α-Carboxy-β-(1-methyl-5-imidazole)-acrylic Acid.—A solution of 5.5 g. (0.05 mole) of 1-methyl-5-imidazolealdehyde and 6.1 g. (0.05 mole) of malonic acid monohydrate in 25 ml. of water was heated on the steam-bath for one-half hour. The mixture was cooled and the white crystalline precipitate was collected and air dried. It weighed 9.4 g. (96% yield); m. p. 224–226° dec.

Anal. Calcd. for $C_8H_8N_2O_4$: C, 48.90; H, 4.06; N, 14.28; neut. equiv., 98.09. Found: C, 49.02; H, 4.28; N, 14.14; neut. equiv., 97.95.

The compound failed to take up any hydrogen when the sodium salt in aqueous solution was shaken with Adams platinum catalyst under 60 lb. hydrogen pressure.

Benzoyl-(1-methyl-5-imidazoyl)-methane.—To a solution of 28 g. (0.20 mole) of methyl 1-methyl-5-imidazolecarboxylate in 200 ml. of dry toluene was added 17.2 g (0.30 mole) of sodium methylate followed by 36 g. (0.30 mole) of acetophenone. The thick mixture was heated on the steam-bath for two hours and then allowed to stand overnight at room temperature. The mixture was shaken with 500 ml. of cold water, and the aqueous layer was separated and acidified with 18 ml. of glacial acetic acid. This mixture was extracted with ether, and the ether solution was dried and evaporated leaving a pale yellow crystalline solid which was washed with petroleum ether and air-dried; yield, 35.5 g. (78%). A sample recrystallized from ethyl acetate–petroleum ether melted at 116.5–117.5°.

Anal. Calcd. for $C_{13}H_{12}N_2O_2$: N, 12.28. Found: N, 12.14.

Summary

A number of 1-substituted-5-imidazolecarboxylic acid esters have been converted to the aldehydes by the McFadyen–Stevens method.

1-Substituted-5-hydroxymethylimidazoles have been synthesized by catalytic hydrogenation of the corresponding aldehydes, and also by reduction of the corresponding esters with lithium aluminum hydride.

Five 1-substituted analogs of histidine have been synthesized and characterized.

1-Methyl-5-(β-aminoethyl)-imidazole dihydrochloride has been prepared.

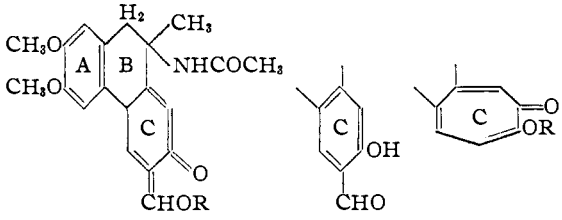
INDIANAPOLIS 6, INDIANA RECEIVED JANUARY 6, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ROCHESTER]

Studies in the Structure of Colchicine. The Structure of Ring C[1,2]

BY H. R. V. ARNSTEIN,[3] D. S. TARBELL,[4] G. P. SCOTT AND H. T. HUANG

The structure suggested for ring C of colchicine (I) by Windaus[5] has not been completely satisfactory in accounting for the unusual properties of colchicine and its hydrolysis product, colchiceine (II). Neither colchicine nor colchiceine give any



I, R = CH_3
II, R = H

III

IV, R = CH_3
V, R = H

carbonyl derivatives; furthermore, the structure II makes colchiceine a tautomeric form of an ortho-hydroxyaldehyde (III). The latter would be expected to be the stable form, rather than II, but the properties of colchiceine differ significantly from those of an ortho-hydroxyaldehyde. Dewar[6] proposed that colchicine and colchiceine had structures IV and V, respectively, and pointed out that V would be chelated, which would allow resonance stabilization between two nearly equivalent bond structures. The present paper records results which give strong support to the Dewar structure for ring C.[7]

Catalytic reduction of ring C in colchiceine (assuming that no rearrangement occurred) would lead to a 1,2-diol (VI) on the basis of structure V, whereas on the Windaus formulation (II), a 1,3-diol (VII) would be obtained. The diol, hexahydrocolchiceine, actually obtained by catalytic reduction of colchiceine with Raney nickel has been found to be a 1,2-diol, because it reacted with exactly one mole of periodate to form an aldehydic product. The same behavior toward periodate was shown by several derivatives of hexahydrocolchiceine.[8]

Reduction of pure colchiceine with hydrogen and Raney nickel in methanol at room temperature and atmospheric pressure resulted in an uptake of three moles of hydrogen, with the formation of hexahydrocolchiceine (VI) which could be

(1) This work was aided by a grant from the National Institute of Health.

