of colchicine since on hydrogenation both yield the carbinol IV. This position is either 9 or 10, depending on the relative position of methoxyl and carbonyl in colchicine. The double bond that remains resistant to hydrogenation has been placed at the fusion of rings B and C since this position seems the most hindered toward catalyst adsorption. However, unsaturation between carbons 12 and 12a also might be subject to considerable resistance to hydrogenation, and although 7a–12a appears the most reasonable location for the double bond, the 12–12a alternative certainly obtains for tetrahydrodemethoxycolchicine (III) and any of the succeeding compounds. Both formulations are consistent with the ultraviolet and infrared absorption spectra (Figs. 2 and 3) which indicate the presence of a conjugate double bond and benzene ring and an unconjugate carbonyl.

Conversion to the dimethylmercaptole[23] and desulfurization proceeded readily and in excellent yield to hexahydrodemethoxydesoxy colchicine (V). Although the olefinic double bond remained resistant to catalytic hydrogenation, its presence was clearly demonstrated by the consumption of one mole of perbenzoic acid in chloroform at 0° and isolation of a crystalline epoxide.[24]

To remove the acetamido group from hexahydrodemethoxydesoxycolchicine (V), it was subjected to

(23) Using the general procedure of M. S. Newman and H. M. Walborsky, THIS JOURNAL, **72**, 4296 (1950).

(24) Although methoxylated benzene nuclei react with perbenzoic acid [H. Fernholtz, *Ber.*, **84**, 110 (1951); S. L. Friess, *et al.*, THIS JOURNAL, **72**, 2611 (1950); **74**, 1305 (1952)] the difference in rate as compared to the alicyclic double bond is of such great magnitude that there is no difficulty in isolating the one mole product in the case of compounds V and VI. After the initial rapid reaction, further consumption of perbenzoic acid continued but at a tremendously decreased rate.

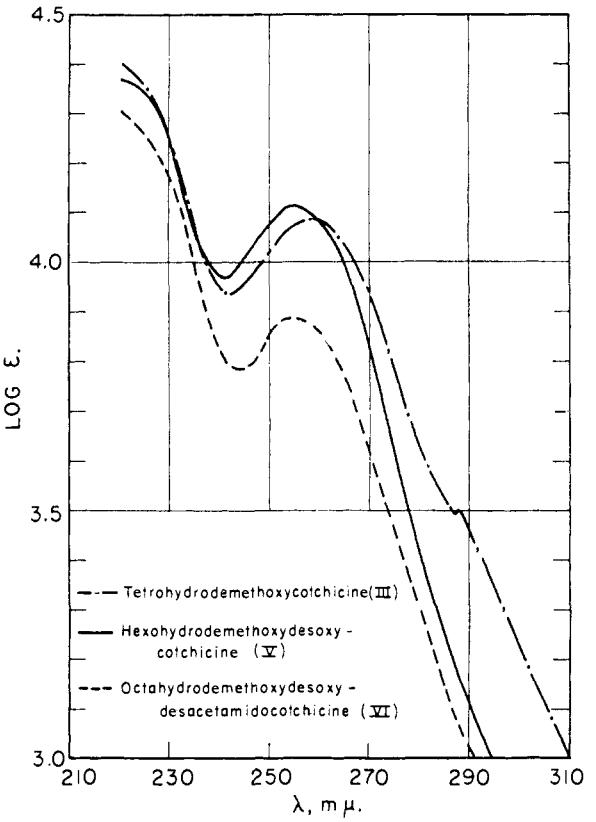


Fig. 2.—Ultraviolet absorption spectra of tetrahydromethoxycolchicine, hexahydrodemethoxydesoxycolchicine, and octahydrodemethoxydesoxydesacetamidocolchicine in 95% ethanol.

