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CO-OCCURRENCE OF Δ^5- AND Δ^7-STEROLS IN TWO *GLEDITSIA* SPECIES. A REASSESSMENT OF THE STEROL COMPOSITION IN OILS RICH IN Δ^7-STEROLS

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Key Word Index—*Gleditsia triacanthos*, *G macracantha*, *Spinacia oleracea*, *Thea sinensis*, *Medicago sativa*, Leguminosae, seeds, Δ^7-sterols, Δ^5-sterols, spinasterol, Δ^7-stigmastenol

Abstract—The composition of the sterol fraction of *Gleditsia triacanthos*, *G macracantha*, *Thea sinensis*, *Medicago sativa* and *Spinacia oleracea* has been determined using GC and GC/MS The sum of Δ^7-sterols ranges from 67 to 95 % Among them 24ξ-ethyl-5α-cholest-7,*trans*-22-dien-3β-ol (28–50 %) and 24ξ-ethyl-5α-cholest-7-en-3β-ol (23–49 %) are the major components The co-occurrence of Δ^5- and Δ^7-sterols has been observed in all species The possible biosynthetic pathway of the phytosterol nucleus leading to these sterols is discussed

INTRODUCTION

The type and degree of unsaturation in the ring system of sterols, in addition to variations in the side chain, have taxonomic and phylogenetic importance Tracheophytes rich in Δ^5-sterols, especially 24α-ethyl-Δ^5-sterols such as sitosterol have been most frequently encountered While Tracheophytes containing $\Delta^{5,7}$-sterols together with Δ^5-sterols have been found such as *Lycopodium complanatum* [1] and the roots of *Rauwolfia serpentina* [2], the sterols of the few plants rich in Δ^7-sterols are reported to contain almost exclusively the Δ^7-sterol Studies on the sterol composition of seed oils of several genera of the Cucurbitaceae family have shown them to possess only Δ^7-sterols [3–7] The sterol fraction of the unsaponifiables from three Theaceae oils and alfalfa, garden balsam and *Spinacia oleracea* seed oils and shea fat were alike in their compositions, consisting exclusively of Δ^7-sterols, such as spinasterol and Δ^7-stigmastenol as predominant components [8, 9] The occurrence of spinasterol in *S oleracea* leaves [10, 11], alfalfa leaves and seeds [12] and garden balsam [13] have also been reported The presence of Δ^5-sterols such as stigmasterol in alfalfa and *S oleracea* seed oils have been observed in small quantities and the occurrence of sitosterol in minute proportions was presumed [8] No detectable Δ^5-sterol was found in *S oleracea* by Nes *et al* [1] In the case of pumpkin (*Cucurbita pepo*) seed oil, no Δ^5-sterols could be detected either in the ester or the free sterol pools by Nes *et al* [1], but Jeong *et al* [14] and Bastik *et al* [15] have detected them in trace amounts

This paper describes a study on the sterol composition of two *Gleditsia* (Leguminosae, Caesalpinioideae sub family) seed oils *G triacanthos* (honey locust) and *G macracantha* These two species belong to the category of plants rich in Δ^7-sterols However, the Δ^5-sterol content ranges from 13 to 32 % showing therefore that the co-occurrence of Δ^5- and Δ^7-sterols in some Tracheophytes exists Faced with these observations and the contradictory results given above, we have reinvestigated the sterol composition of *Thea sinensis*, *S oleracea* (spinach) and *Medicago sativa* (alfalfa) seed oils with particular reference to Δ^5- and Δ^7-sterols

RESULTS AND DISCUSSION

The sterol fractions, separated from the unsaponifiable lipids of the seed oil samples of the two *Gleditsia* species and other seed oils were transformed into their TMS-derivatives as described previously [16, 17] The capillary gas chromatogram showed the presence of at least 17 different sterols The identity of the sterols was determined by comparison of RR_ts with previously published retention data of the silyl-, free and acetyl-standards [17–20] and by gas-liquid chromatography–mass spectrometry (GC/MS) Seven sterols present in minute amounts were left unidentified The RR_t and the sterol composition of the six seed oil samples are given in Table 1 The determination of the configuration at C-24 of 24-methyl and 24-ethylsterols is possible using ^{1}H NMR [21–23] and ^{13}C NMR spectroscopy [7, 24], or using GLC [25] In this study, the absolute configuration of the C-24 alkylsterols was not examined 24ξ-Methylcholest-5-en-3β-ol, 24ξ-ethylcholest-5,*trans*-22-dien-3β-ol and 24ξ-ethylcholest-5-en-3β-ol are commonly supposed to be campesterol, stigmasterol and sitosterol since the 24α-alkylsterols are usual in terrestrial plants [26]

