Natural Products of Thailand High Δ^1-THC-Strain *Cannabis*. The Bibenzyl-spiran-dihydrophenanthrene Group: Relations with Cannabinoids and Canniflavones

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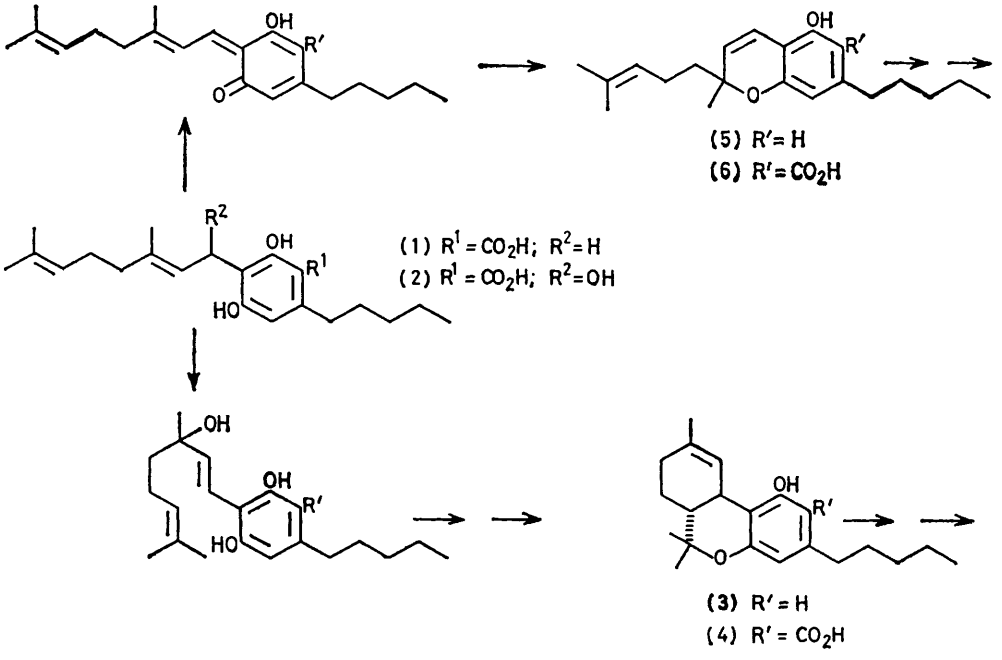
Since non-cannabinoids may influence the pharmacological profile of *Cannabis*-leaf drug, a detailed examination of the acidic fraction from leaf extractive has been made. Twelve non-cannabinoids have been isolated crystalline from a single high Δ^1-THC-strain of Thailand *Cannabis* grown in Nottingham under controlled conditions: nine of the compounds were not previously known as natural products and their structures have been determined. The extractives comprise three bibenzyls, six spirans, two 9,10-dihydrophenanthrenes, and two prenylated flavones.

The bibenzyls, spirans, and dihydrophenanthrenes may be linked together in a biogenetic scheme in which one-electron oxidation and reductive processes play important parts: the scheme is particularly supported by the discovery of a new spiran, cannabispiradienone, which holds a key position and undergoes a dienone–phenol rearrangement to give one of the new dihydrophenanthrenes. Relations between bibenzyl, cannabinoid, and flavone pathways are briefly considered.

LIMITED biosynthetic information [1] supports the view that cannabigerolic acid (1) is the parent of the cannabinoid group of compounds found in *Cannabis sativa*.[2,3] It is likely that oxidase activity leads to the formation of the allylically hydroxylated intermediate (2) which is further elaborated in a bifurcated manner (Scheme 1). latter two decarboxylate rather readily they are frequently handled in decarboxylated form.

The major psychotomimetic of *Cannabis* Δ^1-THC (3) has been extensively studied pharmacologically but not all the biological effects of a complex leaf drug, administered commonly by smoking, are likely to be explained by



SCHEME 1 Outline scheme for cannabinoid biosynthesis

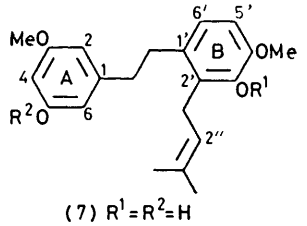
On the one hand S_N2'-displacement or intervention of an allylic carbonium ion leads *via* cyclisation to the *p*-menthane branch which includes cannabidiol, and Δ^1-tetrahydrocannabinol (3); on the other, dehydration and electrocyclisation leads to the cannabichromen (5) branch. The products of each of the pathways, *p*-menthane and chromen, undergo further reactions which form the group of compounds collectively known as the cannabinoids. The cannabinoids occur in the plant mainly as the carboxylic acids *e.g.* (4) and (6) but as the

the presence of this single component or even by the whole biogenetic family of cannabinoids. Among effects which are apparently incompletely explained by the presence of Δ^1-THC are aspects of teratogenicity, estrogenic activity, and inhibition of prostaglandin synthetase activity: [4] synergism, as well as inherent activity, may be significant in some cases. It was with this in mind that we undertook the present study of natural products other than cannabinoids produced by *Cannabis*.[5] A series of new compounds has been found,

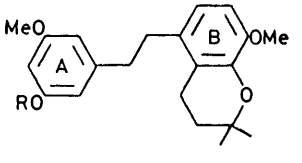
and later in the paper they are arranged in a biosynthetic scheme and compared with the processes which lead to cannabinoids.

Extraction of high-THC type Thailand strain *Cannabis sativa*, greenhouse-grown in Nottingham, led, after purification by column, preparative-layer, and high pressure liquid, chromatography to the isolation of twelve non-cannabinoid compounds. They belong to four types, bibenzyls (dihydrostilbenes), spirans, dihydrophenanthrenes, and terpenylated flavones: the structural investigation is outlined below.[6]

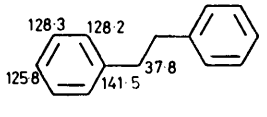
The Bibenzyls.—The new bibenzyl (7), canniprene, $C_{21}H_{26}O_4$, cleaved on electron impact into two fragments $C_{13}H_{17}O_2$ and $C_8H_9O_2$, the former accurately mass-measured: each carried one trimethylsilylatable hydroxy-group as determined by mass spectroscopy. This preliminary work suggested a substituted bibenzyl [7] and was confirmed by n.m.r. investigation. The bismethylene bridge protons resonated at δ 2.81 (4 H) and two aromatic methoxy-groups were present along with two hydroxy-groups, one broad at 4.9, the other sharp at 5.70. A prenyl residue was clearly indicated (benzylic methylene δ 3.41, olefinic proton 5.16, and two olefinic methyls 1.70 and 1.79). Three aromatic protons resonated as a multiplet 6.2—6.4 and the *J* values in canniprene and its dimethyl ether showed *meta*-coupling. A resorcinol monomethyl ether structure was therefore assigned to ring A: a further aromatic resonance appearing as a singlet at 6.71 (2 H) was assigned to ring B.



(7) $R^1 = R^2 = H$
(8) $R^1 = R^2 = Ac$
(9) $R^1 = R^2 = Me$



(10) R = H
(11) R = Ac



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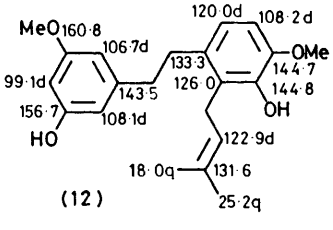


(12)

Methoxy-groups : 56·0q, 55·3q
Bridge : 38·0t 34·3t

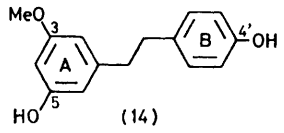
On catalytic hydrogenation canniprene gave a dihydro-derivative, and 1% BF_3 in dichloromethane readily converted canniprene into a chroman (10) demonstrating the relation of the prenyl-function to one of the hydroxy-groups: the sharper hydroxy-group resonance (5.70) of (7) disappeared and it seemed possible that the sharpness was due to hydrogen bonding to an adjacent methoxy-group. The chroman formed a monoacetate (11). The relationship of the two aromatic protons of ring-B became clear when canniprene was converted into a diacetate (8), for the two ring-B protons now appeared as an AB quartet 6.77 (d, 1 H, *J* 8 Hz) and 7.00 (d, 1 H, *J*, 8 Hz) and are clearly *ortho*-coupled. In canniprene itself they fortuitously have the same chemical shift: resolution into the AB form can be seen in the dimethyl ether (9) but not in chroman (10). In accordance with the placing of the prenyl group, the 2''-olefinic proton of the latter is shifted upfield (0.14) on diacetylation of canniprene and there was an upfield shift (0.07) on one methoxy-group.

