nose, xylose, rhamnose, galactose and uronic acid (table 2). These latter sugars were not detected in the monomeric form nor as components of the lower MW oligosaccharides (table 1, mono- to tetrasaccharides). Purification of the largest oligosaccharides (MW $\geqslant$ 2200 D) by anion exchange chromatography revealed that the oligosaccharides in the unbound fraction ($\sim$ 0.5% of total honeydew carbohydrate) were oligoglucans similar to those found in the honeydew of aphids fed on artificial diets containing only sucrose[8] and, hence, were polymerized by the aphid. Conversely, the oligosaccharides in the acid fraction (1.8% of total honeydew carbohydrate) were polymers consisting chiefly of arabinose, galactose, and uronic acid, with significantly less glucose than those oligosaccharides in the unbound fraction (table 2). Furthermore, the neutral sugar and uronic acid composition of these acidic oligosaccharides was qualitatively similar to pectic substances extracted from the same host plant, sorghum variety 'BOK-8' (table 2). Greenbugs cannot synthesize polyuronides or pentans, thus, the acidic oligosaccharides in their honeydew appeared to be of plant origin. To test whether the pectic fragments could have originated in the phloem, cross sections of sorghum leaves were treated with a stain specific for pectin. This staining showed that there was an especially high concentration of pectic substances in the phloem (intracellularly in companion cells and intercellularly between sieve tube elements).

Greenbugs generally cause extensive damage to vascular tissues of susceptible varieties of their host plants[11]. Furthermore, greenbugs feed chiefly from the phloem of their host plants and possess salivary enzymes capable of depolymerizing a wide array of plant matrix polysaccharides (e.g. pectin, arabino-galactan, cellulose, etc.)[9]. Hence, much of the hetero-oligosaccharides in greenbug honeydew are likely to be breakdown products of matrix polysaccharides in the phloem. It is possible, therefore, that pectic fragments which escape being ingested by aphids are translocated elsewhere in the plant.

Oligosaccharides produced from the depolymerization of plant cell walls have been found to elicit phytoalexin synthesis in a number of different plants[12]. More recently, oligouronides were shown to trigger synthesis of proteinase inhibitors[13]. Perhaps phloem translocation of specific hetero-oligosaccharides, produced by polysaccharase activity, is responsible for the variety of systemic responses commonly observed in plants infested by sap feeding insects[14] or infected with bacteria and fungi[15] (e.g. premature senescence, gall formation, hypersensitivity, etc.).

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Cannflavin A and B, prenylated flavones from *Cannabis sativa* L.

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Summary. Two novel prenylated flavones, termed Cannflavin A and B, were isolated from the cannabinoid free ethanolic extract of *Cannabis sativa* L. Both compounds inhibited prostaglandin E_2 production by human rheumatoid synovial cells in culture.
Key words. Cannabis; PGE_2 inhibition; non-cannabinoids.

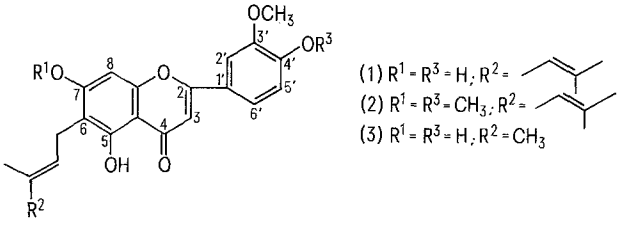
The fact that the use of *Cannabis sativa* in Western medicine has undoubtedly been overshadowed by its use as a social drug[4], has inhibited research into the medicinal properties of this herb. Nevertheless, cannabis does have a tradition for the treatment of a variety of disorders in Indian and Arabian culture[5], including its use as an analgesic and anti-inflammatory agent. The anti-inflammatory action of extracts of cannabis was confirmed pharmacologically by Gill and others[6], but the mechanism of action and the chemical nature of the active principles was unknown. Further work concerned with the anti-inflammatory activity of the hallucinogenic constituent, tetrahydrocannabinol (THC) was inconclusive[7]. Fairbairn and Pickens[8] demonstrated that the cataleptic effects of THC could be inhibited both by cyclooxygenase inhibitors and a cannabinoid free extract of cannabis, thereby providing the first evidence for the presence of noncannabinoid modulators of prostaglandin levels from this plant. That these, unknown agents might be responsible for the analgesic and antiflammatory properties of cannabis was further suggested from the fact that this extract demonstrated appropriate activity in the mouse phenylbenzoquinone writhing test for nonsteroidal anti-inflammatory drugs[9]. The same extract was later shown to inhibit prostaglandin E_2 (PGE_2) release from human rheumatoid synovial cells in culture[10]. This communication describes the structures of Cannflavin A (**1**) together with a lower mol. wt analog, Cannflavin B (**3**) (fig.), isolated from the biologically active ethanolic extract of cannabis following removal of the hallucinogenic cannabinoids.

