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OCCURRENCE OF Δ^5-STEROLS IN PLANTS PRODUCING PREDOMINANTLY Δ^7-STEROLS: STUDIES ON THE STEROL COMPOSITIONS OF SIX CUCURBITACEAE SEEDS

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Key Word Index—*Cucurbita pepo*; *Cucurbita lagenaria*; *Citrullus lanatus*; *Cucumis sativus*; *Cucumis melo*; *Luffa aegyptiaca*; Cucurbitaceae; seeds; Δ^5-sterols; Δ^7-sterols; codisterol; 25(27)-dehydroporiferasterol; clerosterol; isofucosterol; stigmasterol; campesterol; 22-dihydrobrassicasterol; sitosterol; 24-methylenecholesterol; 25(27)-dehydrofungisterol; 25(27)-dehydrochondrillasterol; 24β-ethyl-25(27)-dehydrolathosterol; avenasterol; spinasterol; 24ξ-methyllathosterol; 22-dihydrospinasterol.

Abstract—The sterol compositions of six Cucurbitaceae seeds, *Cucurbita pepo*, *C. lagenaria*, *Citrullus lanatus*, *Cucumis sativus*, *C. melo* and *Luffa aegyptiaca*, were examined by TLC, GLC, HPLC and in some cases by mass spectrometry and ^{1}H NMR spectroscopy. Thirteen components were identified. They were codisterol, 25(27)-dehydroporiferasterol, clerosterol, isofucosterol, stigmasterol, campesterol, 22-dihydrobrassicasterol, sitosterol, 25(27)-dehydrofungisterol, 25(27)-dehydrochondrillasterol, 24β-ethyl-25(27)-dehydrolathosterol, avenasterol, spinasterol, 24ξ-methyllathosterol and 22-dihydrospinasterol. 24-Methylenecholesterol may also have been present. The Δ^5-sterols were observed in all species. The pattern of sterols suggests the existence of four biosynthetic pathways operating from a 24(25)-dehydroprecursor. This work represents only the second time either codisterol or 25(27)-dehydrofungisterol has been isolated from a higher plant.

INTRODUCTION

Variations in the position and degree of unsaturation in the nucleus of sterols, in addition to their side-chain variations, have taxonomic and phylogenetic importance [1, 2]. Two main types of Tracheophyte can be identified in terms of nuclear variations: type A, which represents most higher plants, containing primarily Δ^5-sterols, and type B with Δ^7-sterols. The Cucurbitaceae are the most well studied of the plants of type B. Seeds of several genera of this family have been reported to possess only Δ^7-sterols, and the occurrence of Δ^5-sterols along with Δ^7-sterols has been thought to be rare [1, 3–7]. There are many reports indicating that while plants producing predominantly Δ^5-sterols may frequently also contain Δ^7-sterols, plants having large amounts of Δ^7-sterols do not usually seem to contain any Δ^5-sterols [1, 8, 9] even though co-occurrence of the latter type has been documented [10–16]. Among the cucurbits, however, only a few species have been shown to contain Δ^5-sterols [11–14], and in most cases, a sufficient amount of sterol was not available for adequate identification. It was, therefore, of considerable interest when we found a substantial amount (18% of the total fraction) of sterols with a Δ^5-bond distributed among a large array of different sterols in the seeds of *Cucurbita maxima* (squash) [17]. Furthermore, the relative proportions of the Δ^5-sterols appeared to be dependent on ontogeny. The levels of Δ^5-sterols were highest in the seed, and they rapidly and progressively decreased during seed germination and seedling development to the point where no Δ^5-sterols could be detected at all in mature plants [18, 19].

Thus, in view of the existence of Δ^5-sterols in the Cucurbitaceae and their concentration and probable importance in seeds of this family, we have investigated the sterol compositions of six additional Cucurbitaceae seeds: *Cucurbita pepo* (pumpkin), *Cucurbita lagenaria* (ornamental gourd), *Citrullus lanatus* (water-melon), *Cucumis sativus* (cucumber), *Cucumis melo* (canteloupe) and *Luffa aegyptiaca* (luffa gourd). Our results indicate that as with *Cucurbita maxima* all of the six seeds examined contain some Δ^5-sterols. We have characterized the individual sterol components from each species and have also examined the Δ^7-sterols in each of the species.