Making the restriction of *o*-coupled protons in ring-B, along with adjacent positioning of the prenyl and one hydroxy-group, there are six possible arrangements of the aromatic substituents. These were explored using the ^{13}C n.m.r. spectrum of canniprene (12). Working from a framework of ^{13}C resonances derived from bibenzyl (13), the 2'-prenyl, 3'-hydroxy, 4'-methoxy arrangement gave best agreement between the calculated [8] and experimental sets: experimental values are shown on formula (12). We have since confirmed the correctness of the structure by synthesis of both (10) and (7).[6b,9]

The two other bibenzyls isolated were readily assigned structures. Bibenzyl (14), $C_{15}H_{16}O_3$, cleaved to give benzylic fragments of *m/e* 137 (8%) and 107 (100%) on electron impact, and each carried one hydroxy-group (trimethylsilylation). Four benzylic protons resonated at δ 2.79, there was one methoxy-group (3.74), and the aromatic protons divided into two groups. One, 6.2—6.4 (3 H) formed a *meta*-coupled system similar to those of ring A of compound (7) and other models: the remainder formed two identical AB quartets 6.69 (d, 2 H, *J* 8 Hz) and 7.01 (d, 2 H, *J* 8 Hz) leading to (14). The *p*-hydroxy-group of ring B doubtless accounts for the high abundance of this ring-fragment in the benzylic ions formed on electron impact. Structure (14) was confirmed by synthesis [9] and a similar synthesis has lately been reported in connexion with the naturally occurring, but isomeric, batatasins III and IV.[10]

Bibenzyl (15), $C_{16}H_{18}O_4$, cleaved into two, each half having a value of *m/e* 137: each carried one hydroxy-group (trimethylsilylation). Four benzylic protons resonated at δ 2.80 and the molecule was not symmetrical (methoxys 3.74 and 3.88). Ring A showed the typical pattern of three *meta*-oriented protons whilst those of ring B formed a three-proton multiplet near 6.75. Biosynthetic considerations and the relationship to canniprene led us to the tentative structure (15) which was readily confirmed by synthesis.[9] In an independent investigation (14) and (15) have also been recently reported in a Mexican variety of *Cannabis* by Ketennes-van den Bosch and Salemink,[4] together with a bibenzyl said to be (16). The latter was later shown to be identical with our canniprene and its formula has been revised.[11]

The bibenzyls (14) and (15) are minor components of Thailand *Cannabis sativa* (2 and 5 mg/kg expressed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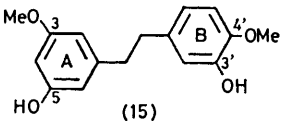
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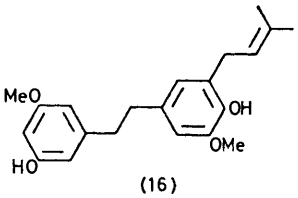
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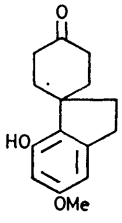
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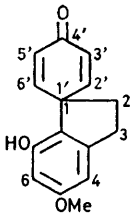
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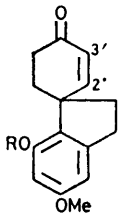
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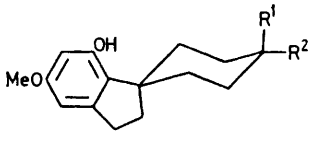
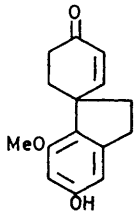
(20) R = Me


(22) R^1=OH, R^2= H
(23) R^1=H, R^2= OH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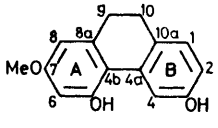
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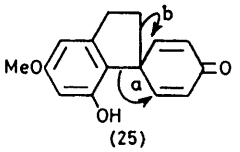
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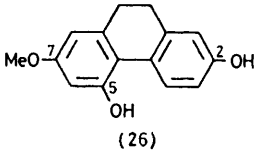
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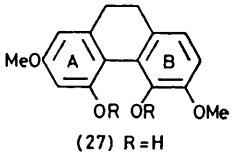
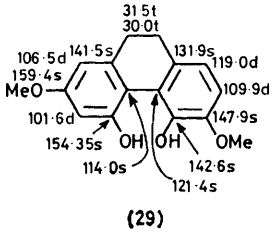

(27) R=H
(28) R=Ac


(29)

respectively as dry weight, by isolation) but canniprene (7) was isolated at a total level of 470 mg/kg. Since the pharmacological profile is at present not known we have analysed a number of samples by g.l.c., following trimethylsilylation, on OV 17 at 240 °C, confirming the nature of the peaks by g.l.c.–m.s. methods. Samples of mature Thailand strain leaves, 16 weeks old or more contained 0.5—1.5 g/kg depending on season and growing conditions (typical cannabinoid analysis of our Thailand strain: Δ^1-THC 6—9 g/kg, cannabidiol + cannabichromen 1.5 g/kg, other cannabinoids including cannabinol *ca.* 0.2 g/kg). Canniprene and cannabispirenone (see later) can be detected by g.l.c. and t.l.c. in stem tissue, but only in small amount: the compounds were not detectable by these methods in roots. Younger Thailand leaves (*ca.* 6 weeks) contained considerably less canniprene, 0.1 g/kg (Δ^1-THC *ca.* 3 g/kg). Mature freshly dried Indian drug-type *Cannabis* contained 0.43 g/kg (Δ^1-THC 2.2 g/kg) whilst a similar S. African type had 0.15 g/kg (Δ^1-THC 10.3 g, kg): in both types, cannabispirone and β-cannabispiranol were present. Kew-strain *Cannabis*,* a high cannabidiol (fibre-type) variety, contained less canniprene (0.1 g/kg) (Δ^1-THC 0.1 g/kg, cannabidiol + cannabichromen 2.19 g/kg), nor could appreciable quantities of any members of the spiran series be detected. Little canniprene, or members of the spiran series, were detectable in various cannabis 'soles', or in one sample of resin, from the United Nations collection. They were, however, all considerably aged as indicated by their high cannabinol content.

The Spirans.—The isolation of cannabispirone (cannabispiran) (17) in 1976 from Indian *Cannabis* by Turner *et al.*[13] and from S. African *Cannabis* by Bercht *et al.*[14] represented the first indication that spirans were formed by this plant. Its structure was established by spectral [14] and single crystal *X*-ray methods.[13] We have also isolated this compound (10 mg/kg), identical with the literature descriptions, from Thailand *Cannabis*. More importantly however, we have now discovered a crystalline spiradienone (cannabispiradienone) (6 mg/kg) in this plant and shown its structure to be (18): it will be apparent later that it holds a key biosynthetic position. The new compound, $C_{15}H_{14}O_3$, contained one hydroxy-group (trimethylsilylation) and a conjugated carbonyl $\nu_{max.}$ (KBr) 1 654 cm^{-1}. In the n.m.r. spectrum there were two *meta*-coupled aromatic protons δ 6.21, 6.45 (*J* 2 Hz) along with an aromatic methoxy-group (3.80) and an exchangeable hydroxy-group (5.35). The four protons of the spiro-bismethylene bridge formed two triplets at 3.09 amd 2.28 and the remaining four protons constituted two degenerate AB systems at 6.37 (2 H d, *J* 10 Hz) and 6.98 (2 H d, *J* 10 Hz) in complete accord with (18). U.v. data reveal the high extinction of the dienone system at 239 nm, and the structure was confirmed by catalytic hydrogenation to cannabispirone (17).

* *Cannabis* has usually been viewed as a monotypic genus containing within it marked variability: more recently it has been viewed as polytypic having at least three species *C. sativa*, *C. indica* and *C. ruderalis* [12] but this view is not accepted by some authorities.[5]

Alone of the spirans discussed in this paper, the cannabispirenone-A* structure (19) is chiral and it apparently originates from, and passes to, compounds having a mirror plane of symmetry by biological reductive processes (Scheme 2). The literature on it is somewhat confusing, one group giving m.p. 163—164 °C,[13b] $[\alpha]_D^{20}$ +34° in methanol, from Indian *Cannabis*, the other m.p. 173—174 °C,[14] with no mention of a rotation, from S. African *Cannabis*. We believe that both statements are accurate in respect of m.p., if incomplete. As isolated by our procedure cannabispirenone-A had m.p. 150—151 °C $[\alpha]_D^{27}$ −231° (methanol $[\alpha]_D^{27}$ −173° (EtOAc). Continued crystallisation gradually raised the m.p. to 172 °C with considerable loss of material and the rotation became zero. Although the two forms have identical solution spectra, their n.m.r. may be differentiated (250 MHz) by the addition of europium chiral shift reagent. The doublet from the 3′-proton is shifted to form a double doublet in the (±)-isomer whereas samples such as the above (−)-compound retain doublet form, only a slight shoulder being observable. The best chiral sample examined had m.p. 145—147 °C, $[\alpha]_D^{23}$ −178.8° (EtOAc) but we are doubtful if this had reached total optical purity.

Since no clear evidence of a racemisation process during crystallisation could be obtained it was speculated that acid or base treatment used during the extraction could be causing racemisation. A new extraction procedure avoiding the use of acid or base (Experimental section) was therefore devised but the product was of similar optical quality to that from our standard extraction and on continued crystallisation (±)-cannabispirenone-A of zero rotation was attained as usual. Our view of the situation is that the material extracted, largely (−) but containing (±)-cannabispirenone-A, is probably representative of the material in the leaves and at least the majority of the partial racemisation has, whatever the causes, occurred in this organ. Cannabispirenone-A was isolated from Thailand *Cannabis* at a level of 90—210 mg/kg dependent on the crop.

β-Cannabispiranol (22) was isolated from Thailand *Cannabis* (60-80 mg/kg) and identified by spectral data and oxidation to cannabispirone (17). It has been isolated from both Indian [17] and other [18] types and the axial β-form established by ^{1}H n.m.r. and ^{13}C n.m.r. studies.[17,18] The present investigation has shown that the equatorial α-form (123), hitherto not known in *Cannabis*, also occurs in Thailand material (0.2 mg/kg). It was separated from the β-isomer chromatographically, being finally purified by h.p.l.c. on a C_{18} reversed-phase column and shown to be identical with an authentic specimen made by reduction of the ketone (17) with sodium borohydride.[14,17,18] This leads to a mixture of α- and β-isomers, the former predominating. Since the α-form has the hydroxy-group and the large aryl ring equatorial, it is the more stable of the two forms and the possibility that it is an artefact arising from the β-must arise. However, it has been isolated from two different years' crops and its actual presence is not in doubt.

All the spiro-compounds mentioned have now been synthesised in our laboratory.[15,16]

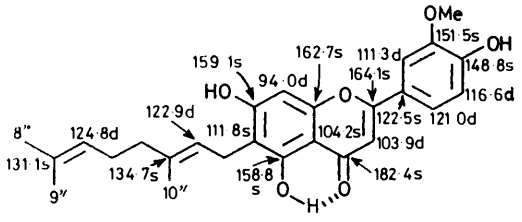
The Dihydrophenanthrenes.—Although bibenzyls and dihydrophenanthrenes have been known for some time to be found in other plants,[7] their occurrence in *Cannabis* is novel. By a combination of preparative plate-chromatography and reversed-phase h.p.l.c. we have isolated two new 9,10-dihydrophenanthrenes and named them cannithrene-1 and -2.