Cannflavin A (**1**) was isolated from the herb alcoholic extract,

following removal of the cannabinoids using the method of Fairbairn and Pickens[8]. The concentrated alcoholic extract was fractionated by means of TLC. Each step of the isolation procedure was monitored by means of a biological assay involving the inhibition of PGE_2 release from human rheumatoid synovial cells. The methods concerning both extraction, isolation and biological analysis have been published elsewhere[8,10]. Compound **1**, a pale yellow waxy solid, m.p. 182 °C, yield 0.019% w/w of dry herb, was shown to have the composition $C_{26}H_{28}0_6$: *Microanalysis,* C, 69.32%; H, 6.68%; N_2, absent. High resolution *EI-MS* (observed values were with 10 ppm of theoretical values), $M^{+\cdot}$, m/z 436.18454 (35%), significant fragment ions at m/z 367 (100%), 351 (19%), 325 (19%), 313 (81%), 165 (32%), 69 (97%). A yellow color reaction to aqueous NaOH, H_2SO_4 and MgHCl indicated that the compound was a flavonoid and belonged to the class of flavones. The *UV* spectral data and shifts were characteristic of a 3′-methoxy-4′,5,7-trihydroxy flavone with an alkyl substitution at the 6 or 8 position[11,12]; λ_{max}^{MeOH}, nm (ε), 220 (38000), 280 (19000), 346 (26000); +NaOMe, 283, 343, 408; +$AlCl_3$, 264 (sh), 290, 370; +$AlCl_3$ and HCl, 264 (sh), 292, 364; +NaOAc, 278, 400, 522; +NaOAc + H_3BO_3, 277, 347, 522. *IR*, $\nu_{max}^{CHCl_3}$, 3 770, 3 460, 1 600, 1 580, 1 520 cm^{-1}. The presence of $M^{+\cdot}+1$ and $M^{+\cdot}+2$ ions in the mass spectrum suggested the presence of an alkyl-benzene group, and the loss of the ion m/z 69 has previously been reported for $C_{10}H_{17}$ alkylene chains attached to a benzene ring[13,14]. *^{1}H-NMR* (250 MHz $CDCl_3$); δ 1.60 (3 Hs, 3H-10″); 1.69 (3 Hs, 3H-8″); 1.84 (3 Hs, 3H-9″); 2.10 (4H, m, 2H-4″, 2H-5″); 3.49 (2 Hd, J = 7.1 Hz, 2H-1″); 3.99 (3 Hs, 3H-3″); 5.06 (1 Hm, H-6″); 5.30 (1 Ht, J = 7.1 Hz, H-2″), 6.49 (1 Hs, H-3); 6.57 (1 Hs, H-8); 7.03 (Hd, J = 8.4 Hz, 1H-5′); 7.32 (1 Hd, J = 2.0 Hz, H-2′); 7.47 (1 Hdd, J = 8.4 and 2.0 Hz, H−6′); 6.04, 6.45, 13.16 (3 × 1Hs, exchangeable D_2O) ppm. The D_2O exchangeable signals were assigned to the phenolic protons of the A and B rings, the latter hydrogen-bonded hydroxyl at C-5. Irradiation of the triplet at 5.30 ppm caused the doublet at 3.49 ppm to collapse to a singlet and these signals were assigned to 2″ and 1″ of the alkylene chain. The signal for the B ring proton 2′ appeared as a doublet at 7.32 ppm as did the signal for the 5′ proton at 7.03 ppm whilst the 6′ proton was exhibited as a double doublet. When the spectrum was obtained from deuteroacetone the relative positions of the signals for the protons of 2′ and 6′ were altered. In this spectrum irradiation of the methoxy signal at 3.99 ppm induced a positive N.O.E. at 7.61 ppm assigned to proton 2′.

