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CANNABIS—VIII

PYROLYSIS OF CANNABIDIOL. STRUCTURE ELUCIDATION OF THE MAIN PYROLYTIC PRODUCT

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Abstract—GLC analysis of the products obtained by pyrolysis of cannabidiol in air at 700° revealed the formation of several components, which are not only the result of a mere cracking process. A peak with a retention time corresponding to the one of Δ1(2)tetrahydrocannabinol has been analysed by mass spectrometry. Next to at least two components with a molecular weight of 314 ($C_{21}H_{30}O_2$), possibly including a small amount of Δ1(2)tetrahydrocannabinol, the major component was shown to have the molecular formula $C_{21}H_{30}O_3$. The structure of this oxidation product of cannabidiol has been established as the decarboxylated product of the naturally occurring cannabielsoic acid A by the identity of its mass spectrometrical fragmentation pattern to that of one of the two decarboxylated cannabielsoic acid A C1-stereoisomers, obtained by photochemical oxidation of cannabidiolic acid.

Since cannabis is known to produce stronger and more immediate effects when smoked—this being in western countries the usual way of administration—than when orally taken in similar amounts, it is important to investigate the products, which are obtained after the smoking process instead of concentrating at the pharmacology of the natural constituents. In our opinion however, the complexity of the starting material hardly allows a proper characterization of changes occurring during pyrolysis. Existing contradictions in literature on this subject[1-4] may in part be ascribed to the complex nature of cannabis or its pyrolysate.

Therefore we decided to first investigate the pyrolysis of single cannabis constituents, noting the inherent differences of this choice as compared to the actual smoking process. Since many cannabis samples are characterized as being of phenotype 2,[5] cannabidiol (CBD) should be considered as an important product for detailed study. Moreover, the structure of CBD suggests a susceptibility for conversion into the psychotomimetic Δ1(2)tetrahydrocannabinol (Δ1(2)THC), as was earlier pointed out[2] in order to explain the increased pharmacological activity of a CBD preparation after smoking. Pyrolysis of pure isolated† (−)-3,4-*trans*-CBD in the apparatus described‡ (Experimental) produced a mixture of products as represented in Fig 1.

†We preferred to use isolated material in order to exclude a possible effect of stereochemical differences with synthetical products.

‡These experiments have also been performed by burning pre-extracted cotton wool, treated with CBD solutions. Thin layer chromatography on this pyrolysate shows a great resemblance to that of a mixture obtained with the procedure described above.

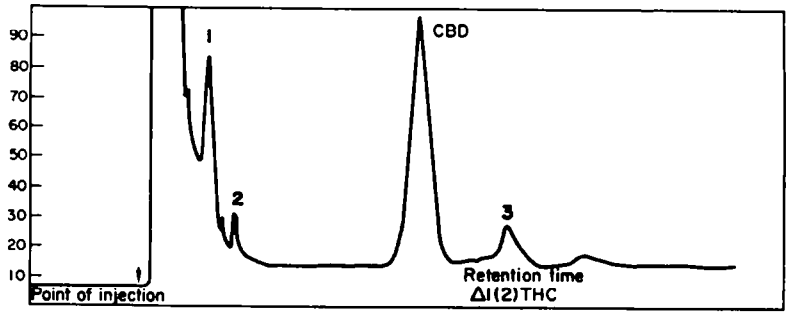

Fig 1. Gaschromatogram of the pyrolystate of CBD.