RESULTS AND DISCUSSION

The total 4-desmethylsterols were obtained from the seeds and the Δ^5- and Δ^7-mixtures were separated from each other as described previously [17, 20]. Separation of the individual components into the various Δ^5- and Δ^7-fractions was then achieved by preparative HPLC [20]. Data from analytical GLC and HPLC, as well as from ^{1}H NMR in some cases, were used for quantitation of the individual components. The identity of the sterols was determined by their chromatographic mobilities in GLC and HPLC and also by mass spectrometry. ^{1}H NMR was also an important tool when enough sterol was available. The distribution of individual sterols in the six seed types studied is given in Tables 1 and 2. All of the seeds contained predominantly Δ^7-sterols as expected, but some Δ^5-sterols were also found in each sample. Based on the

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Table 1. Relative compositions of Δ^5-sterols isolated from the six Cucurbitaceae seeds investigated*

Sterol	Plant species					
	Cucurbita pepo†	*Cucurbita lagenaria*	*Citrullus lanatus*	*Cucumis sativus*	*Cucumis melo*	*Luffa aegyptiaca*
	(% of total Δ^5-sterols)					
Codisterol (**1a**)	25.0	24.3	tr.	1.6	2.9	nd
25(27)-Dehydroporiferasterol (**2a**)	4.1	2.3	28.7	30.0	49.6	tr.
Clerosterol (**3a**)	29.0	19.2	31.2	10.1	30.3	tr.
Isofucosterol (**4a**)	5.1	3.7	7.8	tr.	3.4	nd
Stigmasterol (**5a**)	14.1	16.0	13.9	21.7	7.2	tr.
Campesterol‡ (**6a**)	3.0	4.3	2.9	13.8	4.9	tr.
Sitosterol (**8a**)	19.7	30.2	15.5	22.8	1.7	tr.
Total content of Δ^5-sterols (mg/100 g of seed)	12.30	6.25	8.33	1.24	1.90	0.36
% of total 4-desmethylfraction	24.9	18.0	18.4	3.0	3.8	1.0

*tr. = traces, nd = not detectable. Values for *C. maxima* from our earlier work [17] are (in % of total Δ^5-sterols): **1a**, 35.3; **2a**, 4.3; **3a**, 19.8; **4a**, 7.4; **5a**, 10.9; **6a**, 5.7; **8a**, 16.6.

†A small amount (<1%) of 24-methylenecholesterol (**9a**) may also have been present based on GLC, HPLC and MS.

‡A minute amount of the 24β-epimer, 22-dihydrobrassicasterol (**7a**), was also present.

Table 2. Relative compositions of Δ^7-sterols isolated from the six Cucurbitaceae seeds investigated*

