J. Pharm. Pharmacol. 1980, 32: 21–24 Received March 12, 1979

Cannabinoid compounds in South African *Cannabis sativa* L.

B. I. FIELD AND R. R. ARNDT*

*City Health Department Laboratory, PO Box 1477, Johannesburg and *Department of Chemistry, Rand Afrikaans University, Johannesburg 2000, Republic of South Africa*

Dagga (*Cannabis sativa* L.) samples were collected from various geographical regions of South Africa. These were classified into age, sex and plant part and the cannabinoids were analysed quantitatively by gas-liquid chromatography and mass spectrometry. Analytical results show that there appears to be at least three chemovariants of *Cannabis sativa* growing in South Africa with respect to relative cannabinoid content. One of these variants appears to be unique to Southern Africa. It also appears that South African *C. sativa* ranks among the world's more potent *C. sativa* variants in terms of its Δ^9-tetrahydrocannabinol content.

Cannabis sativa L. is known as dagga in South Africa, and, apart from a few papers published on the constituents of dagga grown in Europe (Nielson 1970; Fairbairn et al 1971; Fairbairn & Liebmann 1974; Krejci et al 1975) and North America (Turner & Hadley 1973, 1974; Turner et al 1973; Holley et al 1975; Small et al 1975) from South African seeds, little was known about its composition, its chemical potency, and chemovariants. Hence we have investigated the composition of dagga in terms of its psycho-active principles and related compounds obtained from various geographical regions of South Africa. The plants collected were classified into age, sex and plant part and subsequently analysed by gas chromatography for cannabinoid content. Representative samples were analysed by the gas-chromatography—mass spectrometry technique for identification of the individual cannabinoids present.

MATERIALS AND METHODS

Samples of dagga plants were obtained from three regions of Southern Africa. These were: the Kokstad district of the Transkei; the Pongola district of Natal, and the Tzaneen district of the Transvaal.

Samples were classified according to the age and sex of the plant samples as well as the various plant parts. For age, the plants were divided into young plants (less than 6 weeks old), medium plants (6 weeks to 3 months) and old plants (3 to 6 months). The sex of the plants was determined as male, female and plants before sex identification. The plants were further divided into their growing or flowering tips, leaves from inflorescence, upper leaves, lower leaves, upper and lower stems, roots, flowers (male) and seeds (female).

* Correspondence.

Extraction of the cannabinoids from fresh plant material

The extraction method was that of Lerner (1969) as modified by Fetterman et al (1971). The air dried sample was powdered in a blender to approximately 60–80 mesh. To a 1 g sample accurately weighed, 40 ml of chloroform was added, and the resulting suspension refrigerated at 4 °C and shaken for 30 s at 10 min intervals for 1 h. The insoluble plant material was removed by vacuum filtration. The filtrate, was evaporated under vacuum at 40 °C, the residue normally a dark green paste was overlayed with nitrogen, stoppered, and stored in a refrigerator at 4 °C until required.

Moisture determination

To obtain meaningful results, all cannabinoid analyses are reported on a dry basis. The method used for moisture analysis is that of the A.O.A.C. (Association of Official Analytical Chemists, 1975) for tobacco.

Quantitative gas chromatographic analysis

A standard solution of androst-4-ene-3,17-dione, for use as an internal standard, was prepared by dissolving 1·00 g in 1 litre absolute ethanol. A suitable aliquot of this solution (usually 10 ml) was added to the dagga extract residue, and continuous vibration from an ultrasonic vibrator was applied to bring the extract into solution.

