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ISOLATION AND IDENTIFICATION OF NEW CANNABINOIDS IN CANNABIS SMOKE

D. P. PAPADAKIS† and C. A. SALEMINK
Department of Organic Chemistry of Natural Products, University of Utrecht, Netherlands

and

F. J. ALIKARIDIS and T. A. KEPHALAS
Department of Biological Chemistry School of Medicine, University of Athens, Greece

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Abstract—Three new cannabinoids, furo[1,2-a] 4-n-pentyl-7,7,10-trimethyl-dibenzopyran **1**, 2-methylfuro[1, 2-a] 4-n-pentyl-7,7,10 trimethyl-dibenzopyran **2** and 2,3 dimethylfuro(1,2-a)-4-n-pentyl-7,7,10 trimethyl-dibenzopyran **3** were identified in the sublimate of cannabis resin smoke by spectroscopic methods. Spectral data of dehydrocannabifuran **4** are also presented.

As reported earlier,[1] a mixture of unknown cannabinoid-like compounds was detected by GC–MS in the n-hexane extract of cannabis smoke sublimate. From the above mixture in the present study the isolation and the determination of the structure of two new cannabinoids **1** and **2** is reported. The structure of a third component **3** is also proposed. The mass spectra of the three compounds are similar with respect to fragment ions and their relative intensities. A difference of 14 mass units among the molecular ions strongly suggests that the three compounds are homologues.

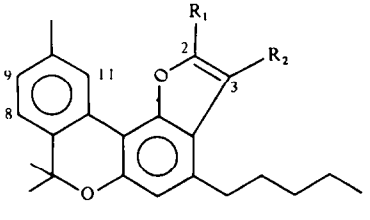
The base peak in the spectra of the compounds is the fragment ion $M^+ - 15$, which corresponds to the elimination of a methyl group mainly from the geminal methyl groups of the molecule. Expulsion of a butyl radical from the pentyl side of chain of the base peak leads to the $M^+ - 72$ fragment.[2] Unsuccessful attempts to acetylate or to silylate the compounds **1** and **2** suggest the absence of hydroxyl groups.

The IR spectra of the compounds, which were similar, confirm the absence of hydroxyl group. The absorption bands at 1390 and 1380 cm^{-1} indicate gem Me groups. The strong absorption bands at 1055 cm^{-1} and 1140 cm^{-1} probably reveal the presence of ether functions.

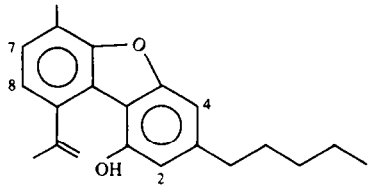
The NMR spectra of both compounds **1** and **2** are similar to that of CBN **5**. From Table 2 there is no doubt that at least the fundamental structure of the two molecules is similar to that of CBN and that there is a difference in the olivetol moiety of their structural formulae.

The resonances of the CBN protons at δ 6.24 ppm and δ 5.44 ppm, which correspond to a m-H olivetyl and a hydroxyl proton respectively are missing from the spectra of compounds **1** and **2**. In the spectra of **1**, two doublets appear at δ 6.72 ppm (H, J = 1 Hz) and δ 7.62 ppm (H, J = 1Hz) which are assigned to the two

†Present address: Department of Biological Chemistry, School of Medicine, Goudi(609), Athens, Greece.



(1) R_1 : H R_2 : H
(2) R_1 : CH_3 R_2 : H
(3) R_1 : CH_3 R_2 : CH_3



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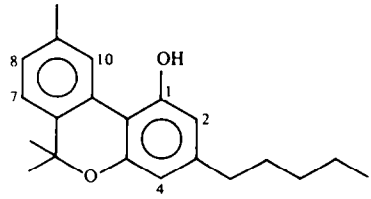
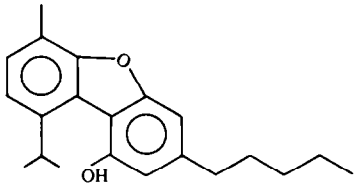


(5)



(6)

Table 1. Mass spectra of compounds (1), (2) and (3)

	Compound (1) (% int)	Compound (2) (% int)	Compound (3) (% int)
M^+	334 (22%)	348 (28%)	362 (32%)
M^+-15	319 (100%)	333 (100%)	347 (100%)
M^+-57	277 (3%)	291 (2%)	305 (2%)
M^+-59	275 (2%)	289 (3%)	303 (2%)
M^+-72	262 (16%)	276 (14%)	290 (12%)
M^+-87	247 (8%)	261 (10%)	275 (9%)

Table 2. Proton chemical shifts of compounds CBN, (1) and (2)