(2) For the previous paper in this series, see Huang, Tarbell and Arnstein, THIS JOURNAL, **70**, 3183 (1948). Part of the material in the present paper has been reported in preliminary form (Arnstein, Tarbell, Huang and Scott, *ibid.*, **70**, 1669 (1948)).

(3) Present address: National Institute for Medical Research, London, England.

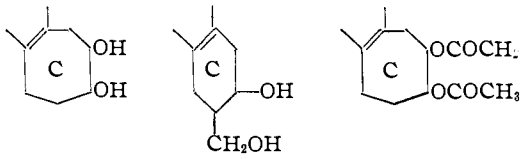
(4) Fellow of the Guggenheim Foundation, 1946-1947.

(5) Windaus, *Ann.*, **439**, 59 (1924).

(6) Dewar, *Nature*, **155**, 141, 479 (1945). The suggestion of Lettre, *Angew. Chem.*, **59**, 223 (1947), that ring C has a pyrone structure seems to be incompatible with the results of the present paper. The interesting observations of Santavy, *Helv. Chim. Acta*, **31**, 821 (1948), on the formation of colchicic acid from colchicine and sodium methoxide are more readily explained on the basis of structure IV than I.

(7) The presence of the same seven-numbered ring as in IV and V, has recently been demonstrated conclusively in purpurogallin ((a) Haworth, Moore and Pauson, *J. Chem. Soc.*, 1045 (1948); Barltrop and Nicholson, *ibid.*, 116 (1948)), and in γ-thujaplicin ((b) Erdtmann and Gripenburg, *Nature*, **161**, 719 (1948)).

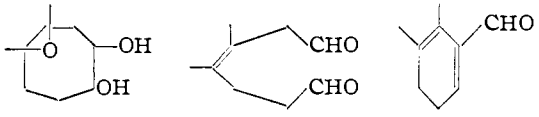
(8) No evidence is so far available as to the position of the oxygen functions in ring C with respect to ring B, and hence structures such as IV, V and VI are uncertain in this respect; work is in progress on this point.



VI VII VIII

readily isolated from the crude product, although the yield of purified material was rather low. The reaction product was evidently a mixture, because there was also isolated a small amount of material which was apparently a stereoisomeric hexahydrocolchiceine.[9] Raney nickel was found to be more satisfactory for the reduction than Adams catalyst, which had been used previously.[10]

The hexahydrocolchiceine (VI) was characterized by the preparation of the diacetate (reported by Bursian[10]) and of a bromo derivative. The presence of a double bond resistant to catalytic reduction, shown by Bursian by titration with perbenzoic acid,[10] was confirmed by titration with monoperphthalic acid, and further by isolation of the corresponding epoxide IX.[11] This compound was very unstable compared to the epoxide from hexahydrocolchicine, and could not be recrystallized satisfactorily, but the analytical data, and



IX X XI

the similarity of the ultraviolet absorption spectrum to that of hexahydrocolchicine epoxide[11] supported the epoxide structure. Further confirmation of the presence of the double bond in hexahydrocolchiceine was obtained by the preparation of the epoxide (which could be readily recrystallized) from hexahydrocolchiceine diacetate (VIII). The double bond in VI was assumed to be between rings B and C because of its failure to be reduced catalytically; steric factors might be expected to prevent reduction of a bond in this position by interfering with the necessary adsorption of the molecule on the catalyst.[12]