A Becker Research Gas Chromatograph Type 3810 with a flame ionization detector was used. The oven was operated isothermally at 210 °C, the injector and detector were set at temperatures 260 °C and 240 °C respectively. Silanized glass columns, 2 m $\times$ 4 mm (i.d.) were packed with 2% OV-17 on 100–120 mesh Supelcoport. Nitrogen at 40 ml min^{-1} was used as the carrier gas. Peak area measurements

were made with a Philips type PM8000 recorder equipped with a disc integrator. In each case 2 μl of the cannabinoid solution was injected into the chromatograph. Each sample was chromatographed in duplicate from which the average peak area was calculated. The chromatograph was calibrated using at least 10 different mass ratios of the internal standard and authentic cannabinoids, cannabidiol (CBD–C_5), cannabinol (CBN–C_5) and Δ^8-tetrahydrocannabinol (Δ^8-THC). The confidence limits were $0{\cdot}96 < b_1 < 1{\cdot}1$ and $-0{\cdot}31 < b_0 < 3{\cdot}6$, where b_1 is the slope and b_0 the intercept of the regression line, at 95% level of confidence ($t = 2{\cdot}228$ for 10 degrees of freedom). These confidence limits include the regression lines of CBD and CBN, hence, because of the errors inherent in the method and the similarity of the calibration lines, it was decided to use the Δ^8-THC calibration line for all calculations.

Gas chromatography—mass spectrometry analysis

Analysis of representative samples from the three geographical regions were carried out on a Finnigan 3200/6100 g.c.-m.s. system equipped with a 20 m $\times 0{\cdot}3$ mm i.d. glass capillary column coated with 2% OV-1. The separator was a glass jet and a split ratio of 1:10 was maintained. The oven temperature was 100 °C isothermal for 2 min and then programmed at 6 °C min^{-1} to 230 °C. The injector, interface and transfer line temperature were kept at 230 °C and the manifold was at 50 °C. The carrier gas was helium at 2 ml min^{-1}. Electron energy was 70 eV, 0·5 μl of the extract, as prepared above, was injected.

Statistical analysis

To ascertain the precision of the analytical results, twenty portions of a sample of dagga were extracted under identical conditions and analysed in duplicate by g.l.c. Table 1 shows the statistical results of those analyses with the mean, standard deviation and coefficient of variation for the cannabinoids present.

RESULTS AND DISCUSSION

The retention times of the cannabinoids, relative to androst-4-ene-3,17-dione (Rt = 1·0) are as follows: cannabidivarin (CBD–C_3) 0·18, Δ^9-tetrahydrocannabivarin (Δ^9-THC-C_8) 0·26, cannabicyclol (CBCL) 0·26, cannabichromene (CBC) 0·34, cannabivarin (CBN-C_3) 0·34, cannabidiol (CBD-C_5) 0·34, cannabigerol monoethyl ether (CME) 0·38, Δ^8-tetrahydrocannabinol (Δ^8-THC) 0·44, Δ^9-tetrahydrocannabinol (Δ^9-THC-C_5) 0·49, cannabigerol (CBG) 0·57, and cannabinol (CBN-C_5) 0·63. The values are in good agreement with those of Fetterman & Turner (1972), Turner & Hadley (1973) and Turner et al (1974).

Table 2 shows a representative selection of the cannabinoid content of dagga from the various areas. The complete results were reported by Field (1976). The cannabinoid content of the roots, stems and seeds have not been reported as the values are low.

Of the 35+ known naturally occurring cannabinoids, nine were analysed routinely. The carboxylic acids of the cannabinoids, claimed to be present in large concentrations in the fresh plant material (Claussen & Korte 1968; Kimura & Okamoto 1970; de Zeeuw et al 1972; Turner et al 1973) were not considered separately for analysis, and as it is known that the cannabinoid acids decarboxylate in the injection port, the results shown in Table 2 refer to the sum of the neutral and acid cannabinoids.

Identification of the cannabinoids was by various methods. Δ^9-THC-C_3 and Δ^9-THC-C_5 were identified by g.c.-m.s. while the others were identified by comparison of the relative retention times with those of authentic samples, and relative retention times published in the literature (Fetterman & Turner 1972; Turner & Hadley 1973; Turner et al 1974).