Cannithrene-1 (24), (4 mg/kg) $C_{15}H_{14}O_3$, contained two hydroxy-groups from the mass spectrum of its trimethylsilyl derivative, and its u.v. data $\lambda_{max.}$ 220, 241sh, 266sh, 274, 300, and 310 nm suggested a dihydrophenanthrene type.[19] This was confirmed by the n.m.r. spectrum which revealed the two hydroxy-groups, a methoxy-group, and two *meta*-coupled protons assigned to C-6 and C-7 of the A-ring (6.49 d, 6.37 d, *J* 2.5 Hz). The four protons of the bismethylene bridge were located (2.65) and an AB quartet was assigned to the 1- and 2-protons of ring B (7.00 d, *J* 8.0 Hz, 6.58 dd, *J* 8.0 and 2.5 Hz): the 2-proton was also *m*-coupled to the characteristically deshielded C-4 proton [19] at 7.94, d, *J* 2.5 Hz. Since a deshielded 5-proton is not present, this positions the three oxygen substituents on the dihydrophenanthrene. Location of the methoxy-group followed readily from the finding that on heating above its m.p. (*e.g.* 180 °C), cannabispiradienone (18) rearranged to cannithrene-1 by a dienone–phenol rearrangement.[20] Migration of the spiro-centre with movement of the electron-rich aryl bond ' a ' in (25) leads to (24). If the migration involved the bismethylene bond ' b ', (26) would be formed and this is ruled out by the n.m.r. data which requires the deshielded 4-proton to be *m*-coupled only. The fact that a precursor is present in *Cannabis* raises the issue of whether cannithrene-1 is to be viewed as a natural product or artefact. All that can be said is that the comparatively mild extraction conditions do not appear to cause rearrangement. Formation of compound (24) does, however, explain why, when cannabispiradienone is trimethylsilylated and examined by g.c., two peaks are formed; one is the trimethylsilylated (TMS) derivative of (18), the other of (24).

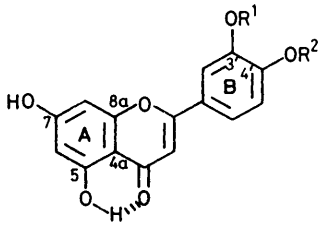
Cannithrene-2 (27) (8 mg/kg), $C_{16}H_{16}O_4$, contains two hydroxy-groups (mass spectrum as TMS-derivative) and like cannithrene-1 forms a diacetate. The sequence of u.v. bands 219, 267sh, 275, 293sh, and 301 nm support a dihydrophenanthrene type.[19] Apart from confirming the two hydroxy-groups, the n.m.r. spectrum showed two methoxy-groups, the four bismethylene bridge protons (2.67), and *meta*-coupled protons in ring A (6.53 d, 6.47 d, *J* 2.5 Hz). Ring B showed *ortho*-coupled aromatic

* The '—A' descriptor is added because a second cannabispirenone, in which the positions of the methoxy- and hydroxy-groups of (19) are interchanged, has been isolated from Mexican *Cannabis*.[4,11] Although we have not isolated it pure from Thailand *Cannabis* the n.m.r. resonances of cannabispirenone-B (21) can be seen in the residues from our preparations of —A: its content in dry Thailand *Cannabis* leaves is *ca.* 5—10 mg/kg. We have synthesised the compound.[15,16]

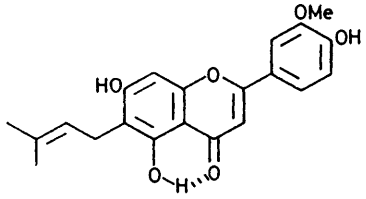
protons (6.85 d, 6.69 d, J 8 Hz). Since there is no appropriate low-field proton [19] in the two sets, the C-4 and C-5 positions must be occupied by oxygen substituents leading to the substitution pattern shown in (27). What is less certain is the location of the two methyl ethers, and this was tackled from the ^{13}C spectrum of cannithrene-2. Using data from other dihydrophenanthrenes and aromatic substituent shift constants,[8] (29) was arrived at as providing the best fit between the substituents to be located at C-3, -4, -5, and -7, and the



(30) 56·8q OCH_3, 21·6t C-1″
39·8t C-4″, 18·1q C-9″
26·9t C-5″, 16·6q C-10″
25·9q C-8″



(31) R^1 = Me, R^2 = H
(32) R^1 = H, R^2 = Me



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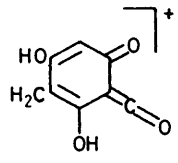
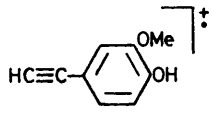
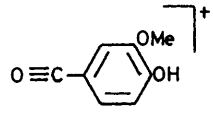


m/e 165
(34)



m/e 148
(35)



(36)

chemical shifts observed. Since determination of the methoxy orientation was particularly important from a biosynthetic point of view, a single-crystal X-ray study of the diacetate (28) has been carried out by Dr. M. Begley in our laboratory. It confirms the structure arrived at on spectroscopic grounds and will be published elsewhere.

The Prenylated Flavones.—Purification of the appropriate column fraction by p.l.c. and reversed-phase h.p.l.c. gave two new pale yellow flavones named canniflavone-1 and -2, formulated as (33) and (30), respectively. Canniflavone-2 (6 mg/kg), $C_{26}H_{28}O_6$, had $\lambda_{max.}$ 242i, 276, 344 nm and $\nu_{max.}$ 1 653 cm^{-1} indicating a flavone type structure. U.v. shift studies (NaOMe, $AlCl_3$, $AlCl_3$–HCl, NaOAc, and NaOAc–H_3BO_3) [21] showed the presence of a chelated carbonyl, and the absence of two adjacent phenolic hydroxy-groups. Very close similarities in the u.v., and in the u.v. shift patterns, between canniflavone-2 and crysoeriol (31) [21] and diosmetin (32) [21] were noted. In the mass spectrum, (30) showed losses of M −43, −69, and −123 (the last two in high abundance: the *m/e* 313 fragment was accurately mass-measured and there is a metastable ion linking it with the 436 parent), characteristic of a geranyl side-chain. The large abundance of the geranyl-cleavage products tends to overshadow other fragmentation but ions for both (34), *m/e* 165 and (35) *m/e* 148, and (36) *m/e* 151, were located and the first two mass measured. This indicates that ring A carries the geranyl group and two hydroxy-groups (one chelated) whilst ring B carries one hydroxy-group and one methoxy-group.

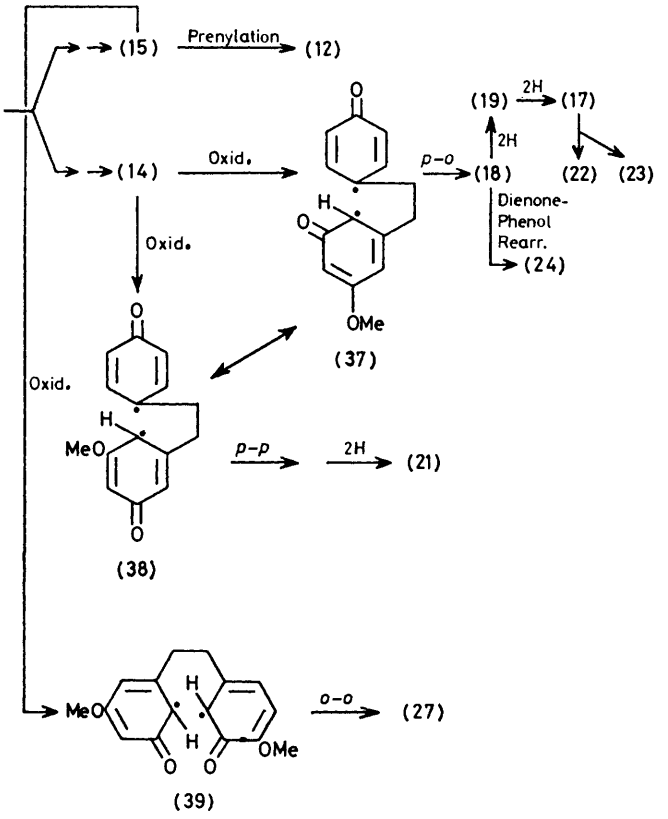
N.m.r. spectra were determined in [$^2H_6$] acetone, [$^2H_6$]dimethyl sulphoxide, and methanol to extract full information. They confirm the presence of a geranyl residue, one methoxy-group, a chelated hydroxy-group and two other hydroxy-groups. In methanol the proton pattern of ring B, on expansion, can be seen to be that of two protons near δ 7.4—7.5, one of which is *m*-coupled and one of which is both *m*-coupled and *o*-coupled to the proton at 6.93, J-8 Hz: this defines the ring-B oxygenation pattern, but leaves the relative orientation of the hydroxy- and methoxy-group in doubt. ^{13}C N.m.r. data favour the ' vanillin ' arrangement in ring-B (30) over the ' isovanillin '. Assignments were made using literature data for flavones.[22] C-2′ Resonates at 111.3 and C-5′ at 116.6 in canniflavone-2 (Δ + 5.3). In the close model chrysoeriol (31),[22a] C-2′ resonates at 110.2 and C-5′ 115.8 (Δ + 5.6). In isomeric diosmetin,[22a] C-2′ resonates at 113.1 and C-5′ at 112.1 (Δ − 1.0). The placing of the geranyl chain at C-6 is also dependent on ^{13}C comparative data. The 8-carbon in ring-A of canniflavone-2 resonates at 94.0 (O.R. doublet). Model compounds [22a] show that in ring-A, 6-protons in flavones resonate in the range δ 98—99 whilst 8-protons are at 94—95 (*meta*-shifts by alkyl groups are very small).[8]

Canniflavone-1 (0.8 mg/kg), $C_{21}H_{20}O_6$, also pale yellow

and occurring as a very minor component, showed mass-spectral losses of −43 and −55 indicating a prenyl side-chain. U.v. and i.r. data were consistent with a flavone structure, and u.v. shifts effected by the reagents mentioned above emphasised a close parallelism of behaviour with canniflavone-2. Except for the differences between a geranyl and a prenyl group, similar parallelism throughout was found in the ^1H n.m.r. spectra in [$^2H_6$]acetone and in [$^2H_6$]dimethyl sulphoxide. It is on these grounds that canniflavone-1 is formulated as the lower prenylogue (33) of canniflavone-2. Synthesis to confirm these two structural proposals is being investigated.

