Canavalia ensiformis Neutral Lipids, a Rich Source of Lupeol

E.M. Gaydou[a,*], J. Viano[b] and P.J.-L. Bourreil[b]

[a]Laboratoire de Phytochimie, Faculté des Sciences et Techniques de Saint Jérôme, Marseille Cédex 13, France and [b]Laboratoire de Systématique et d'Ecophytochimie, Faculté des Sciences et Techniques de Saint Jérôme, Marseille Cédex 13, France

The composition of the neutral lipids of *Canavalia ensiformis*, which represent 2.21% of whole seeds, has been investigated. The fatty acid composition is characterized by the presence of palmitic (15%), oleic (54%), linoleic (7%) and linolenic (8%) acids. The unsaponifiable matter (7.9% of the neutral lipids), was examined for sterol, 4α-methylsterol and triterpene alcohols. The occurrence of lupeol in high amount in the last fraction (96%) constitutes an interesting source for this compound.

KEY WORDS: *Canavalia ensiformis*, fatty acids, Jackbean, lupeol, methylsterols, neutral lipids, sterols, triterpenic alcohols.

The Jackbean, *Canavalia ensiformis* (L.) DC (Leguminosae fam.), a bushy erect annual climber, is widespread in the humid African, Asian and Indian tropics, but its use as vegetable and pulse crop is limited (1). Jackbean is capable, under good conditions, of producing high yields of green matter for forage (2), and the bean gives about twenty large (20 × 13 mm) white seeds. Seed crude polysaccharides (3,4) and protein (3,5) contents are relatively high, but the lipid content is low (3,5). Although the occurrence of canavalin (6,7) and storage proteins like concanavalin A (8,9) and the presence of alkaloids (10), saponins (11) and gibberelins (12) have been reported, the composition of the lipid fraction, to our knowledge, has not been worked out. Ripe, unprocessed *C. ensiformis* beans used as feed have a toxic effect on animals (13). This legume is used as a cover in tobacco, sugar, cocoa, *citrus* and pineapple plantations, and the young leaves and pods, once cooked, are consumed by humans (14).

In this paper, we present the quantitative determination of the fatty acid composition and the unsaponifiable matter of Jackbean neutral lipids, with emphasis on triterpene alcohols, 4α-methylsterols and sterols. The results obtained have been compared to other tropical seed lipids (15–17).

EXPERIMENTAL PROCEDURES

Proximate analyses. C. ensiformis seeds were purchased from a local market in Santo Domingo (Dominican Republic). Crude protein (nitrogen × 6.25) was determined by micro-Kjeldahl nitrogen analysis. Analyses for crude fiber, ash, neutral lipids, unsaponifiable matter, hydrocarbons, triterpene alcohols, 4α-methylsterols and sterols were carried out according to published methods (17,18).

Gas chromatography (GC). A Girdel 300 gas chromatograph (Louisville, KY), equipped with a flame ionization detector (FID), was used for fatty acid methyl esters (FAME) and steryl acetates analyses. FAME were separated on a fused silica capillary column (30 m × 0.32 mm I.D.) coated with Carbowax 20 M (phase thickness 0.15 μm). Column temperature was 190°C, and detector and inlet temperatures were 250°C. Helium was used as carrier gas at a pressure of 0.7 bar. The injections averaged 1 μL of a 2% solution of FAME in hexane. Steryl acetates were separated on a fused silica capillary column (25 m × 0.32 mm I.D.) coated with OV-1 (phase thickness 0.15 μm). Column temperature was 260°C, and detector and inlet temperatures were 290°C. Helium was used as carrier gas at a pressure of 1 bar.

Gas chromatography-mass spectrometry (GC-MS). Spectra of steryl acetates were obtained on a Delsi gas chromatograph (Lyon, France), linked to a Ribermag R10-10B mass spectrometer and coupled with a Sidar data computer, under the following conditions: Ionization energy, 70 eV; ion source, 220°C; trap current, 60 μA; temperature, 250–290°C at 5°C/min; GC column, 50 m × 0.32 mm WCOT OV-1701 fused silica; carrier gas He, p 1.8 bar.