The mass spectrum of the peak ascribed to Δ^9-THC-C_3 at Rt 0·26 showed no signals at *m/e* 314, 299 and 231. These peaks are typical for CBCL. (Novotny et al 1976). The latter cannabinoid has the same retention time as Δ^9-THC-C_3 when gas chromatographed. Three cannabinoids, viz. CBC-C_5, CBN-C_3 and CBD-C_5 could not be resolved and these are reported as a mixture in the peak at Rt 0·34.

It was found that the plants collected from the Transkei have Δ^9-THC-C_5 as the major component mixed with relatively small concentration of the other cannabinoids. The relative cannabinoid content appears to be similar to *Cannabis sativa* of Mexican

Table 1. Statistical results of 20 assays of one dagga sample by gas chromatography.

Statistic	% mass/mass Cannabinoid					
	Δ^9-THC-C_5	Δ^9-THC-C_3	CBN-C_5	CBC-C_5*	CBG	CME
Mean	1·266	0·574	0·029	0·116	0·047	0·031
Standard deviation	0·089	0·055	0·008	0·016	0·009	0·005
% Co-efficient of variation	7·03	9·58	27·6	13·8	19·2	16·1

* CBC-C_5 includes CBD-C_5 and CBN-C_3.

Table 2. Cannabinoid content of *Cannabis sativa* L. from various regions of South Africa.

Description	% Moisture	Cannabinoids % mass/mass (dry basis)‡ Δ^9THC-C_5	Δ^9THC-C_3	CBN-C_5	CBC-C_5†	CBG	CME	Δ^8THC	CBD-C_3
Plants from the Transkei, Kokstad district									
Young plants:									
Growing tips*	9·36	4·24	0·035	0	0·49	0·63	0·023	0	—
Upper leaves*	9·22	1·41	0·024	0	0·28	0·18	0·012	0	—
Lower leaves*	9·33	0·64	0·012	0	0·16	0·007	0·008	0	—
Medium plants:									
Flowering tips ♂	11·41	1·87	Trace	0·024	0·11	0·082	0·028	0	—
Flowering tips ♀	9·72	1·62	Trace	0	0·035	0·077	Trace	0	—
Leaves ♂	10·95	0·66	0·001	Trace	0·036	0·009	0·005	Trace	—
Leaves ♀	9·81	0·72	Trace	0·002	0·018	0·028	Trace	0	—
Flowers ♂	14·23	1·82	0·049	0·038	0·090	0·13	0·023	0	—
Old plants:									
Flowering tips ♂	5·63	1·63	0·005	0·035	0·098	0·059	Trace	Trace	—
Flowering tips ♀	8·73	2·32	0·015	0·047	0·10	0·11	0·030	0·016	—
Leaves ♂ + ♀	10·93	0·95	0·005	0·007	0·10	0·004	0·004	Trace	—
Plants from Pongola district, Natal									
Young plants									
Growing tips*	8·89	2·15	2·22	0	0·26	0·029	0	0	0·007
Leaves from inflorescences*	8·35	1·69	2·32	0	0·48	0·091	0	0	0·25
Upper leaves*	7·93	0·84	1·02	0	0·15	0·013	0·034	0·003	0·11
Lower leaves*	6·58	0·23	0·29	0	0·040	0	0	0	0
Medium Plants:									
Growing tips ♂	10·55	1·41	2·40	0	0·12	0·18	0	0	Trace
Growing tips ♀	11·68	2·51	3·08	0·004	0·20	0·055	0	0	0·016
Flowers ♂	10·60	1·19	2·24	0·056	0·028	0·11	0	0	0·10
Upper leaves ♂	8·53	0·95	2·02	0	0·050	0	0	0	0·037
Lower leaves ♂	8·39	0·45	1·13	0	0·036	0	0	0	0·002
Upper leaves ♀	10·32	0·54	0·87	0	0·012	0	0	0	0·015
Lower leaves ♀	8·61	0·38	0·98	0	0·038	0	0	0	0·070
Leaves from inflorescences ♂	8·86	1·30	1·79	0	0·060	0·048	0	0	0·14
Leaves from inflorescences ♀	10·45	1·26	1·61	0	0·12	0	0	0	0·089
Plants from Tzaneen, District									
Young plants:									
Growing tips*	9·12	1·35	0·31	Trace	0·10	0·039	0·009	0	0·001
Upper leaves*	9·05	0·89	0·35	0·005	0·11	0·005	Trace	0	0·018
Lower leaves*	8·90	0·74	0·31	0·002	0·11	Trace	Trace	0	0·029
Medium plants:									
Growing tips ♂	10·35	4·77	1·05	0·045	0·25	0·29	0·007	0	Trace
Growing tips ♀	9·33	3·83	0·087	0·064	0·034	0·18	0·016	0	Trace
Inflorescences ♂	9·43	3·67	1·20	0·041	0·25	0·23	0·027	0	0·022
Inflorescences ♀	8·24	4·06	0·15	0·062	0·24	0·20	0·015	0	Trace
Upper leaves ♂	9·17	1·62	0·53	Trace	0·10	0·010	0·008	Trace	Trace
Lower leaves ♂	9·37	1·44	0·50	0·003	0·12	0·009	0·009	Trace	0·006
Upper leaves ♀	8·03	2·02	0·12	0·001	0·030	0·032	0	0	0·045
Lower leaves ♀	8·66	1·63	0·064	0·029	0·084	0·008	0·019	0	Trace
Old plants:									
Growing tips ♀	10·28	3·08	1·52	0·057	0·22	0·19	0·01	Trace	0·023
Inflorescences ♀	9·44	3·81	1·27	0·059	0·32	0·28	Trace	Trace	0·028
Upper leaves ♀	10·25	1·66	0·80	0·002	0·12	0·32	Trace	0·001	0·015
Lower leaves ♀	10·51	1·50	0·26	0·020	0·13	0·011	Trace	Trace	Trace

