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Triterpene Alcohols, Methyl Sterols, Sterols, and Fatty Acids in Five Malagasy Legume Seed Oils

Emile M. Gaydou,* Jean-Pierre Bianchini,[1] and Julie V. Ratovohery

This paper reports a study of the chemical composition of the neutral lipids which form 0.8–46.7% of five legumes (*Arachis hypogea*, *Voandzei subterranea*, *Phaseolus lunatus*, *Lens esculentus*, and *Vigna sinensis*). Fatty acid profiles revealed that the major acids were palmitic (13.9–31.5%) linoleic (30.9–46.4%), and oleic (5.5–40.0%). Unsaponifiable matter (1.09–3.67%) was examined for sterol, 4α-methyl sterol, and triterpene alcohol fractions. β-Sitosterol was the prominent component in four legumes (39.8–79.7%), while stigmasterol (41.7%) was the major component in *V. sinensis*. Campesterol, stigmasterol, and Δ^5-avenasterol were detected in all samples. Among the 4α-methyl sterol fractions, obtusifoliol (9.5–39.5%), gramisterol (14.7–58.7%), and citrostadienol (8.1–47.6%) were the main components. Cycloartanol (3.0–26.4%), butyrospermol (1.4–59.1%), cycloartenol (1.4–22.4%), 24-methylenecycloartanol (0.8–42%), and cyclobranol (1.0–14.4%) were the main compounds of the triterpene alcohol fractions.

Leguminous seeds make an important contribution to the diet in many tropical countries. They are used as vegetables and are a good source of protein, lipid, and fatty acids for human nutrition. The fatty acid composition of the endogenous fats plays an important role in determining shelf life, nutrition, and flavor of food products. Although some values have already been presented on numerous foods (Brignoli et al., 1976) and legumes (Exler et al., 1977), data for some tropical vegetable legumes are rarely given. The nature of some Indian legume lipids was investigated by Mahadevappa and Raina (1978). Busson and Bergeret (1958) and Doku et al. (1978) have studied the chemical composition of *Voandzei subterranea*. A number of some tropical vegetable and subtropical vegetable oils have been analyzed for their unsaponifiable fractions, particularly sterols and methyl sterols. It was shown that campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, and Δ^7-stigmastenol were common constituents, β-sitosterol being the most prominent in the sterol fraction (Itoh et al., 1974a). Four sterol-containing lipid fractions were isolated from the chloroform–methanol-extracted lipids of cowpea (*Vigna uguiculata*) by Mahadevappa and Raina (1981). Generally 4α-methyl sterols are minor constituents of the unsaponifiable fractions and their separation from unsaponifiable matter is quite difficult. In common vegetable oils, 4α-methyl sterols were studied by Sawicki and Mordret (1970), Itoh et al. (1974b), and Jeong et al. (1975).

Among the important legumes consumed in Madagascar, five of them are used commonly in this country. They are *Arachis hypogea* (var. Valencia), *V. subterranea* (or *Glycine subterranea*), *Phaseolus lunatus* (var. Inamoenus), *Lens esculentus*, and *Vigna sinensis*. Soybeans are becoming an important industrial legume for oil and protein production but are not, at this time, used very much by the population for human nutrition.

This paper presents results on these five legumes consumed in Madagascar. This investigation was undertaken to obtain more information on the fatty acid composition and the unsaponifiable matter of these oils, especially triterpene alcohols, 4α-methyl sterols, and sterols.

EXPERIMENTAL SECTION

The five legumes were collected from a local market in Antananarivo.

Protein content (nitrogen × 6.25) was determined by micro-Kjeldahl nitrogen analysis. Fat, fiber, and ash were analyzed by the NF-Norms (Wolff, 1968).

Fatty acid methyl esters were analyzed by GLC as described previously (Gaydou et al., 1980).

Extraction of unsaponifiable matter was carried out according to published methods (Wolff, 1968; Pelloquin et al., 1977).

