Plant Antitumor Agents. II. The Structure of Two New Alkaloids from *Camptotheca acuminata*[1,2]

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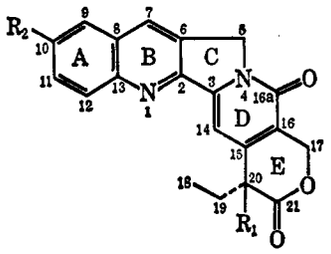
Further fractionation of *C. acuminata* has yielded two new alkaloids, hydroxycamptothecin (2) and methoxycamptothecin (3). The former could be methylated to give a methyl ether identical with 3. In order to establish the position of the hydroxyl group in ring A, nmr spectra of deuterated methoxycamptothecin and of the model compounds 7–10 have been obtained. The syntheses of model compounds have been described.

The isolation and structure of camptothecin 1, an alkaloid with a novel ring system exhibiting potent antileukemic and antitumor activities, has been reported from our laboratory.[2] Further fractionation of *C. acuminata* has resulted in the isolation of two minor and related components hydroxycamptothecin (2) and methoxycamptothecin (3) both possessing good antileukemic activity in L-1210 (Chart I[3]). This report is on the characterization of these two alkaloids.

CHART I



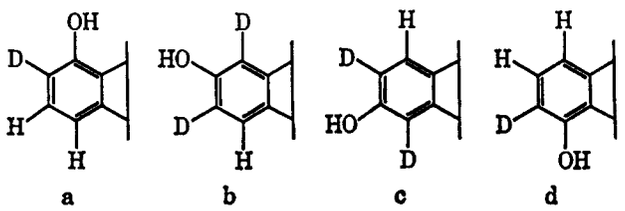
1, R_1 = OH; R_2 = H
2, R_1 = R_2 = OH
3, R_1 = OH; R_2 = OCH_3
4, R_1 = OH; R_2 = O_2CCH_3
5, R_1 = R_2 = O_2CCH_3

The molecular composition of 2 ($C_{20}H_{16}N_2O_5$) as determined by mass spectrometry suggested that it might be a monohydroxy derivative of 1 ($C_{20}H_{16}N_2O_4$). This hypothesis was further supported by the formation of mono (4) and diacetate (5) derivatives. The ultraviolet spectra of compounds 4 and 5 were similar to that of 1 thereby suggesting the presence of the same camptothecin chromophore in these compounds. The phenolic nature of the hydroxyl function was evidenced by the positive ferric chloride test and bathochromic shifts in the ultraviolet spectrum of 2 in the presence of dilute base. Treatment of 2 with diazomethane gave a compound identical with 3 obtained from natural sources. Hence, methoxycamptothecin is the methyl ether of hydroxycamptothecin.

The phenolic nature of the additional hydroxyl function in 2 meant it could be present in only one of the three rings, A, B, or D. The nmr spectra of 1 and 2 were quite similar except for some differences in the aromatic region. In the aromatic region of 1 and 2, the one proton singlet at δ 8.38 has been assigned to the C-7 proton *para* to the quinoline nitrogen.[4] The other one proton singlet at δ 7.22 must therefore be due to the C-14 proton *meta* to the pyridone nitrogen.[5] These assignments are further supported by the nmr spectra of tricyclic model compounds 7–10 (see Figure 1) in which the singlet due to the C-9[6] proton appears around δ 8.20 while the other singlet around δ 7.20 is absent. Therefore, the phenolic hydroxyl in 2 cannot be located at C-7 or C-14 and consequently its location is narrowed to one of the four possible positions in ring A.

In order to determine the exact position of the hydroxyl group in ring A, it was decided to study the deuterium exchange reaction of 2. It is well known that protons adjacent to a phenolic hydroxyl group can be replaced by deuterium under base catalysis.[7] The four possibilities in the present case are shown by partial structures a, b, c, and d (Chart II). The nmr spectra of b and c would show only a one-proton singlet in the aromatic region, whereas a and d would show two-proton doublets in this region.