* Plants before sex identification.
† Analyses of CBC-C_5 refer to a mixture of CBC-C_5, CBN-C_3 and CBD-C_5.
‡ Inclusive of carboxylic acids of the cannabinoids.

origin (Fetterman & Turner 1972). This similarity may be misleading as the Mexican variant contains CBD-C_5 (Fetterman et al 1971) but the South African variant does not (Turner & Hadley 1973).

In the plants collected from Pongola, Natal, the major cannabinoids present are Δ^9-THC-C_3 and Δ^9-THC-C_5, in almost equal amounts. Turner & Hadley (1973) have shown that propyl homologues of the cannabinoids occur in significant quantities (>5% total cannabinoids) in variants from India, Afganistan and Nigeria, but except for the Afganistan variant, concentrations do not appear to be as high as the Pongola variant.

The relative cannabinoid content of the plants from Tzaneen are again different from those above. In this case Δ^9-THC-C_5 is the major cannabinoid present, with the Δ^9-THC-C_3 content higher than for the Transkei variant, but lower than the Pongola variant. The striking difference of these three variants in terms of their Δ^9-THC-C_5 and Δ^9-THC-C_3 content is shown in Table 2.

It therefore appears to be at least three different chemovariants, growing naturally in South Africa, and the variant from Pongola, Natal, appears to be unique to South Africa, on data published to date.

It does appear that South African dagga ranks among the more potent class of *Cannabis sativa* L. in terms of its Δ^9-THC-C_5 content, Table 3. Comparison of these results with *Cannabis sativa* grown outside South Africa show that the Δ^9-THC-C_5 content of dagga is of the same order reported for *Cannabis sativa* grown in South- and South East Asia (Doorenbos et al 1971; Petcoff et al 1971; Fetterman et al 1971) and Central and South America (Lerner & Zeffert 1968; Doorenbos et al 1971; Fetterman et al 1971; Ohlsson et al 1971; Chiesa et al 1973), and in the U.K. where values up to 7% m/m of THC were obtained (Fairbairn & Liebemann 1974). However, these comparisons may be misleading because extraction and analytical methods vary greatly and published results, with a few exceptions, usually refer to the whole plant rather than to the various plant parts as were presented.