Each of the peaks was isolated by preparative gas chromatography and next, consecutive mass spectra were recorded during the slow evaporation of each sample into the ion source. The spectra indicated that most of the GLC peaks constituted a mixture of several compounds. The peaks **1** and **2**, eluted ahead of the starting material (CBD) show mass spectra with a lower molecular ion than that of CBD ($m/e = 314$) (cracking products). We therefore primarily paid our attention to peak **3** (5% of starting material) of which a LRP- (Low Resolving Power-) mass spectrum is presented in Fig 2.

of this sample indicated that the fragment ions at $m/e = 330$, 247, 205, 204, 148, 147 belong to the same product, possessing the molecular formula $C_{21}H_{30}O_3$. Furthermore, this compound is the major product of this fraction as could be derived from the total ion intensity of its ions versus the other molecular and fragment ions. Metastable measurements using the defocusing technique confirmed the above relationship between the principal daughter ions and their precursor ion (Fig 3).

While the peak intensity ratios between daughter and molecular ions in the 330-compound hardly

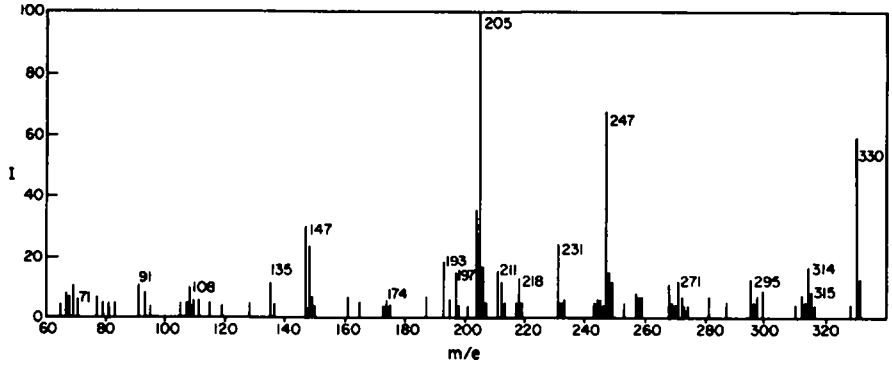

Fig 2. LRP spectrum of GLC peak **3** (Fig 1).

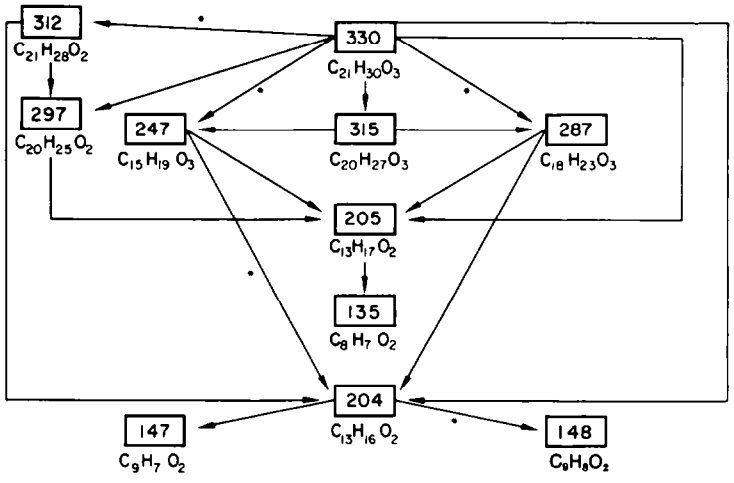

Fig 3. Metastable transitions of the main pyrolytic product.

HRP-(High Resolving Power-) on line exact mass measurements and LRP-spectra obtained from consecutive runs during the evaporation varied during evaporation, the ratio of 314 versus e.g. 299 and 231 varied substantially. This is certainly not due to an effect of the ion source temperature on the fragmentation, because this temperature was kept at the low and constant level of 50°. The varying peak intensity ratios should therefore be ascribed to minor co-products in

*Metastable transitions marked with an asterisk denote corresponding transitions in the monosilylated 330-compound.

addition to the major 330-compound. Standard spectra of CBD, Δ1(2)THC, Δ1(6)THC and Δδ(9)isoTHC, obtained from several measurements at this temperature resumed peak intensity ratios between molecular and fragment ions that remained fairly constant. It, therefore, appears very well feasible to identify the above mentioned cannabinoids on a few peak intensity ratios from their LRP-mass spectra*.[6,7]