Reaction of compound **1** with diazomethane produced (**2**), the dimethyl analog of Cannflavin A. The presence of two UV maxima between 240 and 285 nm was indicative of 3′, 4′-dimethyl substitution. The absence of a UV shift with NaOMe implied methylation of the hydroxyl groups at 4′ and 7′. The ^{1}H-NMR spectrum differed from **1** most obviously due to the appearance of two additional methoxy signals at 3.93 and 3.97 ppm concomitant with the absence of the signals at 6.04 and 6.45 ppm. Compound **2** failed to form the chroman by means of facile acid catalyzed cyclization[17]. The ^{13}C-NMR spectrum of Cannflavin A (**1**) was interpreted by reference to previous work on flavone[15]; *^{13}C-NMR,* (62.5 MHz, deutero acetone, the carbon state was determined by means of separate INEPT spectra), 16.3 (CH_3), 17.7 (CH_3), 22.0 (CH_2), 25.8 (CH_3), 27.4 (CH_2), 40.5 (CH_2), 56.6 (CH_3), 94.1 (CH), 104.4 (CH), 105.3 (C), 110.5 (CH), 112.4 (C), 116.3 (CH), 121.2 (CH), 123.2 (CH), 123.7 (C), 125.2 (CH), 131.6 (C), 135.4 (C), 148.8 (C), 151.3 (C), 156.6 (C), 160.2 (C), 162.4 (C), 164.7 (C), 183.2 (C) ppm. The resonance of unsubstituted C-6 and C-8 have been found to be distinct in 5,7-dihydroxy and methoxy flavones as they do not overlap. Substitution of C−6 or C−8 does not result in a marked shift of the signal of the other carbon and therefore substitution at C-8 or C-6 can be determined[16]. The assignment of the diprenyl moiety to C-6 was evident from the chemical shift of 94.1 ppm of the C-8.

Of the two isolated compounds, Cannflavin B (**3**) was the minor component, yield 0.003% w/w of dry herb; m.p. 234 °C; *EI-MS* (70 eV, 195 °C), m/z 368 ($M^{+\cdot}$, 56%), 325 (100%), 313 (98%), 165 (12%); *UV* λ_{max}^{MeOH}, (ε), 220 (23,000), 244 (sh), 278 (12,000), 346 (18,000); *^{1}H-NMR* (250 MHz, $(CD_3)_2CO$), 1.65 (3 Hs, 3H-4″); 1.78 (3 Hs, 3H-5″); 3.36 (Hd, J = 7.2 Hz, 2H-1″); 3.99 (3 Hs, OCH_3); 5.28 (1 Hm, H-2″); 6.63 (1 Hs, H-3); 6.69 (1 Hs, H-8); 7.01 (1 Hd, J = 8.2 Hz, H-5′); 7.58 (1H dd, J = 2.2 and 8.2 Hz, HG-6′); 7.61 (1 Hd, J = 2.2 Hz, H-2′); 6.0, 6.4, 13.3 (3 × 1 Hs, exchangeable D_2O) ppm. Both the mass spectrum and the ^{1}H-NMR of **3** indicated that Cannflavin B differed from **1** only in the absence of the five carbon alkyl unit at C-4″ of **1** (fig.). When both these compounds were assayed for their ability to inhibit PGE_2 release from human rheumatoid cells they exhibited an IC_{50} of 31 ng/ml and were approximately 30 times more potent than aspirin in the same system. Cannflavin A is the first of a new group of diprenylated flavones, although similar compounds are known from the isoflavonoid series of natural products[17]. Cannflavin B is a new member of a limited series of prenylated flavones previously isolated from the genera *Artocarpus* and *Morus* of the family Moraceae[18]. Such compounds have not previously been detected in *Cannabis sativa* L. of the family Cannabinaceae.



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