	CBN	Compound (1)	Compound (2)
ω-CH_3	0,89 (t)	0,85 (t)	0,90 (t)
$(CH_2)_3$ -pentyl chain	1,20-1,40 (m)	1,20-1,40 (m)	1,20-1,40 (m)
-CH_2 benzylic	2,49 (t)	2,75 (t)	2,75 (t)
-CH_3 aromatic	2,39 (s)	2,35 (s)	2,45 (s)
gem-CH_3	1,61 (s)	1,61 (s)	1,63 (s)
C^1(11)-H	8,20 (s)	8,30 (s)	8,30 (s)
C^2(8)-H, C(9)-H	7,10 (s)	7,13 (s)	7,13 (s)
m-H olivetyl	6,24 (d)	-	-
m-H olivetyl	6,45 (d)	6,68 (s)	6,68 (s)
OH-group	5,44	-	-
α-Furo-H	-	7,62 (d)	-
β-Furo-H	-	6,72 (d)	6,38 (m)
β-Furo-CH_3	-	-	2,53 (d)

1. The chemical shift for the C(11)-proton corresponds to C(10) proton of CBN according to the dibenzopyran nomenclature.

2. The chemical shifts for the C(8) and C(9) protons coresponds to C(7) and C(8) protons of CBN.

coupled protons of the furan ring. The resonance at the lower field corresponds to the α-proton of the furan ring. In compound **2** the α-proton of the furan ring is replaced by a Me group. This is confirmed by the fact that the doublet at δ 7.62 ppm is missing and a doublet corresponding to three protons is observed at δ 2.53 ppm (J = 1 Hz) coupled to a proton at δ 6.38 ppm.

According to the similarities of their mass spectra, mentioned above, the structure proposed for compound **3** is that of the dimethyl homologue of compound **1** resulting from substitution of the two hydrogens of the furan ring.

EXPERIMENTAL

Instrumentation

All mass spectrometrical measurements were performed using a Jeol JMS-D 300/Hewlett Packard 5710A GCMS combined with an on-line JMA-2000 data system, nominally operating at 70 eV electron energy and 50–70° ion chamber temp. Elemental compositions were derived from element tests obtained by on-line measurement of exact masses with the AEI-MS 902-Argus 500 computer combination at a dynamic resolving power of 10,000. NMR spectra were recorded on a Bruker spectrospin 90 MHz with FT or a Varian EM-390 90 MHz spectrometer depending upon the quantity. IR spectra were taken with a Perkin–Elmer 257 IR spectrophotometer introducing the sample as a thin film between NaCl plates. Gas chromatography was carried out with a Becker 409 equipped with FID. For analytical work a glass column 3 mm × 2 m 3% OV-17 on Chromosorb WHP 100/120 mesh was used, col. temp. 230°. For preparative work a glass column 6 mm × 2 m 10% SE-30 on Chromosorb GHP 80/100 mesh was used, col. temp. 250°. In general, the injection temperature was 270°, whereas the detector operated at 320°. For the collection of the sublimate of the cannabis resin smoke, an Ethel MK II cigarette smoking machine was used. The frequency of puffs was regulated to one puff per min, with a duration of 8 s for each puff.

Materials

The cannabis resin used was the United Nations reference sample UNC 351. All solvents used were purified by elution over Al_2O_3 (neutral, activity V) followed by distillation. A detailed description of the experimental device used for the smoking of cannabis resin as well as for the separation of the sublimate fractions has been reported elsewhere.[1,3] Sublimate (5 g) was

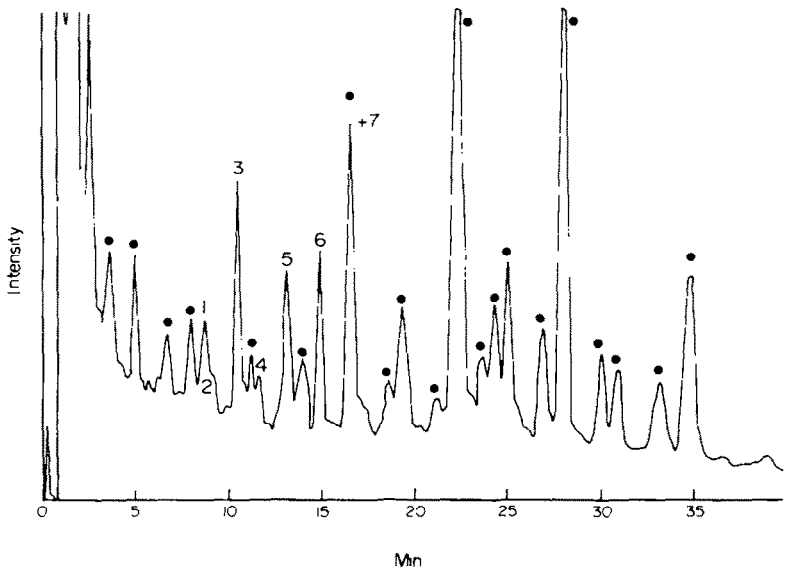


Fig. 1. Gas chromatogram of the yellow zone. The black spots in the top of the peaks correspond to the hydrocarbon like substances. The peaks with numbers correspond to cannabinoid like compounds. Conditions: Col. SE-30. Programme 200°–250°, 2° min

chromatographed on silica gel G (Merck 7734) column (60 × 3 cm) using a mixture of n-hexane–ether (99:1) as eluent. The mixture of the less polar substances, which was eluted from the column as a yellow band, was rechromatographed through a silica gel G (Merck 7731) column (20 × 2 cm) using the same eluent. Fractions of 5 ml were collected and examined by GC. The similar fractions were combined.