The mass spectrometrical analysis of the preparatively isolated fraction revealed that we were dealing with several components with a molecular weight of 314. At the beginning of the evaporation of the sample the ion intensity distribution of peak 3 is alike the one of Δ1(2)THC. In later scans the ratio 314/231 is enlarged to about 10, which indicates the presence of a mass 314 component which is neither CBD, nor Δ1(2)THC, or Δ1(6)THC and Δδ(9)isoTHC. Further analysis of these components remains to be done and is presently in progress.

With regard to the structural analysis of the 330 compound it was necessary to assign the smaller fragment ions in the mixture. In order to do so the compound was first isolated in pure form through preparative thin layer chromatography (TLC) of the mixture represented by peak 3 of the gas chromatogram. The purely isolated product (R_f value 0·19) was submitted to mass spectrometrical analysis (Table 1) and further information was obtained from measurements on its silylated derivatives. Whereas the phenolic OH groups of CBD could be rapidly silylated under mild conditions (Experimental) yielding a dislylated derivative, it was found that silylation of the 330 compound afforded under the same conditions only a mono-substituted product (parent ion m/e = 402). From the mass spectrum of this mono-substituted product it could be concluded that only one phenolic OH group remained available for silylation in the original product.

Since the molecular formula requires an additional O atom to be present in the molecule and an $(M—H_2O)^+$-ion at $m/e = 312$ (metastable confirmed) denotes the presence of an additional OH group in the terpene moiety of the molecule, a more rigorous silylation was also performed. (Thus, a small amount of a disilylated product was finally formed and its mass spectrum was recorded.)

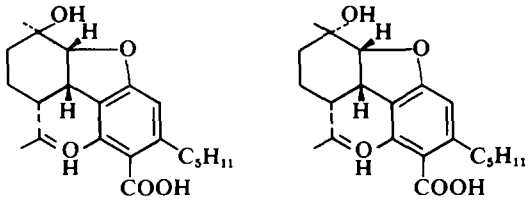

Fig 4. Cl-stereoisomers of cannabiëlsoic acid A.

The above mass spectrometrical information provided a substantial amount of structural information, which was indicative for the decarboxylated product of cannabiëlsoic acid A, a naturally occurring cannabinoid earlier isolated by Shani and Mechoulam.[8] As it was also possible to obtain this compound from a photo-oxidative cyclisation starting with cannabidiolic acid, we decided to synthesize this product as a reference compound.

*Ratios 314/231 as obtained from standard spectra are:
CBD: 0·40 standard deviation: 0·07
Δ1(2)THC: 2·7 standard deviation: 0·5
Δ1(6)THC: 1·7 standard deviation: 0·2
Δδ(9)isoTHC: 0·19 standard deviation: 0·01.

Table 1. Mass spectrum of the product with $R_f = 0·19$

Mass	Relative intensity	Formula	Mass	Relative intensity	Formula
330	60	$C_{21}H_{30}O_3$	174	2	$C_{11}H_{10}O_2$
315	6	$C_{20}H_{27}O_3$	161	5	$C_{10}H_9O_2$
312	4	$C_{21}H_{28}O_2$	149	4	$C_9H_9O_2$
297	2	$C_{20}H_{25}O_2$	148	25	$C_9H_8O_2$
287	5	$C_{18}H_{23}O_3$	147	25	$C_9H_7O_2$
274	3	$C_{17}H_{22}O_3$	135	12	$C_8H_7O_2$
249	12	$C_{15}H_{21}O_3$	133	2	$C_{10}H_{13}$
248	13	$C_{15}H_{20}O_3$	126	3	$C_8H_{14}O$
247	55	$C_{15}H_{19}O_3$	125	4	$C_8H_{13}O$
233	3	$C_{14}H_{17}O_3$	111	8	$C_7H_{11}O$
231	4	$C_{15}H_{19}O_2$	108	19	C_8H_{12}
205	100	$C_{13}H_{17}O_2$	93	11	C_7H_9
204	35	$C_{13}H_{16}O_2$	91	5	C_7H_7
193	3	$C_{12}H_{17}O_2$	83	8	C_6H_{11}
187	2	$C_{12}H_{11}O_2$	71	13	85%: C_4H_7O 15%: C_5H_{11}

