Sterols and Fatty Acids of Two Caesalpiniaceae

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Seed oils of *Cassia hirsuta* and *C. tora* L., two Caesalpiniaceae belonging to the Leguminoseae family, contain small amounts of malvalic and sterculic acids. Sterol composition was determined by capillary GLC and GC/MS; 15 sterols were identified. In addition to the usual Δ-5 sterols, high levels of Δ-7 sterols and small amounts of saturated sterols were found.

Previous workers have demonstrated that the seed oil of *Cassia grandis*, belonging to the Leguminoseae family, contains small amounts of sterculic and malvalic acids (1). In a preliminary study on sterol fractions of some *Cassia* species we found a complex composition with the presence of many Δ-7 sterols (2). The presence of these different compounds, which are quite rare in higher plants, led us to investigate the lipid composition of two Senegalese *Cassia* seed oils, *Cassia hirsuta* and *C. tora*.

Cassia hirsuta L. is an undershrub of 2–3 m height with yellow flowers; leaves, stems and fruits are very long pilose or hirsute. Native to tropical America, it has been introduced into Africa (3). *Cassia tora* L. is an undershrub of 1–3 m height with yellow flowers in pairs or singly, and is widespread in tropical areas (3).

EXPERIMENTAL PROCEDURES

Seeds collected in the Dakar area were ground to a powder and extracted with hexane in a Soxhlet apparatus. The solvent was removed at 45°C under reduced pressure and the oil content was determined.

Fatty acids study. The fatty acid methyl esters (FAME) were prepared by transesterification of oil with a solution of 1N sodium methoxide in methanol and treated with anhydrous methanol saturated with silver nitrate (4). The normal methyl esters and the reaction products from cyclopropene fatty acids were recovered and analyzed by gas liquid chromatography (GLC). GLC was performed with a Carlo-Erba model 4130 gas chromatograph equipped with a flame ionization detector. A carbowax 20M fused silica capillary column (0.40 μm phase thickness, 25 m × 0.32 mm i.d.) was used to separate fatty acid methyl esters. Temperatures were: injector and detector, 230°C; column 175°C; inlet pressure of hydrogen used as carrier gas was 0.5 bar; split ratio, 5/100. A sample of *Sterculia foetida* seed oil was used as reference.

Sterols studies. Oils were saponified by KOH/EtOH with usual workup and unsaponifiable matters were recovered by n-hexane. Sterols were extracted by digitonin complexation and recrystallized in methanol. The sterol mixture was separated by preparative thin layer chromatography (TLC) on silica gel (0.5 mm thick), developped four times with C_6H_6/MeOH (98:2, v/v) into two fractions, one containing mainly Δ-5 sterols and another consisting mainly of Δ-7 and saturated sterols. The two fractions were purified by another TLC (0.2 mm thick) under the same conditions and acetylated with Ac_2O/Py at room temperature overnight. The acetates were analyzed by GLC and gas liquid chromatography mass spectrometry (GC-MS).

GLC was performed with the same apparatus used for FAME, on an OV1 fused silica capillary column (0.40 μm thick, 25 m × 0.32 mm i.d.). Temperatures were: injector and detector, 280°C; column, 260°C; inlet pressure of hydrogen used as carrier gas was 0.6 bar; split ratio, 1/100. The relative retention times (RRT) for the acetate derivatives were expressed relative to cholesteryl acetate (RRT = 1.00).

GC-MS equipment included a Girdel Ribermag R10-10B apparatus coupled with a Sidar data computer. The chromatograph was fitted with the same column as described above. Operating conditions were: temperature column was programmed from 250° to 290°C at 5°C/min; helium was used as carrier gas at 0.5 bar; ion source, 150°C; ionizing voltage, 70 eV.

RESULTS AND DISCUSSION

The seeds of *Cassia hirsuta* and *C. tora* yielded 3.0% and 5.4% oil, respectively.

The content and distribution of fatty acids are summarized in Table 1. About 15 compounds were identified in the two oils. A high level of unsaturated fatty acids was observed (76.6 and 68.2%) with a prominence of linoleic (58.0 and 44.6%) and oleic acids (13.3 and 21.6%). The cyclopropenoid acids, malvalic and sterculic, were found in small amounts in *C. hirsuta*, respectively 0.4 and 0.6%, but only a trace of sterculic (0.2%) was found in *C. tora*. Among the saturated acids, palmitic acid was the