The combined fraction 31–43 (28 mg) was chromatographed on TLC plates (Merck 5715) using a mixture of n-hexane–diethylamine (97:3) as eluent. Two bands (Rf: 0.33, 0.47) were separated and observed under UV. The lower one, which was also stained violet after spraying with Fast Blue B salt, consisted of pure dehydrocannabifuran[4] (2 mg). GC: R_f: 21.3 min, R_x: 1.42 (R_{CBD}: 1). High resolution mass measurement: Found 308.1779; Calc. for $C_{21}H_{24}O_2$ 308.1776.

M/S *m/e* (%): 308 (M^+, 77), 293(6), 266(9), 265(12), 253(18), 252(100), 251(50), 237(16), 235(10), 223(10), 208(12). PMR(C_3D_6O) δ: 0.89 (3H, t, J = 6 Hz) ω-CH_3; 1.10 – 1.40 (6H, m) $(CH_2)_3$-pentyl chain; 2.73 (2H, t, J = 8 Hz)-CH_2 benzylic; 5.24 (1H, m) 5.65 (1H, m) (C=CH_2); 2.59 (3H, d, J = 1 Hz)CH_3 aromatic; 2.31 (3H, dd, J = 1 Hz), CH_3 isopropylene; 6.67 (1H, d, J = 2Hz) m-H olivetyl; 7.03 (d, J=2Hz), 7.11 (d, J = 2 Hz) and 7.21 (d, J = 2 Hz) 7.34 (d, J = 2 Hz) overlapping resonances which corresponds to four protons. Decoupling experiments showed that the resonances consist of an AB pattern of C(8) and C(7) respectively as well as to the m-H-olivetyl (7.03 ppm) and to the hydroxyl proton (7.11 ppm). IR (cm^{-1}): 3555 (OH), 1640, 1600, 1580, 1375, 1315, 1060, 1010, 910, 815.

Silylation and acetylation of dehydrocannabifuran led to the formation of derivatives with MW 380 (R_x: 1.83) and 350(R_x: 2.15) respectively.

Hydrogenation of dehydrocannabifuran led to the formation of cannabifuran **6** (R_x: 1.77).

Isolation of compound **1**

The upper band (R_f: 0.47), which was separated from the above mentionned preparative TLC, consisted of a mixture of compounds. Preparative TLC, consisted of a mixture of compounds. Preparative GC led to the isolation of pure compound **1** (1.5 mg). GC: R_t: 28.2 min, R_x: 1.91 (R_{CBD}: 1). High resolution mass measurement: Found 334.1927; Calc for $C_{23}H_{26}O_2$ 334.1933. IR (cm^{-1}): 1620, 1610, 1540, 1500, 1390 and 1380 (gem-Me), 1360, 1195, 1155, 1140, 1130, 1090, 1055, 1030, 870, 810.

Isolation of compound **2**

The column fraction 56–67 (11.2 mg) containing compounds **2** and **3** as well as a number of hydrocarbons, was subjected to preparative GC. Compound **2** was isolated in a pure form (1.5 mg). GC: R_t 34.8 min, R_x: 2.30 (R_{CBD}: 1). High resolution mass measurement : Found 348.2090, Calc for $C_{24}H_{28}O_2$ 348.2089. IR (cm^{-1}): 1625, 1610, 1590, 1500, 1395, and 1380 (gem-Me), 1360, 1195, 1155, 1125, 1100, 1050, 870, 810.

A few μg of the sample were dissolved in dried over KOH pyridine (10 μl) and silylated by BSTFA (5 μl) containing 1% trimethylchlorosilane at room temperature for 2 h. The reaction product was analysed by GCMS.

A few μg of the sample were dissolved in dried over KOH pyridine (3 μL) and acetylated by acetic anhydride (7 μl) at room temperature for 24 h. The reaction product was analysed by GC–MS.

Dehydrocannabifuran (1 mg) was disolved in ethanol (3 ml) and a few mg of PtO_2 was added to the solution. The mixture was hydrogenated under pressure. Quantitative hydrogenation required 48 h. Hydrogenation using acetic acid and Pd/C was unsuccessful.

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