When hexahydrocolchiceine was treated with periodic acid in 50% aqueous methanol at low *p*H, there was an initial rapid consumption of periodic acid, followed by a slow uptake over a period of forty hours, accompanied by a marked darkening of the solution. The final consumption was about 1.4 moles of periodic acid. The chloroform extract of the solution gave, after removal of the solvent, a strongly positive Schiff reaction. It seemed likely that the low *p*H of the oxidation solution might have caused side reactions, such as hydrolysis of the N-acetyl group, which accounted for the slow periodate consumption. The oxidation was therefore carried out with sodium metaperiodate at a *p*H of 4–5. These runs showed a consumption of almost exactly one mole of periodate within ten minutes, and no further consumption after standing many hours. The optical rotation of the solutions showed a parallel rapid change, followed by a constant value. A suitably prepared blank showed no periodate consumption by the solvent.

The product obtained by extracting the solution with chloroform was a pale yellow mobile sirup, which gave positive Schiff and Tollens reactions and reduced Fehling solution. Attempts to prepare a semicarbazone or a dimedone derivative were unsuccessful, but 2,4-dinitrophenylhydrazine yielded an amorphous derivative, which, after purification by chromatography, had the composition of the 2,4-dinitrophenylhydrazone of the aldehyde XI, or an isomer thereof, which would be formed by aldol condensation from the primary dialdehyde X. The dinitrophenylhydrazone may have been derived from the benzenoid dehydrogenation product from XI.

Since the lack of success in isolating a derivative of the dialdehyde X might have been due to the activating effect of the double bond on a methylene group, the epoxide of hexahydrocolchiceine (IX) was used as a starting material in the oxidation. In this case, also, almost exactly one mole of periodate was consumed, and the reaction product was oxidized with peracetic acid with the hope of getting the epoxy dibasic acid. The product was very heterogeneous, however; it could be separated into neutral, acidic and lactonic fractions, but no pure compounds could be isolated. The lactonic material was probably caused by action of a carboxyl group on the epoxide ring.

The fact that the periodate uptake of hexahydrocolchiceine (VI) and its epoxide (IX) was actually due to the 1,2-glycol grouping and not to attack on some other portion of the molecule, was demonstrated by periodate treatment of hexahydrocolchicine and its epoxide.[10] These compounds showed no appreciable periodate consumption after many hours, under the same conditions which gave an immediate consumption of one mole with VI and IX.

From the mother liquors from the preparation of hexahydrocolchiceine (VI) was obtained by acetylation a small amount of crystalline material, which was apparently the monoacetate of a stereoisomer of VI. Hydrolysis of this acetate gave a compound melting at about the same point as VI, but the mixed melting point was strongly depressed; the analysis corresponded to a stereoisomer of VI, and a periodate run, which was carried out on the few milligrams of material available, showed a consumption of 0.7 mole of periodate.

(9) γ-Thujaplicin, which contains the seven-membered ring as in V, gives on catalytic reduction a crystalline diol and an oil; the latter is believed to be a stereoisomer (or mixture of stereoisomers) of the solid.[7b]

(10) Bursian, *Ber.*, **71**, 245 (1938).

(11) *Cf.* Fernholz, *Angew. Chem.*, **60**, 62 (1948), for the action of perbenzoic acid on colchicine and some of its derivatives, which gives extensive oxidation with ring cleavage under some conditions.

(12) *Cf.* Waldeland, Zartman and Adkins, THIS JOURNAL, **55**, 4234 (1933); Linstead, *et al.*, *ibid.*, **64**, 1985 (1942).

Although colchicine and colchiceine do not form any carbonyl derivatives, as mentioned above, partially hydrogenated colchicine and colchiceine do show carbonyl properties. Thus, amorphous "tetrahydrocolchicine," prepared by reduction with palladium-charcoal in methanol, gave a yellow precipitate (which could not be obtained crystalline) when treated with 2,4-dinitrophenylhydrazine. A similar amorphous tetrahydro product from colchiceine also gave a yellow precipitate with 2,4-dinitrophenylhydrazine, which, after purification by chromatography, yielded a small amount of crystalline product with a fairly satisfactory analysis for tetrahydrocolchiceine 2,4-dinitrophenylhydrazone.