CHART II



a b c d

In our studies, deuterated hydroxycamptothecin was prepared by treatment with aqueous sodium deuterioxide and converted into the corresponding methyl ether for reasons of solubility. In the nmr spectrum of the deuterated methoxycamptothecin (*cf.* Figure 1) the signals due to the C-7 and C-14 protons in the aromatic region appeared unaltered as expected. However, the multiplets centered at δ 8.0 and 7.44 in the undeuterated sample were replaced by a pair of doublets located precisely as in the undeuterated sample, suggesting partial structure a or d. The results of deuteration therefore tend to favor position 9 or 12 for the location of the hydroxyl (methoxy in 3) group in 2.

A priori it was felt that the aromatic regions of the nmr spectra of model compounds having methoxy

(1) This investigation was conducted under Contract SA-43-ph 4322, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health. Presented in part before the Organic Division at the 19th Southeastern Regional Meeting of the American Chemical Society, Atlanta, Ga., Nov 1967.

(2) Previous paper in this series: Plant Antitumor Agents. I: M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **88**, 3888 (1966).

(3) The numbering system is based on the probable relationship of camptothecin to indole alkaloids, particularly those of the ajmalicine type; *cf.* M. Shamma, *Experientia*, **24**, 107 (1968).

(4) W. Seiffert, *Angew. Chem. Intern. Ed. Engl.*, **1**, 215 (1962); Varian Spectra Catalog, Vol. 2, Spectrum No. 579.

(5) J. A. Elvidge and L. M. Jackman, *J. Chem. Soc.*, 859 (1961).

(6) The numbering system for the model compounds is different from that of 1 and is based on similar compounds reported in the literature; *cf.* G. Kempter and S. Hirschberg, *Chem. Ber.*, **98**, 419 (1965).

(7) G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 6914 (1965).

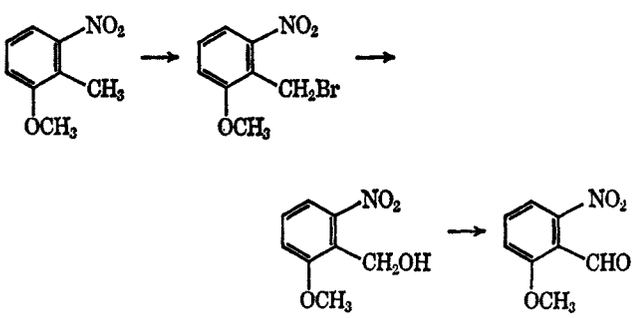
groups at the two positions suggested by deuterium-exchange reactions should be significantly different. A comparison of the nmr spectra of these compounds with that of **3** should therefore allow one to choose between the two possible positions suggested by deuteration studies.

With this objective, pyrroloquinolines **7** and **8** with a methoxy group at positions 5 and 8 of ring A were synthesized (see below) using procedures already developed in our laboratory in connection with studies on the total synthesis of camptothecin.[8] The aromatic regions of the nmr spectra of **7** and **8** were indeed different; however, these did not bear any resemblance with that of **3** (*cf.* Figure 1). Therefore, the remaining two methoxypyrroloquinolines **9** and **10** were also synthesized (see below). A comparison of their spectra with that of **3** (*cf.* Figure 1) left no doubt as to the identity of the splitting patterns of **9** and **3**, thereby establishing beyond any reasonable doubt that the methoxy group in methoxycamptothecin and therefore the hydroxy group in hydroxycamptothecin must be located at position 10 as shown in formulas **3** and **2**, respectively. Such a placement is in accord with biogenetic considerations.[9,10]

In view of the above findings, the deuteration of **2** was repeated under base and also acid catalysis using drastic conditions. However, no deuterium exchange at the other *ortho* position was observed. It appears therefore that the 11 position in pyrroloquinolines like the 3 position in 2-naphthol is inert toward electrophilic substitution.

Synthesis of Pyrroloquinolines.—The isomeric 3-, 4-, and 5-methoxy-2-nitrobenzaldehydes[11-13] were synthesized using methods reported in the literature. The fourth isomer 2-nitro-6-methoxybenzaldehyde was prepared from 2-nitro-6-methoxytoluene as shown in Chart III. Bromination of the latter with N-bromo-

CHART III



succinimide gave 2-nitro-6-methoxybenzyl bromide which on boiling with aqueous sodium carbonate solution gave the corresponding alcohol. Oxidation of the alcohol with potassium dichromate and sulfuric acid yielded the desired aldehyde.

