PHYTOSTEROLS, TRITERPENOID AND OTHER LIPIDIC CONSTITUENTS FROM *Cajanus cajan* L. MILLSP. LEAVES*

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Dedicated to late Dr G. S. Gupta.

Received December 13th, 1976

The light petroleum extract of the leaves of the *Cajanus cajan* L. MILLSP. was examined by chromatographic and spectrometric methods. Neutral portion afforded n-alkanes (16·6%; C_{26}—C_{37}), simple esters (8·4%; C_{36}—C_{62}), free alcohols (26·4%; C_{20}—C_{34}), the triterpenic lupeol (19·2%) and phytosterols (29·4%) (β-sitosterol 52·1%; stigmasterol 39·3%; campesterol 8·3%; cholesterol 0·3%).

Cajanus cajan L. MILLSP. *syn. C. indicus* SPRENG (*Leguminosae*) grows as a crop plant in the plains of India. The small leaves are used in diseases of mouth, against jaundice and for other purposes[1-3]. Since only their essential oil[4], sugars[5] and amino acids[6] have been analysed so far in view of the medicinal importance, the plant material was taken up for a systematic chemical investigation by chromatographic and spectroscopic methods.

EXPERIMENTAL

Extract. The dried and powdered leaves of *C. cajan* L. MILLSP. (1 kg, Agra district, U.P., India) were exhaustively extracted successively with light petroleum (60—80°C), benzene and ethanol (5 l each) at room temperature and their boiling points respectively.

Chromatography. Alumina and silica gel used for column and thin layer were obtained from N.C.L. (India), S. Merck (India) and E. Merck (German Federal Republic). Plates for thin layer chromatography were coated with silica gel and silica gel + $AgNO_3$ respectively. A 20% aqueous solution of perchloric acid was used as spraying agent. Elution of the plate with benzene and light petroleum (80 : 20) gave the following R_F values: Alkanes 1·00, esters 0·75 and alcohols 0·61; elution with benzene showed R_F values 0·42 for lupeol and 0·38 for phytosterols. Gas chromatography was performed on PYE series 104 chromatograph Model 124 with two flame ionization detectors at 250°C or at programmed temperature 170—260°C (2°C min^{-1}). Columns were

* Presented partly at the "Convention of Chemists", Madurai (India), December, 1974.

usualy packed with 3% SE-30 on Gas Chrom Q, phytosterols were analysed on the columns (2·5 m × 4 mm) containing 1% Dexsil 300GC on Diatomite CQ (100—120 mesh) over 260°C, flow rate 50 ml He per min. Homologues were identified using the graphical method[7]. For a quantitative evaluation area under the peaks have been calculated. Standards were injected for identification of the chromatographic peaks by comparison of the retention values. Gas chromatography — mass spectrometry. The assembling[8] of PYE Model 104 chromatograph, splitter, A.E.I. Membrane Separator Interface Type WM-075, spectrometer A.E.I. MS 9, total ion monitor recorder and solvent dump valve was used for providing the mass spectra of individual chromatographic peaks at 220°C and a trap current of 100 μA. Trimethylsilylation[8] of alcohols was done by the reaction with N,O-bis(trimethylsilyl)acetamide.

Spectroscopy. IR spectra were recorded in a Perkin Elmer Spectrometer in KBr discs. The ^{1}H-NMR spectra were run at 60 MHz with Varian A 60 spectrometer using CCl_4 as solvent and tetramethylsilane as internal standard.

Separation and identification. The light petroleum extract was dissolved in diethyl ether and treated with aqueous KOH (10%). No identifiable product could be isolated from the alkali-soluble portion. The alkali-insoluble part was taken in chloroform–diethyl ether (1 : 1) and subjected to column chromatography on alumina. Initial light petroleum eluate afforded a crude product (16·6% of the extract residue), which was subjected to further chromatography on silica gel (+15% $AgNO_3$). Crystallisation from tetrachloromethane yielded a waxy solid, m.p. 65—67°C, whose IR spectrum exhibited bands at 2890, 2850 cm^{-1} (C—H saturated), 1470, 1376 cm^{-1} (C—CH_3) and 730, 720 cm^{-1} $(CH_2)_n$. The subsequent fraction (8·4% of the extract residue) was obtained on elution with light petroleum–benzene (1 : 1), m.p. 68—70°C; it exhibited IR absorption bands of the ester grouping (1745, 1175 cm^{-1}) and of the normal aliphatic chain (735, 725 cm^{-1}). After transesterification[9] it was analysed by gas chromatography. n-Alcohols (26·4% of the extract residue) were obtained on elution with benzene. They melted at 82—83°C and showed IR absorption bands at 3332, 1064 cm^{-1} (OH) and 735, 725 cm^{-1} $(CH_2)_n$. A crystalline TLC homogeneous fraction (19·2% of the extract residue) melting at 206°C, $[\alpha]_D^{17}$ +23·6° was obtained on elution with benzene and subsequent crystallisation. It afforded an acetate m.p. 215—218°C, $[\alpha]_D^{17}$ +47·2°. The spectral data (IR (ref.[10]) PMR (ref.[11,12]) in addition with the comparative TLC, GLC and mixed melting points characterised the isolated alcohol as lupeol. Elution with benzene–chloroform (1 : 1) yielded an another crystalline fraction (29·4% of the extract residue), m.p. 156—158°C, $[\alpha]_D^{17}$ −53·5°, which was positive in Liebermann–Burchardt