Isolation of lupeol acetate. Unsaponifiable matter (0.6 g) was fractionated over silica gel 60 (240 g, 230 mesh, Merck, Darmstadt, Germany) in a column of 40 cm (25 mm I.D.). Elution was carried out by applying a 0–100% gradient of isopropyl ether in hexane. Fractions were monitored by thin-layer chromatography (TLC) as previously described (17). The triterpene alcohol fraction (143 mg) was acetylated with acetic anhydride and pyridine. Needles of pure lupeol acetate (98 mg), m.p. 216–217°C, lit. 218°C (19) were obtained.

Nuclear magnetic resonance (NMR). ^{13}C NMR spectra were recorded on a Bruker AMX-200 (Faculté de Pharmacie de Marseille, France). Samples were prepared in a 5-mm o.d. tube by mixing the lupeol acetate with $CDCl_3$ in a volume ratio of 1:4. Tetramethylsilane (TMS) was used as internal standard. The FT ^{13}C NMR were measured under the following conditions: Frequency, 25.2 MHz; spectral width, 6000 Hz; pulse delay, 5 seconds, acquisition time, 1.4 seconds; number of data points, 16 K.

RESULTS AND DISCUSSION

Proximate analysis of Jackbean seeds is given in Table 1. The content of neutral lipids is relatively low, in the same range of *Lens esculentus* (syn. *L. culinaris*) (17), *Vigna sinensis* (syn. *V. unguiculata*) (17), *Prosopis chilensis* (20) or *Cassia siebberiana* (20). The neutral lipids are characterized by a high amount of unsaponifiable matter (7.9%) in comparison with other legumes (20), showing that *C. ensiformis* has an important proportion of sterol-containing lipids.

Fatty acid composition (Table 2) indicates the presence of oleic acid (54%) as the major compound. Palmitic (14.8%), linoleic (7.4%) and linolenic (7.8%) acids are the other main components, thus giving an interesting unsaturated/saturated ratio.

TLC examination of the unsaponifiable matter on silica gel F showed four major spots. The compounds' families were identified by comparison of the R_f values with those standards. The quantitation of hydrocarbons, triterpenic alcohols, 4α-methylsterols and sterols (Table 1) was achieved by column chromatography and showed the high amount of the triterpenic alcohol family (29.3%).

*To whom correspondence should be addressed at Laboratoire de Phytochimie, Faculté des Sciences et Techniques de Saint Jérôme, avenue Escadrille Normandie Niémen 13397 Marseille Cédex 13, France.

TABLE 1

Proximate Analysis[a] of *Canavalia ensiformis* Neutral Seed Lipids

	Composition (%)	
	In the fraction	In whole seed
Crude fiber		7.8
Crude protein		27.2
Ash		2.6
Neutral lipids		2.21
Unsaponifiable matter[b]	7.91	0.175
Hydrocarbons[c]	47.7	0.083
Triterpenic alcohols[c]	29.3	0.051
4α-Methylsterols[c]	5.2	0.009
Sterols[c]	17.8	0.031

[a]In dry matter, average of triplicate analyses.

[b]In neutral lipid fraction.

[c]In unsaponifiable matter fraction, determined by column chromatography.

TABLE 2

Fatty Acid Composition of *Canavalia ensiformis* Neutral Seed Lipid

Fatty acid		ECL[a]	%[b]
Lauric	12:0	12.00	0.2
Myristic	14:0	14.00	0.4
Palmitic	16:0	16.00	14.8
Palmitoleic	16:1(n-7)	16.29	2.2
Stearic	18:0	18.00	1.4
Oleic	18:1(n-9)	18.33	54.2
Linoleic	18:2(n-6)	18.77	7.4
Linolenic	18:3(n-3)	19.45	7.8
Arachidic	20:0	20.00	0.7
Eicosenoic	20:1(n-9)	20.26	2.4
Behenic	22:0	22.00	0.3
Docosenoic	22:1(n-9)	22.41	3.0
Lignoceric	24:0	24.00	1.6
Other fatty acids[c]			3.6
Total saturated			19.4
Total unsaturated			77.0

[a]Equivalent chainlengths of FAME on a Carbowax 20 M fused silica capillary column at 190°C.