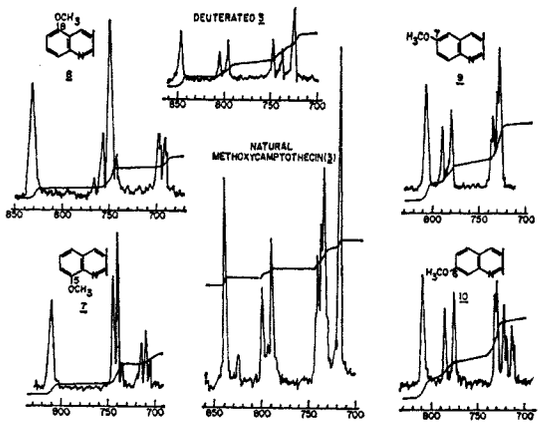
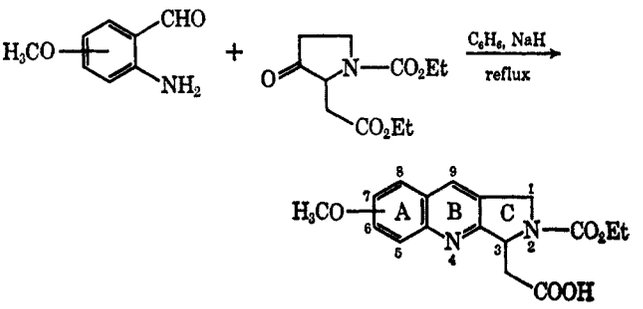


Figure 1.—Aromatic regions of the 100-MHz nmr spectra of **3**, **7**, **8**, **9**, **10**, and deuterated **3** run in DMSO-d_6–TMS.

Reduction[14] of the methoxy-2-nitrobenzaldehydes gave the corresponding methoxy-2-aminobenzaldehydes.

Base-catalyzed Friedlander condensations of the methoxy-2-aminobenzaldehydes with ethyl 1-ethoxycarbonyl-3-oxopyrrolidin-2-ylacetate[15] (**6**) gave the corresponding methoxypyrroloquinolines **7**–**10** (Chart IV) isolated as carboxylic acids.

CHART IV



7, 5-methoxy
8, 8-methoxy
9, 7-methoxy
10, 6-methoxy

Experimental Section[16]

Isolation of Hydroxycamptothecin (2).—Compound **2** was present in the material that was eluted after methoxycamptothecin during the large-scale isolation of camptothecin from *C. acuminata* by the Squibb group. This material was further fractionated by a second adsorption chromatography on a silicic acid column (300 g). A solution of the mixture (500 mg) in 5% methanol in chloroform (75 ml) was applied to the column. The column was eluted with 5% methanol in chloroform. All fractions were investigated by means of tlc using chloroform–acetone–methanol (7:2:1) as the solvent system. Compound **2** (85 mg) was crystallized by the dropwise addition of ethyl acetate to a boiling solution of **2** in 20% methanol–chloroform until turbidity was observed at which point it was allowed to cool slowly: mp 268–270°; ir (KBr) 3480 (OH), 1740–1755 cm^{-1} (lactone C=O);

(8) J. A. Kepler, M. C. Wani, M. E. Wall, and S. G. Levine, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., 1968, Paper No. ORGN 027.

(9) E. Wenkert, K. G. Dave, R. G. Lewis, and P. W. Sprague, *J. Amer. Chem. Soc.*, **89**, 6741 (1967).

(10) E. Wenkert, *ibid.*, **84**, 98 (1962).

(11) P. Friedlander and O. Schenck, *Ber.*, **47**, 3040 (1914).

(12) R. B. Woodward, F. Bader, H. Bickel, A. Frey, and R. Kierstead, *Tetrahedron*, **2**, 1 (1958).

(13) H. H. Hodgson and H. G. Beard, *J. Chem. Soc.*, 147 (1926).

(14) L. I. Smith and J. W. Opie, *Org. Syn.*, **28**, 11 (1948).

(15) J. W. Clark-Lewis and P. I. Mortimer, *J. Chem. Soc.*, 191 (1961).