TABLE 1

Analytical Data on *Cassia hirsuta* and *C. tora* Seed Oils

	Cassia hirsuta	*Cassia tora*
Oil content in seeds (%)	3.0	5.4
Unsaponifiable matter (%)	5.4	2.6
Sterol content in oil (%)	2.7	1.2
Fatty acids:		
12:0	0.4	—
14:0	0.3	0.2
16:0	16.9	22.8
16:1ω7	0.6	0.7
17:0	0.2	0.2
18:0	2.9	6.7
18:1ω9	13.3	21.4
18:2ω6	58.0	44.6
18:3ω3	3.3	1.1
20:0	0.8	1.3
20:1ω9	0.4	0.2
22:0	1.1	0.6
24:0	0.8	—
Malvalic	0.4	—
Sterculic	0.6	0.2
Total saturated	23.4	31.8
Total unsaturated	76.6	68.2

TABLE 2

RRT[a]	Sterol[b]	% Composition	
		C. hirsuta	*C. tora*
1.00	cholest-5-en-3β-ol	0.4	0.6
1.03	5α-cholestan-3β-ol	0.2	0.4
1.13	5α-cholest-7-en-3β-ol	0.3	1.2
1.29	24-methylcholest-5-en-3β-ol	7.5	6.0
1.33	24-methylcholestan-3β-ol	0.8	0.8
1.40	24-ethylcholest-5,22(E)-dien-3β-ol	18.8	20.1
1.43	24-methylcholest-7,24(28)-dien-3β-ol	2.0	0.9
1.46	24-methylcholest-7-en-3β-ol	1.7	2.2
1.53	24-ethylcholest-5,23(E)-dien-3β-ol	0.4	0.2
1.58	24-ethylcholest-5,22(E)-dien-3β-ol	2.5	1.4
1.62	24-ethylcholest-5-en-3β-ol	40.1	38.2
1.65	24-ethylcholestan-3β-ol	0.6	0.8
1.67	24-ethylcholest-5,24(28)-dien-3β-ol	3.5	2.1
1.81	24-ethylcholest-7-en-3β-ol	15.1	19.9
1.89	24-ethylcholest-7,24(28)-dien-3β-ol	6.1	5.2

[a]Relative retention time of steryl acetates (cholesteryl acetate = 1.00).

[b]Determined as steryl acetates.

main component (16.9 and 22.9%). These results are in good agreement with the fatty acids composition found in *Cassia grandis,* and it seems that small or trace amounts of cyclopropenoid acids, malvalic and sterculic acids, are widely distributed in the *Cassia* genus.

The sterol fractions (total, Δ-5, and Δ-7 sterols) obtained from the unsaponifiable lipids of the seed oils of the two *Cassia* species were identified as acetates based on comparisons of the GLC and GC-MS data with those of authentic compounds. Results were in agreement with known data (5–7). Absolute configurations of C-24 alkylsterols were not examined in this study. Table 2 summarizes the sterol compositions of the two *Cassia* species. Fifteen sterols were identified; among these compounds we found three saturated sterols rarely reported in the plant kigdom, six Δ-5 sterols and six Δ-7 sterols. The three major sterols identified, at similar relative concentrations, in the seeds of *Cassia hirsuta* and *C. tora* were: 24-ethylcholest-5-en-3β-ol (40.1 and 38.2%); 24-ethylcholest-7-en-3β-ol (15.1 and 19.9%) and 24-ethyl-cholest-5,22(E)-dien-3β-ol (18.8 and 20.1%). A high level of Δ-7 sterols was observed in the two seed oils, 27.7 and 32.2%, respectively, for *C. hirsuta* and *C. tora.* Such data appear to be more common among species belonging to the Leguminoseae and Cucurbitaceae families (8). Small amounts of sterols with saturated skeletons were identified recently in some Cucurbitaceae (9).

REFERENCES

1. Daulatabad, C.D., K.M. Hosamani, V.A. Desai and K.R. Alagawadi, *J. Am. Oil Chem. Soc. 64*:1423 (1987).
2. Miralles, J., and E.M. Gaydou, *Rev. Fr. Corps Gras 33*:381 (1986).
3. Hutchinson, J., and J.M. Dalziel, in *Flora of West Tropical Africa,* Whitefriars Press Ltd., London and Tonbridge, Vol. 1, 1958.
4. Schneider, E.L., S.P. Loke and D.T. Hopkins, *J. Am. Oil Chem. Soc. 45*:585 (1968).
5. Itoh, T., H. Tani, K. Fukushima, T. Tamura and T. Matsumoto, *J. Chromatogr. 234*:65 (1982).
6. Knights, B.A., and W. Laurie, *Phytochemistry 6*:407 (1967).
7. Miralles, J., K. Noba, A.T. Ba, E. Gaydou and J.M. Kornprobst, *Biochem. Syst. Ecol. 16*:1475 (1988).
8. Goad, L.J., and T.W. Goodwin, in *Progress in Phytochemistry,* Vol. 1, Pergamon Press, Oxford, New York, 1972.
9. Akihisa, T., N. Shimizu, P. Ghosh, S. Thakur, F.U. Rosenstein, T. Tamura and T. Matsumoto, *Phytochemistry 26*:1693 (1987).

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