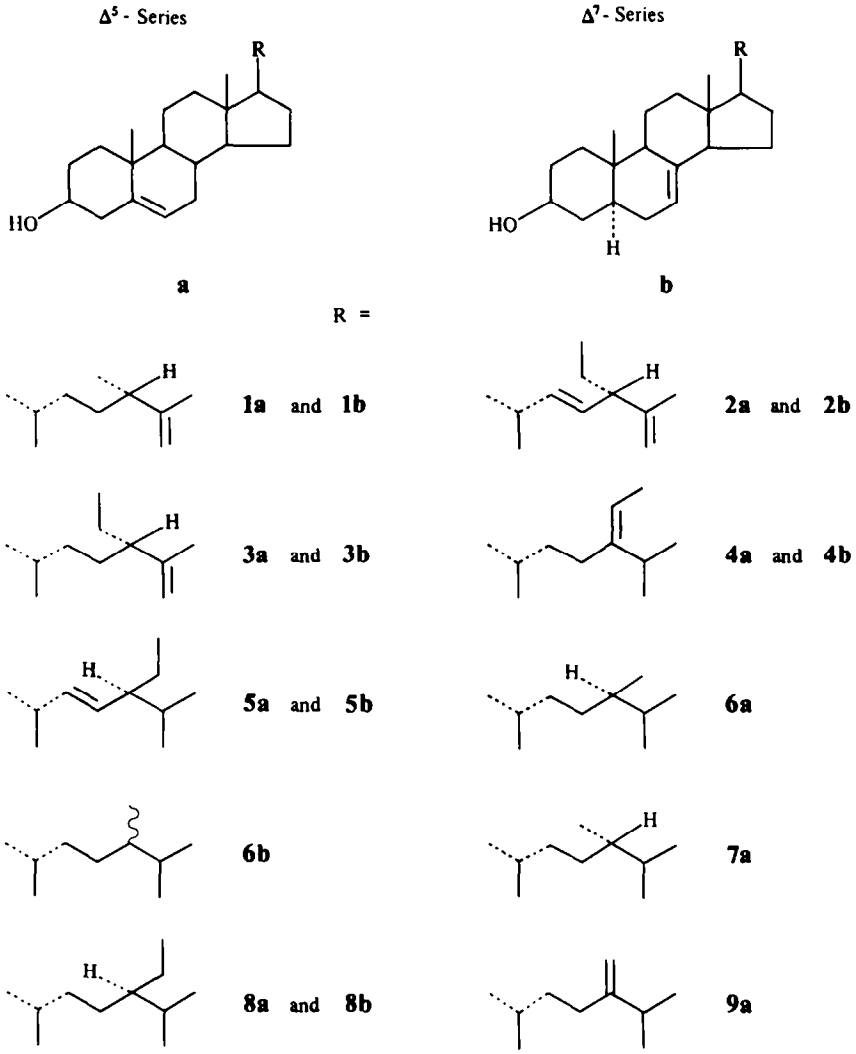
Sterol	Plant species					
	Cucurbita pepo	*Cucurbita lagenaria*	*Citrullus lanatus*	*Cucumis sativus*	*Cucumis melo*	*Luffa aegyptiaca*
	(% of total Δ^7-sterols)					
25(27)-Dehydrofungisterol (**1b**)	0.4	0.5	tr.	nd	nd	nd
25(27)-Dehydrochondrillasterol (**2b**)	28.3	31.6	40.1	38.5	45.2	50.2
24β-Ethyl-25(27)-dehydrolathosterol (**3b**)	30.6	35.2	32.3	21.0	33.1	16.7
Avenasterol (**4b**)	10.8	7.9	11.0	7.6	5.0	7.0
Spinasterol (**5b**)	23.2	19.0	9.8	31.2	15.9	24.6
24ξ-Methyllathosterol (**6b**)	1.0	tr.	1.8	0.7	tr.	tr.
22-Dihydrospinasterol (**8b**)	5.7	5.8	5.0	1.0	0.8	1.5
Total content of Δ^7-sterols (mg/100 g of seed)	37.10	28.45	36.83	40.06	48.32	35.96
% of total 4-desmethyl fraction	75.1	82.0	81.6	97.0	96.2	99.0

*tr. = traces, nd = not detected. Values for *C. maxima* from our earlier work [20] are (in % of total Δ^7-sterols): **1b**, 0.2; **2b**, 20.0; **3b**, 21.1; **4b**, 12.7; **5b**, 32.0; **6b**, 1.0; **8b**, 13.0.

relative proportions of the Δ^5- vs. Δ^7-sterols, the six species can be divided into two groups. One group (*Cucurbita pepo*, *Cucurbita lagenaria* and *Citrullus lanatus*) contains a substantial proportion (18–25% of the total 4-desmethyl fraction) of Δ^5-sterols. The other (*Cucumis sativus*, *Cucumis melo* and *Luffa aegyptiaca*) contains a low proportion (only 1–4%) (Table 1).

The Δ^5-sterols

In the group with the most Δ^5-sterols, the two members of the genus *Cucurbita* (*C. pepo* and *C. lagenaria*) showed similar sterol profiles; each of the two species contained seven major components (Table 1). Of these, three sterols, codisterol (**1a**), 25(27)-dehydroporiferasterol (**2a**) and clerosterol (**3a**) possessed a 24β-alkyl stereochemistry and together accounted for most (50–60%) of the Δ^5-fraction. The rest of the mixture was comprised of a 24-ethylidene-sterol, isofucosterol (**4a**) and three 24α-alkylsterols, stigmasterol (**5a**), campesterol (**6a**) and sitosterol (**8a**). In earlier work we found that a very similar sterol profile exists in the seeds of another member of the genus *Cucurbita*, *C. maxima* [17]. In all three species not only were the sterols themselves the same, but the proportions of the individual sterol components were markedly similar. A striking peculiarity of this genus (*Cucurbita*) is the presence of a very high proportion of the otherwise unusual 24β-methyl-$\Delta^{5,25(27)}$-sterol, codisterol (**1a**). In the present investigation this sterol was present in substantial proportions (*ca* 25% of the total Δ^5-fraction) in the two *Cucurbita* species, as we found earlier for *C. maxima* [17], while in seeds from *Cucumis*, *Citrullus* and *Luffa* species codisterol was present either in a very small proportion (<3% of the Δ^5-fraction) or only in trace amounts (Table 1). This indicates a relatively specific association of **1a** with the members of the genus *Cucurbita*. The other



