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Chemical composition and potential activity of Argentine marihuana

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The occurrence and amount of Δ^1-tetrahydrocannabinol, cannabidiol and cannabinol in Argentine marihuana have been investigated, and its potential activity assessed. The analytical method was applied to thirteen different samples obtained from the Argentine Police or from domestic cultivations of *Cannabis sativa L.* The Δ^1-tetrahydrocannabinol content including decarboxylated precursors ranged from 0·1 to 8·3 g per 100 g of the dried drug according to the source.

This work responds to the need for information about the cannabinoid content of marihuana from different parts of the world (Asuni, Booij & others, 1971), to know the chemotypes used and their potential biological activity. Samples seized by the police and samples from plants cultivated either for their fibre or for illicit purposes have therefore been examined to give a general idea of the type and quality of marihuana in Argentina.

While the active compounds of marihuana are Δ^{1-6}-tetrahydrocannabinol* (Δ^{1-6}-THC), Δ^1-tetrahydrocannabinol** (Δ^1-THC), propyl and methyl homologues, and the acids related to them, its main component is Δ^1-THC (Asuni & others, 1971; Gill, 1971). We decided to determine it as the major active component, cannabidiol (CBD) as the major inactive component, and cannabinol (CBN) as the component indicating the degree of decomposition of each sample (Ohlsson, Abou-Chaar & others, 1971).

MATERIALS AND METHODS

Materials

The standard Δ^1-THC (UNC 332) was provided by the Argentine Federal Police and obtained from Interpol. It was kept in a refrigerator.

Most of the marihuana samples analysed were also provided by the Argentine police. Some other samples were obtained from cultivations made for the fibre or for illicit purposes.

Preparation of extract. The material was thoroughly mixed and dried at 40° to constant weight. An accurately weighed aliquot (about 2 g) was exhaustively extracted with light petroleum (b.p. 40°–60°) for 6 h in a Soxhlet apparatus. This extract was evaporated to dryness in a rotary evaporator and the oily residue redissolved in a small volume of chloroform and transferred quantitatively to a test tube previously calibrated to contain 2 ml. Further dilutions were made when an excessive chromatographic response was obtained.

* Δ^6-THC according to IUPAC rules.

** Δ^9-THC according to IUPAC rules.

Standard preparation. An accurately weighed amount of Δ^1-THC (40 mg) was dissolved in chloroform to make 2 ml total volume. This solution was kept in a refrigerator.

Quantitative g.l.c.

A Beckman GC-M Research instrument with flame ionization was used with direct injection, all glass column 6 mm $\times$ 3·0 m, packed with 3% OV-17 on DMCS-treated Chromosorb W-AW, 80–100 mesh; carrier gas nitrogen, 40 ml min^{-1}, inlet pressure 2 kg cm^{-2}; injection block 290°; oven 240°; detector 290°. A 1 mV/25 cm Beckman recorder was used at 5 mm min^{-1}. The total attenuation was maintained between 1×10^3 and 5×10^5.

In each case 5 μl of extract was injected and its chromatogram recorded. Each sample was chromatographed three times and the area average of each peak calculated. The areas were calculated by triangulation. In the case of overlapping peaks the normal shape of each peak was drawn and the common area divided between both peaks according to their relative height.

A sample of the standard preparation was chromatographed between two injections of the same extract. The cannabinoids were determined (as Δ^1-THC) using the following equation:

$$\text{g cannabinoid per 100 g marihuana} = \frac{A_u\, C_s\, At_u\, 100}{A_s\, C_u\, At_s} \qquad (1)$$

where A_u was the area under each peak of unknown extract; C_s the concentration of the standard preparation in g of THC per ml solution; At_u the attenuation used to record the chromatogram of the unknown; A_s the area under the peak (Δ^1-THC) of the standard preparation; C_u the concentration of the unknown extract in g of marihuana per ml extract, and At_s the attenuation used to record the standard preparation.

RESULTS

The retention times of the cannabinoids analysed were as follows: CBD, 10 min; Δ^1-THC, 15 min; CBN, 18 min.

The origin of each batch and its CBD, Δ^1-THC and CBN content is shown in Table 1. The potential hallucinogenic potency of each batch is tentatively expressed in Table 2 as the number of cigarettes necessary to obtain a "normal biological high response."