(16) Melting points were determined on a Kofler hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 221 spectrophotometer. Ultraviolet spectra were determined in methanol on a Cary Model 14 spectrophotometer. Nuclear magnetic resonance spectra were obtained in deuteriochloroform or dimethyl sulfoxide-d_6 with TMS as an internal standard on a Varian HA-100 apparatus; results are expressed in parts per million on a δ scale. Mass spectra were determined with A. E. I. MS-902 mass spectrometer through the valued assistance of Dr. David Rosenthal of our laboratory. Thin layer chromatography was performed on silica gel HF plates.

uv max 222 mμ (ε 50,300), 267 (27,400), 330 (12,100), 382 (28,000); nmr (DMSO-d_6) δ 0.88 (t, 3, C-18), 1.85 (m, 2, C-19), 5.15 (s, 2, C-5), 5.35 (s, 2, C-17), 6.40 (s, 1, C-20, OH), 7.22 (s, 1, C-14), *ca.* 7.28 (m, 2, C-11 and C-12), 7.96 (d, 1, C-9), 8.38 (s, 1, C-7), 10.3 (s, br, C-10, OH).

Anal. Calcd for $C_{20}H_{16}N_2O_5 \cdot H_2O$: C, 62.82; H, 4.75; N, 7.33. Found: C, 62.97; H, 4.55; N, 7.29. *Anal.* Calcd for $C_{20}H_{16}N_2O_5$: *m/e* 364. Found: *m/e* 364.

Isolation of methoxycamptothecin (3) was accomplished by the fractional crystallization of the material that was eluted immediately after camptothecin during the large-scale isolation of camptothecin from *C. acuminata* by the Squibb group. The material (12.5 g) was dissolved in a mixture of hot acetonitrile (1000 ml) and methanol (300 ml). Traces of insoluble brown material were removed by filtration and the solution was slowly cooled to about −10° to give precipitate A (1.61 g) and filtrate B.

A tlc of precipitate A on Eastman Chromagram (Type K 301R, silica gel) in benzene–acetone–methanol (18:2:0·5) indicated the presence of **1** as the major impurity in **3**. It was triturated with hot 20% ethanol in acetonitrile (100 ml). The insoluble material was extracted with hot 50% ethanol in acetonitrile (80 ml). Evaporation of the filtrate gave **3** (140 mg) identical with the one obtained by the methylation of **2**. Concentration of filtrate B to about 500 ml gave precipitate C (300 mg). Precipitate C was processed in a manner analogous to precipitate A to yield an additional 60 mg of **3**.

Compound **3** was crystallized in the same manner as **2**: mp 254–255°; ir (KBr) 3330 (OH), 1750 cm^{-1} (lactone C=O); uv max 220 mμ (ε 49,900), 264 (29,800), 293 (5700), 312 (8600), 328 (12,000), 365 sh (27,500), 379 (31,500); nmr (DMSO-d_6) δ 3.96 (s, 3, OCH_3), 7.38 (m, 2, C-11 and C-12), 7.94 (d, 1, C-9). Rest of the spectrum was similar to that of **2**.

Anal. Calcd for $C_{21}H_{18}N_2O_5$: C, 66.66; H, 4.80; N, 7.40. Found: C, 66.41; H, 4.88; N, 7.16.

Hydroxycamptothecin Monoacetate 4.—To a suspension of **2** (75 mg) in 50 ml of dry benzene was added 0.4 ml of acetic anhydride and 0.3 ml of pyridine and the reaction mixture was refluxed for 24 hr. The solvent and excess reagents were removed under vacuum at 50°. The product was crystallized from methanol: mp 255–257°; ir (Nujol) 3470 (OH), 1750 cm^{-1} (lactone C=O); uv max 218 mμ (ε 24,400), 253 (17,500), 290 (4500), 365 (13,400).

Anal. Calcd for $C_{22}H_{18}N_2O_6$: C, 65.02; H, 4.46; N, 6.89. Found: C, 65.26; H, 4.48; N, 6.77.

Hydroxycamptothecin diacetate 5 was prepared in the same manner as **4** by using a large excess of acetic anhydride and pyridine and increasing the reflux period to 3 days: mp 270–273°; ir (Nujol) absence of OH, 1750 cm^{-1} (lactone C=O); uv similar to that of **4**.

Anal. Calcd for $C_{24}H_{20}N_2O_7$: *m/e* 448. Found: *m/e* 448.