Thin-Layer Chromatography of the Unsaponifiable Matter. Thin-Layer chromatography (TLC) was performed on silica gel 60 (F-254) (0.2-mm layer thickness) plates (E. Merck, Darmstadt, GFR) using chloroform–diethyl ether (9:1 v/v) as the developing solvent. The approximately relative R_f values of the fractionation by TLC were hydrocarbons, 0.97, tocopherols, 0.66, 4,4-dimethyl sterols, 0.45, 4-monomethyl sterols, 0.35, and 4-demethyl sterols, 0.27. The unsaponifiable matter was dissolved in carbon tetrachloride (5%) and 150 μL was deposited as a streak of 15-cm length on the plate. Squalene, α-tocopherol, lanosterol and cholesterol, used as standards, were spotted (20 μg) on the left-hand and right-hand side of the plate, respectively. After ascending TLC with the eluting solvent mixture, the standards were visualized with Rhodamine-B by using ultraviolet (UV) light at 360 nm, and the corresponding bands of terpene alcohols (4,4-dimethyl sterols), 4-monomethyl sterols, and sterols (4-demethyl sterols) were scraped off the plate and extracted with dichloromethane.

Gas–Liquid Chromatography (GLC). An Intersmat IG 12 DFL gas chromatograph equipped with a flame ionization detector and a glass injector was used for fatty acid and sterol analyses. A Carbowax 20 M glass capillary column (0.15-μm phase thickness, 45 m × 0.35 mm i.d.) was used to separate fatty acid methyl esters (temperatures were injector, 220 °C, detector, 200 °C, and column, 190 °C; flow rate of hydrogen used as carrier gas 5 mL/min; split ratio, 5/100). For the identification of fatty acid methyl esters, vegetable oils (e.g., olive, peanut, and sunflower) were used as standards. An OV-17 glass capillary column (30 m × 0.36 mm i.d.) was used to separate the trimethylsilyl (Me_3Si) ethers of the terpene alcohols, 4α-methyl sterols, and sterols (temperatures were injector, 270 °C, detector, 280 °C, and column, 260 °C; flow rate of

Laboratoire de Phytochimie and Ecole Supérieure de Chimie de Marseille, Université de Droit, d'Economie et des Sciences, Centre de Saint Jérôme, 13397 Marseille Cédex 13, France.

[1]Present address: Etablissment d'Enseignement Supérieur des Sciences Agronomiques, Département des Industries Agricoles et Alimentaries, Université de Madagascar, B.P. 175—Antananarivo, République Démocratique de Madagascar.

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Table I. Proximate Analysis of Five Malagasy Legumes

	composition[a] of legume				
	A. hypogea	*V. subterranea*	*P. lunatus*	*L. esculentus*	*V. sinensis*
moisture	3.8	6.8	7.8	6.8	6.9
fat	46.7	6.4	0.8	2.0	1.4
protein	28.6	14.3	21.6	32.3	27.9
ash	1.7	2.8	2.9	4.5	2.5
fiber	4.1	3.5	5.1	5.4	5.8
carbohydrate	19.2	69.7	66.9	54.4	61.3
carbohydrate/protein	0.7	4.9	3.1	1.7	2.2
protein/fat	0.6	2.2	27.0	16.2	19.9

[a] Percent of whole seed.

Table II. Physical Properties of Five Malagasy Seeds Oils

	properties of seed oil species				
	A. hypogea	*V. subterranea*	*P. lunatus*	*L. esculentus*	*V. sinensis*
density (d^{20}_4)	0.9175	0.9177	0.9534	*a*	*a*
refractive index (n^{20}_D)	1.4715	1.4715	1.4845	*a*	1.4814
acidic value[b]	0.5	2.1	*a*	*a*	*a*
saponification value[b]	188	190	157	*a*	170
Wijs iodine value[c]	102	89	103	121	118
unsaponifiable matter, %	1.09	2.44	3.26	3.67	2.36

[a] Not determined. [b] Expressed in mg of KOH for 1 g of oil. [c] Expressed in g of iodine for 100 g of oil.

Table III. Fatty Acid Composition of Lipids from Five Malagasy Legumes

		composition[a] of legume				
fatty acid	ECL[b]	*A. hypogea*	*V. subterranea*	*P. lunatus*	*L. esculentus*	*V. sinensis*
myristic (14:0)	14.00	*c*	*c*	0.3	0.7	0.3
palmitic (16:0)	16.00	13.9	24.1	26.3	15.4	31.5
heptadecanoic (17:0)	17.00	*c*	*c*	0.4	*c*	0.4
stearic (18:0)	18.00	3.2	5.2	5.8	3.7	6.4
arachidic (20:0)	20.00	1.3	1.2	0.9	2.1	1.7
behenic (22:0)	22.00	3.0	2.5	0.8	0.5	3.4
lignoceric (24:0)	24.00	1.0	0.5	1.8	0.2	1.0
total saturated		22.4	38.5	36.3	22.6	44.7
palmitoleic (16:1ω9)	16.19	tr[d]	tr	*c*	*c*	*c*
oleic (18:1ω9)	18.14	40.0	21.6	7.4	19.1	5.5
vaccenic (18:1ω7)	18.30	0.2	1.7	1.7	0.4	0.6
linoleic (18:2ω6)	18.60	36.3	40.4	39.2	46.4	30.9
linolenic (18:3ω3)	19.25	tr	2.2	14.6	10.6	17.3
gadoleic (20:1ω9)	20.16	1.1	0.6	0.7	0.7	0.9
total unsaturated		77.6	66.5	63.6	77.2	55.2
unsaturated/saturated		3.5	2.0	1.8	3.4	1.2

