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Prostaglandins and cannabis—III. Inhibition of biosynthesis by essential oil components of marihuana

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In previous publications [1, 2], we reported that several cannabinoids were inhibitors *in vitro* of the conversion of precursors into prostaglandins E_1 and E_2 (PGE_1 and PGE_2). These findings have since been confirmed and extended by Crowshaw and Hardman [3], who also found that PGF production was stimulated by certain cannabinoids. Since this activity was not confined to Δ^1-tetrahydrocannabinols (THC), the major psychoactive principle of cannabis, we thought it worthwhile examining other fractions of the plant for possible effects on PG synthetase activity.

Marihuana contains an essential oil fraction, which can be obtained from it by steam distillation, which consists of a complex mixture of terpenes and other volatiles [4]. Neither Δ^1-THC nor any other cannabinoids are found in this fraction and, aside from odor, it is not believed to contribute to the properties of the drug. Nevertheless, we decided to examine the volatile fraction, since previous conclusions on its inactivity were based on gross pharmacological measurements [5].

A botanically characterized sample of 50 g marihuana* designated as "Mexican Variant" was subjected to steam distillation until 1·5 litres condensate was collected. This was extracted with ether (500 ml) and concentrated by evaporation of the solvent to yield 120 mg pungent oil. Gas–liquid chromatographic analysis (g.l.c.) showed that no Δ^1-THC or other cannabinoids were present in amounts that would interfere with the inhibitor assay.

The oil was then resolved into four crude fractions by chromatography on Silica gel plates.† Each of these was recovered and assayed for inhibitory activity. The conditions for the assay were the same as those previously

Table 1. Inhibition of prostaglandin E_1 biosynthesis by essential oil constituents of *Cannabis sativa* and related substances

Substance	Occurrence		ID_{50}* (μg/ml)
Fraction 1	*C. sativa*		2·3
Fraction 5	*C. sativa*		†
Eugenol	*C. sativa*	*M. fragrans*	5·6
Safrole		*M. fragrans*	47
Myristicin		*M. fragrans*	170
Methoxy eugenol		*M. fragrans*	2·6
Borneol	*C. sativa*		> 100
Fenchyl alcohol	*C. sativa*		> 100
Linalool	*C. sativa*	*M. fragrans*	NI‡
α-Terpineol	*C. sativa*	*M. fragrans*	NI
Limonene	*C. sativa*	*M. fragrans*	NI
p-Cymeme	*C. sativa*	*M. fragrans*	NI
Carvacrol			4·1
2-Cymidine	*C. sativa*		12·5
β-Caryophyllene	*C. sativa*		910
Δ^1-THC	*C. sativa*		100
Cannabigerol	*C. sativa*		95

* ID_{50} = dose which causes 50 per cent inhibition (see also Ref. 2).

† Fraction 5 was a mixture; therefore, an ID_{50} could not be calculated. However, it appears to be of the same order of activity as fraction 1.

‡ NI = non-inhibitory.

* The marihuana used in this study was kindly provided by Dr. Carlton E. Turner, University of Mississippi, under a program of the National Institute on Drug Abuse. The material was grown on campus and was about 18 weeks old when harvested. Thin-layer chromatographic assay was reported to show a 1·56% Δ^1-THC content.

† Thin-layer chromatography was done on 0·5 mm Merck EM Silica gel plates with fluorescence indicator. Hexane–ether (1:1) was used as the developing mixture, and the zones were visualized with a u.v. lamp. R_f values were: 0·3, 0·4, 0·5 and 0·8.

‡ High pressure liquid chromatography was done on a Waters liquid chromatograph using a 2 ft Corasil column with u.v. monitor (280 nm). The elution mixture was hexane–tetrahydrofuran (200:1) at 1·0 ml/min.

described [2], which were based on the conversion of ^{14}C-8,11,14-eicosatrienoic acid to PGE_1 by bovine seminal vesicle microsomes. Only the least mobile fraction showed appreciable inhibitory activity; g.l.c. of this fraction revealed a mixture of several components.

A further resolution of the active fraction was achieved by means of high pressure liquid chromatography (h.p.l.c.).‡ The ultraviolet detection system showed seven major substances which were collected in five fractions. Only the first and last fractions were inhibitory when assayed (Table 1). G.l.c. of the first fraction indicated a pure substance, while the last fraction still consisted of three major components.

The ultraviolet absorption spectrum of the monocomponent active fraction gave some evidence for its identity. A triple maximum was observed at 275, 280 and 285 nm which compared well with the spectrum reported for eugenol [6]. Direct comparison of authentic material (Aldrich Chemical Co.) with the cannabis fraction showed identical mass and ultraviolet spectra, g.l.c. retention times and h.p.l.c. elution volumes.

The occurrence of eugenol in cannabis has been reported by Obata and Ishikawa [7], who isolated it from freshly harvested Japanese hemp. This catechol derivative, along with a variety of analogs, has also been isolated from the essential oil of *Myristica fragrans* [8]. The spices nutmeg and mace are derived from *M. fragrans* and have long been used both for culinary and medicinal purposes as well as being a mood-altering drug. We have tested several of the reported nutmeg principles, including eugenol, for prostaglandin inhibitory activity (Table 1). The range of activities obtained suggests a definite structure–activity relationship analogous to what we found previously for the cannabinoids [2]. Two examples of cannabinoids, Δ^1-THC and cannabigerol, are given in Table 1 for comparison.

Several of the reported terpene constituents of both cannabis and myristica were also assayed for PG synthetase inhibition (Table 1). The lack of activity of this group of substances shows that inhibition depends upon specific structural features. For example, a comparison of *p*-cymene (methylisopropylbenzene) with carvacrol (2-hydroxy-*p*-cymene) and 2-cymidine (2-amino-*p*-cymeme) shows the dramatic effect on inhibition of heteroatom substituents on an aromatic nucleus.

The role that eugenol and other volatiles may have in the pharmacology of cannabis will have to be considered in view of our findings. Segelman *et al.* [9] have recently shown that the volatile fraction of cannabis exhibits pharmacological activity in rats. The possibility that these and other effects of cannabis may be mediated by an alteration of PG synthesis seems not unlikely.

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