2-Nitro-6-methoxybenzyl Bromide.—To a solution of 1 g of 2-nitro-6-methoxytoluene in 75 ml of carbon tetrachloride were added 2.13 g of N-bromosuccinimide and 30 mg of benzoyl peroxide. The reaction mixture was refluxed for 18 hr, cooled to 0°, and filtered. The product (1.05 g, 71%) from the filtrate was crystallized from ether–hexane, mp 68–69°.

Anal. Calcd for $C_8H_8NO_3Br$: C, 39.46; H, 3.27. Found: C, 39.44; H, 3.37.

2-Nitro-6-methoxybenzyl Alcohol.—2-Nitro-6-methoxybenzyl bromide (5.59 g) was added to a solution of 3.03 g of sodium carbonate in 240 ml of water. The reaction mixture was refluxed for 16 hr, cooled, and extracted with chloroform. The product (3.17 g, 76%), obtained from the dry chloroform extract, was crystallized from ether–hexane, mp 72–73°.

Anal. Calcd for $C_8H_9NO_4$: C, 52.46; H, 4.95. Found: C, 52.62; H, 5.00.

2-Nitro-6-methoxybenzaldehyde.—A solution of 2.63 g of sodium dichromate dihydrate in 22 ml of 20% sulfuric acid was added during 1 hr to 3.17 g of 2-nitro-6-methoxybenzyl alcohol. The reaction mixture was then heated at 60–65° for 3 hr, cooled, and quenched with 25 ml of water. The product (2.41 g, 77%) was crystallized from ether–benzene, mp 101–103°.

Anal. Calcd for $C_8H_7NO_4$: C, 53.04; H, 3.90. Found: C, 53.14; H, 3.93.

Table I gives the melting points and literature references to the preparation of **3-, 4-, and 5-methoxy-2-nitrobenzaldehydes.**

Preparations of 3-, 4-, 5-, and 6-methoxy-2-aminobenzaldehydes from the corresponding nitro compounds were carried out following a procedure[14] reported for the preparation of O-aminobenzaldehyde. These were obtained in 67, 81, 29, and 45% yield, respectively. Owing to the tendency of the O-aminobenzaldehydes to undergo self-condensation,[14] these were used without further characterization.

TABLE I

Compd	Mp, °C	Ref
3-Methoxy	102	11
4-Methoxy	95–96	12
5-Methoxy	83	13

1,3-Dihydro-2-ethoxycarbonyl-3-carboxymethyl-5-methoxy-2H-pyrrolo[3,4-*b*]quinoline (7).—A solution of 1.69 g of 2-amino-3-methoxybenzaldehyde and 2.38 g of **6**[15] in 100 ml of benzene was refluxed until no more water collected in the Dean–Stark trap. It was cooled to 45° and 420 mg of 56% sodium hydride in mineral oil added. The reaction mixture was allowed to stand overnight at room temperature. A 50-ml portion of water was added and the mixture shaken vigorously for 10 min. The water layer was acidified to pH 5 with 1 *N* hydrochloric acid, saturated with sodium chloride, and extracted several times with ethyl acetate. The product (1.03 g, 28%) was crystallized from ethyl acetate: mp 213–215°; ir (KBr) 3450 (OH), 1750 (monomeric carboxyl C=O), 1690 cm^{-1} (N-carbethoxy C=O); uv max 253 mμ (ε 35,500), 321 (5500); nmr (DMSO-d_6) δ 1.28 (t, 3, CH_2CH_3), 3.06 (s, br, 2, $>CHCH_2CO_2H$), 3.94 (s, 3, OCH_3), 4.16 (q, 2, CH_2CH_3), 4.74 (q, 2, C-1), 5.17 (t, 1, C-3), 7.14 (m, 1, C-7), 7.24 (m, 2, C-6 and C-8), 8.19 (s, 1, C-9).

Anal. Calcd for $C_{17}H_{18}N_2O_5$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.79; H, 5.64; N, 8.42.

1,3-Dihydro-2-ethoxycarbonyl-3-carboxymethyl-8-methoxy-2H-pyrrolo[3,4-*b*]quinoline (8) was prepared in exactly the same manner as **7** from 2-amino-6-methoxybenzaldehyde and **6** in 37% yield: mp 202–204°; ir (KBr) 3450 (OH), 1750 (monomeric carboxyl C=O), 1690 cm^{-1} (N-carbethoxy C=O); uv max 232 mμ (ε 31,700), 321 (4600); nmr (DMSO-d_6) δ 6.96 (m, 1, C-6), *ca.* 7.56 (m, 2, C-5 and C-7). The rest of the spectrum was similar to that of **7**.

