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CANNABISPIRONE AND CANNABISPIRENONE, TWO NATURALLY OCCURRING SPIRO-COMPOUNDS

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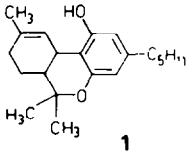
Abstract—Structure elucidation of two naturally occurring acidic compounds from Cannabis gave two spiro-compounds. The spectroscopic properties (^{1}H-NMR, ^{13}C-NMR, MS, IR and UV) and interpretation of these spectra are given.

The chemical research on hemp, *Cannabis sativa* L., resulted in the isolation and structure elucidation of Δ^1-THC (1) and many well known related compounds.[1] These so-called cannabinoids can be thought to be derived from 5-alkyl-2-geranyl-resorcinol-4-carboxylic acid (2),[2] in which the side-chain is methyl, n-propyl or n-pentyl. Decarboxylation easily takes place. Except for the carboxylic acid derivatives, the cannabinoids are very poorly soluble in aqueous alkaline solution.



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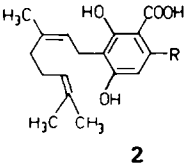

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TLC analysis of various hemp samples revealed the presence of some compounds which were more polar than the usual cannabinoids. Isolation of these polar components was carried out from hemp cultivated in France from seeds of South African origin.[3] The major cannabinoids of this material were Δ^1-THC-C_5 acid and Δ^1-THC-C_3 acid and the corresponding decarboxylated products.[4] Preliminary experiments indicated that the desired polar compounds were soluble in aqueous alkali and it was thus thought that the compounds were carboxylic acids of cannabinoidal nature.

Isolation and purification (see below) yielded two pure products (A and B, 0.003 and 0.001%, resp.). The assumption that the compounds were cannabinoid acids was opposed by the finding that after heating the products for 1 hr at 110°—a temperature at which the cannabinoid acids decarboxylate—the R_f values of A and B remained unchanged. Moreover, the patterns of the NMR spectra indicated that we were dealing with compounds, totally different from cannabinoids. The complete absence of methyl functions except for one methoxy group was incompatible with structures of the cannabinoidal type.

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The elemental compositions of compounds A and B were determined by high resolution mass spectrometry: $C_{15}H_{18}O_3$ and $C_{15}H_{16}O_3$, resp. Reduction of B with hydrogen and a palladium catalyst yielded a compound, $C_{15}H_{18}O_3$, identical with A according to TLC, GLC and MS-analysis. Thus, the presence of an extra double bond in B is the only difference between the compounds.

The structures of A and B could be established from their NMR spectra, supported by UV and IR spectroscopic data. The ^{1}H-NMR spectrum of A (Fig. 1) strongly indicated the presence of a four-fold substituted aromatic nucleus. The resonance positions of the two, meta-coupled, aromatic protons suggested a 4,5-carbon substituted resorcinol derivative. One of the OH groups then has to be methylated (signal at δ3.72 ppm); the presence of the other phenolic group is supported by the acidity of the compound and a signal at δ6.4 ppm (in $CDCl_3$). This structural element was confirmed by ^{13}C-NMR, since the observed resonance positions of the aromatic carbon atoms (Table 1) closely resemble the shifts, calculated for this structure from the substituent effects given by Levy.[5]

On examining the remaining signals in the carbon spectrum the signal at δ214.0 ppm has to be explained as being a CO group, not conjugated with the aromatic nucleus. The other signals are all aliphatic in nature. With the knowledge of the multiplicity of the signals as determined from the off-resonance ^{1}H-decoupled ^{13}C-NMR spectrum they have to be assigned to two times two identical CH_2-carbons (δ34.3 and 38.9 ppm, double intensity), two other CH_2-carbons (δ30.9 and 35.5 ppm) and one quaternary C atom (δ47.6 ppm). Therefore, two alicyclic rings have to be present, connected through only one C atom, so compound A is a spiro-compound (cf. Ref. 6).