DISCUSSION

In the present work we have isolated twelve non-cannabinoid compounds in crystalline form and a thirteenth, of known structure, is shown to be present: nine of the compounds were previously not known either in *Cannabis* or as natural products. In view of their co-occurrence in a single type of *Cannabis* plant grown under strictly controlled conditions, and formed alongside the cannabinoid set of compounds, it seems appropriate to assess their biogenetic connexions. The bibenzyls, spiro-compounds and dihydrophenanthrenes



SCHEME 2 Proposed biogenetic connections among the bibenzyls, spiro-compounds and dihydrophenanthrenes of cannabis

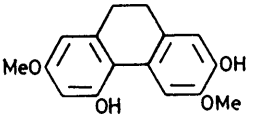
may be arranged as in Scheme 2 and it is suggested that this be referred to as the bibenzyl pathway of *Cannabis* to distinguish it from the cannabinoid pathway with its division into *p*-menthane and chromen branches.

The bibenzyls (14) and (15) probably originate from a common stock, the *p*-coumaryl ring of (14) having undergone ring B *ortho*-hydroxylation at some stage and both have been partially methylated. Prenylation, probably a late-stage process,[23] links (15) with (12). One-electron oxidations of (14) give (37) which, coupling in the *p*-*o*-mode, produces cannabispiradienone (18). Successive reductions then lead to cannabispirenone-A, cannabispirone and β-cannabispiranol apparently accompanied by lesser amounts of the α-form. There is no stereochemical evidence impressed on the latter two structures as they contain mirror planes of symmetry. Only in cannabispirenone-A does the chiral reduction become apparent and here there is the complication that (±)-material is isolated with it. The postulated diradical intermediate (37) may be written in the alternative form (38), and this leads by *p*-*p*-coupling to a spirodienone which has not been isolated, but whose existence would seem to be manifest in the formation of cannabispirenone-B (21). The dienone (18) undergoes a dienone-phenol *in vitro* rearrangement on heating or under conditions of acid catalysis giving the dihydrophenanthrene cannithrene-1 (24) and, particularly in view of biosynthetic evidence from the alkaloid field,[20] this appears to be the likely *in vivo* pathway. One-electron oxidations of the tetra-oxygenated bibenzyl (15) lead to the biradical (39). This has four possible coupling modes and the dihydrophenanthrene cannithrene-2, found in *Cannabis*, is derived from *o*-*o*-coupling. It is of interest to note that the dihydrophenanthrene (40) found in *Combretum* sp.[19a] is the expected product of one mode of *o*-*p*-coupling and presumably these processes are under enzymic control.

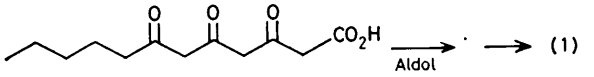
On the Birch-Donovan hypothesis [24,25] the roots of the bibenyzl and cannabinoid pathways have common elements. Both involve triple acetate/malonate extensions, followed by aldol cyclisation, but commencing in one case with a short chain fatty acid as starter (hexanoic acid: this occurs as an ester in *Cannabis;* [26] butyric and acetic acids are also involved as THC's with shortened chains occur), and in the other probably with *p*-coumaric acid (this also occurs in *Cannabis* [27]). The two polyketides (or equivalents) are (41) and (42) but the latter is also likely to be the precursor of the two prenylated flavones canniflavones-1 (33) and -2 (30) found in this work. Flavone formation involves Claisen condensation of (42) [25] and elaborative steps of *o*-hydroxylation in the shikimate-derived ring, methylation, prenylation, oxidation *etc.* Bibenzyl production requires steps of stilbene formation,[28] reduction and decarboxylation.

As mentioned at the outset of this paper, geranylated olivetolic acid (1) is the precursor of the cannabinoids and it is of interest that analogous bibenzyls (and stilbenes) have been recently found in nature. Thus compounds (43) and (44) occur in the liverworts *Radula variabilis* [29] and *R. takiensis*.[30] Of still more interest is the recent report that (44) and (45) co-occur together with cannabigerol (1) and cannabigerolic acid (2) in the higher plant *Helichrysum umbraculigerum* (Compositae).[31] No other

cannabinoids were reported in this plant so it presumably lacks the enzyme or enzymes necessary for the 1''-hydroxylation and subsequent steps outlined in Scheme 1. One may contemplate the prospect of finding series of natural compounds having the products of the various cannabinoid manipulations of the geranyl chain (*p*-menthanes, chromens, cyclols, and citrans, *etc.*) [2] attached to bibenzyls, stilbenes, and related metabolites, thus hybridising the bibenzyl and cannabinoid pathways.



(40)



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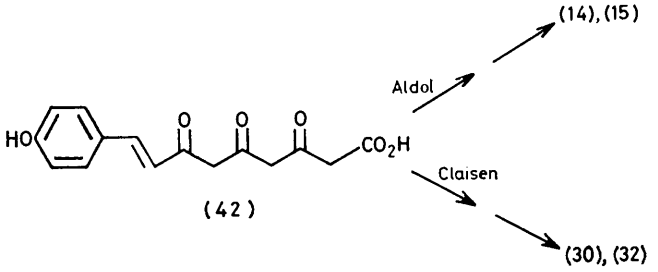
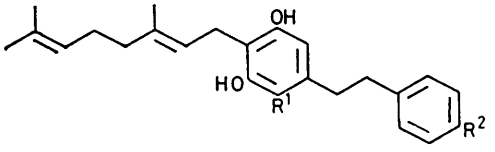


(42)



(43) $R^1 = H$, $R^2 = OH$
(44) $R^1 = R^2 = H$
(45) $R^1 = CO_2H$, $R^2 = H$

EXPERIMENTAL

Extraction of Thailand Cannabis.—Extractions were made on material grown (greenhouse) in Nottingham, in three different years. Methods varied slightly in attempts to improve yields and separations, but the following (1979) is typical. Leaves were stripped from well-grown but non-flowering plants and air dried, finally at 40 °C. Stem and petioles were removed and the leaves ground in a ball mill (the fine powder causes severe eye irritation). Ground leaf (2.65 kg) was continuously stirred at 20 °C for 24 h with (i) 6 l dichloromethane: the solvent was decanted and replaced by (ii) 2 l of dichloromethane for a similar time. The procedure was repeated as follows: (iii) and (iv) each 2 l of dichloromethane, (v)—(viii) each 2 l of n-hexane, (ix)—(xii) each 2 l of ether. The combined extracts were filtered, evaporated (40 °C) and dissolved in ether (2 l) to give a black-green solution which was extracted with an aqueous solution containing 2% sodium hydroxide and 2% sodium metabisulphite (3 × 500 ml; 2 × 250 ml). The neutral extract was dried and evaporated to a dark green sludge (84.2 g) containing 'neutral' cannabinoids: it was not investigated further. After cautious acidification (sulphuric acid) of the alkaline solution and extraction (4 × 500 ml), the combined ether extracts were washed with water (2 × 500 ml), dried ($MgSO_4$), and evaporated to yield the crude acid extract (3.4 g). This contained cannabinoid acids, spirans, and related phenolic compounds.

The extract (3.4 g), mixed with dry silica, was applied to the top of a dry silica column in nylon tubing (1½ in × 18 in) and eluted with ether–hexane (3 : 1) until the solvent reached the bottom of the column. The column was divided into 10 sections, each section being eluted with ether–methylene chloride with, in the case of bands 1—3 only, a little methanol. Components were monitored by t.l.c. Band 1 (brown-green) and band 2 (yellow) at the top of the column (0.10 g) contained many compounds in trace amounts. Band 3 (light yellow) (0.21 g) and band 4 (light yellow) (0.45 g) both contained cannabispiranol and yellow compounds. On evaporation to low volumes in ether, bands 3 and 4 deposited crystals of almost pure β-cannabispiranol (0.20 g, 0.008%). Bands 5 and 6 (yellow and lighter yellow-green) combined (0.60 g) contained cannabispiran, cannabispiradienone and (predominantly) cannabispirenone. Band 7 (yellow-green) plus 8 (deep blackish-green), together (1.10 g) gave mainly canniprene (0.61 g, 0.023%) on crystallisation from ether–hexane. Some cannabinoid acids were present. The fastest eluting bands 9 (deep blackish-green) and 10 (deep yellow) (together 0.61 g) contained canniprene together with cannabinoid acids and green compounds.

On other occasions, a first crude separation of the acidic components was effected by p.l.c. (40 × 40 cm HF 254 Silica G plates) with benzene–methanol–acetic acid (44 : 5 : 1) as eluant. The crude compounds thus isolated were further purified by p.l.c. on 40 × 40 or 20 × 20 cm plates using ether–hexane (3 : 1) or chloroform–methanol (19 : 1) as eluants and recombining appropriate fractions. In a number of cases h.p.l.c. using a reversed-phase C_{18} column, elution with methanol–water mixtures was employed for final purification: details are given later for individual compounds.