two 24β-alkylsterols in *Cucurbita* species were 25(27)-dehydroporiferasterol (**2a**) and clerosterol (**3a**). These have previously been isolated from several plant species including *C. maxima* [1, 17, 21–24]. In *C. maxima* [17], as well as in *C. pepo* and *C. lagenaria*, **2a** was only a minor component (accounting for $< 5\%$ of the total Δ^5-fraction), however, **3a** was present in substantial amounts in all three species and represented $25 \pm 6\%$ of the total Δ^5-fraction (Table 1). Of the other four sterols, the three with a 24α-alkyl group (**5a**, **6a** and **8a**) represented a 'main line' distribution in which a homologous alkyl series at C-24 (CH_3, C_2H_5) occurred together with the Δ^{22}-derivative of the 24-ethylsterol. The 24-ethyl components, sitosterol (**8a**) and stigmasterol (**5a**), were exclusively 24α, whereas in the 24-methyl components, the 24α-sterol, campesterol (**6a**), was accompanied by a small amount of the 24β-epimer, 22-dihydrobrassicasterol (**7a**). The 24-ethylidene-sterol, isofucosterol (**4a**), is probably the precursor to the 24α-ethylsterols [8]. In *C. pepo*, a small amount ($< 1\%$) of 24-methylenecholesterol (**9a**) seemed to accompany the seven major sterols.

The other member of the seed group with the higher proportion of Δ^5-sterol, *Citrullus lanatus*, showed a slightly different Δ^5-sterol profile from that in the *Cucurbita* species. The differences were mainly in the 24β-alkyl series. While the total 24β-alkylsterols still represented approximately 60% of the Δ^5-fraction, in this case there were only two instead of three 24β-alkylsterols present in measurable amounts (Table 1). Codisterol (**1a**) was only a trace component in *C. lanatus*, and its place was taken in a quantitative sense by 25(27)-dehydroporifera-sterol (**2a**). This (**2a**) and the other 24β-ethylsterol (**3a**) were both present in substantial proportions, each accounting for *ca* 30% of the total Δ^5-fraction. The relative proportions of the three 24α-alkylsterols (**5a**, **6a** and **8a**) and isofucosterol (**4a**) in *C. lanatus* were more or less the same as those in the *Cucurbita* species (Table 1).

In the seed group with a smaller amount of Δ^5-sterols, enough of the individual Δ^5-sterol components was present for quantitation only in two species, *Cucumis sativus* and *Cucumis melo*. In the third species, *Luffa aegyptiaca*, the Δ^5-sterol content was too low to allow anything more than a qualitative identification of the individual components. As with *C. lanatus*, both *C. sativus* and *C. melo* contained a very small proportion of codisterol (**1a**) (*ca* 2–3%), whereas the two 24β-ethylsterols, 25(27)-dehydroporiferasterol (**2a**) and clerosterol (**3a**), were both present in substantial proportions (Table 1). In

C. sativus, **2a** and **3a** accounted for *ca* 30 and 10% of the total Δ^5-fraction, respectively. *C. melo*, on the other hand, was unusual in that **2a** alone accounted for 50% of the total Δ^5-fraction in this species. Since **3a** also accounted for 30% of the total Δ^5-fraction, the 24β-ethylsterols together represented as much as 80% of the Δ^5-sterols in *C. melo* (Table 1). In *C. sativus* the 'main line' 24α-alkylsterols (**5a**, **6a** and **8a**) accounted for *ca* 60% of the total Δ^5-fraction, whereas in *C. melo* these represented only about 14% of the Δ^5-sterols (Table 1). Isofucosterol (**4a**) was a trace component in *C. sativus*, but represented about 3% in *C. melo*.

