

ISOLATION, CHARACTERIZATION, AND SYNTHESIS OF 3,5,4'-TRIHIDROXYBIBENZYL FROM CANNABIS SATIVA

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ABSTRACT.—The title compound (**1**) was isolated from a locally confiscated sample of hashish. Its structure was established by its spectra and those of the triacetate **2**, and by total synthesis.

Examination of the ethanolic extract of a locally confiscated sample of hashish (*Cannabis sativa* L.) revealed the presence of a noncannabinoidal phenol. The structure elucidation and synthesis of this compound are the subject of this paper.

RESULTS AND DISCUSSION

Ethanolic extraction of the hashish sample followed by solvent partitioning and flash chromatography (**1**) provided **1** as colorless needles, C₁₄H₁₄O₃, mp 156-157°, and was optically inactive. The ir spectrum (see Experimental section) established the phenolic nature of the compound, while its ¹H-nmr spectrum provided more information about its gross structure. It exhibited an AA'BB' system as a pair of doublets at δ 7.05 and δ 6.71 (*J*=8.3 Hz) due to H-2', H-3', H-5', and H-6', a four-proton singlet at δ 2.77 assigned to the two methylene groups, and a three-proton singlet at δ 6.23 assigned to H-2, H-4, and H-6. The ¹H-nmr spectrum of the triacetate derivative **2** exhibited similar patterns that failed to distinguish between the protons of the two methylene groups or the two types of aromatic protons in ring A.

The ¹³C-nmr spectra of **1** (Table 1) and its triacetate **2** were in full agreement with their assigned structure, as they accounted for all carbon atoms in each compound. The structure of **1** was further confirmed, unambiguously, by total synthesis in a 27% overall yield (Scheme 1). This was accomplished by benzylating 3,5-dihydroxybenzoic acid (**4**) to the ester **5**, followed by lithium aluminum hydride reduction to the benzyl alcohol **6**. Pyridinium chlorochromate (2) oxidation to the aldehyde **7** followed by

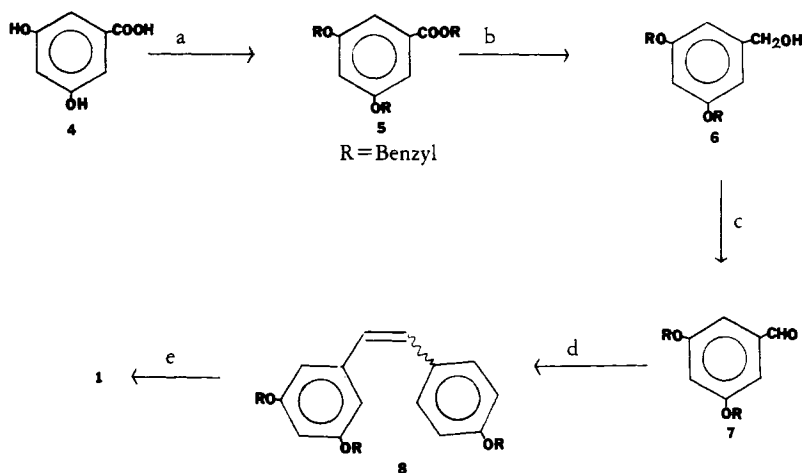
TABLE 1. ¹³C-nmr Assignments of **1**^a

Carbon	1	Carbon	1
1	145.7 (<i>s</i>)	β-C	37.0 (<i>t</i>)
2,6	108.3 (<i>d</i>)	1'	134.2 (<i>s</i>)
3,5	158.7 (<i>s</i>)	2',6'	130.3 (<i>d</i>)
4	101.1 (<i>d</i>)	3',5'	116.0 (<i>d</i>)
α-C ^b	38.5 (<i>t</i>)	4'	155.7 (<i>s</i>)

^aAssignment of the aromatic carbons was based on chemical shift values and comparison to the spectrum of orcinol (3,5-dihydroxytoluene) and that of *p*-cresol. The former exhibited signals at δ 141.4, 108.9, 158.7, and 100.8 (due to C-1, C-2, C-3, and C-4, respectively), while the latter showed signals at δ 129.7 (C-1), 130.6 (C-2), 115.8 (C-3), and 154.9 (C-4).