The colours obtained by spraying with Fast Blue Salt B (FBSB) are helpful in separating bands of similar R_F. All the spirans gave a slightly bluish-pink, the 9,10-dihydrophenanthrenes a deep purple, whilst the bibenzyls gave more muted colours, *e.g.* canniprene a brownish-pink.

Canniprene (7).—After initial separation as above *canniprene* crystallised from ether—hexane as needles, m.p. 112—113 °C (M^+, 342.1833. $C_{21}H_{26}O_4$ requires *M*, 342.1831); $\lambda_{max.}$ (EtOH) 206 (ε 43 000), 224infl. (11 400), 277infl. (2 400), and 281 (2 400) nm; $\nu_{max.}$ (KBr) 3 368br s, 2 933, 2 866, 1 627s, 1 598s, 1 497s, 1 459, 1 356, 1 315, 1 288, 1 261, 1 200, 1 161, 1 089, 1 063, 991, 935, 840, 798, and 711 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 6.71 (2 H, s, 5'- and 6'H), 6.2—6.41 (3 H, m, 2,4, and 6-H), 5.70 (1 H, s, D_2O exch.), 5.16 (1 H, br t, *J* 7, ~ 1 Hz, 2''-H), 4.9 (1 H, br s, D_2O exchg.), 3.90 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.41 (2 H, d, *J* 7 Hz, 1''-CH_2), 2.81 (4 H, s, bridge methylenes), and 1.79

(3 H, s, olefinic Me), 1.70 (3 H, s, olefinic Me). For ^{13}C n.m.r. data ($CDCl_3$) see (12); *m/e* 343 (8%), 342 (38), 285 (5), 273 (5), 206 (16), 205 (100), 191 (13), 189 (8), 173 (13), 163 (29), 161 (11), 148 (19), 145 (14), 137 (21), 131 (19), 103 (19), 91 (10), and 77 (11). Mass measured ions: 205.1250 ($C_{13}H_{17}O_2$ requires 205.1228), 173.0980 ($C_{12}H_{13}O$ requires 173.0966). Metastables *m**: 123 (342→205) 146 (205→173), 237.5 (342→285). Canniprene in ether–n-hexane (2 : 1) had R_F 0.54; in chloroform containing 1.5% methanol R_F 0.34; in benzene–methanol (88 : 10) R_F *ca.* 0.5: colour with FBSB dull pinkish purple. Retention time after trimethylsilylation (heating with BSTFA + 1% TMCS for 1 h. at 90 °C) on 2% OV17 (5 ft × ¼ in) at 240 °C, 18.1 min (He flow 20 cm³/min).

Canniprene formed a *diacetate* (8) (Ac_2O–pyridine) as an oil, M^+ 426.2029 ($C_{25}H_{30}O_6$ requires 426.2042); ^1H n.m.r. ($CDCl_3$) δ 1.67 (s, 3 H, olefinic Me), 1.73 (s, 3 H, olefinic Me), 2.33 (s, 6 H, s acetate Me), 2.83 (s, 4 H, bridge methylenes), 3.44 (d, 2 H, *J* 7 Hz, 1''CH_2), 3.80 (s, 3H, OMe), 3.83 (s, 3 H, OMe), 5.02 (t, 1 H, *J* 7 Hz, 2''-olefinic H), 6.5—6.6 (m, 3 H, 2, 4, and 6-H), 6.77 (d, 1 H, *J* 8 Hz), and 7.00 (d, 1 H, *J* 8 Hz) 5',6'-AB quartet). The *dimethyl ether* (9) (NaH, MeI, THF), an oil, M^+ 370.2146 ($C_{23}H_{30}O_4$ requires 370.2144) also showed the 5',6'-AB quartet: 6.64 (d, 1 H, *ca.* 7 Hz) and 6.80 (d, 1 H, *J ca.* 7 Hz).

Dihydrocanniprene.—Hydrogenation of canniprene (21.4 mg) in methanol over 5% Pd/C gave the *dihydrocanniprene* (18.6 mg, 87%), m.p. 112—113 °C (from ether–hexane), M^+ 344.1998 ($C_{21}H_{28}O_4$ requires 344.1987). ^1H N.m.r. confirmed that the side chain was saturated.

Canniprene Chroman (10).—Canniprene (23 mg) was stirred in 5 ml of 1% boron trifluoride–diethyl ether in CH_2Cl_2 at 20 °C for 15 min. Neutralisation (Na_2CO_3) and extraction gave an oil (22.1 mg, 96%) which crystallised from ether–hexane to give the *chroman*, m.p. 144—145 °C, M^+ 342.1851 ($C_{21}H_{26}O_4$ requires 342.1831). The mass spectra of the silylated and unsilylated chroman, and the ^1H spectrum ($CDCl_3$), were concordant with structure (10). The *chroman acetate* (11) (Ac_2O–pyridine) (81%) had m.p. 70—71 °C from ether–hexane, M^+ 384.1939 ($C_{23}H_{28}O_5$ requires 384.1937) (m.s. and ^1H n.m.r.). The *methyl ether* of (10) (NaH, MeI–THF) (64%) crystallised from ether, m.p. 113—114 °C, M^+ 356 ($C_{22}H_{28}O_4$ requires 356) (m.s., ^1H n.m.r., i.r., u.v.). The *5-bromofuroyl derivative* of (10), m.p. 108—109 °C and the *p-bromobenzenesulphonyl derivative of* (10), m.p. 73—74 °C were also prepared and characterised (m.s. and ^1H n.m.r. spectra).

4',5-Dihydroxy-3-methoxydihydrostilbene (14).—The compound was detected by t.l.c. and g.l.c. investigation of the mother liquors from cannabispirenone crystallisations. Purification was effected by p.l.c. on HF 254 Silica G using 3% methanol in chloroform as eluant. The *dihydrostilbene* had m.p. and mixed m.p. with a synthetic speciman [9] 109—110 °C (lit.,[11] m.p. 112.5—113.0 °C), M^+, 244.1140 ($C_{15}H_{16}O_3$ requires 244.1099), ^1H n.m.r. ($CDCl_3$) δ 2.79 (s, 4 H, bridge methylenes), 3.74 (s, 3 H, OMe), 6.22—6.35 (m, 3 H, 2, 4, 6-H's), 6.69 (d, 2 H, *J* 8 Hz, 3',5'-H's), and 7.01 (d, 2 H, *J* 8 Hz, 2', 6'-H's); *m/e* 244 (24%), 138 (20), 137 (8), 107 (100), and 77 (16); *m/e* of trimethylsilylated (14): 389 (10), 388 (29), 181 (6), 180 (22), and 179 (100). Compound (14) had R_F (ether–hexane, 3 : 1) 0.50 (cannabispirenone R_F 0.46) and gave a dull purply orange colour with FBSB.

3',5-Dihydroxy-3,4'-dimethoxydihydrostilbene (15).—The presence of a second dihydrostilbene in the cannabispirenone mother liquors was detected by g.l.c., the trimethylsilyl-(15) having much longer retention times on OV17 columns [1.60 relative to Δ^6-THC(1.00)] than cannabispirenone (1.18). Its R_F value both in ether–n-hexane and methanol in chloroform mixtures was closely similar to that of cannabispirenone, but repeated p.l.c. in ether–n-hexane (3 : 1) gave the pure *dihydrostilbene* (15) as needles, m.p. 133—134 °C and mixed m.p. with a synthetic speciman,[9] m.p. 132—133 °C. It had M^+ 274.1232 ($C_{16}H_{18}O_4$ requires *M*, 274.1205); ^1H n.m.r. ($CDCl_3$) δ 2.80 (s, 4 H, bridge methylenes), 3.74 (s, 3 H, OMe), 3.88 (s, 3 H, OMe), 6.2—6.3 (m, 3 H, ring A aromatic H's), and *ca.* 6.74 (m, 3 H, ring B aromatic H's); *m/e* 274 (16%), 138 (10), 137 (100), and 122 (4); for trimethylsilyl (14) *m/e* 419 (7), 418 (20), 403 (3), 211 (5), 210 (17), 209 (100), 179 (8), and 137 (5). Spectral comparison confirmed the identity of the natural and synthetic compound (15): comparison with synthetic 4',5-dihydroxy-3,3'-dimethoxydihydrostilbene [9] showed recognisable ^1H n.m.r. differences on expansion of the ring B proton resonances.

Cannabispirone (17).—This compound, giving a bluish pink colour with FBSB reagent, was present mainly in bands 5 and 6 of the original dry column. It was purified by p.l.c. on Silica G HF 254 using ether–hexane (3 : 1) as eluant. Crystallised from ether–hexane it had m.p. 179—180 °C (lit.,[13] m.p. 178—179 °C and [14] 181—182 °C). Cannabispirone had $\nu_{max.}$ (KBr) 1 685 cm^{-1}; M^+, 246.1292 (Calc. for $C_{15}H_{18}O_3$: *M*, 246.1256), and its mass-spectrum and ^1H n.m.r. spectrum ($CDCl_3$) agreed with the data given by Bercht *et al.*[14] In trimethylsilylated form it had *m/e* 319 (12), 318 (40), 263 (9), 262 (31), 261 (100), 247 (9), 246 (29), 233 (11), and 75 (12).