[b]Percent by weight of total fatty acids.

[c]Every fatty acid detected represented less than 0.1%.

The sterolic compounds were acetylated, and the identification and the relative quantitation were obtained from relative retention times (RRT) and GC-MS (21). The relative composition of the sterolic fraction (Table 3) reveals the presence of campesterol (10%), stigmasterol (41.5%) and sitosterol (29.2%), showing that this sterolic profile is quite similar to that of *V. sinensis* (17). The 4α-methylsterol fraction of *C. ensiformis* contains gramisterol (14.4%) and citrostadienol (82.5%), in the same range with those observed for other legumes (17). The triterpenic alcohol fraction (Table 3) was composed mainly of lupeol (96.3%), with the minor component being β-amyrin. The identification of lupeol was confirmed by ^{13}C NMR analysis. The assignments of carbon resonances for lupeol acetate are given in Table 4. The chemical shifts observed are in agreement with those described for lupeol by Wenkert *et al.* (22).

TABLE 3

Composition of the Sterol, 4α-Methylsterol and Triterpenic Alcohol Fractions of *Canavalia ensiformis* Neutral Seed Lipids

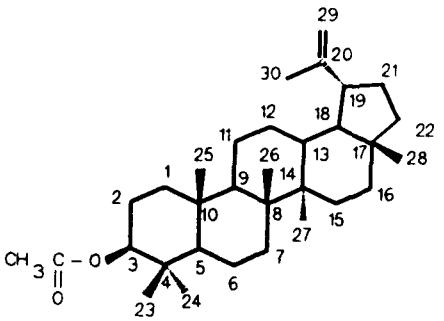
Compound	RRT[a]	%[b]
Sterols		
Cholesterol	1.00	trace
Campesterol	1.29	10.0
Stigmasterol	1.40	41.5
Sitosterol	1.60	29.2
Δ5-Avenasterol	1.67	6.8
28-Isoavenasterol	1.82	5.2
Δ7-Avenasterol	1.89	6.1
Unidentified		1.2
4α-Methylsterols		
Gramisterol	1.63	14.4
Citrostadienol	2.16	82.5
Unidentified		3.1
Triterpenic alcohols		
β-Amyrin	1.52	3.7
Lupeol	1.66	96.3

[a]Relative retention times of corresponding acetates expressed against cholesterol acetate on an OV-1 fused capillary column at 260°C.

[b]Percent by weight for each fraction.

TABLE 4

Carbon-13 NMR Shifts of Lupeol Acetate Isolated from the Triterpenic Alcohol Fraction of *Canavalia ensiformis* Neutral Seed Lipids



Carbon number	δ (ppm)[a]	Carbon number	δ (ppm)[a]
1	38.38	17	43.16
2	23.68	18	48.15
3	79.16	19	48.64
4	37.43	20	150.81
5	55.63	21	30.12
6	18.51	22	39.00
7	34.59	23	28.13
8	40.17	24	16.47
9	50.77	25	16.18
10	37.08	26	15.42
11	20.92	27	14.70
12	25.49	28	18.13
13	37.95	29	109.36
14	43.06	30	19.45
15	27.68	CH_3	21.17
16	35.54	CO	174.71

[a]δ Values from TMS ($CDCl_3$ solution).

The presence of lupeol was first discovered in *Lupinus albus* (23). The occurrence of this triterpenic alcohol in plants has been reviewed (24), showing that this pentacyclic alcohol is widespread in the leaves, barks and roots of Leguminosae, Apocynaceae, Moraceae, Rutaceae, Asclepiadaceae and Sapotaceae families. Since the content of lupeol in *C. ensiformis* neutral lipids is relatively high, Jackbean may be a convenient source of this pentacyclic triterpenic alcohol.

ACKNOWLEDGMENTS

This work was initiated by R. Sansoucy, Food and Agriculture Organization of the United Nations. C. Charlot performed the GC-MS and R. Faure the NMR analyses.

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[Received July 9, 1991; accepted January 14, 1992]