^bDistinction between the α-C and the β-C was achieved by introducing a deuterium on the α-C of synthetic **1** (see Experimental section). While the signal at δ 37.0 stayed the same, that at δ 38.5 underwent splitting into a weak triplet.

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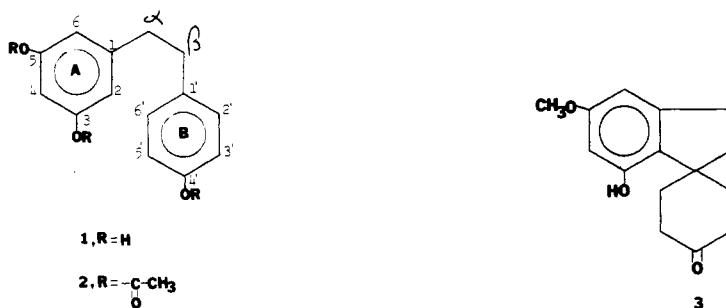


Scheme 1.

(a) Benzyl bromide/ K_2CO_3 ; (b) Lithium aluminum hydride; (c) Pyridinium chlorochromate; (d) *p*-Benzyloxybenzyltriphenylphosphonium chloride/*n*-butyl Li; (e) H_2 -5% Pd/C.

Wittig's reaction with the phosphorane prepared from *p*-benzyloxybenzyl chloride provided a mixture of *Z* and *E* stilbenes (**8**). Catalytic hydrogenation of this mixture converted it to a product identical with **1** (same mp, undepressed mmp, and indistinguishable ir and 1H -nmr spectra).

The bibenzyl **1** is to be added to the long list of related compounds and their cyclic analogs such as **3** that have been reported to occur in *Cannabis sativa* L. (3). Some of them were found to contribute to the estrogenic activity of cannabis; for example, the 3-methyl ether of **1** (4). Besides, compounds of this type are known to exert plant growth regulating activity (5).



EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The hashish sample used in this investigation was confiscated from a passenger traveling from India in 1980. Because it was moist, it was kept for several days in a desiccator over dry $CaCl_2$, then stored. All starting compounds and reagents used in the synthetic scheme were obtained from Alrich Chemical Co., 940 W. Saint Paul Avenue, Milwaukee, WI 53233. All melting points were taken in capillaries on a Gallenkamp apparatus and are uncorrected. Ir spectra were determined on a Perkin-Elmer 580 B infrared spectrophotometer; 1H -nmr spectra were determined on a Varian T-60A nuclear magnetic resonance spectrometer at 60 MHz using $CDCl_3$ as solvent, unless otherwise specified, and TMS as an internal standard with chemical shifts reported as values. ^{13}C -nmr spectra were recorded on Varian FT-80A nuclear magnetic resonance spectrometer using $CDCl_3$ as solvent, unless otherwise specified, and TMS as internal standard. Mass spectra were taken on a Finnigan 3200 mass spectrometer; elemental analyses were done by Galbraith Laboratories, Inc., Knoxville, TN 37921; spot detection on tlc

plates was achieved by spraying with 0.3% aqueous KMnO_4 , 0.5% fast blue salt B, or by viewing under uv light.

EXTRACTION.—The hashish sample (57 g) was powdered and extracted by cold percolation with 95% EtOH. The extract (21 g) was partitioned between 500 ml of CHCl_3 and four 75-ml portions of H_2O . The CHCl_3 solubles (19 g) were similarly partitioned between 10% aqueous MeOH and *n*-hexane to yield 11 g of *n*-hexane solubles and 8 g of aqueous MeOH solubles.

