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CANNABINEROLIC ACID, A CANNABINOID FROM *CANNABIS SATIVA**

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Key Word Index—*Cannabis sativa*; Moraceae; biosynthesis; cannabinoid; cannabinerolic acid; Δ^1-tetrahydrocannabinolic acid.

Abstract—Investigation of the leaves of *Cannabis sativa* resulted in the isolation of a new cannabinoid, cannabinerolic acid. The structure of the new cannabinoid was established on the basis of spectroscopic and chemical evidence.

INTRODUCTION

Numerous cannabinoids have been hitherto isolated from *Cannabis sativa* L. and their structures have been well characterized [2]. Among them, Δ^1-tetrahydrocannabinol has long been known to be the most psychoactive cannabinoid [3]. Therefore, pharmacological and biological investigations on Δ^1-tetrahydrocannabinol have been rigorously conducted [4–7]. In contrast, only a few studies have been carried out with respect to the biosynthesis of cannabinoids. Our previous investigation showed that Δ^1-tetrahydrocannabinolic acid (**1**), cannabidiolic acid and cannabichromenic acid (**2**) are biosynthesized from cannabigerolic acid (**3**) [8], thus suggesting that **3** plays an important role in the biosynthesis of cannabinoids. We have now isolated a new cannabinoid named cannabinerolic acid (**4**), which seems to be involved in the biosynthesis of **1**, from the leaves of *C. sativa* (Mexican strain). This paper describes the isolation and characterization of the new cannabinoid.

RESULTS AND DISCUSSION

Repeated chromatography of the acetone-soluble fraction of air-dried *Cannabis* leaves (Mexican strain) on silica gel and Fujigel ODS-G3 afforded four cannabinoids (**1**–**4**). The known cannabinoids Δ^1-tetrahydrocannabinolic acid (**1**), cannabichromenic acid (**2**) and cannabigerolic acid (**3**) were identified by comparison of their physical and spectral data with those of authentic samples [9, 10].

Cannabinerolic acid (**4**) gave an orange coloration with diazotized benzidine reagent [9]. The FAB-mass spectrum of **4** exhibited the same $[M - H]^-$ ion peak at m/z 359 as **3**. The ^1H NMR spectrum of **4** showed signals due to a pentyl group [δ0.89 (3H, *t*, $J = 8$ Hz), 1.30 (4H, *m*), 1.59 (2H, *m*), 2.88 (2H, *t*, $J = 8$ Hz)] along with an aromatic signal [δ6.27 (1H, *s*)]; these signal patterns being almost identical with those arising from the olivetolic acid moiety in **3**. In addition, signals attributable to three methyls [δ1.62, 1.74, 1.77 (each 3H, *s*)], three methylenes [δ2.19 (2H, *m*), 2.27 (2H, *m*), 3.53 (2H, *d*, $J = 8$ Hz)] and two olefinic methines [δ5.18 (1H, *t*-like), 5.28 (1H, *t*-like)] were observed, and the assignment of these signals by ^1H–^1H and ^1H–^{13}C COSY spectroscopy revealed the existence of a 3,7-dimethyl-2,6-octadiene moiety in **4**. Accordingly, **4** was found to be closely related to **3**. The signal patterns of the olefinic protons were, however, somewhat different from those [δ5.05 (1H, *m*), 5.29 (1H, *t*-like)] found in **3**, thus suggesting that the configuration of the C-2,3 double bond was different in **3** and **4**. The configuration of this double bond was determined to be *Z* in the case of **4** by NOESY spectroscopy, which clearly indicated the correlation between the methylene protons assignable to H-1 and H-4. Therefore, the 3,7-dimethyl-2,6-octadiene moiety in **4** was found to possess the same configuration as nerol.

In order to confirm definitively the structure of **4**, an attempt was made to prepare this compound. Mechoulam and Yagen reported that cannabigerol (**3a**) can be synthesized by coupling geraniol and olivetol in the presence of *p*-toluenesulphonic acid [11]. Reaction of nerol and olivetol yielded a major product (**4a**). Thereafter, carboxylation of **4a** was carried out with methyl magnesium carbonate [12] to yield **4**. From these spectral and chemical findings, cannabinerolic acid is **4**.

Since our previous investigation of the biosynthesis of cannabinoids revealed that nerol is incorporated into **1** [8], compound **4** may be also involved in the biosynthesis of **1**. We have now purified an enzyme which catalyses the conversion of **4** to **1**.