Cannabispiradienone (18).—The compound was present in column bands 4, 5, and 6 and crystallised from ether along with cannabispirenone and cannabispiranol. The crystallisation fractions were purified by chromatography on silica G HF 254 (20 × 20 cm or 40 × 40 cm plates) with ether–n-hexane (3 : 1) as eluant. Cannabispiradienone (R_F 0.32) elutes between cannabispiranol and cannabispirenone (R_F 0.44), having a similar bluish pink colour with FBSB. Further purification was effected by p.l.c., with 5% methanol in chloroform as eluant, and finally by h.p.l.c. on a C_{18} reversed-phase column, with methanol–water (85 : 15) as eluant at 1.5 ml/min (R_t 22.6 min). Yields were between 4 and 6 mg/kg for three different years' extractions. *Cannabispiradienone* formed slightly yellowish needles from ether–dichloromethane, m.p. 172—174 °C, with darkening, M^+ 242.0945 ($C_{15}H_{14}O_3$ requires *M*, 242. 0943); $\lambda_{max.}$ (EtOH) 212 (ε 28 600), 239.5 (23 100), 277 (3 100), 285 (3 050), and 307-infl. nm. (600); $\nu_{max.}$ (KBr) *ca.* 3 600, 3 280, 3 140, 2 900, 2 820, 1 654s, 1 602, 1 590, 1 512, 1 455, 1 441, 1 403, 1 365, 1 330, 1 320, 1 185, 1 155, 1 133, 1 080, 1 047, 950, 865, and 847 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 6.98 (d, 2 H, *J* 10 Hz, 2',6'-H), 6.45 (m, 1 H, 4-H), 6.37 (d, 2 H, *J* 10 Hz, 3',5'-H), 6.21 (d, 1 H, *J ca.* 2, 6-H), 5.35 (1 H, D_2O exchg., OH), 3.80 (s, 3 H, OMe), 3.09 (t, 2 H, *J ca.* 7, 3-H), and 2.28 (t, *J ca.* 7, 2-H); *m/e* 243 (18%), 242 (100), 241 (21), 227 (13), 214 (18), 213 (19), 199 (12), 187 (13), 181 (11), 153 (8), 128 (10), 115 (15), 77 (8), 69 (10), 63 (8), 58 (11), 55(10), and 51 (10); *m/e* for trimethylsilylated (18) 316 (7%), 315 (27), 314 (100), 299 (12), 287 (7), 286 (26), and 271 (10).

Hydrogenation of Cannabispiradienone.—The dienone (18) (4.9 mg) was hydrogenated over 5% Pd–C (17.1 mg) in ethyl acetate (5.5 ml). The product was purified by p.l.c. on Silica G HF 254 with ether–n-hexane (3 : 1) as eluant to give cannabispiran, m.p. and mixed m.p. 178—180 °C with

natural (17). It had M^+ 246 (17%) and *m/e* 176 (100): further comparisons by i.r. (KBr) spectroscopy and t.l.c. using ether–n-hexane (3 : 1) and 5% methanol in chloroform confirmed the identity with cannabispiran: it gave the same FBSB colour as the latter.

Cannabispirenone-A (19).—This compound was present mainly in bands 5 and 6 from the original dry column. Crystallisation from ether–hexane gave a mixture of spirans which were further purified by p.l.c. on Silica G HF 254 plates with ether–n-hexane (3 : 1) as eluant. It gave a bluish red FBSB colour and yields varied between 90 and 210 mg/kg for crops grown in three different years. As first isolated it had m.p. 150—152 °C from ethyl acetate–hexane $[\alpha]_D^{27}$ −231° (*c* 0.132, methanol) and $[\alpha]_D^{27}$ −173° (*c* 0.044, EtOAc). Continued crystallisations raised the m.p. to 172 °C, $[\alpha]_D^{27}$ 0: the lack of optical activity was confirmed by an o.r.d. spectrum and the finding that this material was identical in all respects with our synthetic (±)-cannabispirenone A.[15,16] Solution spectral data were virtually identical for the specimens m.p. 150—152 °C and 172 °C as were i.r. data in KBr discs (Found: M^+, 244.1099 ($C_{15}H_{16}O_3$ requires 244.1099), $\lambda_{max.}$ 204.5 (28 600), 228 (15 300), and nm 284 (2 000); $\nu_{max.}$ (KBr) 3 233, 2 959, 2 947, 2 851, 1 657, 1 651, 1 616infl., 1 599, 1 513, 1 468, 1 449, 1 332, 1 302, 1 263, 1 195, 1 150, 1 095, 1 072, 1 050, 1 029, 846, 831, and 804 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 6.98 (dd, 1 H, *J* 10 Hz 2 Hz, 2′-H), 6.36 (m, 1 H, 4-H), 6.21 (d, 1 H, *J* 2 Hz, 6-H), 6.01 (d, 1 H, *J* 10 Hz, 3′-H), 3.78 (s, 3 H, OMe), *ca.* 2.9 (m, 2 H), *ca.* 2.5 (m, 4 H), and *ca.* 2.0 (m, 2 H) (8 alicyclic protons); ^{13}C n.m.r. ($CDCl_3$) δ 201.7s (C-4′), 160.8 (s, C-5), 159.8 (d, C-2′), 153.8 (s, C-7), 146.4 (s, C-3a), 126.7 (d, C-3′), 125.8 (s, C-7a), 101.9 (d, C-4), 100.6 (d, C-6), 55.4 (q, OMe, 48.2 (s, C-1), 35.7 (t, C-2), 35.4 (t, C-6′), and 31.2 (t, C-5′ and C-3); *m/e* 245 (12%), 244 (100), 216 (14), 201 (9), 190 (12), 189 (84), 188 (12), 187 (71), 173 (12), 161 (10), and 115 (12); *m/e* for trimethysilyl compound 317 (19), 316 (77), 301 (10), 289 (22), 288 (100), 242 (11), 233 (13), 187 (15), 77 (64), and 73 (11).

The chiral and (±)-forms of cannabispirenone-A could be differentiated by n.m.r. (250 MHz) spectroscopy using optishift reagent [tris-3-trifluoromethylhydroxymethylene-(+)-camphoratoeuropium(III)]. The reagent was added portionwise to (±)-cannabispirenone, m.p. 172—173 °C, in $CDCl_3$ in a standard n.m.r. tube: addition of 9 mg reagent resolved a pair of doublets of equal intensity at δ 6.85 (original position δ 6.05, doublet). Addition of further reagent caused peak broadening. Material of m.p. 149—152 °C $[\alpha]_D^{23}$ −173° (*c*, 0.185, EtOAc) was not resolved from doublet form, only a slight shoulder being observable. The best sample, m.p. 145—147 °C, unchanged material recovered from the mother liquors of a derivatisation reaction (*p*-bromophenacyl ester), had $[\alpha]_D^{23}$ −178.8° (*c*, 0.085, EtOAc) and showed little discernible splitting of the 3′-proton doublet. Samples of intermediate rotation could be semi-quantitatively analysed by this procedure.

On hydrogenation over 5% Pd/C natural cannabispirenone-A gave cannabispirone-A, m.p. and mixed m.p. 179—181 °C and i.r., t.l.c. and n.m.r. comparison.

Extraction of Thailand Cannabis *for Cannabispirenone-A avoiding Acid and Base Treatment.*—Ground and dried *Cannabis* leaves (1.5 kg) were extracted with hexane, dichloromethane, and ether as described above. The extracts (79.8 g) were dissolved in ether (1.4 l) and divided into two equal portions, one of which was worked up as above, the other as follows. The extract was chromatographed on Florisil (180 g) in an 8-cm diam. column with the following eluants: light petroleum (b.p. 60—80 °C) (700 ml) (8.2 g); 25% ether in light petroleum (b.p. 60—80 °C) (1 l) (12.6 g); more of the same eluant (500 ml) (0.6 g), 50% ether in light petroleum (b.p. 60—80 °C) (1 l) (4.1 g); and ether (1 l) (3.3 g). T.l.c. indicated that the cannabispirenone was located in the fraction eluted with 50% ether in light petroleum (4.1 g).

The latter was chromatographed on a dry silica column in nylon tubing (1 in × 18 in) with ether–light petroleum (b.p. 60—80 °C) (3 : 1) for development; the column was cut arbitrarily into portions, those containing spirenones (t.l.c., visualised by FBSB) being combined and eluted to give a mixture of spiran, spirenone, and a yellow compound. Crystals which were deposited from an ether solution of the mixture were further purified on one 40 × 40 cm HF 254 Silica G plate with ether-light petroleum (b.p. 60—80 °C) as eluant (3 : 1) to give cannabispirenone-A (30 mg), m.p. 164—165 °C, $[\alpha]_D^{22}$ −104° (*c* 0.16, EtOAc). The ether mother-liquors gave a further 15.4 mg, m.p. 149—155 °C, $[\alpha]_D^{22}$ −163° (*c* 0.3, EtOAc). Recrystallisations of the spirenone, m.p. 164—165 °C, from ether gave crystals of the racemate, m.p. 171—172 °C identical with that mentioned above.

The other half of the original extract, worked up as given earlier in the paper, gave a total of 90 mg cannabispirenone-A as a mixture of (−)- and (±)-compound.

Cannabispirenone-A Methyl Ether (20).—Cannabispirenone-A [m.p. 161—163 °C, $[\alpha]_D^{23}$ −159° (*c* 0.218, MeOH)] (19.6 mg) was warmed to 50 °C in acetone (150 μl) containing dry potassium carbonate (13.6 mg) and dimethyl sulphate (10 μl) and then stirred for 2 h at 60 °C. P.l.c. of the product, with ether–n-hexane (2 : 1) as eluant gave *cannabispirenone-A methyl ether* (20) (17.6 mg, 85%), m.p. 110—112 °C from ether, $[\alpha]_D^{23}$ −135° (*c* 0.034, EtOAc). It had *m/e* 259(10), M^+ 258 (64), 231 (12), 230 (100), 216 (8), 215 (14), 201 (10), 188 (10), 187 (84), 175 (16), 128 (12), and 115 (32). Metastable M^* 205.3 (258→230); ^1H n.m.r. ($CDCl_3$) 6.89 (dd, 1 H, *J* 10.1, 1.8 Hz, 2′-H), 6.39 (m, 1 H, 4-H), 6.29 (d, 1 H, *J* 2.1 Hz, 6-H), 5.95 (d, 1 H, *J* 10.1 Hz, 3′H), 3.80 (s, 3 H, OMe), 3.72 (s, 3 H, OMe), *ca.* 2.94 (m, 2 H), *ca.* 2.5 (m, 4 H), and 2.1—1.8 (m, 2 H) (alicyclic methylenes).