ISOLATION OF 3,5,4'-TRIHYDROXYBIBENZYL (1).—The aqueous MeOH solubles (3 g) obtained above were flash chromatographed (1) using 4% EtOH in CHCl_3 as solvent to yield 34 mg of a product, Rf 0.35 on silica gel G tlc plates using 8% EtOH in CHCl_3 as solvent. It was crystallized from $\text{Et}_2\text{O}-\text{CHCl}_3$ to give colorless needles, mp 156-157°; ir ν max (KBr) 3300 (OH) and 1595 (C=C) cm^{-1} ; $^1\text{H-nmr}$ ($\text{Me}_2\text{CO}-d_6$) δ 2.77 (4H, s, 2CH_2), δ 6.23 (3H, s, ring A protons) and δ 6.71 and δ 7.05 (4H, d, $J=8.3$ Hz, AA'BB' of ring B); ms M^+ at m/z 230 (10%). For the $^{13}\text{C-nmr}$ spectrum see Table 1.

Anal. Calcd. for $\text{C}_{14}\text{H}_{14}\text{O}_3$; C 73.03, H 6.13. Found; C 72.88, H 6.33%.

ACETYLATION OF 1.—Phenol **1** (60 Mg) was stirred for 24 h with 1 ml of pyridine and 1 ml of Ac_2O . After usual work-up, the triacetate **2** (62 mg) was obtained as colorless needles from $\text{Et}_2\text{O}-n$ -hexane, mp 58-59°; ir ν max (CHCl_3) 1765 (CH_3COO) cm^{-1} , and no OH bands; $^1\text{H-nmr}$ (CDCl_3) δ 2.19 (9H, s, CH_3COO); $^{13}\text{C-nmr}$ (CDCl_3) δ 169.4 and 168.9 (CH_3-COO), four aromatic singlets at δ 151.0, 149.0, 143.9, and 138.6, four aromatic methine signals at δ 129.3, 121.5, 119.0, and 113.1, two methylene signals at δ 37.5 and 36.6, and an intense signal (q) at δ 21.0 (CH_3COO); ms M^+ at m/z 356 (2%); uv λ max (MeOH) 224 and 278 nm (ϵ 4600 and 900, respectively).

Anal. Calcd. for $\text{C}_{20}\text{H}_{20}\text{O}_6$; C 67.41, H 5.66. Found; C 67.61, H 5.55%.

BENZYLATION OF 3,5-DIHYDROXYBENZOIC ACID (4) TO THE ESTER 5.—Benzyl bromide (100 g)², dry K_2CO_3 (200 g), and 3,5-dihydroxybenzoic acid (**4**, 50 g) were refluxed in Me_2CO (500 ml) solution for 10 h. Work-up produced an oily residue that crystallized from chilled MeOH to give 89 g of **5** as colorless needles, mp 64-65°; ir ν max (CHCl_3) 1710 cm^{-1} , and no OH bands; $^1\text{H-nmr}$ ($\text{Me}_2\text{CO}-d_6$): δ 5.13 (4 H, s, 2CH_2), 5.35 (2H, s, CH_2), and 7.33 (18 H, m, aromatic protons); ms M^+ at m/z 424 (11%).

Anal. Calcd. for $\text{C}_{28}\text{H}_{24}\text{O}_4$; C 79.23, H 5.69. Found; C 79.18, H 5.74%.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF THE ESTER 5 TO THE ALCOHOL 6.—The ester **5** (50 g) in 250 ml of Et_2O was added gradually to a stirred solution of lithium aluminum hydride (LAH) (24 g) in 1 liter of Et_2O . The mixture was stirred at room temperature for 6 h then worked up to produce **6** (31 g) as colorless needles from Et_2O , mp 79-81°; ir ν max (CHCl_3) 3600 (OH) and 1595 (C=C) cm^{-1} ; $^1\text{H-nmr}$ (CDCl_3) δ 7.33 (10H, s, arom. H of BZO), 7.26 (1H, s, H-4), 6.53 (2H, s, H-2 and H-6), 4.95 (4H, s, 2CH_2 of BZO), and 4.50 (2H, s, $-\text{CH}_2\text{OH}$); ms M^+ at m/z 320 (23%).