The two signals at δ30.9 and 35.5 ppm in the ^{13}C-spectrum could be ascribed to two methylene carbons which are also revealed by the ^{1}H-spectrum by two inter-coupled triplets (A_2B_2) of 2 H each appearing at δ2.93 and 2.25 ppm. Due to the position in the ^{1}H-spectrum, this ethylene moiety has to be attached directly to the aromatic nucleus while at the other side either a CO group or a quaternary C atom must be present.

In the ^{1}H-spectrum, the correlation between the signals at δ2.70 (H_1), 2.53 (H_2), 2.36 (H_3) and 1.82 (H_4) ppm was found by decoupling experiments to be an ABCD-system

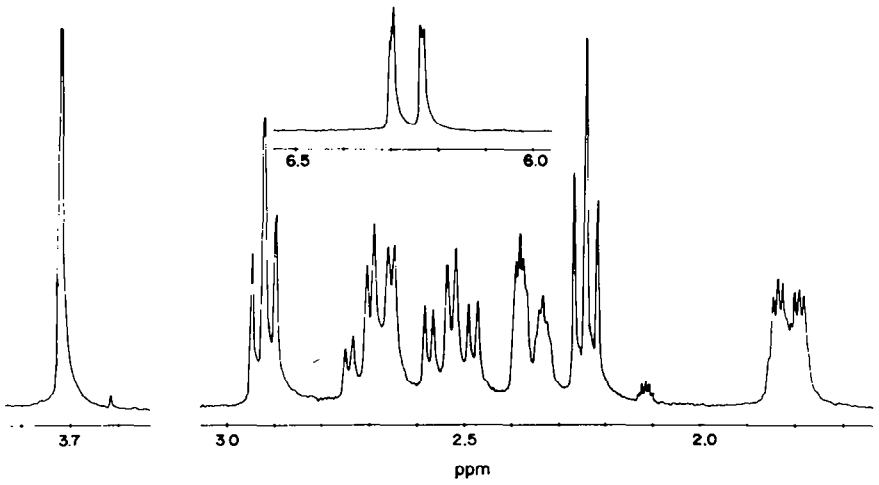


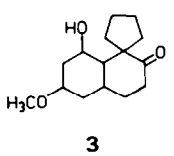
Fig. 1. 300 MHz ^{1}H-NMR spectrum of compound A, taken in $CDCl_3$.

Table 1. The ^{13}C-NMR spectra of compounds A and B, taken in $CDCl_3$*

Carbon atom	compound A			compound B		
	ppm	signal height	multiplicity	ppm	signal height	multiplicity
1	47.6	58	s	48.0	71	s
2	35.5	130	t	35.5	113	t
3	30.9	130	t	31.0	80	t
4	101.9	132	d	102.0	94	d
5	160.3	32	s	160.8	59	s
6	100.8	108	d	100.5	99	d
7	153.3	42	s	153.3	40	s
8	126.8	30	s	125.6	22	s
9	146.3	44	s	146.4	53	s
10	55.4	108	q	55.3	90	q
2'	34.3	122	t	158.5	98	d
3'	38.9	129	t	127.2	97	d
4'	214.0	40	s	200.6	36	s
5'	34.3	122	t	31.0	80	t
6'	38.9	129	t	35.3	135	t

*) The spectra were referred to $CDCl_3$ at 77.0 ppm, relative to TMS. Peak heights were determined for the conditions: total delay time 1.2 sec, flip angle 70°.

with coupling constants $J_{1,2} = 14.0$ Hz, $J_{1,3} = 4.7$ Hz, $J_{1,4} = 13.1$ Hz, $J_{2,3} = 14.5$ Hz, $J_{2,4} = 5.5$ Hz and $J_{3,4} = 2.7$ Hz. Interpretation results in a system of two C atoms, one with H_1 and H_4 and one with H_2 and H_3. Because each multiplet represented an integrated area of two protons there must be a place of symmetry in this part of the molecule resulting in two identical ABCD-patterns. This confirms the conclusion obtained from the ^{13}C-spectrum. Combination of all the spectral data leads to two structural proposals, 3 and 4. The positions of the OH and OMe groups will be discussed below.