Performance of this experiment on cannabidiolic acid isolated from natural sources gave a reaction mixture, which, after decarboxylation and TLC, yielded two spots with R_f values of 0·19 and 0·39. The GLC retention time of the compound with $R_f = 0{\cdot}19$ appeared to be identical to the one of the pyrolysate fraction under investigation. Moreover, its mass spectrum was identical to that of the 330 compound in the CBD pyrolysate.

The structure proposed is therefore the decarboxylated form of cannabielsoic acid A, which can occur as its C1-stereoisomers 1 and 2 (Fig 4).

The second product obtained from synthesis ($R_f = 0{\cdot}39$) possessed a mass spectrum, which showed great similarity to that of the compound at $R_f = 0{\cdot}19$, except for the intensity ratio of the fragment ions at $m/e = 247$ and $m/e = 205$.* The fragmention mechanism of both compounds, by which

*Ratios 247/205 as obtained from LRP-spectra are:
compound at $R_f = 0{\cdot}19$: 0·6
compound at $R_f = 0{\cdot}39$: 1·2.

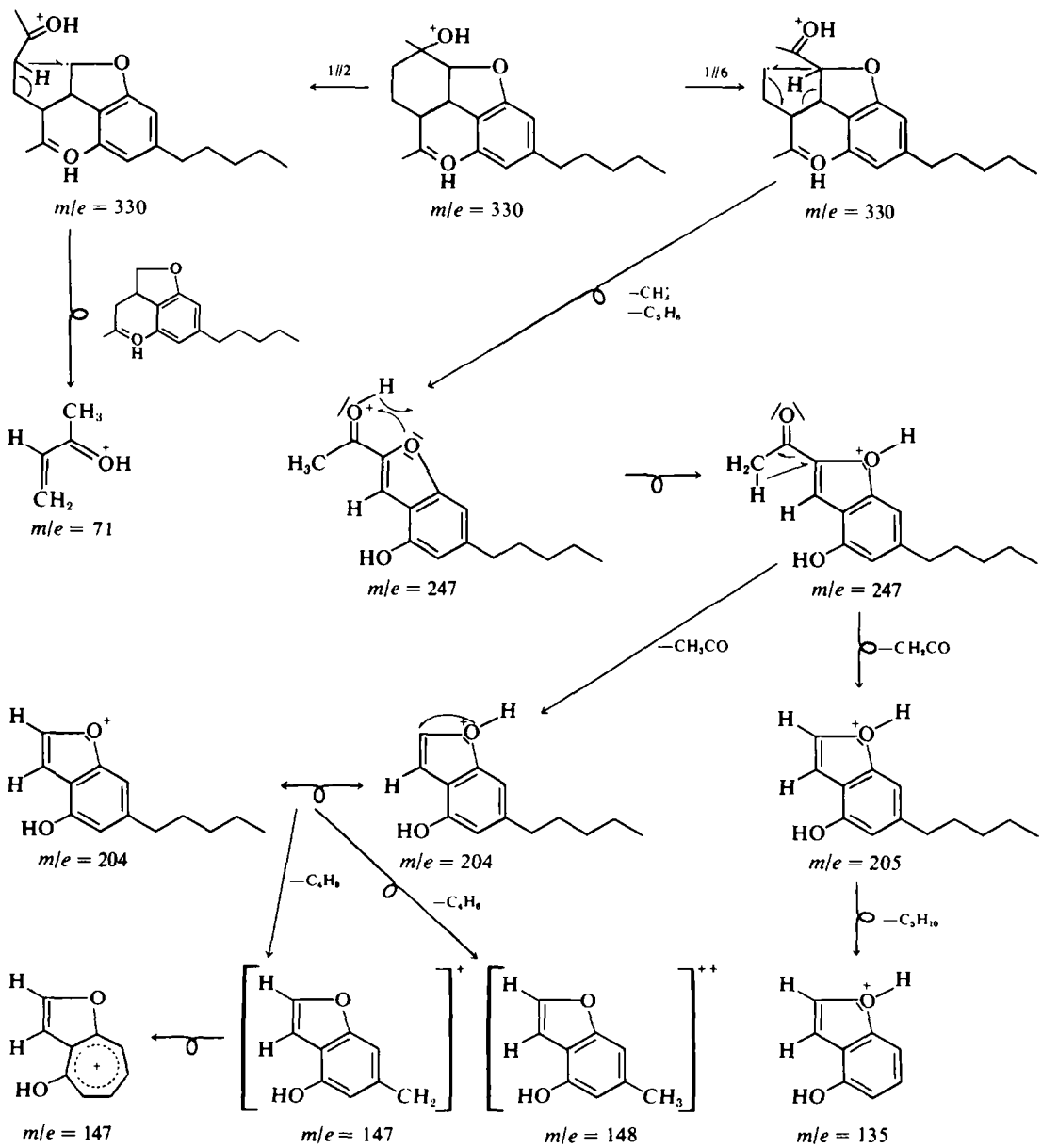

Fig 5. Proposed fragmentation mechanism of cannabielsoin.

the most abundant ions will be obtained, is proposed to proceed as represented in Fig 5.

Many of the breakdown reactions of the molecular ion correspond with the mass spectral fragmentations of cyclic alcohols.[7] The fragmentation starts in the terpene moiety of the molecule by ring opening, hydrogen shifts and successive elimination of different neutral fragments. Fragmentation of the aromatic part of the molecule is not an energetically favourable process. The abundance of the fragment ions can be explained