Table 3. Ratios of Δ^9-THC-C_5 to Δ^9-THC-C_3 for the three South African chemovariants of *Cannabis sativa*.

Origin	$\frac{\Delta^9\text{-THC-}C_5}{\Delta^9\text{-THC-}C_3}$	No. of samples analysed
Transkei	45·3	33
Tzaneen	5·9	32
Pongola	1·0	38

Acknowledgements

We thank Prof. J. Offermeier and Mrs S. Fourie of the Dept. of Pharmacology, Potchefstroom University for supplying and classifying the dagga samples; Dr M. Braude of the N.I.M.H. of the U.S.A. for authentic samples of various cannabinoids; and Finnigan A.G., Switzerland for running g.c.-m.s. on the dagga extracts.

REFERENCES

Association of Official Analytical Chemists (1975) Official Methods of Analysis 12 edn. p. 52 Horwitz W. (ed.)

Chiesa, E. P., Rondina, R. V. D., Coussio, J. D. (1973) J. Pharm. Pharmacol. 25: 953–956

Claussen, U., Korte, F. (1968) Natur wissenschaften 53: 541–546

de Zeeuw, R. A., Malingre, Th. M., Merkus, F. W. H. M. (1972) Ibid. 24: 1–6.

Doorenbos, N. J., Fetterman, P. S., Quimby, M. W., Turner, C. E. (1971) Ann. N.Y. Acad. Sci. 191: 3–14

Fairbairn, J. W., Liebmann, J. A., Simic, S. (1971) J. Pharm. Pharmacol. 23: 558–559

Fairbairn, J. W., Liebmann, J. A. (1974) Ibid. 26: 413–419

Fetterman, P. S., Turner, C. E. (1972) J. Pharm. Sci. 61 (9) 1476–1477

Fetterman, P. S., Keith, E. S., Waller, C. W., Guerrero, O., Doorenbos, N. J., Quimby, M. W. (1971) Ibid. 60: 1246–1249

Field, B. I. (1976) M.Sc. Dissertation, Rand Afrikaans University, Johannesburg

Holley, J. H., Hadley, K. W., Turner, C. E. (1975) J. Pharm. Sci. 64 (5): 892–894

Kimura, M., Okamoto, K. (1970) Experientia 27: 988

Krejci, Z., Hanus, L., Yoshida, T., Braenden, O. J. (1975), Acta Palacki Olomuc., Fac. Med. 74: 147–160

Lerner, P. (1969) Bull. Narcot. 21 (3): 39–42

Lerner, M., Zeffert, J. T. (1968) Bull. Narcot. 29 (2): 53–54

Nielson, E. (1970) Dan. Tidsskri. Farm. 44: 359–364

Novotny, M., Lee, M. L., Chow-Eng, L. Raymond, A. (1976) Analyt. Chem. 48 (1): 24–29

Ohlsson, A., Abou-Chaar, C. I., Agurell, S., Nilsson, I. M., Olofsson, K., Sandberg, F. (1971) Bull. Narcot. 23 (1): 29–32

Petcoff, D. G., Strain, S. M., Brown, W. R., Ribi, E. (1971) Science 173: 824–827

Small, E., Beckstead, H. D., Chan, A. (1975) Econ. Bot. 29 (3): 219–32

Turner, C. E., Hadley, K. (1973) J. Pharm. Sci. 62 (2): 251–254

Turner, C. E., Hadley, K. (1974) Arch. Invest. Med. 5 (Suppl. 1): 141–150

Turner, C. E., Hadley, K., Fetterman, P. S. (1973) J. Pharm. Sci. 62 (10): 1739–1741

Turner, C. E., Hadley, K. W., Mole, L., Henry, J. (1974) Ibid. 63 (12): 1872–1876