Acknowledgment.—We are indebted to Dr. G. E. Ullyot, of Smith, Kline and French Laboratories, for his kindness in furnishing some samples of reduced colchicine derivatives and for interesting discussions of the colchicine problem.

Experimental[13]

Preparation of Anhydrous Colchiceine.—Colchicine (7.1 g.), purified by chromatography on alumina,[14] was dissolved in 425 cc. of 0.25% hydrochloric acid, and heated at 100° for two hours, when crystals began to separate. The solution was cooled, and allowed to stand in the ice-box for several hours. The product, colchiceine hydrate, was collected and dried *in vacuo;* weight, 4.1 g. Anhydrous colchiceine was best obtained by dissolving the hydrate in hot anhydrous ethyl acetate, adding an equal volume of dry ether and allowing the solution to cool slowly. Anhydrous colchiceine melted at 175–175.5°, and was light sensitive and slightly hygroscopic.[15]

Anal. Calcd. for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.65; H, 6.06; N, 3.45.

Colchiceine Benzoate.—Benzoyl chloride (1 cc.) was added to a solution of colchiceine (0.75 g.) in 10 cc. of dry pyridine. The mixture stood at room temperature for fifteen hours, was heated for one hour at 100°, and was poured into water; the oily layer was extracted with ethyl acetate. The extract was washed with dilute sodium bicarbonate solution, with dilute hydrochloric acid and with water, then dried, and the solvent removed under reduced pressure. The gum so obtained was triturated with dry ether, and the ether solution decanted; after standing overnight, colchiceine benzoate separated as clusters of laths, m. p. 205–207°. After two crystallizations from ethyl acetate–ether and benzene–ether, the product (285 mg.) had the m. p. 206–207°.

Anal. Calcd. for $C_{28}H_{27}O_7N$: C, 68.70; H, 5.56; N, 2.86. Found: C, 68.87; H, 5.90; N, 2.78.

Reduction of Colchiceine with Raney Nickel. Hexahydrocolchiceine (VI).—Colchiceine (2.12 g.) in 25 cc. of dry methanol was hydrogenated at room temperature (19.5°) and atmospheric pressure (745 mm.) using Raney nickel[16] as catalyst. After twenty-four hours, the uptake of hydrogen amounted to 413 cc. (calcd. for 3 moles, 405 cc.) and further uptake was extremely slow. The solution was filtered through a sintered glass funnel and evaporated to dryness under reduced pressure, giving 1.9 g. of crude product. Crystallization from anhydrous methanol by the addition of an equal volume of ether, afforded crude hexahydrocolchiceine (VI) (0.5 g., m. p. 195.5–197°). Several recrystallizations from the same solvents yielded an analytical sample, m. p. 205.5–206°. Bursian[10] reported a m. p. of 202–203°, and Windaus[5] gave 198–200° for a so-called octahydrocolchiceine, obtained by reduction of colchiceine in acetic acid with hydrogen and platinum.

Anal. Calcd. for $C_{21}H_{29}O_6N$: C, 64.43; H, 7.47; N, 3.58. Found: C, 63.68, 63.86; H, 7.39, 7.33; N, 3.65.

Hexahydrocolchiceine did not form an acetone compound when treated with acetone and hydrochloric acid, zinc chloride or anhydrous copper sulfate, for prolonged periods of time.

The **diacetate** (VIII) of hexahydrocolchiceine was prepared by acetylation with acetic anhydride in pyridine, as described by Bursian,[10] and melted at 167–167.5° (reported, 166°).

Anal. Calcd. for $C_{25}H_{33}O_8N$: C, 63.14; H, 7.00; N, 2.95; acetyl, 27.15. Found: C, 63.20, 63.01; H, 7.09, 6.83; N, 3.37; acetyl, 28.54.

The same diacetate was obtained by acetylation with acetic anhydride–sodium acetate at 100°. This procedure was used by Windaus[5] to prepare "octahydrocolchiceine monoacetate," m. p. 160–161°, from "octahydrocolchiceine," and it appears probable that his compound was the hexahydrocolchiceine diacetate.