EXPERIMENTAL

General. Fuji-gel ODS-G3 (43–65 μm) was obtained from Fujigel Hanbai Co., Ltd. Olivetol and nerol were

*Part 23 in the series 'Cannabis'. For Part 22 see ref. [1].
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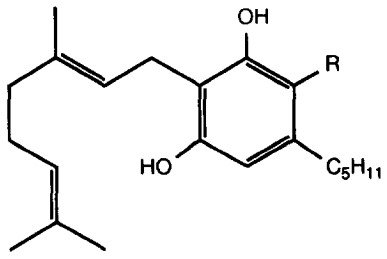
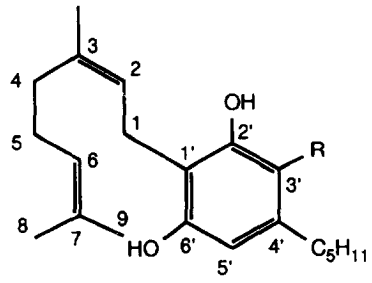


3 : R=COOH
3a : R=H



4 : R=COOH
4a : R=H

purchased from Sigma. The details of the instruments and chromatographic conditions were essentially the same as described in the previous paper [13].

Extraction and isolation. The air-dried leaves (300 g) of *C. sativa* (Mexican strain) were extracted with 2 l of C_6H_6 at room temp. The C_6H_6 extract was concd to dryness by evapn *in vacuo*, and the residue was dissolved in Me_2CO. After removal of insoluble materials (waxes etc.) by filtration, the Me_2CO-soluble portion was concd and applied to a silica gel column. Elution with C_6H_6–Me_2CO (9:1) afforded frs 1 and 2, and further elution with C_6H_6–Me_2CO (1:1) gave fr. 3. Silica gel CC for fr. 1 with *n*-hexane–EtOAc (3:1) gave **1** (4.3 g). Separation of fr. 2 by repeated CC on silica gel with *n*-hexane–EtOAc (3:1) and Fujigel ODS-G3 with MeOH–H_2O (7:3 → 1:9) as solvent system yielded **3** (12 mg) and **4** (2.3 mg). Fr. 3 was sepd by silica gel CC with $CHCl_3$–MeOH–H_2O (20:1:0.01) to afford **2** (153 mg).

Cannabinerolic acid (**4**). Needles (*n*-hexane–EtOAc), mp 132° (Found: C, 73.3; H, 8.9. $C_{22}H_{32}O_4$ requires: C, 73.3; H, 9.0%). Negative FAB-MS *m/z*: 359 $[M-H]^-$. ^{1}H NMR (270 MHz, $CDCl_3$): δ0.89 (3H, *t*, *J* = 8 Hz, H-ω), 1.30 (4H in total, *m*, H-γ and H-δ), 1.59 (2H, *m*, H-β), 1.62 (3H, *s*, H-8), 1.74 (3H, *s*, H-10), 1.77 (3H, *s*, H-9), 2.19 (2H, *m*, H-5), 2.27 (2H, *m*, *H*-4), 2.88 (2*H*, *t*, *J* = 8 Hz, H-α), 3.53 (2H, *d*, *J* = 8 Hz, H-1), 5.18 (1H, *t*-like, *J* = 8 Hz, H-6), 5.28 (1H, *t*-like, *J* = 8 Hz, H-2), 6.27 (1H, *s*, H-5'); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ14.1 (C-ω), 17.7 (C-8), 21.8 (C-1), 22.5 (C-δ), 23.5 (C-10), 25.8 (C-9), 26.4 (C-5), 31.4 (C-β), 32.0 (C-4 and C-γ), 36.5 (C-α), 103.3 (C-3'), 111.2 (C-5'), 111.7 (C-1'), 122.1 (C-2), 123.8 (C-6), 132.4 (C-7), 139.1 (C-3), 147.5 (C-4'), 160.3 (C-6'), 163.6 (C-2'), 176.3 (CO_2H).

Preparation of compound **4**. A mixture of olivetol (1.2 g), nerol (1.1 g) and *p*-toluenesulphonic acid (250 mg) in $CHCl_3$ (250 ml) was stirred at room temp. for 12 hr in the dark. The reaction mixture was washed with satd $NaHCO_3$ soln and then repeatedly with H_2O. The $CHCl_3$-soluble fraction was concd *in vacuo* and applied to a silica gel column. The column was eluted with *n*-hexane–EtOAc (39:1) to give **4a** (563 mg) as needles (*n*-hexane), mp 115°. Negative FAB-MS *m/z*: 315 $[M-H]^-$. ^{1}H NMR (270 MHz, $CDCl_3$): δ0.87 (3H, *t*, *J* = 8 Hz, H-ω), 1.32 (4H, *m*, H-γ and H-δ), 1.56 (2H, *m*, H-β), 1.64 (3H, *s*, H-8), 1.74 (3H, *s*, H-10), 1.77 (3H, *s*, H-9), 2.15 (2H, *m*, H-5), 2.27 (2H, *m*, H-4), 2.45 (2H, *t*, *J* = 8 Hz, H-α), 3.38 (2H, *d*, *J* = 8 Hz, H-1), 5.12 (1H, *t*-like, *J* = 8 Hz, H-6), 5.28 (1H, *t*-like, *J* = 8 Hz, H-2), 6.24 (2H, *s*, H-3' and H-5'). Carboxylation of **4a** (120 mg) was conducted according to the method described in ref. [12] to afford **4** (48 mg).

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