TABLE III

ADSORPTION OF SOME SEVEN-RING KETONES IN THE CARBONYL REGION (1750–1600 CM.$^{-1}$)[a]

Compound	Band minima, cm.$^{-1}$
Cycloheptanone	1699
2-Chlorocycloheptanone	1715
2,6,6-Trimethylcycloheptadiene-2,4-one (X)	1661
	1603
2,3-Benzocycloheptanone	1683
	1602
4,5-Benzocycloheptadiene-	1641
2,7-one (IX)[b]	1603

[a] Measurements made on the pure liquid except as indicated. [b] Measured in carbon tetrachloride.

TABLE IV

γ-THUJAPLICIN AND COLCHICEINE IN THE 2800–3800 CM.$^{-1}$ REGION (BAND MINIMA (CM.$^{-1}$))

γ-Thujaplicin in carbon tetrachloride[a]	Colchiceine in chloroform[b]	Colchiceine in benzene[c]
2958	3010	2990
3196	3265	3265

[a] Concentration, 3.9 and 1.2%. [b] Concentration, 6.5%. [c] Saturated (1%).

It would appear, therefore, that the lowered frequency of the carbonyl band in the tropolone compounds is due to conjugation rather than to the presence of a chelate ring like XI. It is true that the curves of colchiceine and γ-thujaplicin in the higher frequency region (Fig. 2A and B, and Table IV) show no bands above 3500 cm.$^{-1}$ (below 2.86μ) as would be expected of a completely unbonded O–H. However, the β-diketones mentioned above, gave bands only at much lower wave numbers (2703 cm.$^{-1}$) than the bands found in colchiceine and γ-thujaplicin at 3196 and 3265 cm.$^{-1}$, respectively.

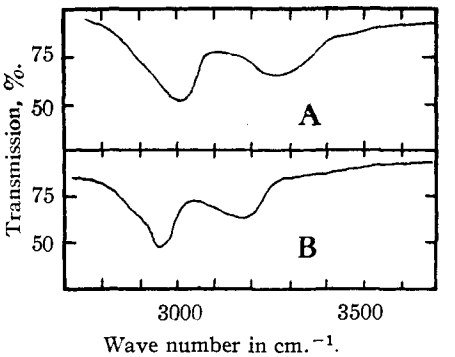


Fig. 2.—A, Colchiceine in chloroform and B, γ-thujaplicin in carbon tetrachloride in the 3μ region.

The bands at 1547–1552 cm.$^{-1}$ (6.89μ) in the tropolone compounds may be analogous to bands at about 1570 cm.$^{-1}$ (6.37μ) found by Gunthard and Plattner[18] in azulenes. The bands in the region 1250 to 1300 cm.$^{-1}$ (7.69 to 8.0μ) may arise from the enol C–O bond, the frequency being raised still higher than is the case in phenols (1200 to 1250 cm.$^{-1}$)[12a] by conjugation.

Summary

Infrared absorption spectra of colchicine and five of its derivatives have been investigated together with those of γ-thujaplicin and β-methyltropolone. Some characteristic bands have been found, and it is considered that the results are in agreement with the Dewar formula for ring C of colchicine. The carbonyl bands for several seven-ring ketones have also been measured.

(18) Gunthard and Plattner, *Helv. Chim. Acta*, **32**, 284 (1949).

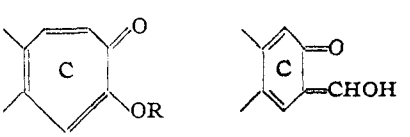
ROCHESTER, NEW YORK RECEIVED APRIL 27, 1949

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

Studies on the Structure of Colchicine. Reduction Products from Ring C[1]

BY ALEXANDER D. KEMP AND D. STANLEY TARBELL*

Evidence has been presented in preceding papers[2] which supports the Dewar "tropolone" structure (I)[3] for ring C of colchicine, as against the Windaus formula (III).[4] It was shown by periodate oxidation studies that hexahydrocolchiceine is a 1,2-glycol[2a]; furthermore, infrared studies on colchicine derivatives showed marked similarities to the spectra of compounds known to contain the tropolone ring.[2a,5] However, direct degradative studies on ring C to establish the position of the oxygen functions relative to ring B, and to provide additional evidence of the presence of the seven-membered ring, were obviously desirable.