DISCUSSION

Nine determinations of Δ^1-THC solution run in the same day gave a mean of 20·0 mg of Δ^1-THC found per ml with a standard deviation of 2·9%. Nevertheless, the area obtained for the same sample in different days, that is the detector response, was significantly different from one day to the other. In addition to this, it was difficult to use an internal standard because of the presence of numerous peaks in some batches. So we decided to inject a standard sample (kept in a refrigerator) each time a new unknown extract was chromatographed, and to diminish the error in the sample measurement by means of the injection of the same amount of solution in every trial. A single direct calculation was possible because, by using the method

Table 1. *Content of cannabinoids of 13 different samples of marihuana.*

Batch No.	Sample: Source	Sample: Origin	Cannabinoids† % of dry weight: THC	CBD	CBN
1	Buenos Aires	unknown	1·1	2·2	0·06
2	—*	Province of Santa Fe	0·9	0·02	0·02
3	Buenos Aires	unknown	8·3	0·2	0·00
4	Buenos Aires	unknown	0·2	0·00	1·8
5	—*	Río Cuarto, Cba.	3·3	0·01	3·6
6	I. Casanova, B. A. **	I. Casanova, B. A.	0·1	0·002	0·00
7	Buenos Aires**	Buenos Aires	4·5	0·05	0·00
8	Posadas, Mns.	Posadas, Mns.	1·1	0·3	0·2
9	Mar del Plata, B.A.**	Mar del Plata, B.A.	4·2	0·07	0·00
10	Mercedes, B.A. (1972)**	Mercedes, B.A.	0·8	0·02	0·1
11	Mercedes, B.A. (1973)**	Mercedes, B.A.	3·6	0·02	0·00
12	— (female)*	Pasco, B.A.	0·5	0·7	0·00
13	— (male)*	Pasco, B.A.	0·5	0·5	0·00

* Cultivated for their fibre.

** Cultivated for illicit purposes.

† Inclusive of carboxylated precursors.

as described, the concentration of a pure Δ^1-THC solution was shown to be linear with the corresponding peak area.

In spite of the low relative standard deviation obtained through the repeated analysis of the same solution, the determination of Δ^1-THC in eight samples of the same batch gave a mean value of 8·34 (Δ^1-THC % of dry weight) and a standard deviation of 15·7%. Nevertheless, we consider our results representative of the composition of their corresponding batches, and useful to give a general idea of probable activity. Different results in the analysis of the same marihuana have also been obtained by other workers and biological activity was not connected to the Δ^1-THC content (Karniol & Carlini, 1972).

Table 2. *Theoretical amount of Δ^1-THC absorbed and potential 'activity' if smoked.*

Batch No.	Δ^1-THC Absorbed mg per cigarette (0·2 g marihuana per cigarette): Smokers*	Δ^1-THC Absorbed mg per cigarette (0·2 g marihuana per cigarette): Experienced smokers†	Number of cigarettes needed to obtain a "normal biological high response" (total of 4 mg THC available): Smokers*	Number of cigarettes needed to obtain a "normal biological high response" (total of 4 mg THC available): Experienced smokers†
1	0·44	1·7	9	2½
2	0·36	1·4	11	3
3	3·3	13·2	1¼	⅓
4	0·08	0·32	50	13
5	1·3	5·2	3	¾
6	0·04	0·16	100	25
7	1·8	7·2	2¼	½
8	0·44	1·7	9	2½
9	1·7	6·8	2½	½
10	0·32	1·3	12	3
11	1·4	5·6	3	¾
12	0·2	0·8	20	5
13	0·2	0·8	20	4

* 20% of available THC absorbed.

† 80% of available THC absorbed.

Gas chromatography under the described conditions does not detect the acidic cannabinoids, because they decarboxylate in the injection chamber. But instead of being a problem this becomes an advantage because of the greater similarity to marihuana smoking conditions (Mechoulam, 1970) since the total Δ^1-THC available to the smoker is determined.

Table 2 has been made taking into account the fact that while cigarette smoking makes only about 20% of the cannabinoid constituents of marihuana available to the smoker, this figure may be increased to about 80% by experienced smokers (Mikes & Waser, 1971; Agurell & Leander, 1972). The absorbed dose of Δ^1-THC necessary to achieve a "normal biological high response" was considered to be about 4 mg according to Agurell & Leander (1972) and is equivalent to a cigarette having about 2 g of drug.

There was a difference in quality between the various marihuanas analysed. For instance, batches 4 and 6 must be considered harmless but batch 3 could be dangerous.

The high Δ^1-THC content of batches 9 and 11, which were cultivated in a temperate climate, and the similar cannabinoid content between batches 12 (female) and 13 (male) plants agree with the work of Ohlsson & others (1971) who have demonstrated that Δ^1-THC content is related neither to the climate nor to the sex of the plant, but to the particular chemotype of each sample.

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