Bromohexahydrocolchiceine.—To a solution of hexahydrocolchiceine (214.4 mg., m. p. 204–205°) in 10 cc. of chloroform, a solution of 0.1 cc. of bromine in 10 cc. of chloroform was added dropwise with shaking. After 2.9 cc. had been added, the solution was permanently colored, and was allowed to stand for one hour. The solvent was removed under reduced pressure, and the gummy residue shaken with ethanol, which caused the separation of 160 mg. of crystals, m. p. 262–270°, with decomposition. The product was recrystallized from hot acetic acid by the addition of an equal volume of water; cooling yielded rectangular prisms, m. p. 275–283°, with decomposition from 260°. Recrystallization from acetic acid–ethanol afforded an analytically pure sample of rectangular prisms, m. p. 281–283° (marked decomposition at 275°).

Anal. Calcd. for $C_{21}H_{28}O_6NBr$: C, 53.62; H, 6.00; N, 2.98; Br, 16.99. Found: C, 53.60; H, 6.06; N, 3.02; Br, 17.00.

The **diacetate** was prepared from 35 mg. of the compound in 3 cc. of dry pyridine with 0.8 cc. of acetic anhydride. After three days at room temperature, the solution was concentrated under reduced pressure, and water was added. The crystalline product (m. p. 223–225°) was recrystallized from ethyl acetate–ligroin (b. p. 100°), and formed clusters of needles, m. p. 225–226°. One further crystallization from the same solvents afforded an analytically pure sample, m. p. 226–227°.

Anal. Calcd. for $C_{25}H_{32}O_8NBr$: C, 54.15; H, 5.82; Br, 14.41. Found: C, 54.38; H, 5.75; Br, 14.57.

Periodate Titration of Hexahydrocolchiceine.—Hexahydrocolchiceine (241 mg., m. p. 204–205°) in 50% aqueous methanol was treated at 0° with 180 mg. of periodic acid (by analysis) in an aqueous solution which had been brought to a *p*H of 5 by the addition of sodium bicarbonate; the total volume was 25 cc. Titrations of aliquots for the periodic acid content, using sodium arsenite and iodine–potassium iodide solutions, showed, after one hundred fifty minutes, a periodate consumption of 0.971 and 0.968 mole of periodate per mole of substrate, in duplicate runs.

Isolation of the 2,4-Dinitrophenylhydrazone.—In a similar experiment, hexahydrocolchiceine (395.3 mg., m. p. 201–202°) in 25 cc. of 50% aqueous methanol was oxidized with periodate solution at *p*H 5. The uptake of periodate proceeded as follows (time in minutes in parentheses): 0.856 mole (10); 0.92 mole (95); 0.925 mole

(13) Melting points are uncorrected; analyses by Microtech Laboratories and Mrs. G. Sauvage. Many of the compounds described held water very tenaciously, and low carbon values were obtained unless the sample was dried *in vacuo* at 140° directly before analysis.

(14) Ashley and Harris, *J. Chem. Soc.*, 677 (1944).

(15) Meyer and Reichstein, *Pharm. Acta Helv.*, **19**, 27 (1944), reported colchiceine to melt at 178–179.5°.

(16) Prepared according to Mozingo, *Org. Syn.*, **21**, 15 (1941).

(1070). A control solution of 50% aqueous methanol gave no periodate uptake.

After seventeen hours, the reaction mixture was diluted to 32 cc. with water and extracted with three 15-cc. portions of chloroform. Removal of the solvent from the chloroform extract yielded about 300 mg. of a mobile yellow sirup, which gave a strong Schiff reaction and a precipitate with 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The control solution, when treated in the same way, gave a negative Schiff test and no precipitate with 2,4-dinitrophenylhydrazine.