I, R = CH_3
II, R = H

III

Some catalytic reduction products from ring C described by Bursian[6] appeared to be suitable starting materials for such degradative studies.

* Harvard University Ph.D. 1937.

(1) Aided by a grant from the National Institute of Health.

(2) (a) Arnstein, Tarbell, Scott and Huang, THIS JOURNAL, **71**, 244 (1949); (b) Scott and Tarbell, *ibid.*, **72**, 240 (1950).

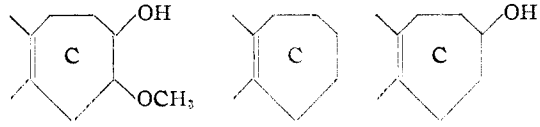
(3) Dewar, *Nature*, **155**, 141, 479 (1945).

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

(5) (a) Erdtman and co-workers, *Acta Chem. Scandinavia*, **2**, 625, 639, 644 (1948); (b) Haworth, Moore and Pauson, *J. Chem. Soc.*, 1045 (1948).

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

By reduction with hydrogen and Adams catalyst in glacial acetic acid, he obtained a hexahydrocolchicine, m. p. *ca.* 125° (IV)[7] and a compound



IV, m. p. 125° V, m. p. 171° VI VII

$C_{21}H_{29}NO_4$ (VI)[7] from which the oxygen in ring C had apparently been completely removed. Reduction with Adams catalyst in methanol, on the other hand, gave a compound $C_{21}H_{29}NO_5$, which had apparently lost one methoxyl group from ring C.[7]

These compounds, particularly the latter, offer various opportunities for degradations, which should furnish the desired information about ring C.

In the present work, we have found that the reduction of colchicine with Adams catalyst is a more complicated process than indicated[6]; the reaction is apparently very sensitive to changes in conditions, and the products are difficult to separate and purify. For these reasons, we have not yet been able to utilize the reduction products for the degradations planned originally.

Reduction of colchicine in glacial acetic acid with hydrogen and platinum yielded the 125° hexahydro compound IV reported by Bursian; because this compound has a rather indefinite m. p., it was characterized by preparation of the known bromo derivative.[6] In addition to IV, this reaction also formed the compound $C_{21}H_{29}NO_5$ which was proved to be the desmethoxy compound VII by elementary analysis and a methoxyl determination. A trace of a new hexahydrocolchicine, m. p. 171° (V) was also obtained.

The latter compound was obtained more readily by the reduction of colchicine with platinum in methanol solution; this reaction yielded, after a laborious separation by crystallization and chromatography, a trace of the 125° hexahydro compound, 2% of the desmethoxy compound VII and 16% of the 171° hexahydro compound V. The remainder of the material was amorphous, from which nothing crystalline could be isolated. In spite of careful search, none of the $C_{21}H_{29}NO_4$ hydrogenolysis product VI could be isolated, and apparently it was not formed in appreciable amount in any of the reductions which we carried out.

Two runs, in which a different sample of catalyst was used, gave about 17% of the 125° hexahydro compound, and apparently no other crystalline material. It seemed possible that the 125° compound, which appeared to be solvated and in general was not a well-defined compound, might represent a dimorph or an impure form of the 171° compound. It was shown, however, that the two are different, and hence must be considered to be stereoisomers, whose existence is due to the formation of two new asymmetric carbons in the reduction of ring C. The 125 and 171° compounds were shown to be different by the fact that they yielded different *p*-nitrobenzoates and different bromo derivatives.

It seemed possible that the purification of the two stereoisomers might be facilitated by conversion to the *p*-phenylazobenzoates, which could be separated by chromatography. The 125° compound was converted to the phenylazobenzoate, but the yield was small, and the purification difficult.

Oxidation experiments using the small amounts of material available, on the 171° compound with permanganate in acetone and on the desmethoxy compound with chromic anhydride in acetic acid,[8] gave no appreciable amounts of useful degradation products.