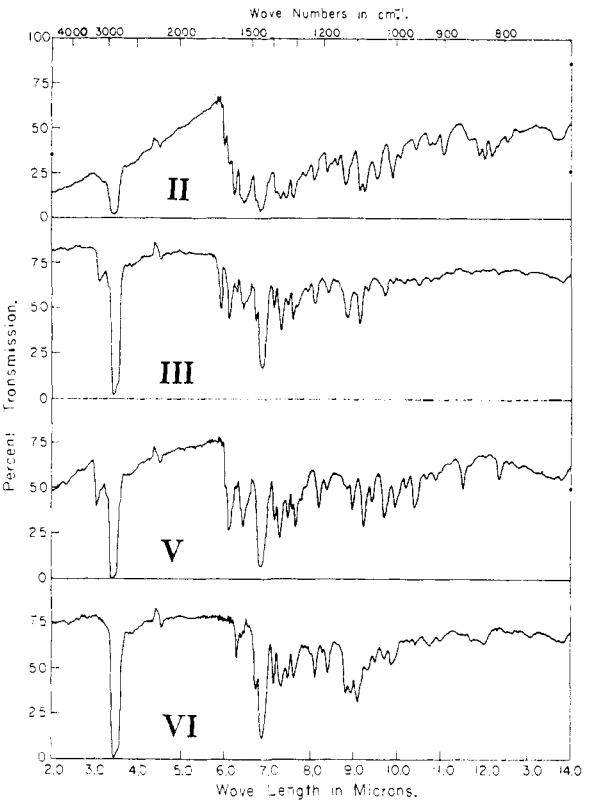


Fig. 3.—Infrared absorption spectra of N,N-dimethylaminocolchicide (II), tetrahydrodemethoxycolchicine (III), hexahydrodemethoxydesoxycolchicine (V), and octahydrodemethoxydesoxydesacetamidocolchicine (VI) as oil mulls (Baird spectrophotometer with NaCl prism).

the action of phosphorus pentoxide in refluxing xylene.[25] By strict adherence to reaction conditions, reproducible results could be obtained, and the reaction product on immediate hydrogenation absorbed one mole of hydrogen and gave octahydrodemethoxydesoxydesacetamidocolchicine (VI). Again, the persistence of a double bond was shown by consumption of one mole of perbenzoic acid and isolation of an epoxide.

The ultraviolet and infrared[26] absorption spectra of the compounds in the degradation scheme are shown in Figs. 1, 2 and 3. In every case, the spectra are consistent with the structures presented, although they do not permit a unique structure assignment (*e.g.*, as to whether the compounds are $\Delta^{7a(12a)}$ or Δ^{12}). Considering the method of degradation employed and the avoidance of rearrangement, octahydrodemethoxydesoxydesacetamidocolchicine (VI) appears to be a compound whose synthesis would establish definitively the nature of ring C in colchicine.

Experimental[27]

Colchicine (I).—Colchicine (U.S.P.) was purified by chromatography[28] followed by crystallization from ethyl acetate, m.p. 154–155°.

N,N-Dimethylaminocolchicide (II).—A solution of pure dimethylamine in methanol was prepared by adding saturated methanolic potassium hydroxide to dimethylamine hydrochloride, purified by several crystallizations from absolute ethanol, and absorbing the liberated amine in methanol. To 80 ml. of such a solution (3 *N*) was added 31.1 g. (78 mmoles) of colchicine and the resulting solution was heated in a sealed tube completely immersed in an oil-bath at 105° for 18 hours. After cooling, the contents of the tube were evaporated to a thick sirup which was dissolved in 150 ml. of benzene and then extracted with three portions of 2 *N* hydrochloric acid and two portions of water (50 ml. each). The combined aqueous extracts, after washing with benzene, were made strongly alkaline with sodium hydroxide solution and extracted with three 100-ml. portions of benzene. Evaporation of the washed (water) and dried benzene layers gave a residue which was chromatographed on alumina (Merck) using benzene–chloroform (1:1) as eluant. Crystallization of the eluted material from ethyl acetate gave 23.7 g. (58 mmoles, 74% yield) of N,N-dimethylaminocolchicide, m.p. 175–177°. Recrystallization gave material of m.p. 178–179°, $[\alpha]^{25}D$ +69.4° (*c* 1.03, ethanol), $[\alpha]^{17}D$ +508° (*c* 1.04, chloroform), $[\alpha]^{27.5}D$ +465° (*c* 1.00, chloroform); reported m.p. 204–206°,[16] 203–205°,[15] 205–207°,[17] $[\alpha]^{21}D$ +510° (*c* 0.79, chloroform).[17]

Anal. Calcd. for $C_{23}H_{28}N_2O_5$: C, 67.0; H, 6.8; N, 6.8; OCH_3, 22.6. Found: C, 66.9; H, 6.9; N, 7.0; OCH_3, 22.2.