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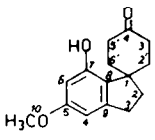

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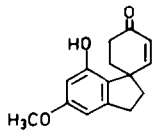

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Definite proof of the structure could be obtained through the spectroscopic properties of compound B. The double bond, introduced in A to give B, has to be conjugated with the CO function. This can be concluded from the shift of the CO absorption from 1690 cm^{-1} in the IR spectrum of A to 1645 cm^{-1} in the spectrum of B. Also the carbonyl signal at δ200.6 ppm and the double bond signals at δ158.5 and 127.2 ppm in the ^{13}C-NMR spectrum of B implicate an α,β-unsaturated ketone. The presence of four different atiphatic CH_2-carbons in the ^{13}C-spectrum of B already denotes 5 as the structure of B and not the corresponding unsaturated compound of 3. Moreover, the UV spectrum of B shows the absence of conjugation of the unsaturated part with the aromatic nucleus. In addition, the ^{1}H-NMR spectrum of B still shows the presence of two benzylic protons (δ2.9 ppm). The A_2B_2-pattern in the spectrum of A was changed into an ABCD-pattern in B, because the spiro quaternary carbon atom has become asymmetric.

The positions of the resonances at δ6.91 and 5.71 ppm are in good agreement with those for 2-cyclohexenone (δ6.91 and 5.88 ppm).[7] The long-range coupling in the signal at δ6.91 ppm was refound in the multiplet at δ2.0 ppm as was shown by decoupling experiments. An explanation for this coupling can be found in the Dreiding model of the structure. The H_2' and one of the H_6' atoms are in so-called W-position which enables the possibility for a long-range 4δ-bond coupling of 1.5–2 Hz.[8]

The configuration of the left part of the structure proposals for A and B was found in the ^{1}H-NMR and the IR-spectrum. The long-range coupling on the signal of one aromatic H-atom which was refound in the broadening of the signal at δ2.93 ppm, is only explainable when position 4 is unsubstituted. The very low absorptions for the carbonyl group in the IR spectra of both compounds implicate strong internal H-bonding with the phenolic group. The H-bonding is only possible when the OH group is situated on the 7 position.

As the final conclusion we propose 7-hydroxy-5-methoxyindan-1-spiro-cyclohexan-4'-one (4) and 7-hydroxy-5-methoxyindan-1-spiro-(2'-cyclohexen-4'-one) (5) as the structures for the compounds A and B. We suggest the trivial names Cannabispirone for compound A and Cannabispirenone for compound B.

The proposals for the molecular structures can be fully accounted for by their mass spectra (Table 2) and the fragmentation schemes (Figs. 3 and 4). Spiroalkanones give ring opening at one of the bonds of the quaternary C atom or at the bond adjacent to the CO function.[9] The

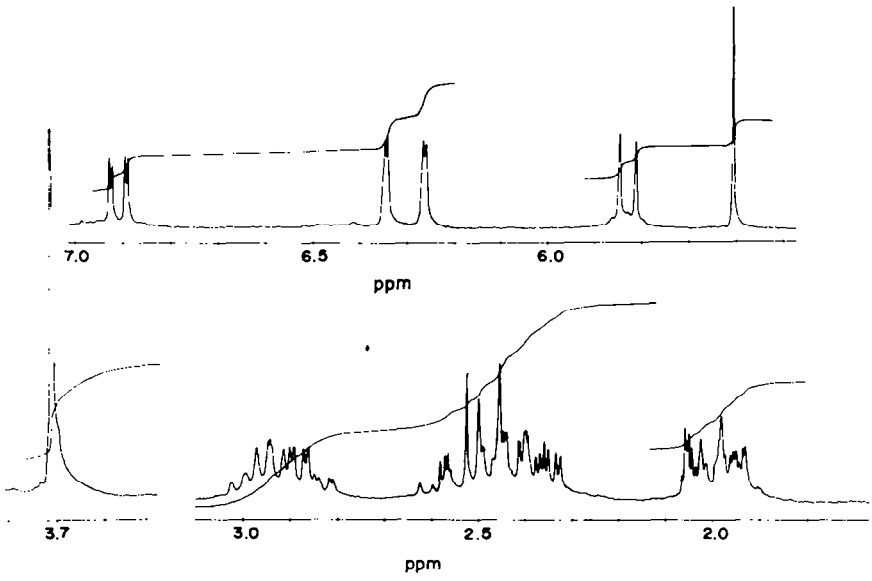

Fig. 2. 300 MHz ^{1}H-NMR spectrum of compound B, taken in acetone-d_6.