Experimental[9]

Hydrogenation of Colchicine in Methanol with Adams Catalyst.—Colchicine (3 g., purified by chromatography)[10] in 100 cc. of pure methanol was hydrogenated, using 1 g. of Adams catalyst, at room temperature and pressure. Three moles of hydrogen was absorbed rapidly, and the reaction had stopped after the uptake of 3.4 moles. Removal of the catalyst by filtration and evaporation of the filtrate under reduced pressure gave a frothy gum, from which was obtained by Bursian's procedure[6] 518 mg. of the 125° hexahydrocholchicine IV, m. p. 114–120°. One recrystallization from ethyl acetate raised the m. p. to 121–123°, with previous sintering. No other crystalline material could be isolated by this procedure.

171° Hexahydrocolchicine (V) and Desmethoxyhexahydrocolchicine (VII).—The crude reduced colchicine, obtained by hydrogenation as above, was boiled with 50 cc. of ethyl acetate for ten minutes, which dissolved all of the material, with the exception of about 300 mg. of white solid. The latter was collected, and it commenced to char in the range 200–300°. It was insoluble in most organic solvents, but was soluble in ethanol, methanol and hot water. Its character is not obvious, but it crystallized from hot water to give slender prisms of 125° hexahydrocolchicine, m. p. 122–124°.

The ethyl acetate filtrate, mentioned above, was evaporated under reduced pressure to a volume of about 15 cc., and, after standing at 0° for several days, it deposited a hard crystalline mass (600–900 mg.) which consisted of a mixture of 171° hexahydrocolchicine and desmethoxyhexahydrocolchicine in proportions that varied from run to run. In one run, the filtrate from this crystalline material contained 70 mg. of the desmethoxy compound, but it usually contained much less than this of impure material.

The hard crystalline mass could be resolved into its components, with much loss, by fractional crystallization from ethyl acetate; in one run, 720 mg. of the mixture yielded, after six crystallizations, 27 mg. of pure desmethoxy compound and 41 mg. of pure 171° hexahydro compound.

The separation was achieved much more economically by chromatography. Thus, 1200 mg. of the crystalline mixture described above (obtained by reduction of 6 g. of colchicine) was dissolved in 3 cc. of solvent consisting of 60% chloroform and 40% benzene, and adsorbed on a

(7) Based on the Dewar structure, with the position of the oxygen functions assigned arbitrarily.

(8) *Cf.* Fieser and Szmuszkovicz, THIS JOURNAL, **70**, 3352 (1948).

(9) Melting points corrected; microanalyses by Micro-tech Laboratory and Clark Micro-analytical Laboratory.

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

column of Grade I alumina.[11] The column was eluted with the same solvent until most of the hexahydrocolchicine had been removed, and the solvent was then changed to pure chloroform, which eluted the desmethoxyhexahydrocolchicine. The progress of the elution was followed by observing the optical rotation of the eluent.[12]

One crystallization of the **hexahydrocolchicine** from ethyl acetate gave 985 mg. of the pure material as stout colorless prisms, m. p. 170–170.5°. The analytical sample was obtained by a further crystallization, followed by drying four hours at 110° in vacuum, m. p. 170.5–171°. It was soluble in chloroform, benzene, alcohol and warm water.

Anal. Calcd. for $C_{22}H_{31}NO_6$: C, 65.18; H, 7.65; N, 3.46; OCH_3, 30.61. Found: C, 64.90; H, 7.85; N, 3.47; OCH_3, 30.76; optical rotation, $[\alpha]^{24}D$ − 126° (*c* 1.217, chloroform); · −137.7° (*c* 0.3043, chloroform).

The desmethoxyhexahydrocolchicine after one crystallization from ethyl acetate was obtained as 16 mg. of rosettes of thin colorless plates, m. p. 130–140° after drying one hour at 24° in vacuum. Further drying in vacuum at 110° for several hours raised the m. p. to 168.5–171.5°; the mixed m. p. with the 171° hexahydro compound was 157–170°. The analytical sample, prepared by further crystallization from ethyl acetate, melted, after thorough drying, at 173°.

Anal. Calcd. for $C_{21}H_{29}NO_5$: C, 67.20; H, 7.73; N, 3.73; OCH_3, 24.80. Found: C, 67.10; H, 7.47; N, 4.03; OCH_3, 24.76.