Anal. Calcd for $C_{17}H_{18}N_2O_5$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.66; H, 5.57; N, 8.21.

1,3-Dihydro-2-ethoxycarbonyl-3-carboxymethyl-7-methoxy-2H-pyrrolo[3,4-*b*]quinoline (9) was prepared in exactly the same manner as **7** from 2-amino-5-methoxybenzaldehyde and **6** in 38% yield: mp 198–200° (with prior softening); ir (KBr) 3450 (OH), 1725 (carboxyl C=O), 1710 cm^{-1} (N-carbethoxy C=O); uv max 217 mμ (41,500), 242 sh (21, 800), 330 (6300); nmr (DMSO-d_6) δ 7.29 (m, 2, C-6 and C-8), 7.86 (d, 1, C-5). The rest of the spectrum was similar to that of **7**.

Anal. Calcd for $C_{17}H_{18}N_2O_5$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.88; H, 5.48; N, 8.42.

1,3-Dihydro-2-ethoxycarbonyl-3-carboxymethyl-6-methoxy-2H-pyrrolo[3,4-*b*]quinoline (10) was prepared in exactly the same manner as **7** from 2-amino-4-methoxybenzaldehyde and **6** in 84% yield: mp 198–202° (with previous softening around 186°); ir (KBr) 3450 (OH), 1725 (carboxyl C=O), 1705 cm^{-1} (N-carbethoxy C=O); uv max 224 mμ (ε 33,900), 335 (8700); nmr (DMSO-d_6) δ 7.18 (m, 1, C-8), 7.34 (d, 1, C-5), 7.82 (d, 1, C-7). Rest of the spectrum was similar to that of **7**.

Anal. Calcd for $C_{17}H_{18}N_2O_5$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.64; H, 5.62; N, 8.73.

Deuterated Methoxycamptothecin. Base Catalysis.—A solution of 40 mg of **2** in 1 ml of 1 *N* sodium deuterioxide was heated at 100° in a nitrogen-filled sealed tube for 9 days. Traces of insoluble material were removed by filtration and the deep orange filtrate was acidified to pH 4 under cooling. The suspension was freeze dried and the residue was extracted thrice with 10 ml of 20% methanol–chloroform. The solution was treated with excess diazomethane solution in ether. The product (33 mg) was crystallized from chloroform–ethyl acetate: nmr (DMSO-d_6) δ 7.45 (d 1, C-11), 8.0 (d, 1, C-12). The rest of the spectrum was similar to that of **3**.

Deuterated Hydroxycamptothecin. Acid Catalysis.—A solution of 20 mg of **2** in 0.3 ml of DMSO-d_6 containing 2 drops of 1 *N* deuterium chloride was heated at 100° for 24 hr in a nmr tube. The progress of the reaction was followed by nmr spectroscopy: (DMSO-d_6–DCl) δ 7.45 (d, 1, C-11), 8.05 (d, 1, C-12). The rest of the spectrum was similar to that of **2**.

Registry No.—**2**, 19685-09-7; **3**, 19685-10-0; **4**, 19685-11-1; **5**, 19685-12-2; **7**, 19713-66-7; **8**, 19713-67-

8; **9**, 19689-84-0; **10**, 19689-85-1; 2-nitro-6-methoxybenzyl bromide, 19689-86-2; 2-nitro-6-methoxybenzyl alcohol, 19689-87-3; 2-nitro-6-methoxybenzaldehyde, 19689-88-4.

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Thurberin, a New Pentacyclic Triterpene from Organ Pipe Cactus