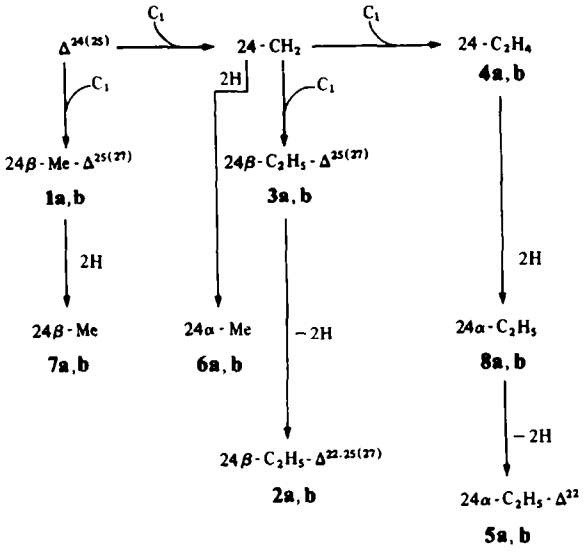
The Δ^7-sterols

Unlike the Δ^5-sterols, the Δ^7-sterols from Cucurbitaceae seeds have been previously reported by many investigators [3–6, 11, 13, 14, 20, 25, 26], and the compositional data are also available for many Cucurbitaceae species [3, 5, 6, 14, 20]. The Δ^7-sterol compositions of the six species investigated (Table 2) were more or less similar to those reported by the earlier investigators. However, there were certain differences that are worth noting. From *Cucurbita pepo* and *Cucurbita lagenaria* we isolated small proportions of the unusual 24β-methyl-$\Delta^{7,25(27)}$-sterol, 25(27)-dehydrofungisterol (**1b**) (Table 2). This is the Δ^7-analogue of codisterol (**1a**), and, like **1a**, this sterol is also believed to be rare in nature. Since **1b** was previously reported also to occur in *Cucurbita maxima* [17], these results emphasize further the importance of the 24β-methyl pathway in the genus *Cucurbita*. In the 24β-ethyl series, the two sterols, 25(27)-dehydrochondrillasterol (**2b**) and 24β-ethyl-25(27)-dehydrolathosterol (**3b**), both constituted approximately equal amounts in *C. pepo* and *C. lagenaria*, each representing about 30% (Table 2). However, in the other four species the amount of **2b** was always higher than that of **3b**. This effect was most striking in *Luffa aegyptiaca*, in which **2b** (50%) was about three times more abundant than **3b** (17%) (Table 2). In the 24α-ethyl series it was interesting to note that the Δ^{22}-derivative of the 24-ethylsterol, spinasterol (**5b**), was always more abundant than the Δ^7-monoene, 22-dihydrospinasterol (**8b**). In fact, **8b** was a relatively minor component (<6%) in all six species (Table 2). This finding represents a situation contrary to the 'main line' type of distribution that is generally observed in most higher plants [8, 27]. The level of the 24-ethylidenesterol, avenasterol (**4b**), was about the same (5–10%) in all species (Table 2), perhaps owing to its intermediacy in the 24-ethyl pathway [8]. Finally, a 24-methyl-Δ^7-monoene, 24-methyllathosterol (**6b**), was also detected in all six species; however, it was present in very small amounts (Table 2) and this prevented us from determining its C-24 configuration by ^{1}H NMR spectroscopy.

In our previous [17, 20] and the present work, we have examined the seeds of seven species in the family Cucurbitaceae and have shown the co-occurrence of Δ^5- and Δ^7-sterols in all of them. Clearly, the occurrence of Δ^5-together with the dominant Δ^7-sterols appears to be a general feature of the family Cucurbitaceae. Since these sterols are particularly abundant in the seeds, it is tempting to suggest that the Δ^5-structure is specifically required for some seed function(s). One possibility is a role in seed germination as indicated by our earlier work [18, 19], in which the Δ^5-sterols were found to disappear during germination. Alternatively, these sterols may reflect a developmental requirement of the seed. Whatever the significance might be, it is clear that the biochemical mechanisms for the introduction of the Δ^5-bond are present in the family Cucurbitaceae, and that the normally assumed route from Δ^7- to $\Delta^{5,7}$- to Δ^5-sterols [8, 9] is probably operating. However, the relative amounts of Δ^5- and Δ^7-sterols together with the absence of $\Delta^{5,7}$-sterols indicate that the rate of introduction of the 5(6)-double bond must be limited, while the reduction of the Δ^7-bond of the intermediate $\Delta^{5,7}$-sterols proceeds readily.