When a different batch of Adams catalyst was used, the only product which could be isolated from the hydrogenation of 6 g. of colchicine was 1.55 g. of 125° hexahydrocolchicine, m. p. 115–122°, raised to 123–125° by recrystallization from ethyl acetate.

125° Hexahydrocolchicine Bromide.—Dry, crystalline hexahydrocolchicine (IV) (190 mg., m. p. 118–124°) was brominated in chloroform with 3 cc. of a solution of 0.1 cc. of bromine in 10 cc. of chloroform; after standing overnight the solution was washed twice with 5% alkali, then with water, and the solution dried and evaporated. The residue crystallized from ethyl acetate to give 61 mg. of colorless plates of the bromide, m. p. 218–219°. Two further crystallizations yielded an analytical sample, m. p. 220–222° (sintering from 216°). Bursian[6] reports a m. p. of 221°.

Anal. Calcd. for $C_{22}H_{30}BrNO_6$: C, 54.55; H, 6.20; Br, 16.53. Found: C, 54.74; H, 6.48; Br, 16.55.

125° Hexahydrocolchicine *p*-Phenylazobenzoate.—Hexahydrocolchicine (420 mg., m. p. 118–124°) was heated with 302 mg. of pure *p*-phenylazobenzoyl chloride[13] in 15 cc. of dry pyridine for about an hour on the steam-bath and 10 cc. of pyridine was removed under reduced pressure. The reaction mixture yielded 320 mg. of yellow precipitate on dilution with 8 cc. of water; this product was washed with water and the combined filtrate and washings yielded 170 mg. of impure hexahydrocolchicine. The yellow precipitate was dissolved in dry ether and adsorbed on Grade I alumina, the chromatogram being developed with 700 cc. of ether. Two zones were apparent; the lower brownish-red zone of the ester was eluted by ether–ethyl acetate, and was purified by rechromatographing followed by two crystallizations from benzene–hexane. The *p*-phenylazobenzoyl ester was obtained as clusters of orange-yellow needles, m. p. 232.5–233.5°.

Anal. Calcd. for $C_{35}H_{39}N_3O_7$: C, 68.50; H, 6.36. Found: C, 68.50; H, 6.45.

The upper red zone on the original chromatogram was eluted with hot dilute acetic acid, and found to contain 134 mg. of *p*-phenylazobenzoic acid, m. p. and mixed m. p. 244–245°.

125° Hexahydrocolchicine *p*-Nitrobenzoate.—Dry crystalline hexahydrocolchicine (304 mg., m. p. 116–119°) was heated with 279 mg. of *p*-nitrobenzoyl chloride in 2 cc. of dry pyridine at 115–125° for three hours. The reaction mixture was worked up in the usual way, using chloroform to extract the product; the gummy product crystallized on trituration with ethyl acetate to give a mixture of the desired ester and hexahydrocolchicine. The mixture was finally separated by adsorption on alumina from benzene solution; elution with benzene containing up to 25% chloroform gave a small yield of the ester which after crystallization from ethyl acetate formed clusters of long, slender, fairly yellow needles and weighed 25 mg., m. p. 247.5–249°. (A mixed m. p. with *p*-nitrobenzoic acid was strongly depressed.) The analytical sample obtained by another crystallization from ethyl acetate, melted at 250–251°.

Anal. Calcd. for $C_{29}H_{34}N_2O_9$: C, 62.82; H, 6.14; N, 5.06. Found: C, 63.26; H, 6.25; N, 5.16.

171° Hexahydrocolchicine *p*-Nitrobenzoate.—Pure 171° hexahydrocolchicine (100 mg.) was refluxed for fifteen minutes with 50 mg. of *p*-nitrobenzoyl chloride in 0.3 cc. of pyridine; 2 cc. of water was added after the mixture had cooled, and the ester, which separated as a dark oil, was washed with dilute base, with water, dried and recrystallized from ethyl acetate–hexane. The product was obtained as lemon needles (48 mg., m. p. 208–211°). The analytical sample, obtained by another crystallization from the same solvent pair, melted at 212–213°.

Anal. Calcd. for $C_{29}H_{34}N_2O_9$: C, 62.82; H, 6.14; N, 5.06. Found: C, 62.72; H, 6.13; N, 4.95.