[a] Percent by weight of total fatty acids. [b] Equivalent chain lengths of fatty acid methyl esters on a Carbowax 20 M glass capillary column at 190 °C. [c] Not detected. [d] Trace.

hydrogen used as carrier gas, 5 mL/min; split ratio, 5/100). The relative retention time (RRT) for the Me_3Si ether derivatives of terpene alcohols, 4α-methyl sterols, and sterols were expressed against β-sitosterol. A Perkin-Elmer Model 56 recorder was used and peak areas were integrated by an Intersmat Minigrator integrator.

RESULTS AND DISCUSSION

Proximate analyses of the five legumes investigated are shown in Table I. *A. hypogea*, *L. esculentus* and *Vigna sinensis* have high protein content. The content of neutral lipids in these five legumes ranged from 0.8 to 46.7%. *A. hypogea* was characterized by the highest oil content. *Voandzei subterranea* with 6.4% fat has an interesting protein/fat ratio, showing therefore that this legume is a good source of protein and lipid for human nutrition. *P. lunatus*, *L. esculentus*, and *V. sinensis* have a low fat content and the protein/fat ratio ranges from 16 to 27. Some minor differences in ash, fiber, and carbohydrate contents are also noted for the five legumes.

The physical properties of the seed oil are given in Table II. The acidic value are small for the samples analyzed, showing a low free fatty acid content in these oils. High Wijs iodine values were obtained for the five legumes in relation to the high unsaturated fatty acid contents. The unsaponifiable matter of *A. hypogea* is the lowest of the five legumes. For the other legumes, unsaponifiable matter ranged from 2.44 to 3.67%, which is relatively important, showing that these legumes have high proportions of sterol-containing lipids.

Methyl esters of fatty acids were identified by using standard oils and comparing their equivalent chain lengths with previous results (Gaydou et al., 1980). We investigated 13 fatty acids as reproduced in Table III. The fatty acid compositions were characterized for all samples by a high proportion of unsaturated fatty acid which ranged from 55 to 77%. The lowest unsaturated/saturated ratios were obtained for *V. sinensis* and *P. lunatus*. Palmitic acid is the main saturated component in all legumes and ranged from 13.9 to 31.5%. The presence of arachidic (0.9–2.1%), behenic (0.5–3.4%), and lignoceric (0.2–1.8%) acids was observed in all species. Among the unsaturated fatty acids, linoleic was the main component (30.9–46.4%) except for *A. hypogea*. For three species (*P. lunatus*, *L. esculentus*, and *V. sinensis*) linolenic represents a high percentage (10.6–17.3%) of the fatty acid profile. Oleic acid was the

Table IV. Compositions of Sterol Fractions of Five Malagasy Legumes Determined by Gas-Liquid Chromatography

sterol	RRT[b]	composition[a] of legume				
		A. hypogea	*V. subterranea*	*P. lunatus*	*L. esculentus*	*V. sinensis*
cholesterol	0.61	*c*	*c*	*c*	0.3	2.7
campesterol	0.80	16.6	16.6	7.3	10.8	10.7
stigmasterol	0.88	6.3	28.5	36.0	6.2	41.7
β-sitosterol	1.00	72.0	39.8	46.2	79.7	29.2
Δ^5-avenasterol	1.11	5.1	12.9	5.8	2.1	13.1
Δ^7-stigmastenol	1.17	*c*	1.1	2.5	0.9	1.1
Δ^7-avenasterol	1.30	*c*	1.1	1.5	*c*	0.5

[a] Percent by weight. [b] RRT (relative retention time) of Me_3Si ether derivatives of sterols is expressed against the Me_3Si ether derivative of β-sitosterol on an OV-17 glass capillary column at 260 °C. [c] Not detected.