Further chromatography on alumina, sublimation, or recrystallization from ether, acetone, ethanol, methyl isobutyl ketone or xylene had no effect on the properties of N,N-dimethylaminocolchicide, and an alumina-impregnated paper chromatogram showed only one spot. A mixed melting point determination with N-methylaminocolchicide[18] showed a depression of 20°.

(25) J. W. Cook, W. Graham, A. Cohen, R. W. Lapsley and C. A. Lawrence, *J. Chem. Soc.*, 322 (1944).

(26) We are indebted to Dr. N. K. Freeman of the Radiation Laboratory for the infrared spectra and helpful discussions concerning their interpretation.

(27) All melting points are corrected and those above 200° were taken in evacuated capillaries; microanalyses were performed by the Microchemical Laboratory, University of California.

(28) J. N. Ashley and J. O. Harris, *J. Chem. Soc.*, 677 (1944).

The picrate, prepared with saturated ethanolic picric acid, was crystallized from absolute ethanol, m.p. 186–188°, $[\alpha]^{25}D$ +171° (*c* 1.08, chloroform).

Anal. Calcd. for $C_{29}H_{31}N_5O_{12}$: C, 54.3; H, 4.9; OCH_3, 14.5. Found: C, 54.3; H, 5.0; OCH_3, 14.3.

Hydrolysis of N,N-Dimethylaminocolchicide to Colchiceine.—A mixture of 50 mg. (0.12 mmole) of N,N-dimethylaminocolchicide and 5 ml. of 0.1 *N* sodium hydroxide was heated on the steam-bath for four hours, then cooled, washed with 5 ml. of benzene, and acidified with 1 ml. of 1 *N* hydrochloric acid. The mixture was extracted with three 5-ml. portions of chloroform and evaporation of the combined chloroform extracts after drying gave a residue which was digested on the steam-bath with 1 ml. of saturated sodium bicarbonate solution and filtered. Acidification of the bicarbonate solution with 1 *N* hydrochloric acid and cooling several hours precipitated material, which was crystallized from ethyl acetate–*n*-butyl ether to yield 27 mg. (0.07 mmole, 59%) of colchiceine, m.p. and mixed m.p. with an authentic sample, 178–179°.

Tetrahydrodemethoxycolchicine (III).—Hydrogenation of 4.0 g. (9.7 mmoles) of N,N-dimethylaminocolchicide in 120 ml. of glacial acetic acid proceeded rapidly in the presence of 500 mg. of 5% palladized carbon and 250 mg. of platinum oxide at 20° and a pressure of 30 p.s.i. Three moles of hydrogen was absorbed in about 19 minutes, and an additional 0.1 mole in the next two minutes after which the hydrogenation was stopped and the reaction mixture was filtered. The filtrate was concentrated under reduced pressure, basified with 6 *N* sodium hydroxide, extracted with three 30-ml. portions of benzene, and the separate benzene extracts were in turn extracted with two 25-ml. portions of 2 *N* hydrochloric acid and two 20-ml. portions of distilled water. Evaporation of the combined, dried benzene extracts left a yellow viscous oil which was digested for successive 30-minute periods on a steam-bath with two 50-ml. portions of 20% aqueous sodium bisulfite and one 50-ml. portion of water. The combined aqueous digests were basified with potassium carbonate and extracted with four 30-ml. portions of benzene which were then washed, dried, concentrated to 25 ml., and applied to a column (30 × 1 cm.) of alumina (Merck). Elution was accomplished with 500 ml. of chloroform, from which on evaporation the ketone was obtained as a white amorphous solid, 1.91 g. (5.1 mmoles, 53%). This material was of suitable purity for use in the degradative sequence; however, crystallization from a mixture of ethyl acetate and *n*-butyl ether (2:1) gave a total of 1.61 g. (4.3 mmoles, 44%) of tetrahydrodemethoxycolchicine in several crops, melting variously between 140–144°. A recrystallized sample melted at 143–144°, $[\alpha]^{25}D$ −174° (*c* 1.11, ethanol).

Anal. Calcd. for $C_{21}H_{27}O_5N$: C, 67.5; H, 7.3; N, 3.8; $-OCH_3$, 24.9. Found: C, 67.5; H, 7.3; N, 3.8; $-OCH_3$, 24.8.