by a stabilization due to the presence of the aromatic part in the ion, which can also be expanded to a tropylium ion structure. The assignment of the relationship between precursor and daughter ions is restricted to ions differing not more than a factor 4 in mass, due to instrumental conditions. Therefore, the precursor ion of the small but important fragment ion at $m/e = 71$ (C_4H_7O) is assumed to be the molecular ion, which corresponds to the known fragmentation of cyclic alcohols.

In order to establish which isomer was obtained through pyrolysis of CBD we decided to isolate cannabielsoic acid from natural sources. The decarboxylated natural cannabielsoic acid isomer,* which possessed the same R_f value and identical mass spectrometrical data as the 330 compound from the CBD-pyrolysate was further studied in PMR-decoupling experiments.

The C2-proton ($\delta = 3{\cdot}97$, doublet, $J = 5{\cdot}2$ c/s) appeared as a singlet on irradiation of the C3-proton, whereas the C3-proton ($\delta = 3{\cdot}24$, doublet doublet) collapsed to a doublet ($J = 9$ c/s) on irradiation of the C2-proton.

The structure of cannabielsoic acid as proposed by Shani and Mechoulam can be visualized to originate from a reaction of the phenolic OH group with the double bond at C2, resulting in a furan-type ring as opposed to ring closure at C1, which would give a pyran-type ring. PMR-data provided evidence on the furan-type derivative rather than on the pyran-type derivative. Calculations on Dreiding models of the possible skeleton-structures using a suitable Karplus equation[9] yielded the following results (Table 2).