Table V. Composition of 4α-Methyl Sterol Fractions of Five Malagasy Legumes Determined by Gas-Liquid Chromatography

4α-methyl sterol	RRT[b]	composition[a] of legume				
		A. hypogea	*V. subterranea*	*P. lunatus*	*L. esculentus*	*V. sinensis*
lophenol	0.87	6.4	3.2	2.9	5.8	9.2
obtusifoliol	0.97	17.2	20.1	9.5	39.9	14.5
31-norcycloartenol	1.03	2.9	*c*	1.7	10.6	7.1
cycloeucalenol	1.12	14.4	*c*	1.4	5.0	5.9
gramisterol	1.15	14.7	58.7	23.4	16.3	22.3
not identified	1.23	11.7	*c*	1.6	4.8	1.5
not identified	1.28	8.2	4.6	1.9	2.4	3.7
24-ethyllophenol	1.37	2.1	*c*	6.6	2.6	1.5
not identified	1.47	1.6	*c*	3.3	4.7	2.1
citrostadienol	1.55	20.5	13.4	47.6	8.1	32.0

[a] Percent by weight. [b] RRT (relative retention time) of Me_3Si ether derivatives of 4α-methyl sterols is expressed against the Me_3Si ether derivative of β-sitosterol on an OV-17 glass capillary column at 260 °C. [c] Not detected.

Table VI. Composition of Triterpene Alcohol Fractions of Five Malagasy Legumes Determined by Gas-Liquid Chromatography

triterpene alcohol	RRT[b]	composition[a] for legume				
		A. hypogea	*V. subterranea*	*P. lunatus*	*L. esculentus*	*V. sinensis*
not identified	<1.00	*c*	6.2	5.2	2.2	13.3
not identified	1.01	3.3	5.6	5.5	72.1	1.6
cycloartanol	1.02	15.0	8.4	26.4		3.0
lanosterol	1.07	*c*	7.0	6.7	tr[d]	4.7
β-amyrin	1.12	1.5	9.3	*c*	3.2	1.6
butyrospermol	1.17	1.4	13.0	9.3	13.3	59.1
α-amyrin	1.24	*c*	3.2	38.3	3.1	4.5
cycloartenol	1.27	22.4	9.3	5.5	2.1	1.4
lupeol	1.32	10.1	*c*	*c*	*c*	*c*
14-methylene-cycloartanol	1.36	42.0	9.3	*c*	*c*	0.8
not identified	1.42	*c*	6.5	1.0	3.3	1.0
not identified	1.49	*c*	7.5	1.0	0.6	7.5
cyclobranol	1.62	4.3	14.4	1.0	tr[d]	1.0

[a] Percent by weight. [b] RRT (relative retention time) of Me_3Si ether derivatives of triterpene alcohols is expressed against the Me_3Si ether derivative of β-sitosterol on an OV-17 glass capillary column at 260 °C. [c] Not detected. [d] Trace.

main fatty acid in *A. hypogea* (40%) but represents only 7.4% for *P. lunatus*. These results are in good agreement with the fatty acid composition for various *Phaseolus* species (Korytnyk and Metzler, 1963; Shah, 1975; Busson, 1965) and for *V. subterranea* (Busson, 1965).

TLC on silica gel F examination of the unsaponifiable matter of the five legume seed oils, using a mixture of chloroform–ether as the developing solvent, showed many spots. The compound families were tentatively identified by comparison of the R_f values with those of standards. The increasing order of the R_f values were 4-demethyl sterols (sterols), 4α-methyl sterols, 4,4-dimethyl sterols (and terpenic alcohols), tocopherols, and hydrocarbons. The bands of the different sterol, methyl sterol, and terpenic alcohol fractions were identified and scrapped off the chromatoplate. Two methods are generally used for the sterol GLC analysis: acetylation of the hydroxyl group (Prevot and Barbati, 1968; Jeong et al., 1974, 1975; Itoh et al., 1974a, 1975) and trimethylsilyl (Me_3Si) ether derivatives (Homberg and Seher, 1977). We chose the silylating derivatization method already used in our laboratory (Gaydou et al., 1980). The products were tentatively identified both by comparison of their retention times with those of standards and by comparison with results obtained by Jeong et al. (1975) and Itoh et al. (1974a). The relative retention time (RRT) expressed against β-sitosterol and the compositions of the sterol fractions for the five legume seed oils are shown in Table IV. β-Sitosterol is prominent in four legumes (39.8–79.7%), while stigmasterol (41.7%) is the major compound in *V. sinensis*. Cholesterol was detected in only two legumes (*L. esculentus* and *V. sinensis*) in small amounts. Campesterol, stigmasterol, and Δ^5-avenasterol were detected in all samples (7.3–16.6, 6.2–41.7, and 2.1–13.1%, respectively). Δ^7-Stigmastenol was detected in four samples but not in *A. hypogea*. Δ^7-Avenasterol was not detected in *A. hypogea* and *L. esculentus*. Among the 10 4α-methyl sterols detected by GLC, three were not identified. The compo-