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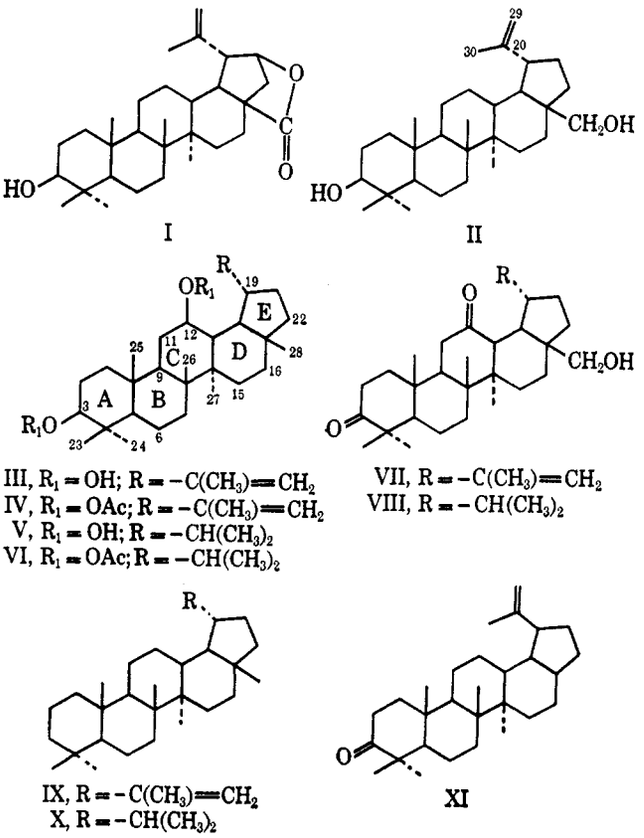
A chemical analysis of the alcoholic extract of the fresh cortical (pulp) portion of the Organ Pipe cactus (*Lemaireocereus thurberi*) affords, besides thurberogenin (I), two more triterpenes, betulin (II) and a new triterpene, thurberin (III), hitherto unidentified in this species. The new triterpene belongs to the lupeol class and is an isomer of betulin. Based on the mass, nmr, spectral, and optical rotatory dispersion (ORD) data, the hydroxyl groups are assigned to positions 3 and 12.

In the course of our studies of the biosynthesis of wound tissue formation in giant cacti,[1,2] we examined the ethanol extract of the Organ Pipe cactus (*Lemaireocereus thurberi*) cortex. Acid hydrolysis of the extract yielded a neutral fraction, which had a very high lipid and steroid content. From this fraction, we isolated three crystalline compounds by column chromatography on alumina. The first was identified as thurberogenin (I), previously found by Djerassi[3] in Organ Pipe. The second compound was betulin (II), hitherto undetected in this species. The third substance (mp 206–208°) had the same molecular formula as betulin $C_{30}H_{50}O_2$, gave a positive tetranitromethane test and had an ir spectrum almost identical with that of betulin. It appeared to be a new triterpene of the lupane class, and was given the trivial name, thurberin (III). Evidence to support the structural assignment (III) is given below.

Thurberin formed a diacetate (IV) and absorbed 1 mol of hydrogen (PtO_2) to yield dihydrothurberin (V), which also could be converted into a diacetate (VI). Oxidation of thurberin with CrO_3–pyridine led to the diketone, thurberindione (VII), which on catalytic reduction (H_2/PtO_2) yielded dihydrothurberindione, VIII. When VII was submitted to Wolff–Kishner conditions, α-lupene (IX) was formed; under identical conditions VIII gave rise to lupane (X).[4]

These transformations established that thurberin is a lupenediol, isomeric with betulin (II). The two hydroxyl groups were assigned the positions C-3 and C-12 on the basis of ir, nmr, mass, and ORD spectral studies, as outlined in the following sections.

Nmr Measurements.—The nature of the two hydroxyl groups was deduced from the 100-Mc CAT nmr spectra of thurberin (III) and its diacetate (IV). The spectrum of III contained a one-proton quartet centered at τ 6.76 with splitting of 10 and 4.5 cps (X component of ABX system), characteristic of the C-3 proton resonance, adjacent to an OH moiety. This



pattern is almost identical in the spectrum of the diacetate, IV, but is displaced downfield to τ 5.6. This evidence also substantiates the equatorial orientation of the C-3 hydroxyl group.

Information regarding the second OH group also was obtained from the above spectra, which showed a C–X proton quartet centered at τ 6.37 (characteristic of an axial proton attached to an OH-substituted carbon atom).[5] Similar results with appropriate downfield shifts (τ 5.2) were observed in the spectrum of the diacetate, IV. Since the ir spectrum of thurberindione, VII, showed only a single band at 5.88 μ, indicating no cyclopentanone moiety[5] or aldehyde moiety, the nmr data confirm the existence of a second OH group in one of the cyclohexane rings.

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