The dinitrophenylhydrazone of the oxidation product was prepared by adding 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid to an aqueous alcoholic solution of the above sirup. The orange-red precipitate was collected after standing overnight in the ice box, washed with water and dried, first on porous plate, then *in vacuo* over phosphorus pentoxide (yield, 280 mg.). The product was chromatographed on alumina, using ethyl acetate and benzene as eluants. The early fractions, containing nearly all of the product (unchanged 2,4-dinitrophenylhydrazine was left at the top of the column) were combined and evaporated under reduced pressure. The residue was dissolved in benzene; addition of ligroin yielded a gum, and, after standing a little longer, a solid, which was collected. Reprecipitation of the solid from ether solution by adding ligroin gave an amorphous product, m. p. 105° with decomposition (darkening at 96°), and repetition of this process gave a sample, m. p. 103–107° (darkening and decomposition from 93°).

Anal. Calcd. for $C_{27}H_{29}O_8N_5$ (DNP of XI): C, 58.79; H, 5.30; N, 12.70. Calcd. for $C_{27}H_{27}O_8N_5$ (DNP of the benzenoid compound corresponding to XI): C, 59.01; H, 4.95; N, 12.75. Found: C, 59.65; H, 5.39; N, 12.84.

An attempt to isolate a derivative of the dialdehyde X following a procedure developed for a similar reactive dialdehyde[17] yielded a small amount of crystalline DNP, which was very unstable, and decomposed when recrystallized or merely on standing. Attempted oxidation of the dialdehyde to the corresponding diacid with permanganate in acetone or with bromine water and strontium carbonate, was unsuccessful.

Hexahydrocolchicine Epoxide LX.—A solution of monoperphthalic acid[18] in chloroform (6 cc., containing 42.54 mg. per cc.) was added to 482.8 mg. of hexahydrocolchiceine in chloroform; the total volume was adjusted to 25 cc., and the solution kept at 0°. After twenty-four hours, the amount of peracid consumed, as determined by iodide-thiosulfate titration, corresponded to 216.2 mg. or 0.965 mole. After a further five hours, the solution was washed with aqueous sodium bicarbonate, then with water, dried and evaporated under reduced pressure. A little ethyl acetate was added to the resulting gum, and after heating on the steam-bath, the product crystallized. The crystals were collected, dried and analyzed without recrystallization (m. p. 185–186°). Recrystallization from methanol–ether or chloroform–ethyl acetate–ligroin resulted in decomposition and lowering of the melting point.

Anal. Calcd. for $C_{21}H_{29}O_7N$: C, 61.90; H, 7.18. Found: C, 62.23; H, 7.05.

The epoxide (145 mg.) was oxidized in aqueous solution with sodium metaperiodate at 0°; after twenty minutes the uptake amounted to 0.905 mole of periodic acid.

Epoxide from Hexahydrocolchiceine Diacetate.—A slight excess of monoperphthalic acid in chloroform was added to 263.3 mg. of the diacetate VIII at 0° in chloroform. After twenty-four hours, the peracid consumption was 1.15 moles. The solution was extracted with aqueous sodium bicarbonate and water; after drying, the chloroform was evaporated and the residue was recrystallized from ethyl acetate. Four recrystallizations from ethyl acetate–heptane afforded the pure epoxide, m. p. 202.5–203.5°.

Anal. Calcd. for $C_{25}H_{33}O_9N$: C, 61.08; H, 6.77; N, 2.85. Found: C, 61.10; H, 6.70; N, 2.78.

Hexahydrocolchicine epoxide for the absorption curve measurements and periodate blank runs was prepared by oxidation of hexahydrocolchicine with monoperphthalic acid in chloroform,[10] and purified by recrystallization from methanol–ether; m. p. 203–204° (reported,[10] 204°).

Isolation of a Stereoisomer of Hexahydrocolchiceine.—The crude product (4.25 g.) from a Raney nickel reduction of colchiceine was crystallized once from methanol–ether, giving 2.2 g. of hydrated hexahydrocolchiceine (VI). The mother liquors from this crystallization were chromatographed on alumina, and eluted with alcohol, giving two fractions (2.1 g.). Fraction 1 was acetylated with 5 cc. of acetic anhydride in 20 cc. of pyridine at room temperature for three days. Evaporation under reduced pressure yielded 1.2 g. of crude crystalline product, m. p. 206–213°, which was chromatographed on alumina, from solution in 25 cc. of benzene containing a few drops of ethanol. Elution with 50 cc. of benzene yielded the main fraction; further elution with benzene, benzene–alcohol (9:1, 160 cc.), alcohol (100 cc.) and chloroform (250 cc.) yielded no significant amount of material.