sition of 4α-methyl sterol fractions from five legume seed oils is given in Table V. Obtusifoliol (9.5–39.5%), gramisterol (14.7–58.7%), and citrostadienol (8.1–47.6%) were the prominent components of this fraction. Our results for *A. hypogea* are quite similar with those given by Itoh et al. (1974 a) since these authors have found in peanut oil 25% obtusifoliol, 28% cycloeucalenol plus gramisterol, and 24% citrostadienol.

Table VI shows approximate composition of triterpene alcohol (and 4,4-dimethyl sterol) fractions from the five legume seed oils. Tentative identification was based on the comparison of their RRT by GLC. Among these compounds, cycloartanol (3.0–26.4%), butyrospermol (1.4–59.1%), cycloartenol (1.4–22.4%), 24-methylenecycloartanol (0–42%), and cyclobranol (trace–14.4%) were the main components of this fraction. α-Amyrin (38.3%) was found as the prominent component in *P. lunatus*. Butyrospermol (59.1%) was the highest 4,4-dimethyl sterol of *V. sinensis*. In some cases it was difficult to determine precisely the peak area of individual GLC peaks, particularly for the RRT range of 1.0–1.1, as in the case of *L. esculentus* (Table VI). β-Amyrin was found in all legume seed oils (1.5–9.3%) except in *P. lunatus*. The results observed for *A. hypogea* are quite similar to those given by Itoh et al. (1974b) since these searchers found 33% cycloartenol, 46% 24-methylenecycloartanol, 2% cycloartanol, and 8% cyclobranol for peanut oil. Jeong et al. (1975) showed, in a study of the 4,4-dimethyl sterol fraction from 20 vegetable oils, that cycloartanol, 24-methylenecycloartanol, β-amyrin, and cycloartenol are common in most of the oils. Butyrospermol, α-amyrin, lupeol, and cyclobarnol occur in some of these oils.

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Registry No. Cholesterol, 57-88-5; campesterol, 474-62-4; stigmasterol, 83-48-7; β-sitosterol, 83-46-5; Δ^5-avenasterol, 18472-36-1; Δ^7-stigmasterol, 481-19-6; Δ^7-avenasterol, 23290-26-8; lophenol, 481-25-4; obtusifoliol, 16910-32-0; 31-norcycloartenol, 60485-38-3; cycloeucalenol, 469-39-6; gramisterol, 1176-52-9; 24-ethyllophenol, 36735-29-2; citrostadienol, 474-40-8; cycloartanol, 4657-58-3; lanosterol, 79-63-0; β-amyrin, 559-70-6; butyrospermol, 472-28-6; α-amyrin, 638-95-9; cycloartenol, 469-38-5; lupeol, 545-47-1; 24-methylenecycloartanol, 1449-09-8; cyclobranol, 25692-13-1.

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Free Amino Acids from Different Cultivars of *Vicia faba*

Francesco De Simone, Patrizia Morrica, Eliseo Ramundo,* Felice Senatore, and Water Taccone

The free amino acid composition of the seeds of some *Vicia faba* L. and *Vicia faba minor* cultivars was determined by using gas chromatography. Very high levels of L-Dopa and methionine are found, whereas the alanine, threonine, lysine, and serine contents are relatively high in certain samples. The observed variability in the L-Dopa and methionine contents of the variants is briefly discussed in relation to the potential for selecting *V. faba* genotypes with improved seed amino acid quality.

Leguminous seeds constitute an important sources of food protein and energy for a large sector of the world population. The increasing interest in the food legumes has been demonstrated by several international symposia on this topic in the last decade (Milner, 1973; Wall, 1973; Jaffé, 1977).

The *Vicia faba* seeds, widely cultivated in Europe and in remarkable development in Africa and Asia in these last few years, contain a high percent of proteins (up to 25% dry weight) so they are very advantageous as human and animal food. Therefore, the economic interest in the cultivation could be very great, in spite of the presence of

Istituto di Biorganica-Facoltà di Farmacia-Università di Napoli-Via L. Rodinò, 22, 80138 Napoli, Italy.

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