With regards to the relative compositions of the individual Δ^5- and Δ^7-sterol components, several distinct patterns were evident. The most striking was the presence of a large amount of the unusual 24β-methyl-$\Delta^{5,25(27)}$-sterol, codisterol (**1a**), in the members of the genus *Cucurbita* (Table 1). Sterol **1a** is believed to be rare in nature and has hitherto been shown to occur mainly in marine invertebrates [28, 29] and some siphonous marine algae [30, 31]. To our knowledge, this work represents only the second time this sterol has been isolated from a higher plant. While **1a** and each of the other sterols probably play distinctive roles, at this stage it is not clear how the roles might differ from each other. Concerning **1a**, it is also interesting to note that the species with a high proportion of **1a** contained a low proportion of 25(27)-dehydroporiferasterol (**2a**), while the ones with low **1a** showed higher **2a** (Table 1). Again, while the precise significance of such distinct compositional patterns must remain an open question, it seems likely that the pattern may be a reflection of the functional significance of the different sterols. In terms of the sterol side-chain, the structures of all major Δ^5-sterols were analogous to those of the Δ^7-sterols, indicating that structurally similar sterols were being synthesized in both the Δ^5- and Δ^7-series. The configurations at C-24 of the side chain represented two separate series, viz. the 'main line' 24α-alkyl series without a $\Delta^{25(27)}$-bond and the 24β-alkyl series with a $\Delta^{25(27)}$-bond. Clearly, the distribution of sterols in the family Cucurbitaceae constitutes one of the most complex patterns known in the plant kingdom. The Cucurbitaceae profile includes the dominant Δ^7-sterols, typical 'main line' Δ^5-sterols, and the sterols characteristic of organisms (algae) much lower on the evolutionary scale. This, together with the ontogenetic relationships observed in our earlier work [18, 19] in which the 24β-alkylsterols are replaced by 24α-alkylsterols during maturation, adds further weight to the suggestion [2, 8, 9] that in terms of sterols plants of the family Cucurbitaceae represent an evolutionary recapitulation.

From a biosynthetic point of view there appear to be four types of pathway operating for metabolism of the side chain. They begin with a $\Delta^{24(25)}$-sterol as shown in Scheme 1. C_1-Transfer would yield either a 24β-methyl-$\Delta^{25(27)}$-sterol (**1a**, **b**), which by reduction would give a 24β-methylsterol (**7a**, **b**), or C_1-transfer would yield a 24-methylenesterol. The latter, or possibly a double-bond isomer, by reduction would proceed to a 24α-methylsterol (**6a**, **b**), or it could undergo two types of further C_1-transfer. One of the two latter routes would lead through a 24β-ethyl-$\Delta^{25(27)}$-sterol (**3a**, **b**) and dehydrogenation at C-22(23) to **2a**, **b**. In the other type of C_1-transfer, the 24-methylenesterol would be converted to a *Z*-24-ethylidenesterol (**4a**, **b**) which on sequential reduction and dehydrogenation would give the 24α-ethylsterols which either have a saturated side-chain (**8a**, **b**) or one with a Δ^{22}-



Scheme 1. Probable biosynthetic pathways for the metabolism of the side chain of sterols in Cucurbitaceae.

bond (**5a, b**). In the Δ^7-series of sterols the alternative routes involving two successive C_1-transfers are the dominant pathways for the synthesis of sterols found in all of the seeds examined in this and our earlier work with *C. maxima* [20]. They also account for the major Δ^5-sterols except in the three *Cucurbita* species examined here and earlier [17]. In the *Cucurbita* species the route from the $\Delta^{24(25)}$-sterol by a single C_1-transfer to a 24β-methyl-$\Delta^{25(27)}$-sterol becomes quite important in the Δ^5-series yielding codisterol (**1a**) as a major constituent (Table 1) replacing the 24-ethyl-$\Delta^{5,22,25(27)}$-trienol.