Table 2. High resolution mass spectra of compounds A and B

m/e	relative intensity A	B	composition
246	18		$C_{15}H_{18}O_3$
244		85	$C_{15}H_{16}O_3$
243		2.0	$C_{15}H_{15}O_3$
229		1.5	$C_{14}H_{13}O_3$
216		5.2	$C_{14}H_{16}O_2$
		7.1	$C_{13}H_{12}O_3$
202		2.5	$C_{13}H_{14}O_2$
201		7.8	$C_{13}H_{13}O_2$
190	9.2		$C_{12}H_{14}O_2$
189	27	100	$C_{12}H_{13}O_2$
187	1.3	74	$C_{12}H_{13}O_2$
176	100	3.0	$C_{11}H_{12}O_2$
175	5.3	2.0	$C_{11}H_{11}O_2$
174	9.1	8.2	$C_{11}H_{10}O_2$
173		6.0	$C_{11}H_9O_2$
163	3.6	5.3	$C_{10}H_{11}O_2$
161	3.7	10	$C_{10}H_9O_2$
145		4.8	$C_{10}H_9O$
144		4.3	$C_{10}H_8O$
131	1.7	4.3	C_9H_7O
128	1.9	7.0	$C_{10}H_8$
115	2.9	15	C_9H_7
91	2.3	8.0	C_7H_7
77	2.3	4.0	C_6H_5

fragmentation behaviour also excluded structure 3 for compound A, since primary fragmentation of compound A always involves the elimination of a neutral containing the CO moiety.

The larger stability of compound B, caused by the extra double bond, is reflected in its molecular ion abundance. This double bond also prevents fragmentation via the McLafferty rearrangement as observed in compound A. The formation of the ion at *m/e* 187 deserves attention. It is not produced in a single step $C_3H_5O^{\cdot}$ loss from the molecular ion, but it turned out to originate from *m/e* 216. This ion appeared to be a doublet, produced by CO and ethylene elimination from the molecular ion. Without specific labelling experiments it cannot be ascertained whether one or both components of this doublet contribute to the *m/e* 187 formation.

Finally, the corresponding alcohol of cannabispirone is present in nature as well. During the isolation procedure we already observed a compound which gave a front shoulder (relative retention time 1.00 towards cannabidiol) on the peak of cannabispirenone in the gas chromatogram. On reducing cannabispirone with LAH we obtained the two expected stereo-isomers with RRT 1.04 (95%) and 0.98 (5%), so it is very acceptable that the mentioned shoulder is the alcohol.

EXPERIMENTAL

^{1}H-NMR spectra were recorded on a Varian HR 300 instrument equipped with a FT accessory. The ^{13}C-NMR spectra were recorded on a Varian XL 100/15 FT and on a Varian CFT 20. High resolution mass spectra were recorded with an AEI-MS 902 mass spectrometer, nominally operating at 70 eV and 60°C ion chamber temp. Samples were introduced via the direct inlet system. Elemental compositions were derived from element lists obtained by on line measurements with the AEI-MS 902–Argus 500 computer combination at a dynamic resolving power of 10,000. Metastable transitions have been traced by the defocussing technique of Jennings.

The IR spectra were recorded on a Perkin Elmer 377 spectrophotometer.

Dried leaves of hemp (5 kg), cultivated in France from South African seeds (UNC 255) were extracted exhaustively with MeOH. This soln was concentrated and the residue was mixed up with silica gel. This mixture was transferred to two columns of silica gel (75 × 6.4 cm). Gradient elution with hexane-diethyl ether afforded a rough separation. The fractions with the polar components were combined and concentrated. The residue was dissolved in diethyl ether and extracted with 5% NaOH. The aqueous soln was acidified with H_2SO_4 and extracted with diethyl ether. The ethereal soln was dried on Na_2SO_4 and evaporated. The residue was chromatographed several times on columns of silica gel Merck with mixtures of 12% dioxane in hexane. Three constituents could be isolated of which the compounds A and B in a pure form.