Further evidence on the furan-derivative structure was obtained by carrying out the Lucas test,[10,11] which indicated a tertiary OH group to be present (Experimental), while the ease of dehydration would be in strong contradiction with Bredt's Rule in the case of a pyran-type derivative.[12] The extreme difficulty to silylate this tertiary OH group is somewhat unexpected, but it is in good agreement with the findings of Mechoulam, who reported, that acetylation of this group could not be performed. A remaining aspect is the stereochemical assignment of the OH-group at C1 and the configuration of the H atoms at C2 and C3. The tertiary OH-group is probably situated in an equatorial position, since in 1-methoxy-CBD the C1-Me protons appear at 1·19 ppm and 1·39 ppm for its equatorial and axial position, respectively.[13] In our case a value of 1·41 ppm was observed for the protons in question.

The measured coupling constants indicate a *cis* orientation† of the H atoms at C2 and C3 in the isolated product, which is in agreement with the proposal of Mechoulam as based upon the fact that radical processes, which form related tricyclic dihydrobenzofuran systems[14,15] give the more stable *cis*-compounds.

Preliminary pharmacological assays on the CBD-free mixture of pyrolysis products revealed significant effects in mice at a dosis of 50 mg/kg body weight. Remarkable seems the increase (1·5–2°) in body temperature as opposed to the usually observed hypothermal effect of cannabis. Further observations were a general ataxia and nervous reactions when touched.

EXPERIMENTAL

Instrumentation. All mass spectrometrical measurements were performed using an AEI-MS 902 mass spectrometer, nominally operating at 70 eV electron energy and 50–70°C ion chamber temp. Samples were admitted via the direct introduction system. Elemental compositions were derived from element lists 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. Metastable transitions have been traced by the defocusing technique of Jennings.

PMR spectra were recorded on a Varian HA-100 apparatus, using CCl_4 as a solvent and TMS as the internal reference standard. IR spectra were taken with a Perkin-Elmer 257 infrared spectrophotometer. Gas

*It should be noted that the above stereoisomers could also clearly be indicated to be present in natural cannabis isolates as was determined by combined gas chromatography-mass spectrometry (GC—MS).

†In 2,3-*cis* pyran derivatives the values for $J_{2,3}$ and $J_{3,4}$ are found reversed as compared with the 2,3-*cis* furan derivatives.

Table 2. Coupling constants as calculated on Dreiding models of cannabielsoin

	330 Compound	2,3-*cis*-Furan derivative		2,3-*trans* Furan derivatives	
		Boat	Chair	Boat	Chair
$J_{2,3}$	5·2	6·3	3·2	<3	<3
$J_{3,4}$	9·0	11·9	9·3	<4	<3

chromatography (analytical) was carried out with a Becker 409, equipped with flame ionization detection; column: glass, 0·003 × 2 m, 2% OV-17 on chromosorb G AW-DMCS, 100/120 mesh, temp: 250°C. Gas Chromatography (preparative) was performed on a Varian 1800 'autoprep'; conditions: injection temp: 270°, column temp: 265° (column: glass, 20% SE-30 on chromosorb W 30/50 mesh, 0·006 × 3 m), collector temp: 270°, detector temp: 320°.

Materials. Cannabidiol (99·5% by GLC) and cannabidiolic acid (80% Uy GLC) were extracted from Dutch cannabis material according to the methods of Korte and Schultz, respectively.[16,17] All solvents used were purified by elution over Al_2O_3 (neutral, activity grade V), followed by repeated distillation. Bis(trimethylsilyl)trifluoroacetamide ('Regisil© '), containing 1% trimethylchlorosilane, was obtained from Regis Chemical Company.

Pyrolysis of CBD. The inner wall of a quartz tube (inner diameter 1 cm) is uniformly rinsed with a soln of 2·5 mg CBD in 1 ml n-pentane over a length of 5 cm. After evaporation of the solvent a CBD layer of approximately 0·002 cm thickness is obtained. The quartz tube is connected with a spiral cooler (inner diam 0·4 cm, effective length 170 cm), which is cooled at −80°C. After instantaneous heating of the quartz tube to 700°, it is kept at this temp for 5 more seconds without sucking air through (estimated warming up time of the tube wall). Next, air is sucked through during 5 min in a rate of 1 ml/sec (the air used was non-medical grade, temp ±25°, relative humidity ±60%). The products, which deposited on the tube wall and in the cooler were extracted with n-pentane.