Cannabispirenone-B (21).—Although we have not been able to isolate this compound, evidence of its presence was noticed in some samples of cannabispirenone-A. The contaminant had ^1H n.m.r. ($CDCl_3$) δ 6.91 (dd, *J ca.* 10, *ca.* 2 Hz), 6.32 (m), 6.25 (d, *J ca.* 2 Hz), 5.96 (d, 10 Hz), 3.71 (s, OMe). [lit.,[4,11] ($CDCl_3/CD_3COCD_3$) δ 6.88 (dd, *J* 10.2, 1.8 Hz), 6.33 (br d, *J* 1 Hz), 6.26 (d, *J* 1 Hz), 5.89 (d, *J* 10.2, Hz), and 3.68 (s)].

From n.m.r. peak heights the amount present was *ca.* 5—*ca.* 7% of the total cannabispirenones.

β-*Cannabispiranol* (22).—The compound was located in bands 3 and 4 from the crude dry column and deposited readily as crystals from ether. Further amounts were obtained by p.l.c. of the mother-liquors using silica gel G HF 254 plates and eluting with 5% methanol in chloroform or ether–n-hexane (3 : 1); total yield 60—80 mg/kg after crystallisation. It gives a bluish pink colour with FBSB. β-Cannabispiranol crystallised from ethyl acetate–ether, m.p. 192—193 °C (lit.,[17] m.p. 179—183 °C, lit.,[18] m.p. 194—197 °C) (Found: M^+, 248.1417. $C_{15}H_{20}O_3$ requires 248.1413), $\lambda_{max.}$ 206 (ε 30 400), 223infl. (9 200), 276 (2 050), and 282 nm (2 010); $\nu_{max.}$ ($CHCl_3$) 3 450, 3 270, 2 930, 2 860,

1 625, and 1 598 cm^{-1}; ^1H n.m.r. (CD_3COCD_3) δ 8.09 (1 H, D_2O exchg. OH), 6.25 (m, 1 H, 4-H), 6.21 (d, 1 H, *J* 2 Hz, 6-H), 4.00 (m, 1 H, 4'-H), 3.68 (s, 3 H, OMe), and 3.32 (br, 1 H), 2.77 (sextet, 4 H), 1.99 (t, 2 H, *J* 8 Hz), *ca.* 1.72 (m, 4 H), and *ca.* 1.15 (br, d, 2 H) (alicyclic methylenes); ^1H n.m.r. ($CDCl_3$) δ 6.33 (1 H, *J ca.* 1.5 Hz, 4-H), 6.16 d (1 H, *J ca.* 1.5 Hz, 6-H), 4.14 (br m, 1 H, 4'-H), 3.76 (s, 3 H, OMe), and 2.83 (t, 2 H, *J* 7.5 Hz), 2.68—2.16 (m, 2 H), 2.00 (t, 2 H, *J* 7.5 Hz), and 1.86—1.46 (m, 6 H) (alicyclic methylenes); the spectrum was also determined in CD_3OD; *m/e* 248 (7%) 190 (9), 189 (100), 176 (65), 175 (7), 174 (7), 161 (9), 149 (9), 115 (13), 105 (7), and 91 (17); *m/e* for trimethylsilylated (22) 393 (11), 392 (26), 262 (21), 261 (100), 248 (12), and 131 (16). The diacetate (Ac_2O–pyridine) had m.p. 133—135 °C from chloroform (lit.,[18] 141—142 °C; lit.,[17] m.p. 127—128 °C), M^+ 332; two acetyl methyls resonating at δ 2.36 and 2.08 ($CDCl_3$).

To confirm identity, β-cannabispiranol (13.6 mg) was oxidised with pyridinium chlorochromate (20.4 mg) in dry dichloromethane (0.5 ml) containing a little powdered anhydrous sodium acetate. Work-up by t.l.c. gave a product identical in R_F and FBSB colour with natural cannabispirone.

α-Cannabispiranol (23).—A further compound, giving a FBSB colour similar to the β-form was observed in the main fraction containing the latter. Its R_F in ether–hexane and methanol–chloroform was almost identical with that of β-cannabispiranol but g.l.c. of the trimethylsilylated material on an OV 17 SCOT column at 225 °C (N_2 5 ml/min) showed the presence of a new compound having R_t 10.2 min (β-cannabispiranol R_t 11.4 min). The mass spectrum of the trimethylsilylated compound (g.c./m.s.) was similar to the derivative of β-cannabispiranol. Isolation was effected by p.l.c., with chloroform–ethyl acetate (1 : 1) as eluant: it had R_F 0.47 (β-cannabispiranol R_F 0.33). This was followed by purification by h.p.l.c. on a reversed-phase C_{18} column with methanol–water (9 : 1) as eluant. It had m.p. 176—177 °C from ethyl acetate–ether, undepressed by an authentic synthetic specimen,[16] m.p. 176—177 °C (lit.,[17,18] m.p. 176 °C, 177—180 °C). It had M^+ 248.1431 ($C_{15}H_{20}O_3$ requires *M*, 248.1412); $\lambda_{max.}$ (EtOH) 221, 227, and 283 nm; $\nu_{max.}$ (KBr) 3 300 br, 1 610, and 1 590 cm^{-1}; ^1H n.m.r. (CD_3COCD_3) 8.13 (br s, D_2O exch, 1 H, OH), 6.26 (m, 1 H, 4-H), 6.20 (d, 1 H, *J* 2.4 Hz, 6-H), 3.68 (s, 3 H, OMe), *ca.* 3.54 (br, m, 2 H, 4'-H and OH), 2.79 (t, 2 H, *J* 8 Hz, 3-H_2), 2.35 (br, m, 2 H), 2.00 (t, 2 H, *J* 8 Hz, 2-H_2), 1.84 (m, 2 H), and 1.35—1.5 (m, 4 H). α-Cannabispiranol was isolated from leaves cropped in two different years (0.3 mg/kg).

3,5-Dihydroxy-7-methoxydihydrophenanthrene (*Cannithrene*-1) (24).—The chief source was the mother-liquors from fractions 5 of the original dry column, after crystallisation of the spiran components from ether. It was distinguished from spiran compounds by its different colour (purple) with FBSB. Careful p.l.c. on silica G HF 254 with ether–n-hexane (3 : 1) as eluant followed by similar p.l.c. with 3% methanol in chloroform as eluant gave *ca.* 10 mg of compound (4 mg/kg) which was difficult to crystallise.

Cannithrene-1 had m.p. 189 °C after darkening, M^+ 242.0950 ($C_{15}H_{14}O_3$ requires *M*, 242.0943), $\lambda_{max.}$ (MeOH) 220, 241i, 266i, 274, 300, and 310 nm; $\nu_{max.}$ (mull) 3 400, 3 270br, 1 612, and 1 590 cm^{-1}; ^1H n.m.r. (CD_2COCD_3) δ 8.58 (1 H, D_2O exchg., OH), 7.94 (d, 1 H, *J* 2.5 Hz, 4-H), 7.88 (s, 1 H, D_2O exch., OH), 7.00 (d, 1 H, *J* 8 Hz, 1-H), 6.58 (dd, 1 H, *J* 8 and 2.5 Hz, 2-H), 6.42 (br s., 2 H, H-6 and H-8), 3.77 (s, 3 H, OMe), and 2.65 (4 H, 9 and 10-H's) [on addition of D_2O the broad 2H singlet at 6.42 could be seen as one resonance at 6.37 (d, *J* 2.5 Hz, 6-H) and a second at 6.49 (d, *J* 2.5 Hz, 8-H)]; *m/e* 242 (100), 241 (16), 227 (7), 213 (7), 181 (39), 169 (7), 153 (12), and 152 (22); *m/e* in trimethylsilylated form 386 (100) and 371 (6). The diacetate (Ac_2O–pyridine) showed acetyl resonances at δ 2.28 and 2.26.

Thermal Conversion of Cannabispiradienone into Cannithrene-1.—T.l.c. of a sample of cannabispiradienone after m.p. determination showed formation of a compound having a slightly higher R_F value than the original [eluant ether–hexane (3 : 1)] and staining purple with FBSB. The material formed by heating the spiradienone (2 mg) in glass at 180 °C (20 min) was compared with cannithrene-1. U.v. maxima, mass spectra, and R_t value on g.l.c. using a 2% OV 17 column were identical, as were t.l.c. comparisons and FBSB colours.

It had previously been noted that on g.c./m.s. after trimethylsilylation (heating with BSTFA + 1% TMCS), two peaks having M^+ 386 were formed from cannabispiradienone. The second peak had an R_t value identical with that of trimethylsilylated cannithrene-1 (1.46; Δ^6-THC, trimethylsilylated = 1.00) and it seems that partial conversion occurs during trimethylsilylation or on the column.