Compound A (*cannabispirone*). M.p.: 181–182° (recrystallized from hexane-ethyl acetate). Chromatography: relative retention time 1.30 towards cannabidiol on a column of 3% OV-17 on

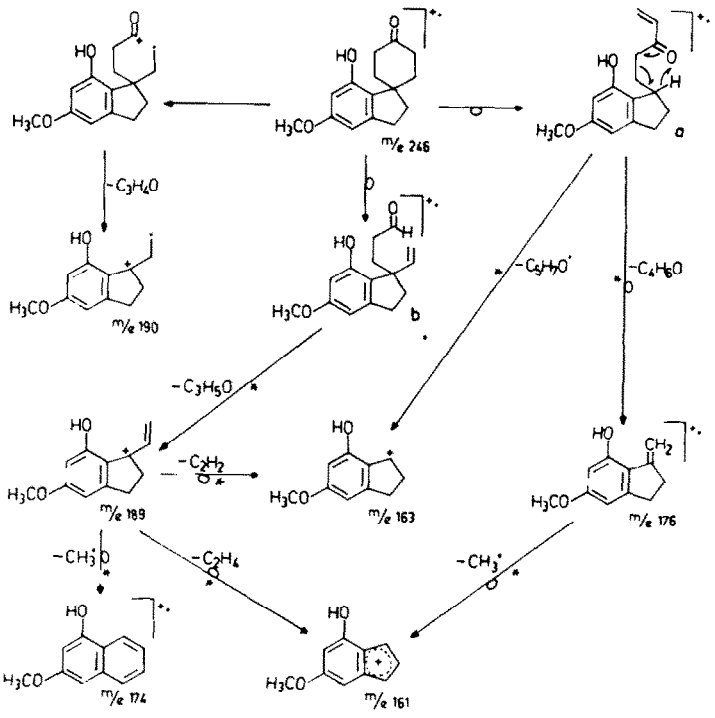

Fig. 3. Proposed fragmentation scheme of compound A.

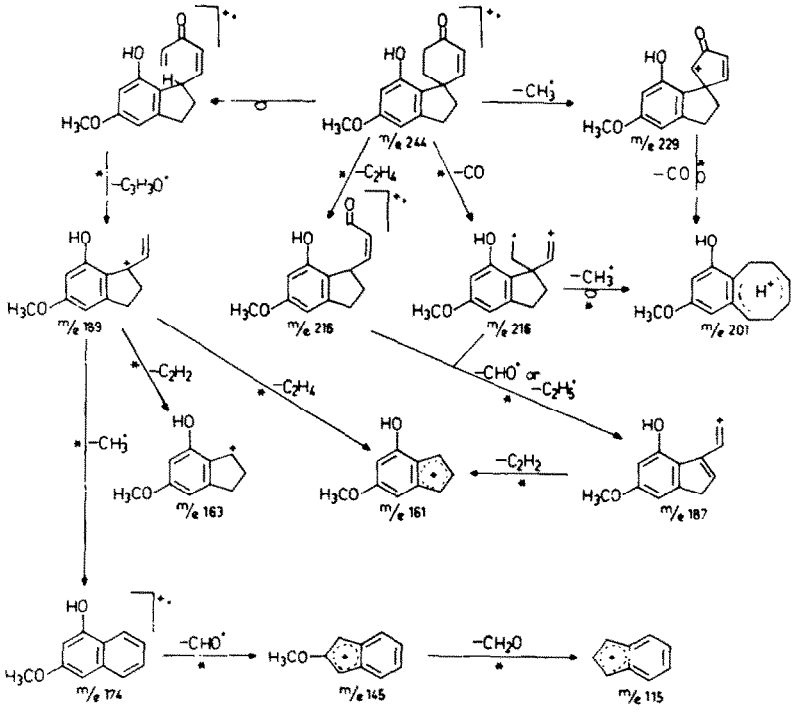

Fig. 4. Proposed fragmentation scheme of compound B.