The gum obtained by evaporation of the first benzene eluate was rubbed with a little ethyl acetate, and 0.56 g. of crystals, m. p. 200–201° (softening from 190°), was obtained. The mother liquors were evaporated, yielding 0.65 g. of solid, m. p. 210–220° (softening from 200°). Two recrystallizations of this material from ethyl acetate–ligroin (b. p. 90–100°) and ethyl acetate–ether yielded a product, m. p. 241–242°. Two further recrystallizations from ethyl acetate–heptane did not change the m. p. appreciably.[19]

Anal. Calcd. for $C_{25}H_{33}O_8N$ (hexahydrocolchiceine diacetate): C, 63.14; H, 7.00; N, 2.95; acetyl (three groups per molecule), 27.18. Calcd. for $C_{23}H_{31}O_7N$ (the monoacetate): C, 63.72; H, 7.21; N, 3.23; acetyl (two groups per molecule), 18.10. Found: C, 62.48, 62.96, 62.94; H, 7.02, 7.12, 7.17; N, 3.00; acetyl, 17.18.

The acetyl value indicates a monoacetate; it is shown above that hexahydrocolchiceine diacetate (VIII) gives the acetyl value calculated for three groups (two on oxygen and one on nitrogen).

Hydrolysis of Monoacetate.—A sample of the above acetate, recovered from mother liquors (51.5 mg., m. p. 229–236°), was hydrolyzed in 50% aqueous methanol with two equivalents of 0.1 *N* sodium hydroxide; after one day at room temperature, the solution was evaporated in an air stream. A little water was added, and the crystalline residue collected (25 mg., m. p. 188–189°). Crystallization from ethyl acetate gave clusters of needles (21 mg., m. p. 201–201.5°) which, after another recrystallization from ethyl acetate, weighed 14 mg. and melted at 201–202°. The m. p. of this material was depressed to 183° when it was mixed with hexahydrocolchiceine of m. p. 200°. Periodate titration on a few mg. of the analytical sample gave an uptake of 0.7 mole.

Anal. Calcd. for $C_{21}H_{29}O_6N$: C, 64.43; H, 7.47. Found: C, 64.36; H, 7.30.

The compound is therefore regarded as a stereoisomer of hexahydrocolchiceine.

Tetrahydrocolchiceine.—Colchiceine (8.0 g.) was reduced with palladium charcoal in methanol in a low pressure shaker; the uptake was 2.0 moles of hydrogen over a period of one hour. The catalyst and solvent were removed, leaving an amorphous material which could not be obtained crystalline.

To a solution of 1.6 g. of 2,4-dinitrophenylhydrazine in 500 cc. of water, 100 cc. of methanol, and 40 cc. of concentrated hydrochloric acid was added 361 mg. of the above amorphous colchiceine reduction product. After a few minutes, 219 mg. of yellow-orange amorphous powder was collected, and an additional 63 mg. was ob-

(17) Fischer and Dangschat, *Helv. Chim. Acta*, **18**, 1204 (1935).

(18) Böhme, *Org. Syn.*, **20**, 70 (1940).

(19) We are indebted to Dr. A. D. Kemp for some of the experiments on the purification of this compound.

tained from the mother liquors. The combined product was dissolved in dry chloroform and chromatographed on alumina. After washing two narrow brown bands down the column with additional chloroform, a third dark-red band was separated mechanically and eluted with methanol. Evaporation of the methanol *in vacuo* to a small volume yielded 17 mg. of yellow crystals, m. p. 228–230°. One recrystallization from a mixture of ethyl acetate and high-boiling petroleum ether yielded 11 mg. of fine yellow needles, m. p. 230–231°.