Isolation of cannabielsoin from the pyrolysate. 5 mg pyrolysate was chromatographed on a thin layer plate (20 × 20 cm 'Merck SiO_2 Fertigplatte', layer thickness 0·025 cm) in the solvent system benzene/chloroform 3/7 (three consecutive runs over 15 cm). The area between $R_f = 0·17$ and 0·22 was removed from the plate and the adsorbent extracted three consecutive times with diethyl ether, yield: 0·1 mg cannabielsoin (98·5% by GLC).

Isolation of cannabielsoin from a cannabis sample. Dutch cannabis-extract (±1 g), containing approximately 5% cannabielsoic acid, was dissolved in 2 ml MeOH. 50 μl of this soln was injected in the preparative gas chromatograph described above. After 30 injections, the yield of the cannabielsoin peak ($R_x = 1·30$, R_X CBD = 1·00) amounted approximately 6 mg (80% by GLC). Preparative TLC of this sample (system described above) yielded 2 mg cannabielsoin A (98·5% by GLC), PMR: 0·88 (t, 3 H), 1·41 (s, 1 H), 1·82 (s, 1 H), 3·24 (dd, 1 H), 3·97 (d, 1 H), 4·98 (m, 2 H), 6·10 (s, 2 H); IR: 3620 cm^{-1} (s), 3480 cm^{-1} (s), 2960 cm^{-1} (s), 2930 cm^{-1} (s), 1840 cm^{-1} (w), 1375 cm^{-1} (s), 1250 cm^{-1} (s), 1070 cm^{-1} (w), 895 cm^{-1} (s).

Silylation of CBD, Δ1(2)THC and cannabielsoin A. A soln of 0·25 ml bis(trimethylsilyl)trifluoroacetamide (+1% trimethylchlorosilane) in 1 ml n-hexane was added to solns of CBD and Δ1(2)THC. After 1 hr at 60°, a GLC analysis of the mixture showed complete di- resp. monosilylation of CBD and Δ1(2)THC. Under the same conditions cannabielsoin A yielded a mono-substituted product. With a 50/50 mixture of bis(trimethylsilyl)trifluoroacetamide and pyridine and after 5 hr at 100°, cannabielsoin A yielded a mixture, which showed by GLC the following products: di-trimethylsilylcannabielsoin (50%), monotrimethylsilylcannabielsoin (45%) and cannabielsoin (5%).

Irradiation of cannabidiolic acid. Irradiations for the photochemical synthesis were carried out in an apparatus, consisting of an outer jacket (300 ml, Pyrex), in which a dipper well (water cooled Hanovia 51 flak immersion well, quartz) could be placed. Cannabidiolic acid (200 mg) in cyclohexane (150 ml) was irradiated during 36 hr. (Hanau Q 81 mercury arc, 125 Watt). O_2 was bubbled through the soln during the irradiation time. At the end of the reaction the solvent was evaporated and a 5 mg sample of the residue was chromatographed by the TLC system described above, yield: cannabielsoin "I" ($R_f = 0·19$): 0·4 mg, cannabielsoin "II" ($R_f = 0·39$): 0·3 mg.

Lucas test. 1·6 g $ZnCl_2$ (anhydrous) was dissolved in 1·0 ml conc HCl. 0·1 ml of this soln was added to 0·1 ml MeOH, 0·1 ml isopropanol, 0·1 ml tertiary butanol and 0·1 ml 50% cannabielsoin in methanol, respectively. After 5 min a precipitate was only obtained with tertiary butanol and the cannabielsoin solution.

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