Chromosorb G AW-DMCS 80-100 mesh. Gives a bright red colour with Fast Blue salt B.

IR spectrum (KBr): 3370, 3300, 2960, 1690, 1618, 1595, 1505, 1450, 1330, 1295, 1240, 1215, 1195, 1150, 1055, 1035, 940, 835 and 740 cm^{-1}.

300 MHz ^{1}H-NMR spectrum (in $CDCl_3$): δ6.38 (s, OH), 6.35 (t, 1H), 6.18 (d, 1H), 3.74 (s, 3H), 2.93 (t, 2H), 2.68 (sextet, 2H), 2.53 (sextet, 2H), 2.44 (m, 2H), 2.22 (t, 2H) and 1.83 (m, 2H) ppm.

300 MHz ^{1}H-NMR spectrum (in acetone-d_6): δ7.35 (s, OH), 6.30 (t, 1H), 6.24 (d, 1H), 3.69 (s, 3H), 2.89 (t, 2H), 2.65 (sextet, 2H), 2.56 (sextet, 2H), 2.26 (t, 2H), 2.23 (m, 2H) and 1.78 (m, 2H) ppm.

300 MHz ^{1}H-NMR spectrum (15% acetone-d_6 in $CDCl_3$): δ7.95 (s, OH), 6.31 (t, J = 2.2 and 1.0 Hz, 1H), 6.25 (d, J = 2.2 Hz, 1H), 3.72 (s, 3H), 2.93 (t, J = 7.5 Hz, 2H), 2.70 (sextet, J = 14.0, 13.1 and 4.7 Hz, 2H), 2.53 (sextet, J = 14.5, 14.0 and 5.5 Hz, 2H), 2.37 (m, J = 14.5, 4.7, 2.7 and 2.5 Hz, 2H), 2.25 (t, J = 7.5 Hz, 2H) and 1.82 (m, J = 13.1, 5.5, 2.7 and 2.5 Hz, 2H) ppm.

^{13}C-NMR spectrum (in $CDCl_3$): see Table 1.

Mass spectrum: see Table 2.

UV spectrum (in ethanol): $\lambda_{max}(\epsilon)$ 213 (17,250), 225 (11,635), 279 (2000), *and* 283 (2205) *nm*.

Compound B (*cannabispirenone*). M.p.: 173–174° (recrystallized from hexane-ethyl acetate).

Chromatography: relative retention time 1.10 towards cannabidiol on a column of 3% OV-17 on Chromosorb G AW-DMCS 80-100 mesh. Gives a bright red colour with Fast Blue salt B.

IR spectrum (KBr); 3200, 3000, 2940, 1645, 1615, 1595, 1505, 1465, 1440, 1330, 1300, 1195, 1140, 1070, 1050, 1030, 925, 850, 830 and 780 cm^{-1}.

300 MHz ^{1}H-NMR spectrum (in acetone-d_6): δ6.91 (double doublet, J = 10.0 and 1.9 Hz, 1H), 6.34 (t, J = 1.9 and 1.2 Hz, 1H), 6.26 (d, J = 1.9 Hz, 1H), 5.83 (d, J = 10.0 Hz, 1H), 5.61 (s, OH), 3.71 (s, 3H), 2.9 (m, 2H), 2.4 (m, 4H) and 2.0 (m, 2H) ppm.

^{13}C-NMR spectrum (in $CDCl_3$): see Table 1.

^{13}C-NMR spectrum (in acetone-d_6; CD_3 at δ29.2 ppm relative to TMS): 32.2($C_{3'}$ or C_3), 32.4 (C_3 or $C_{3'}$), 36.7 ($C_{6'}$ or C_2), 36.8 (C_2 or $C_{6'}$), 49.6 (C_1), 56.1 (C_{10}), 101.7 (C_6), 102.6 (C_4), 127.6 ($C_{3'}$) and 159.5 ($C_{2'}$) ppm. The other signals were not measured.

Mass spectrum: see Table 2.

UV spectrum (in ethanol): $\lambda_{max}(\epsilon)$ 213 (18,240), 232 (19,495) and 284 (2900) nm.

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