Bromination of 171° Hexahydrocolchicine.—Hexahydrocolchicine (192 mg., m. p. 168–170°) in 10 cc. of chloroform was brominated to a permanent color by dropwise addition of a dilute chloroform solution of bromine. After twelve hours, 80 mg. of product was collected by filtration; it melted, after drying at 100°, at 163° with frothing and decomposition. Repeated crystallization from ethyl acetate gave long white needles, m. p. 162–163° with decomposition. Solutions of the compound were red, and it appeared that the compound decomposed in solution; its behavior was entirely different from that of the 125° hexahydro bromination product.

Anal. Calcd. for $C_{22}H_{30}BrNO_6$ (the monobromide): C, 54.55; H, 6.20; Br, 16.53. Calcd. for $C_{22}H_{29}Br_2NO_6$ (the dibromide): C, 46.89; H, 5.15; Br, 28.42. Calcd. for $C_{22}H_{29}Br_2NO_6 \cdot CH_3COOC_2H_5$: C, 47.93; H, 5.68; Br, 24.58. Found: C, 48.22; H, 5.62; Br, 24.30.

Hydrogenation of Colchicine in Acetic Acid with Adams Catalyst. 125° Hexahydrocolchicine (IV).—Colchicine (3 g., m. p. 149–153°, purified by chromatography[10]) was hydrogenated in 50 cc. of glacial acetic acid at room temperature and pressure, using 1 g. of Adams catalyst. Hydrogen uptake was rapid, 3.0 moles being absorbed in fifteen minutes; a total of 3.34 moles was taken up. The product, a clear gum, was obtained by Bursian's procedure,[6] and was found to be almost completely soluble in 150 cc. of hot water, leaving a small residue from which no further products could be isolated, in contrast to Bursian.[6] The hot aqueous extract on cooling gave 1.25 g. of hexahydrocolchicine, as faintly yellow needles, m. p. 110–120°. Recrystallization from either ethyl acetate or water, followed by prolonged drying in vacuum at room temperature gave material of m. p. 122–124°, with previous sintering. When dried at 100° in vacuum, the compound changed to a glassy solid; the loss in weight after fifteen hours at 135° in vacuum was 5.8%; calcd. for $C_{22}H_{31}NO_6 \cdot 1.5H_2O$, 6.25%. A sample dried at 79° in vacuum for fourteen hours remained crystalline, but now had m. p. 100–105° (softening from 93°).

Investigation of material from the mother liquors failed to yield any material other than impure hexahydrocolchicine.

The presence of 171° hexahydrocolchicine and the desmethoxy compound in small amounts, in addition to the 125° hexahydro compound, in the crude reduction product, was demonstrated by using a different method of isolation. The clear gum obtained by hydrogenation of

(11) Brockmann and Schodder, *Ber.*, **74B**, 73 (1941).

(12) *Cf.* R. Adams and Govindachari, THIS JOURNAL, **71**, 1180 (1949).

(13) *Cf.* Ladenburg, Fernholz and Wallis, *J. Org. Chem.*, **3**, 294 (1938); Angeli and Valori, *Atti Accad. Lincei*, **22** [1], 132 (1913).

colchicine was boiled with 15 cc. of water, leaving a residue (A) which partly crystallized on cooling. From the filtrate was obtained by evaporation and trituration of the residue with ethyl acetate–ether some 125° hexahydro compound; the ethyl acetate–ether washings on standing slowly deposited crystals of the desmethoxy compound. The residue A was dried and separated into 125° hexahydro and 171° hexahydro by chromatography on alumina, using benzene and chloroform as eluents.

Summary

Hydrogenation of purified colchicine in methanol with Adams catalyst gives two stereoisomeric hexahydrocolchicines, which have been characterized. A desmethoxyhexahydrocolchicine is also formed in small amount.