The co-occurrence of the two epimers of 24-ethyl-5α-cholest-7,*trans*-22-dien-3β-ol spinasterol (24α-epimer) and chondrillasterol (24β-epimer) has been demonstrated in the seeds of Cucurbitaceae [23] The co-occurrence of the two epimers of 24-ethyl-5α-cholest-7-en-3β-ol 22-

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Table 1 Composition of the sterol fractions of the seed oils investigated*

Sterol	RR_t†	*Gleditsia triacanthos* France	*Gleditsia triacanthos* USSR	*G macracantha*	*Thea sinensis*	*Medicago sativa*	*Spinacia oleracea*
Cholest-5-en-3β-ol	1 00	1 7	0 4	0 6	1 0	tr	0 2
Unknown	1 13			—	—	—	0 3
24ξ-Methylcholest-5-en-3β-ol	1 31	2 6	1 4	0 5	0 5	0 2	0 5
Unknown‡	1 33	0 7	0 8	0 1	—	—	1 3
24ξ-Ethylcholest-5,*trans*-22-dien-3β-ol	1 41	8 7	8 2	4 3	1 1	2 1	2 9
Unknown	1 49	1 7	3 0	1 7	1 4	—	0 9
24ξ-Methyl-5α-cholest-7-en-3β-ol	1 51	5 1	5 5	1 9	1 8	3 9	6 9
Unknown	1 56			—	—	—	0 3
24ξ-Ethylcholest-5-en-3β-ol	1 61	16 0	6 6	4 3	12 4	2 5	5 5
24ξ-Ethyl-5α-cholest-7,*trans*-22-dien-3β-ol	1 66	27 7	33 2	30 5	50 1	42 8	33 7
24*E*-Ethylidenecholest-5-en-3β-ol (fucosterol)	1 70	2 2	1 9	3 0	1 4	—	1 0
24*Z*-Ethylidenecholest-5-en-3β-ol (Δ^5-avenasterol)	1 78	0 8	0 1	0 2	0 1	—	—
24ξ-Ethyl-5α-cholest-7-en-3β-ol	1 88	30 4	36 2	49 0	23 4	45 1	38 0
Unknown	1 97	0 1	0 2	0 3	0 3	0 3	0 4
24*Z*-Ethylidene-5α-cholest-7-en-3β-ol (Δ^7-avenasterol)	2 07	2 2	2 3	2 9	3 1	3 1	6 8
Unknown	2 21	0 1	0 2	0 7	3 3	—	0 6
Unknown	2 32			—	0 1	tr	0 7

tr, Denotes that component was detected in a too small amount to quantitate

*Area % by GLC

†Relative retention times of the TMS-ether derivatives of sterols on an OV-17 WCOT glass capillary column (cholesterol-TMS 1 00)

‡Has same RR_t as 24-methylenecholest-5-en-3β-ol, however analysis on OV-1 shows that it is not this sterol

dihydrospinasterol (24α-epimer) and 22-dihydrochondrillasterol (24β-epimer) has been demonstrated in the roots of *Tricosanthes japonica* [7]

Among the ten sterols identified, two Δ^7-sterols were found in highest amounts as shown in Table 1 24ξ-ethyl-5α-cholest-7,*trans*-22-dien-3β-ol (28–50%) and 24ξ-ethyl-5α-cholest-7-en-3β-ol (23–49%) 24ξ-Ethylcholest-5-en-3β-ol was found in all species investigated and in higher content in *G triacanthos* (7–16%) and in *T sinensis* (12%)

The amounts of Δ^5-sterols for the six oil samples are given in Table 2 and they range from 4 8% for *M sativa* to 19–32% for *G triacanthos* The ratios of the amounts of Δ^7-sterols versus Δ^5-sterols are about 2 1–8 5 for the *Gleditsia* species, *T sinensis* and *S oleracea* but higher in the case of *M sativa* (19 8) as shown in Table 2 We have also determined the Δ^7-sterol Δ^5-sterol ratios for each type of C-24-alkyl substituent of the side chain These ratios are in the same order for each species when the sterol concentration is in a significant quantity An abnormal result was

Table 2 Variations in the ratios of the Δ^5- and Δ^7-sterols with different side chain structures in the seed oils investigated