Hexahydrodemethoxycolchicine (IV). A. By Hydrogenation of N,N-Dimethylaminocolchicide (II).—Hydrogenation of 0.5 g. (1.2 mmoles) of N,N-dimethylaminocolchicide in 12 ml. of glacial acetic acid was allowed to proceed for 72 hours at 25° and atmospheric pressure with 50 mg. of 5% palladized carbon and 25 mg. of platinum oxide, at which time hydrogen absorption (4.9 moles) ceased. The neutral fraction (0.21 g.), on crystallization from ethyl acetate gave hexahydrodemethoxycolchicine, m.p. 168–170°, $[\alpha]^{25}D$ −166° (*c* 1.01, ethanol) (reported m.p. 171°[11] and 173°[12]).

Anal. Calcd. for $C_{21}H_{29}O_5N$: C, 67.2; H, 7.8; N, 3.7. Found: C, 67.2; H, 7.8; N, 3.9.

B. By Hydrogenation of Tetrahydrodemethoxycolchicine (III).—A solution of 0.25 g. (0.7 mmole) of tetrahydrodemethoxycolchicine in 5 ml. of glacial acetic acid was hydrogenated over 10 mg. of platinum oxide and 20 mg. of 5% palladized carbon, and resulted in the absorption of 0.95 mole of hydrogen. After removal of the catalyst, the solution was made alkaline with 6 *N* sodium hydroxide and extracted with three 25-ml. portions of benzene. Drying and evaporating the benzene left a residue which was crystallized from ethyl acetate to give 0.16 g. (0.4 mmole, 64%) of hexahydrodemethoxycolchicine, m.p. 168–170° after drying at 100° *in vacuo*. This material was identical with the neutral material from the 5-mole hydrogenation of N,N-dimethylaminocolchicide.

When treated with acetic anhydride, hexahydrodemethoxycolchicine formed an **acetate**, m.p. 206–208° (reported[11] m.p. 210°).

Tetrahydrodemethoxycolchicine Dimethylmercaptole.—A sealed tube containing 0.91 g. of anhydrous fused zinc chloride, 0.91 g. of anhydrous sodium sulfate, 3.19 g. (8.5 mmoles) of tetrahydrodemethoxycolchicine (III), and 36 ml. of methyl mercaptan was shaken until the ketone and zinc chloride dissolved and then allowed to stand at room temperature for 18 hours. The tube was opened, the methyl mercaptan evaporated, and the residue dissolved in chloroform. After being washed with water and 1 *N* sodium hydroxide, the chloroform was evaporated and the crude mercaptole was crystallized from aqueous methanol; yield 3.21 g., 83%; m.p. 190–192°; $[\alpha]^{25}D$ −160° (*c* 0.96, ethanol).

Anal. Calcd. for $C_{23}H_{33}O_4NS_2$: C, 61.2; H, 7.4; S, 14.2. Found: C, 61.4; H, 7.5; S, 14.0.

Hexahydrodemethoxydesoxycolchicine (V).—A vigorously stirred and refluxing mixture of 3.26 g. (7.2 mmoles) of tetrahydrodemethoxycolchicine dimethylmercaptole in 300 ml. of 90% aqueous ethanol, and 64 g. of Raney nickel[29] was filtered after 16 hours. The nickel was digested with two 200-ml. portions of benzene and the combined benzene digests and ethanol filtrate evaporated. Crystallization of the residue from aqueous methanol gave 2.29 g., 88%, of hexahydrodemethoxydesoxycolchicine, m.p. 183.5–184°, $[\alpha]^{25}D$ −162° (*c* 1.10, ethanol).

Anal. Calcd. for $C_{21}H_{29}O_4N$: C, 70.2; H, 8.1; OCH_3, 25.9. Found: C, 70.1; H, 8.2; OCH_3, 26.0.

Hexahydrodemethoxydesoxycolchicine Epoxide.—Using a 0.04 *M* solution of perbenzoic acid in chloroform,[30] the epoxidation of hexahydrodemethoxydesoxycolchicine (V) at 0° was followed by withdrawal of aliquots and determination of unreacted perbenzoic acid by titration in the usual manner.[30] During 3.2 hours, one mole of perbenzoic acid was consumed per mole of compound after which the rate markedly decreased and during the next three hours only an additional 0.1 mole of perbenzoic acid was consumed. A parallel reaction containing originally 100 mg. (0.28 mmole) of hexahydrodemethoxydesoxycolchicine in 25 ml. of chloroform (0.04 *M* in perbenzoic acid) was allowed to continue for four hours during which 105 mole % of perbenzoic acid was consumed as determined by the removal of five 1-ml. aliquots. To quench the reaction, 10 ml. of 1 *N* potassium carbonate solution was then added and the chloroform phase was washed with two 10-ml. portions of water. Evaporation of the dried chloroform solution left 80 mg. of crystalline material which was applied as a benzene solution to an alumina (Merck) column (22 × 1 cm.). Benzene (150 ml.) and 25% chloroform–benzene (150 ml.) eluted 62 mg. (0.17 mmole, 74%) of colorless, crystalline epoxide. Two crystallizations from hexane–benzene gave hexahydrodemethoxydesoxycolchicine epoxide of m.p. 209.5–210° and $[\alpha]^{25}D$ +28.9° (*c* 0.85, ethanol).