Anal. Calcd. for $\text{C}_{21}\text{H}_{20}\text{O}_3$; C 78.73, H 6.29. Found; C 78.58, H 6.39.

PYRIDINIUM CHLOROCHROMATE OXIDATION OF THE ALCOHOL 6 TO THE ALDEHYDE 7.—Pyridinium chlorochromate (21 g) was gradually added to a stirred solution of **6** (21 g) in 200 ml of CH_2Cl_2 . After 6 h, the mixture was worked up to give **7** (18 g) as colorless needles from Et_2O , mp 75-76°; ir ν max (CHCl_3) 1710 (CHO) and 1600 (C=C) cm^{-1} ; $^1\text{H-nmr}$ (CDCl_3) δ 9.78 (1H, s, CHO); ms M^+ at m/z 318 (13%).

Anal. Calcd. for $\text{C}_{12}\text{H}_{18}\text{O}_3$; C 79.23, H 5.70. Found; C 79.00, H 5.92%.

PREPARATION OF THE STILBENE ISOMERS 8.—*p*-Benzyloxybenzyltriphenyl-phosphonium chloride³ (6.4 g) was stirred for 30 min in C_6H_6 solution under N_2 atmosphere with 6.6 ml of 2 M *n*-butyl lithium in *n*-hexane. The aldehyde **7** (2.8 g) was added, the mixture stirred for 4 h, then worked up to yield an oil that was purified by chromatography on silica gel G (40% hexane in C_6H_6 as solvent) to yield 3.16 g of an oily mixture of stilbenes (**8**), Rf 0.75 and 0.66 (silica gel G plates and C_6H_6 as solvent); ir ν max (CHCl_3) 1595 cm^{-1} (C=C) with no OH or CO bands; $^1\text{H-nmr}$ (CDCl_3) δ 5.08 (6 H, s, 3CH_2 of BZ groups) and a multiplet at δ 6.50-7.55 (24H); ms M^+ at m/z 498 (22%).

HYDROGENATION OF THE STILBENE MIXTURE 8 TO 1.—The stilbene mixture **8** (10 g) was dissolved in absolute EtOH, and 5% Pd/C (5 g) was added and the mixture hydrogenated for 10 h at 15 psi. Upon work-up, the oily residue which was obtained crystallized from $\text{Et}_2\text{O}-\text{CHCl}_3$ to give colorless needles.

²The amount of benzyl bromide was found to be very critical for the yield of **5**. Excess benzyl bromide produced mostly 3,5-dihydroxybenzyl benzoate and poor yields of **5**. The kinetics of this reaction are currently under investigation.

³Obtained by refluxing triphenylphosphine (13 g) with *p*-benzyloxybenzyl chloride (11 g) in xylene (100 ml) solution for 10 h. The product (21 g) was obtained as a finely crystalline powder, mp 242-244; ms M at m/z 494 (100%).

dles (3.6 g) of a product indistinguishable from **1** (same mp, undepressed mmp, and superimposable ir and ^1H -nmr spectra).

PREPARATION OF α -MONODEUTERIODIHYDROSTILBENE **1**.—The ester **5** (1.0 g) was reduced with lithium aluminum deuteride (400 mg) as described above. The resulting deuterated alcohol **6** was converted to α -monodeuteriodihydrostilbene **1** using the same sequence of reactions as before to give this compound, colorless needles, ms M^+ at m/z 231 (50%). The ^{13}C -nmr proton-noise decoupled spectrum was identical to **1** except for the splitting of the methylene signal at δ 38.6.

ACKNOWLEDGMENTS

The author would like to thank Dr. Farid J. Al-Muhtadi of the Department of Pharmacognosy, King Saud University, for providing the plant material used in this investigation. The technical assistance offered by Mr. M. Hafez of the same institute was greatly appreciated.

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Received 24 February 1983