Sterol	*Gleditsia triacanthos* France	*Gleditsia triacanthos* USSR	*G macracantha*	*Thea sinensis*	*Medicago sativa*	*Spinacia oleracea*
Δ^5-sterols	32 0*	18 6	12 9	16 5	4 8	10 1
Δ^7-sterols	67 1	80 2	86 0	79 8	94 9	86 3
Δ^7-sterols/Δ^5-sterols	2 1†	4 3	6 7	4 8	19 8	8 5
24-methyl Δ^7-sterol / 24-methyl Δ^5-sterol	2 0	4 0	3 8	3 6	19 5	13 8
24-ethyl Δ^7-sterol / 24-ethyl Δ^5-sterol	1 9	5 5	11 4	1 9	18 0	6 9
24-ethyl $\Delta^{7\ 22}$-sterol / 24-ethyl $\Delta^{5\ 22}$-sterol	3 2	4 0	7 1	45 5	20 4	11 6

*Percentage composition

†Ratio

found with *T sinensis* for the 24-ethyl $\Delta^{7,22}$-sterol versus $\Delta^{5,22}$-sterol (Table 2)

Since in these results the Δ^7-sterol Δ^5-sterol ratios are in the same order for the sum of the sterols investigated and for each C-24 alkylsterol category, it is reasonable to suppose that the reduction of the Δ^7 bond and the Δ^5 introduction occur in the biosynthetic pathway after elaboration of the side chain These results are in agreement with the metabolism of a Δ^{24}-bond which seems to occur primarily as the first step since 24-methylenecycloartanol is formed in cell-free systems [27, 28] and it has been found in a number of plants The final steps in the biosynthetic pathway leading to sterols seems to be the nuclear transformation by Δ^5-dehydrogenation and Δ^7-reduction In the animal kingdom the sequence $\Delta^7 \rightarrow \Delta^{5,7} \rightarrow \Delta^5$ has been very well studied at the enzymologic level [29] and probably the sequence occurs in plants The presence of $\Delta^{5,7}$-sterols in *Lycopodium complanatum* [1] is in agreement with this sequence The occurrence of Δ^5-sterols in plants rich in Δ^7-sterols suggests that, the Δ^5-dehydrogenase and the Δ^7-reductase exist, as is well known in plants rich in Δ^5-sterols The difference between these two Tracheophyte categories may be explained by imperfect kinetic control of the enzymes In the case of a plant rich in Δ^7-sterols the activity of the Δ^7-reductase step may be very low and the Δ^5-sterol concentration is thus low as shown in Table 2 for *M sativa* (5%), *S olearacea* (10%), *G macracantha* (13%) and *T sinensis* (16%) *G triacanthos* represents an intermediate type between the few plants rich in Δ^7-sterols and the most frequently encountered plants rich in Δ^5-sterols, since its Δ^5-sterol content ranges from 19 to 32%

In conclusion, the co-occurrence of Δ^5- and Δ^7-sterols in *Gleditsia* species and other seed oils may help to further clarify the biosynthesis of the phytosterol nucleus and the evolution in higher plants

EXPERIMENTAL

Material Gleditsia triacanthos seeds were obtained from a USSR crop (1973) and a Marseilles (France) crop (1979) *G macracantha* seeds were collected in the Marseilles area in 1971 *Thea sinensis* were collected in Antananarivo (Republic of Madagascar) area in 1980 *Medicago sativa* and *Spinacia oleracea* seeds were commercially available

General procedure Gleditsia seeds were decorticated by treatment with hot conc H_2SO_4 (30 min, 60°) Germ was separated from endosperm, dried and ground Germ (120 g) was extracted with hexane in a Soxhlet for 8 hr The oils were saponified using 2 M KOH in EtOH (25 ml/g) over a period of 2 hr Unsaponifiable material dissolved in CCl_4 (5%) was fractionated using TLC by depositing 200 μl on a silica gel plate (60F 254 Merck, 20 × 10 cm, 250 μm thick) The developing solvent was a $CHCl_3$–Et_2O (9 1) mixture Cholesterol was used as standard for the identification of the sterol band (R_f 0 35) and a Rhodamine B spray for the detection at 366 nm The zones were scraped off and extracted with CH_2Cl_2 (2 ml) Silylation of sterols was carried out with 50 μl of silylating reagent (0 45 ml HMDS, 0 3 ml TMCS, 0 5 ml pyridine) The solvent was evaporated under N_2 and 1 ml of hexane was added to the dry residue prior to GC analyses Relative retention times (RR_t) were expressed against cholesteryl-TMS The WCOT column was a 40 m glass capillary column, 0 29 mm i d, coated with OV-17 (0 15 μm) Temperatures were 250° for column and 300° for inlet and detector ovens Inlet pressure of H_2 used as carrier gas was 0 6 bar, split 55 ml/min Peak areas were integrated by a LTT ICAP 5 integrator