TABLE I

Phytosterols (by GLC)

Peak	Relative retention time	Compound	%	Identified by MS
1	0·76	cholesterol	0·3	—
2	1·00	campesterol	8·3	+
3	1·06	stigmasterol	39·3	+
4	1·24	β-sitosterol	52·1	+

reaction and with tetranitromethane. IR spectrum revealed the presence of OH-group (3340, 1053 cm^{-1}), double bond (1640, 835 cm^{-1}) and $-C(CH_3)_2$ grouping (1377 cm^{-1}). Thus, the spectral values along with combustion data ($C_{29}H_{48}O$) indicated a mixture of sterols which could be separated by gas chromatography (Table I). Mass spectra[8,13] of each chromatographic peak (injected as trimethylsilyl ethers) were scanned several times. The presence of typical ionic species[8,13] in each spectrum confirmed the structure of the sterols established by gas chromatography.

RESULTS

The leaf epicuticular wax contains a homologous series of n-alkanes (C_{26}—C_{37}); odd members are predominating[14,15] with maximum occurrence of C_{31}, C_{29} and C_{33} (Table II).

Simple esters form a series C_{36}—C_{62} with the preponderance of even numbered members, mainly C_{52} and C_{54}. They are composed of higher fatty acids (C_{14}—C_{30})

TABLE II
n-Alkanes, Esters and Free Alcohols

Number of C atoms	Hydrocarbons[a]	Acids[a,b] from esters	Alcohols	
			from esters[a]	free[a]
14	—	1·1	—	—
15	—	5·8	—	—
16	—	29·0	—	—
17	—	12·0	—	—
18	—	3·5	—	—
20	—	4·2	—	—
22	—	9·7	1·4	—
24	—	7·9	1·2	1·0
25	—	traces	0·4	traces
26	traces	18·1	24·2	22·0
27	traces	2·0	10·2	3·7
28	0·9	4·7	37·6	49·5
29	19·2	traces	12·8	1·5
30	3·3	traces	11·3	6·5
31	55·3	—	traces	traces
32	5·7	—	traces	2·8
33	14·8	—	—	traces
34	traces	—	—	1·0
35—37	traces	—	—	—

[a] In percent by weight, obtained by GLC (triangulation); [b] as methyl esters.

esterified with homologous alcohols (C_{22}—C_{32}) (Table II). A homologous series (C_{20}—C_{34}) of free n-alcohols was also identified, in which even homologues are prevailing, mainly C_{28}, C_{26} and C_{30} (Table II). The main component of the extract are isoprenoids; triterpenic alcohol lupeol and a mixture of phytosterols (β-sitosterol, stigmasterol, campesterol and cholesterol) are present (Table I).

Thanks are due to Prof. W. Rahman, Head of Department of Chemistry, Aligarh, for providing facilities, Dr J. A. Ballantine, Swansea, Dr A. M. M. Berrie, Glasgow, Prof. G. S. Saharia, Delhi for GLC, GC-MS, microanalyses and various spectra. C.S.I.R., New Delhi, is thanked for financial assistance to D.P.S.(S.R.F.).

REFERENCES

1. Kirtikar K. R., Basu B. D.: *Indian Medicinal Plants*, p. 450. S. N. Basu, Allahabad 1918.
2. Chopra R. N., Nayar S. L., Chopra I. C.: *Glossary of Indian Medicinal Plants*, p. 44. C.S.I.R., New Delhi 1956.
3. *The Wealth of India* (*Raw Materials*), Vol. II, p. 6. C.S.I.R., New Delhi 1950.
4. Nigam S. S., Gupta G. L.: Perf. Ess. Oil Record *60*, 1 (1969).
5. Gupta G. S., Lal N., Sharma D. P.: Proc. Nat. Acad. Sci. India, Sect. A, *44*, II, 140 (1974).
6. Gupta G. S., Sharma D. P.: Proc. Nat. Acad. Sci. India. Sect. A, *43*, III, 268 (1973).
7. James A. T., Martin A. J. P.: Biochem. J. *50*, 679 (1952).
8. Ballantine J. A., Roberts J. C., Morris R. J.: J. Chromatogr. *103*, 289 (1975).
9. Streibl M., Jiroušová J., Stránský K.: Fette, Seifen, Anstrichm. *73*, 301 (1971).
10. *Elsevier's Encyclopaedia of Organic Chemistry*, Vol. 14, p. 575; Vol. 14 Suppl., p. 1115S. Elsevier, New York and Amsterdam 1940, 1952.
11. Shamma M., Glick R. E., Mumma R. O.: J. Org. Chem. *27*, 4512 (1962).
12. Lehn J.-M., Ourisson G.: Bull. Soc. Chim. Fr. *1962*, 1137.
13. Mercer E. I., London R. A., Kent I. S. A., Taylor A. J.: Phytochemistry *13*, 845 (1974).
14. Stránský K., Streibl M., Herout V.: This Journal *32*, 3215 (1967).
15. Gupta G. S., Sharma D. P.: Phytochemistry *13*, 2013 (1974).