4,5-Dihydroxy-3,7-dimethoxydihydrophenanthrene (*Cannithrene*-2) (27).—The mother-liquors from column fractions 5 and 6, after crystallisation from ether to remove most of the spirans, contained a new compound giving a purple FBSB colour and having an R_F value slightly less than canniprene (in 3% methanol in chloroform): in ether–hexane (3 : 1) the R_F value is very close to that of canniprene. P.l.c., first in the latter, then the former system gave *ca.* 20 mg of the new dihydrophenanthrene (*ca.* 8 mg/kg) which was finally purified by reversed-phase h.p.l.c. on a C_{18} column with methanol–water (9 : 1) at 1.5 ml/min (R_t 13 min). *Cannithrene*-2 had m.p. 170—172 °C from ether–hexane, M^+, 272.1058 ($C_{16}H_{16}O_4$ requires 272.1049), $\lambda_{max.}$ (MeOH) 219 (ε 33 900), 267sh (13 400), 275 (15 600), 293sh, (8 150), and 301 (8 150); $\nu_{max.}$ ($CHCl_3$ 3 500, 3 350br, 1 618, 1 595, and 15 88 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ *ca.* 8.23 (br, 1 H, D_2O exch., OH), *ca.* 6.8 (1 H, D_2O exchg., OH), 6.85 (d, 1 H, *J* 8 Hz, 2-H), 6.69 (d, 1 H, *J* 8 Hz, 1-H), 6.53 (d, 1 H, *J* 2.5 Hz, 8-H), 6.47 (d, 1 H, *J* 2.5 Hz, 6-H), 3.95(s, 3 H, OMe), 3.83 (s, 3 H, OMe), and 2.67 (s, 4 H, 9 and 10 H's); ^1H n.m.r. (CD_3COCD_3) δ 6.86 (s, 2 H, 1- and 2-H's), 6.46 (d, 1 H, *J* 2.5 Hz, 8-H), 6.41 (d, *J* 2.5 Hz, 6-H), 3.89 (s, 3 H, OMe), 3.79 (s, 3 H, OMe), 2.63 (s, 4 H, 9- and 10-H's). For ^{13}C n.m.r. see (29) [in $(CD_3)_2SO$]. No o.r.d. curve was observed for a solution of 3.2 mg of cannithrene-1 in MeOH (2 ml) (0.1-cm cell). The *diacetyl derivative* (28) was made (Ac_2O–pyridine, 24 h at 20 °C) from cannithrene-2 (13.4 mg) in 47% yield, needles, m.p. 174—176 °C (volatilises from 135 °C). It had *m/e* 357 (12%), M^+ 356 (70), 315 (12), 314 (86), 273 (86), 272 (100), 271 (10), 243 (8), 229 (16), 211 (12), 205 (8), 183 (14), 176 (6), and 149 (6). Metastables *m**: 277 (356→314), 355.5 (314→272), 193.7 (272→229); ^1H n.m.r. ($CDCl_3$) δ 7.10 (d, 1 H, *J* 8 Hz, 2 H), 6.84 (d, 1 H, *J* 8 Hz, 1-H), 6.73 (d, 1 H, *J* 2.4 Hz, 8-H), 6.64 (d, 1 H, *J* 2.4, 6-H), 3.82 (s, 6 H, 2 OMe groups), 2.67 (br s, 4 H, 9- and 10- H's), 2.28 (s, 3 H, acetyl Me), and 2.18 (s, 3 H, acetyl Me); ^1H n.m.r. ($CDCOCD_3$) δ 7.15 (d, 1 H, *J* 8 Hz, 2-H), 6.94 (d, 1 H, *J* 8 Hz, 1 -H), 6.82 (d, 1 H, *J* 2.5 Hz, 8-H), 6.70 (d, 1 H, *J* 2.5, 6-H), 3.82 (s, 3 H, OMe) 3.81 (s, 3 H, OMe), 2.66 (br, s, 4 H, 9- and 10-H's), 2.22 (s, 3 H, acetyl Me), and 2.11 (s, 3 H, acetyl Me).

This diacetyl cannithrene-1 was used for the single-crystal *X*-ray structure determination.

Canniflavanone-2 (30).—This flavone was present mainly in band 4 of the dry column, along with cannabispirenone and cannabispiradienone. It was separated by p.l.c. using ether–n-hexane (3 : 1) as eluant and then repeating the process with 5% methanol in chloroform. Final purification was by reversed-phase h.p.l.c. on a C_{18} column (Whatman Partisil M9 10/50 ODS-2) with methanol-water (9 : 1) as eluant at 1.5 ml/min with u.v. monitoring at 234 nm. Small amounts of interfering spirans eluted first, followed by the flavone (*ca.* 6 mg/kg). *Canniflavone*-2 crystallised from methanol as pale yellow needles, m.p. 185—186 °C, M^+ 436.1862 ($C_{26}H_{28}O_6$ requires *M*, 436.1886), $\nu_{max.}$ (KBr) 3 410, 2 967, 2 928, 2 858, 1 653, 1 613, 1 518, 1 491, 1 470, 1 437, 1 354, 1 295, 1 271, 1 210, 1 185, 1 132, 1 089, 1 033, 842, 825, and 797 cm^{-1}; $\lambda_{max.}$ (MeOH) 242sh, (ε 18 750), 276 (19 350), and 344 nm (24 550); $\lambda_{max.}$ (MeOH/NaOMe) *ca.* 242sh, 281, 342, and 409; $\lambda_{max.}$ (MeOH/$AlCl_3$) 260, 296sh, and 371; $\lambda_{max.}$ (MeOH)/$AlCl_3$/HCl) 258, 289, 295sh, and 365; $\lambda_{max.}$ (MeOH/NaOAc) 277 and 406; $\lambda_{max.}$ (MeOH/NaOAc/-HBO_3) 277 and 352 nm; ^{1}H n.m.r. δ (CD_3OD) 7.5—7.4 (m, 2 H, 2′- and 6′-Hs: in expansion it was possible to see one *m*-coupled proton and one double doublet, *m*- and *o*-coupled), 6.93 (d, 1 H, *J* 8 Hz, 5′-H), 6.61, 6.48 (both s, 1 H, 3- and 8-H's), 5.24 (t, 1 H, *J* 7 Hz, 2″-H), 5.05 (t, 1 H, *J* 7 Hz, 6″-H), 3.96 (3 H, OMe), *ca.* 3.3—3.5 (obscured in methanol peak, 1″-H), 2.10—1.9 (m, 4 H, 4″- and 5″-H's), 1.78 (s, 3 H, 10″-Me), and 1.60 and 155 (both s, 3 H, 8″- and 9″-Me's); ^{1}H n.m.r. [$(CD_3)_2SO$] δ 13.33 s (1 H, D_2O exch., chelated OH), *ca.* 9.5—11.0 (1—2 H, D_2O exch., OH's), 7.5—7.6 (m, 2 H, 2′- and 6′-H's), 6.95 (d, 1 H, *J* 8.7 Hz, 5′-H), 6.89, 6.57 (both s, 1 H, 3- and 8-H's), 5.19 (t, 1 H, *J* 7 Hz, 2″-H), 5.04 (t, 1 H, *J* 6 Hz, 6″-H), 3.90 (s, 3 H, OMe), 3.24 (d, 2 H, *J* 7 Hz, 1″-H), 1.9—2.05 (m, 4 H, 4″- and 5″-H's), 1.78 (s, 3 H, 10″-Me), and 1.59 and 1.53 (both s, 3 H, 8″ and 9″-Me's). The spectrum was also run in CD_3-$COCD_3$.[6b] The ^{13}C n.m.r. spectrum (30) was obtained in $(CD_3)_2SO$; *m/e* 437 (5%), 436 (33), 393 (2), 368 (11), 367 (100), 352 (4), 351 (8), 325 (5), 315 (4), 314 (42), 313 (98), 300 (3), 165 (4), 151 (2), 148 (>1), 123 (2), 93 (2), 91 (3), 81 (4), 69 (10), 67 (4), and 55 (4). Mass measurements: 313.0701 ($C_{17}H_{13}O_6$ requires 313.0712), 165.0197 ($C_8H_5O_4$ requires 165.0188), and 148.0522 ($C_9H_8O_2$ requires 148.0524); metastable *M**: *ca.* 224.7 (436→313).

Canniflavone-1 (33).—This flavone was located mainly in band 3 of the dry column, together with cannabispiranol and cannabispiradienone. The isolation procedure was the same as for canniflavone-2 (0.8 mg/kg). It crystallised from methanol as pale yellow needles, m.p. 230—231 °C, M^+ 368.1282 ($C_{21}H_{20}O_6$ requires 368.1260), $\nu_{max.}$ (KBr) *ca* 3 300 br, 2 950, 2 860, 1 645, and 1 615 cm^{-1}; $\lambda_{max.}$ (MeOH) *ca.* 243sh (ε 13 400), 276 (13 600), and 343 nm (16 600) nm; $\lambda_{max.}$ (MeOH/NaOMe) 280, 342, and 409; $\lambda_{max.}$ (MeOH/$AlCl_3$) 261, 284, 296sh, and 373; $\lambda_{max.}$ (MeOH/$AlCl_3$/HCl) 255, 288, and 358; $\lambda_{max.}$ (MeOH/NaOAc) 278 and 405; $\lambda_{max.}$ (MeOH/NaOAc/H_3BO_3) 276 and 354 nm; ^{1}H n.m.r. (CO_3COCD_3): δ 13.28 (s, 1 H, D_2O exchg, chelated OH), 9.5—8.0 (br, 2 H, D_2O exchg, OH's), 7.65—7.5 (m, 2 H, 2′- and 6′-H's), 7.00 (d, 1 H, *J* 8 Hz, 5′-H), 6.68, 6.62 (both s, 1 H, 3- and 8-H's), 5.28 (t, 1 H, *J* 7 Hz, 2″-H), 3.99 (s, 3 H, OMe), 3.36 (d, 2 H, *J* 7 Hz, 1″-H), and 1.79 and 1.65 (both s, 3 H, 4″- and 5″-Me); ^{1}H n.m.r. [$(CD_3)_2SO$] δ: 13.24 (s, 1 H, D_2O exch., chelated OH), *ca.* 9—7 (v br, 2 H, D_2O exch., OH's), 7.5—7.6 (m, 2 H, 2′- and 6′-H's), 6.94 (d, 1 H, *J* 9 Hz, 5′-H), 6.90 and 6.50 both s, 1 H, 3- and 8-H's), 5.19 (t, 1 H, *J* 7 Hz, 2″-H), 3.89 (s, 3 H, OMe), *ca.* 3.4 (obscured in H_2O peak), and 1.73 and 1.63 (both s, 3 H, 4″- and 5″- Me's).

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