Anal. Calcd. for $C_{21}H_{29}O_5N$: C, 67.2; H, 7.8; OCH_3, 24.8. Found: C, 67.2; H, 7.7; OCH_3, 24.0.

Octahydrodemethoxydesoxydesacetamidocolchicine (VI).—To a solution of 0.5 g. (1.4 mmoles) of hexahydrodemethoxydesoxycolchicine in 50 ml. of xylene (purified by distillation from phosphorus pentoxide) heated to 100°, was added 1.0 g. of phosphorus pentoxide with vigorous stirring and in a nitrogen atmosphere. The stirred mixture was heated to reflux in 10 minutes and reflux was continued for 25 minutes. After cooling and decanting the xylene, the residue was digested with two 100-ml. portions of benzene, and the combined digests were allowed to cool, filter aid was added and the solution filtered. Evaporation of the filtrate left a viscous oil which was dissolved in 2 ml. of hexane (in which hexahydrodemethoxydesoxycolchicine is insoluble) and filtered from a small amount of solid. The 370 mg. of oil obtained on evaporation of the hexane was dissolved in 5 ml. of glacial acetic acid and hydrogenated over 20 mg. of platinum oxide and 40 mg. of 5% palladized carbon. Hydrogen absorption ceased at 110 mole %, after which the catalyst was removed by filtration, and the filtrate, made basic with 6 *N* sodium hydroxide, was extracted with two 10-ml. portions of benzene. The combined benzene extracts were washed with 10-ml. portions of water, 2 *N* hydrochloric acid and 1 *N* potassium carbonate and were then

(29) R. Mozingo, *Org. Syntheses*, **21**, 15 (1941).

(30) G. Braun, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1947, p. 431.

dried and evaporated to an oil which was dissolved in 10 ml. of hexane and applied to a column (15 × 1 cm.) of alumina (Merck). The octahydrodemethoxydesoxydesacetamidocolchicine was eluted with 20% benzene–hexane (200 ml.) after hexane (50 ml.) had removed only a trace of material. Slow sublimation at 40°/6 μ of the white solid, m.p. 49–50°, obtained by evaporation of the benzene–hexane eluant gave 250 mg. (60% yield) of material still melting at 49–50°; $[\alpha]^{25}D$ 0° (*c* 1.01, ethanol).

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.7; $-OCH_3$, 30.8; $C-CH_3$, 0. Found: C, 75.4; H, 8.7; $-OCH_3$, 30.9; $C-CH_3$, <0.5.

Octahydrodemethoxydesoxydesacetamidocolchicine Epoxide.—Perbenzoic acid oxidation of octahydrodemethoxydesoxydesacetamidocolchicine (VI) was carried out as described above for hexahydrodemethoxydesoxycolchicine and resulted in the consumption of one mole of perbenzoic acid per mole of compound. An oxidation reaction which consumed 82 mole % of perbenzoic acid and then became very much slower was treated as above in order to isolate epoxide. The chloroform residue was applied to the alumina column in hexane and this was followed by 10% benzene–hexane, 20% benzene–hexane and benzene. Evaporation of the benzene gave crystalline epoxide in 42% yield and this was recrystallized from hexane; m.p. 116–117°.

Anal. Calcd. for $C_{19}H_{26}O_4$: C, 71.7; H, 8.2. Found: C, 72.0; H, 8.2.

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[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE HEBREW UNIVERSITY]

Syntheses of Aspartyl Amides and Peptides through N-Benzyl-*dl*-aspartic Acid

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The α-amides and α- and β-peptides of *dl*-aspartic acid have been synthesized, *via* N-benzyl-*dl*-aspartic acid, from a mixed anhydride prepared by the reaction of phosgene with this amino acid. A new method is given for the determination of the linkage in aspartyl amides and peptides by the biuret reaction.