In recent complementary work by Matsumoto and co-workers [25, 26, 32, 33] a number of Cucurbitaceae seeds have also been examined for their sterols. Among their interesting findings is that using ^{13}C NMR they were able to show that spinasterol is accompanied by a small amount of its 24β-epimer (chondrillasterol) in squash (*C. maxima*) [32], pumpkin (*C. pepo*) [32], water-melon (*Citrullus battich*) [26] and bottle gourd (*Lagenaria leucantha* var. *gourda*) [26]. This is readily accommodated by the biosynthetic pathway shown in Scheme 1. Spinasterol (**5b**) is already shown there in the pathway to 24α-ethylsterol via a 24-ethylidenesterol, and chondrillasterol would reasonably be derived by reduction of 25(27)-dehydrochondrillasterol (**2b**) in the pathway from the 24-methylenesterol leading to 24β-ethylsterols. Itoh *et al.* [33] also measured the co-occurrence of the epimer at C-24 in the 24-methyl series.

EXPERIMENTAL

The seeds of the six species examined, *Cucurbita pepo* (pumpkin), *Cucurbita lagenaria* (ornamental gourd), *Citrullus lanatus* (water-melon), *Cucumis sativus* (cucumber), *Cucumis melo* (canteloupe) and *Luffa aegyptiaca* (luffa gourd), were obtained from W. Atlee Burpee, Co., Warminster, PA 18974, U.S.A. Sterol standards were either purchased from commercial sources or were isolated from *Cucurbita maxima* seeds [17, 20].

The neutral lipids from the seeds were extracted after saponification as previously described [20], and were then chromatographed on thin-layer plates (0.25 and 1.00 mm thick) coated with silica gel G. The plates were developed 4 × in Et_2O–C_6H_6 (1:9) and the separated components were visualized under UV light after spraying with a 0.05% soln of rhodamine 6G in Me_2CO. Two bands co-chromatographed with the standards containing Δ^7- and Δ^5-sterols, respectively. The Δ^7- (slower moving) and Δ^5-regions were scraped separately into glass tubes, and the sterols were extracted from silica gel with several washes of Et_2O. The sterol mixtures thus obtained were analysed by GLC and HPLC, and were finally separated into various components by prep. HPLC. The general methodology and the instrumentation for the GLC and the HPLC were the same as described previously [20]. Mass spectra were obtained by direct probe (EIMS, ionizing energy 70 eV) on a Hitachi–Perkin Elmer Model RMU-6D instrument. ^1H NMR spectroscopy was performed at 400 MHz at ambient temp. on a Bruker instrument, Model WH-400, in $CDCl_3$ with TMS as internal standard. However, mass spectroscopy and ^1H NMR spectroscopy were not performed on the sterols from all six species. Mass spectroscopy was performed on *C. pepo*, *C. sativus* and *C. melo* sterols, whereas ^1H NMR spectroscopy was performed only on the sterols from *C. pepo* and *C. sativus*. The sterols from *C. lagenaria*, *C. lanatus* and *L. aegyptiaca* were identified on the basis of their GLC and HPLC analyses only. The chromatographic mobilities and the spectral data for the individual components from a representative member, *C. pepo*, are given below.

Codisterol (**1a**). *RR*, (GLC) 1.28, α_c (HPLC) 0.69; MS *m/z* (rel. int.): 398 $[M]^+$ (57), 383 (20), 380 (25), 365 (18), 355 (5), 328 (7), 314 (52), 300 (18), 299 (44), 273 (13), 272 (45), 271 (100), 255 (20), 253 (35), 246 (6), 231 (35), 229 (50), 213 (99), 211 (12); ^1H NMR: δ0.671, 1.005 and 1.635 (each 3H, *s*, H-18, 19 and 26), 0.910 and 0.992 (each 3H, *d*, J = 6.5 Hz, H-21 and 28), 3.499 (1H, *m*, H-3), 4.660 (2H, *br s*, H-27), 5.359 (1H, *br s*, H-6).