Anal. Calcd. for $C_{27}H_{30}O_9N_5$ (DNP of tetrahydrocolchiceine): C, 57.03; H, 5.32; N, 12.32. Found: C, 56.42; H, 5.36; N, 11.78.

Summary

Periodate oxidation studies on hexahydrocolchiceine and several of its derivatives indicate that it has a 1,2-diol structure. This furnishes strong support for the Dewar formula for ring C, and is incompatible with the Windaus structure.

ROCHESTER, N. Y. RECEIVED DECEMBER 30, 1948

[CONTRIBUTION FROM THE DIVISION OF CHEMICAL DEVELOPMENT, E. R. SQUIBB & SONS]

Separation of the Streptomycins[1]

BY ANDREW E. O'KEEFFE, MORRIS A. DOLLIVER AND ERIC T. STILLER

Early work in the streptomycin field was hampered by the limitations and vagaries inherent in biological assay methods. Gradually, as more highly purified material became available, many workers turned their attention to the development of chemical assay methods to supplement the basic bioassay. Of the several methods proposed, probably the most popular is that outlined by Schenck and Spielman,[2] involving the alkaline degradation of streptomycin to yield maltol, which could be readily determined either through its characteristic absorption spectrum or by the formation of colored complexes, as with iron.

During the standardization of the maltol method as adopted in these laboratories, it was noted that, as between solid samples of varying degrees of purity, there was an apparent deviation from Beer's law. That this deviation was apparent rather than real was shown by the fact that it was not reproduced when a given solid sample was progressively diluted. These two facts led to the conclusion[3] that a maltol-producing substance other than streptomycin was present in varying degrees in the different samples.

Pursuit of the above hypothesis by Fried and Titus[4,5,6,7] led to the isolation, characterization and identification of "Streptomycin B," later[8] called mannosidostreptomycin.[9]

While Fried and Titus,[4,7] as well as Plaut and McCormack,[10] were able to perform analytical separations of streptomycin and mannosidostreptomycin by the application of the Craig[11,11a] technique of countercurrent extraction, these separations were not directly applicable to preparative work, both because of scale limitations inherent in the method and because their interests stopped short of the recovery of the antibiotics in usable form.

Concurrently and in collaboration with the work mentioned above, the present authors were engaged in parallel exploitation of the basic finding[12] that streptomycin, normally a strongly hydrophilic substance, could be rendered preferentially soluble in organic solvents by the introduction of a "carrier." A "carrier" is defined as an organic compound which is: (1) capable of reacting reversibly with the functional groups of the compound being treated (in the case of streptomycin, such a compound would be an organic acid, which can react with the guanidine groups of the antibiotic); and (2) of sufficient chain length to render its adduct with the compound being treated selectively soluble in organic rather than aqueous media. It will be noted that these criteria approach very closely the definition of a detergent; generally, it was found that any of the common anionic detergents can be used as carriers for streptomycin. Among some of those tested were: (a) fatty acids of varying chain lengths from C_5 to C_{18}; (b) alkyl sulfonic acids (*e. g.*, dodecyl); (c) alkyl sulfuric acids (*e. g.*, *n*-dodecyl, 2-ethylhexyl, 7-ethyl-2-methyl-undecyl-(4), and 3,9-diethyltridecyl-(6)); and (d) aryl or aralkyl sulfonic acids (*e. g.*, sulfonated cumene).

At the time when our attention became centered upon the problem of separating streptomycin from mannosidostreptomycin we used "Pentasol" (mixed synthetic amyl alcohols; Sharples Chemical Co.) as our solvent phase. This choice was dictated by several factors including availability, cost, minimal miscibility with water, minimal emulsification, etc. As our carrier we used a commercial grade of lauric acid, of a purity of about 85%, the remainder being largely myristic acid.

(1) Presented at the First Meeting in Miniature, North Jersey Section, American Chemical Society, January 10, 1949.

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(12) Lott, Braker and O'Keeffe, and Lott, Braker and Heuser, U. S. Patent Applications.