Since N-benzyl-*dl*-aspartic acid (I) is readily synthesized[1] it was used for the preparation of aspartyl amides and peptides, with the expectation that the N-benzyl group would act as a reversible masking group; ring closure would give the corresponding anhydride with which amines or peptide esters would react. Acetic anhydride could not be used, since it removed water intramolecularly and, even at room temperature, acetylated the secondary α-amino group; these results will be published separately. It was found that phosgene reacted with I in dioxane, yielding compound II which, in accordance with analyses and general behavior, is probably a mixed anhydride of I and chloroformic acid (*cf.* Wieland and Bernhard[2]). Since the action of ammonia or benzylamine upon II yielded N-benzyl-*dl*-α-asparagine (III) and N^{α},-N'^{α}-dibenzyl-*dl*-α-asparagine (IV), respectively, with the evolution of carbon dioxide and hydrogen chloride, the α-carboxyl only was involved in the mixed anhydride formation. III and IV were easily converted to *dl*-α-asparagine (V) and N'^{α}-benzyl-*dl*-asparagine (VI) by catalytic hydrogenolysis (Chart I).

α-Amides were formed when II, either in the original dioxane solution or as the isolated compound, reacted with ammonia or benzylamine.

When the coupling reaction with glycine ethyl ester was carried out in dioxane, N-benzyl-α-*dl*-aspartylglycine ethyl ester (VII) was the only product; with isolated II suspended in dry toluene, mainly N-benzyl-β-*dl*-aspartylglycine ethyl ester (VIII) was obtained with a very small amount of VII, from which it could be separated by fractional crystallization. Catalytic hydrogenolysis of VII and VIII gave the free peptide esters IX and X; hydrolysis and subsequent hydrogenolysis the free dipeptides XII and XIII. N-Benzyl-α-*dl*-aspartylglycine (XI) was produced as an intermediary, but the corresponding β-compound could not be isolated. Hydrolysis of VIII and acidification with hydrochloric acid failed to precipitate the N-benzyl-β-aspartylglycine; this was also true for N-benzyl-β-aspartylalanine. This difference may serve as an additional means of distinguishing N-benzyl-α- and β-aspartylpeptides and as a method for their quantitative separation.

II reacted with *dl*-alanine ethyl ester, in dioxane or toluene, to give only N-benzyl-β-*dl*-alanine ethyl ester (XIV), which on direct hydrogenolysis yielded the dipeptide ester (XV), and on hydrolysis followed by reduction β-*dl*-aspartyl-*dl*-alanine (XVI).

Since the melting points of the peptides in the literature generally refer to optically active compounds, the nature of the linkage in each case had to be determined by other means.

N-Benzyl-α-*dl*-asparagine (III) melted at 180°, N-benzyl-β-*dl*-asparagine at 216°.[1] On hydrogenolysis the former yielded *dl*-α-asparagine which differs from *dl*-β-asparagine by (a) its greater water solubility, (b) its purple color with ninhydrin on paper chromatograms, contrasting with the yellowish-brown color given by the β-isomer, and (c) its red biuret reaction, differing from the bluish tinge shown by β-asparagine.[3]

N^{α},N'^{α}-Dibenzyl-α-*dl*-asparagine (IV) melted at 173°, its β-isomer at 215°.[4] On hydrogenolysis, compound VI was obtained (m.p. 235°) which, unlike the β-isomer,[5] did not form a N-carboxy anhydride with phosgene. This shows clearly that the α-carboxyl is not available.

α-*dl*-Aspartylglycine monohydrate (XII) (m.p. 155°), β-*dl*-aspartylglycine monohydrate (XIII) (m.p. 156°), their derivatives (VII–XI), and the peptide obtained with alanine (XVI) (m.p. 232°) were identified as follows: (a) In agreement with

(1) Max Frankel, Y. Liwschitz and Y. Amiel, THIS JOURNAL, **75**, 330 (1953).

(2) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(3) E. Fischer, *Ber.*, **35**, 1095 (1902).

(4) F. H. McMillan and N. F. Albertson, THIS JOURNAL, **70**, 3778 (1948).

(5) Max Frankel, Y. Liwschitz and A. Zilkha, *ibid.*, **75**, 3270 (1953).