Gas-liquid chromatography–mass spectrometry The chromatograph was fitted with a WCOT Si capillary column (25 m, 0 33 mm i d) coated with OV-1701 (0 1 μm) Operating conditions were 250° for column and 270° for inlet, He as carrier gas 0 5 bar, ion source 150° and ionizing voltage 70 eV

Cholesterol-TMS MS *m/z* (rel int) 458 $[M]^+$ (21 5), 443 (53), 370 (3 0) 368 (36 3), 353 (15 3), 329 (21 1), 275 (3 1), 255 (10 0), 247 (10 7), 233 (2 0), 217 (3 3), 213 (4 3), 129 (100)

24ξ-Methylcholest-5-en-3β-ol-TMS MS *m/z* (rel int) 472 $[M]^+$ (47 8), 457 (8 6), 384 (8 6), 382 (43 0), 367 (25 0), 343 (45 8), 269 (7 9), 255 (14 2), 217 (6 9), 213 (3 4), 129 (100)

24-Methylenecholest-5-en-3β-ol-TMS MS *m/z* (rel int) 470 $[M]^+$ (13 0), 455 (7 5), 386 (31 6), 371 (7 0), 365 (8 1), 343 (17 8), 341 (9 3), 296 (7 9), 281 (7 4), 259 (3 4), 257 (11 4), 243 (4 2), 227 (3 6), 213 (6 8), 129 (100)

24ξ-Ethylcholest-5,trans-22-dien-3β-ol-TMS MS *m/z* (rel int) 484 $[M]^+$ (48 7), 469 (5 8), 394 (23 7), 379 (7 5), 356 (5 6), 355 (10 0), 330 (2 5), 282 (3 2), 271 (4 0), 255 (30 4), 239 (3 2), 227 (2 5), 213 (7 0), 129 (58 3)

24ξ-Methyl-5α-cholest-7-en-3β-ol-TMS MS *m/z* (rel int) 472 $[M]^+$ (59 7), 457 (6 3), 367 (9 5), 343 (7 9), 269 (3 1), 255 (36 8), 237 (3 1), 229 (16 8), 213 (20 7)

24ξ-Ethylcholest-5-en-3β-ol-TMS MS *m/z* (rel int) 486 $[M]^+$ (33 8), 471 (6 2), 396 (40 3), 381 (10 5), 357 (36 6), 355 (2 3), 275 (1 8), 255 (8 4), 217 (4 1), 213 (4 2), 129 (100)

24ξ-Ethyl-5α-cholest-7,trans-22-dien-3β-ol-TMS MS *m/z* (rel int) 484 $[M]^+$ (23 5), 469 (12 0), 441 (2 7), 394 (2 8), 372 (9 7), 357 (2 5), 343 (51 8), 329 (3 8), 318 (6 8), 255 (34 7), 230 (3 6), 229 (23 3), 213 (13 7), 201 (7 6)

24E-Ethylidenecholest-5-en-3β-ol-TMS MS *m/z* (rel int) 484 $[M]^+$ (8 0), 469 (5 7), 386 (47 1), 371 (9 5), 343 (4 9), 296 (20 8), 281 (12 0), 258 (3 7), 257 (12 8), 255 (6 1), 243 (3 3), 227 (3 3), 211 (6 2), 129 (87 5)

24Z-Ethylidenecholest-5-en-3β-ol-TMS MS *m/z* (rel int) 484 $[M]^+$ (5 6), 469 (3 4), 386 (60), 371 (9 8), 343 (3 0), 296 (30 6), 281 (20 7), 258 (4 2), 257 (17 1), 255 (7 4), 243 (3 3), 227 (4 7), 211 (9 6), 129 (76 9)

24ξ-Ethyl-5α-cholest-7-en-3β-ol-TMS MS *m/z* (rel int) 486 $[M]^+$ (100), 471 (15 1), 396 (3 7), 381 (9 8), 345 (9 5), 303 (3 0), 255 (44 7), 230 (3 3), 229 (12 8), 213 (17 6), 201 (5 5)

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