ROCHESTER, NEW YORK RECEIVED SEPTEMBER 29, 1949

[CONTRIBUTION FROM THE CHEMOTHERAPY SECTION, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Components of Podophyllin. III. Isolation of α- and β-Peltatin. Structure Studies[1]

BY JONATHAN L. HARTWELL* AND WENDELL E. DETTY[2]

The report[3] that local application of podophyllin causes complete regression of *condylomata acuminata* (venereal warts), along with the observation[4] that this drug produces mitotic abnormalities in cells of rabbit and human skin similar to those produced by colchicine, prompted a test of this drug with the finding[5] that it exerts a strong destructive action on sarcoma 37 in mice. Belkin,[6] also prompted by these reports,[3,4] obtained reduction in size of sarcoma 180 and of a transplated mouse mammary carcinoma following subcutaneous injection of podophyllin, demonstrating for the first time that this crude drug can affect the growth rate of malignant tumors. Reich, *et al.*,[7] described the regression of soft papillomas of the female urethra by local applications of podophyllin.

Podophyllin N. F. (or resina podophylli, U. S. P. XI), a drug previously sued as a purgative, is a brown powder with a peculiar odor, a sharp taste, and an irritating effect on mucous membranes. It is derived, in the United States, from the alcohol-soluble portion of the dried roots and rhizomes of *Podophyllum peltatum* L. (Fam. *Berberidaceae*), the mandrake or May apple. A search of the literature revealed that podophyllin is a complex mixture of oil, pigments, and noncrystallizable resins from which two definite compounds have been isolated, one colorless and one colored, namely, podophyllotoxin and quercetin.[8] Podophyllotoxin has been the subject of several investigations and the structure, I, has been proposed for it independently by Borsche[9] and by Späth.[10] Bioassays with sarcoma 37 showed[5] that podophyllotoxin (prepared as described below) had several times the tumor-necrotizing action of podophyllin while quercetin[5,11] (prepared from rutin[12]) was inactive except in very much higher doses.

In a search for other components it seemed reasonable to try chromatography both because podophyllin is a highly-colored complex mixture and because in all previous chemical studies of the drug this technique had never been employed. Application of this technique has resulted so far in the isolation of podophyllotoxin and two other colorless, crystalline compounds[1a,1b] of roughly equal biological activity[5,11] against tumors, which have been named α-peltatin and β-peltatin. The isolation and progress in the structure determination of the last two substances comprise the subject of this communication.

Structural Studies

Consideration of the elementary analysis and molecular weight values for the peltatins and their derivatives indicates an empirical formula of $C_{22}H_{22}O_8$ as the most likely for both peltatins. The latter would thus be isomeric with podophyllotoxin. It may be mentioned, however, the the analyses of α-peltatin and several of its derivatives are equally consistent with the formula $C_{21}H_{20}O_8$. Until more evidence is obtained on this point, the two peltatins will be considered to be isomers. If the alternate formula for α-peltatin should be correct, the following discussion will not be invalidated.

The functional groups containing all the oxygen are accounted for as follows. Both peltatins

* Harvard University Ph.D. 1935.

(1) Presented, in part, at the Detroit meeting of the American Association for Cancer Research Inc., April 16, 1949. (a) For paper I in this series, see Hartwell, THIS JOURNAL, **69**, 2918 (1947); (b) for paper II, see Hartwell and Detty, *ibid.*, **70**, 2833 (1948).

(2) The isolation studies constituted a thesis presented by Wendell E. Detty to the Department of Chemistry of the Graduate Division of Georgetown University, in partial fulfillment of the requirements for the degree of Master of Science, 1949. Present address: University of Oklahoma, Norman, Oklahoma.

(3) Kaplan, *New Orleans Med. Surg. J.*, **94**, 388 (1942).

(4) King and Sullivan, *Science*, **104**, 244 (1946).

(5) Hartwell and Shear, *Cancer Research*, **7**, 716 (1947). In this paper α-peltatin is referred to as NCI-1074.

(6) Belkin, *Fed. Proc.*, **6**, 308 (1947); *J. Pharm. Exp. Therap.*, **93**, 18 (1948).

(7) Reich, Nechtow and Rubenstein, *Am. J. Obstet. Gyn.*, **53**, 658 (1947).

(8) Podwyssotzki, *Arch. exp. path.*, **13**, 29 (1881).

(9) Borsche and Niemann, *Ber.*, **65**, 1633 (1932).

(10) Späth, Wessely and Nadler, *ibid.*, **66**, 125 (1933).

(11) Leiter, Downing, Hartwell and Shear, *Cancer Research*, **9**, 597 (1949).

(12) Wunderlich, *Arch. pharm.*, **246**, 224, 244 (1908).