25(27)-Dehydroporiferasterol (**2a**). *RR*, 1.38, α_c 0.72; MS *m/z* (rel. int.): 410 $[M]^+$ (13), 395 (4), 392 (4), 381 (12), 377 (4), 363 (12), 309 (11), 300 (40), 273 (19), 272 (40), 271 (100), 255 (76), 253 (21), 231 (10), 229 (14), 227 (11), 215 (22), 213 (34); ^1H NMR: δ0.693, 1.006 and 1.643 (each 3H, *s*, H-18, 19 and 26), 0.834 (3H, *t*, J = 7.3 Hz, H-29), 1.020 (3H, *d*, J = 6.5 Hz, H-21), 4.705 (2H, *s*, H-27), 5.214 (2H, *m*, H-22, 23), the H-3 and H-6 signals were the same as those of **1a**.

Clerosterol (**3a**). *RR*$_t$ 1.58, α_c 0.89; MS *m/z* (rel. int.): 412 [M]$^+$ (61), 397 (15), 394 (5), 379 (25), 371 (3), 328 (5), 314 (51), 300 (30), 299 (31), 273 (15), 271 (100), 255 (10), 253 (34), 231 (22), 229 (52), 213 (47), 211 (50); ^{1}H NMR: δ0.671, 1.007 and 1.569 (each 3H, *s*, H-18, 19 and 26), 0.799 (3H, *t*, *J* = 7.3 Hz, H-29), 0.908 (3H, *d*, *J* = 6.6 Hz, H-21), 4.642 and 4.723 (2H, *s*, H-27, terminal protons resonating at two δ values).

Isofucosterol (**4a**). *RR*$_t$ 1.67, α_c 0.91; the MS data were more or less the same as those of **3a**; ^{1}H NMR: δ0.681 and 1.007 (each 3H, *s*, H-18 and 19), 0.947 (3H, *d*, *J* = 6.3 Hz, H-21), 0.976 (6H, 2*d*, *J* = 6.9 Hz, H-26 and 27), 1.577 (3H, *d*, *J* = 6.5 Hz, H-29), 2.820 (1H, *m*, H-25), 5.116 (1H, *m*, H-28).

Stigmasterol (**5a**). *RR*$_t$ 1.44, α_c 1.08; MS *m/z* (rel. int.): 412 [M]$^+$ (50), 397 (5), 394 (11), 379 (22), 369 (21), 327 (8), 314 (39), 300 (26), 299 (18), 273 (35), 272 (8), 271 (42), 255 (99), 253 (25), 231 (50), 229 (40), 213 (100), 211 (15); ^{1}H NMR: δ0.697 and 1.009 (each 3H, *s*, H-18 and 19), 1.022 (3H, *d*, *J* = 6.5 Hz, H-21), 0.795 and 0.847 (each 3H, *d*, *J* = 6.5 Hz, H-26 or 27), 0.805 (3H, *t*, *J* = 7.1 Hz, H-29), 5.012 and 5.157 (each 1H, *dd*, *J* = *ca* 7.5 Hz, H-22 or 23).

Campesterol (**6a**). *RR*$_t$ 1.31, α_c 1.11; MS *m/z* (rel. int.): 400 [M]$^+$ (50), 385 (22), 382 (31), 367 (30), 315 (25), 289 (44). The base peak and all other peaks were the same as those of **5a**. ^{1}H NMR: δ0.680 and 1.010 (each 3H, *s*, H-18 and 19), 0.911 (3H, *d*, *J* = 6.1 Hz, H-21), 0.771 (3H, *d*, *J* = 6.1 Hz, H-28), the H-26 and 27 signals were the same as those of **5a**.

22-Dihydrobrassicasterol (**7a**). This sterol was identified on the basis of the H-21 NMR signal at δ0.921 (3H, *d*, *J* = 6.1 Hz), which is distinctly downfield from the H-21 signal of its 24α-epimer, **6a**.

Sitosterol (**8a**). *RR*$_t$ 1.61, α_c 1.27; MS *m/z* (rel. int.): 400 [M]$^+$ (50), 399 (22), 396 (40), 381 (15), 329 (50), 303 (50), 273 (51), 272 (5), 271 (5), 255 (50), 231 (55), 229 (24), 213 (100); ^{1}H NMR: δ0.680 and 1.009 (each 3H, *s*, H-18 and 19), 0.920 (3H, *d*, *J* = 6.5 Hz, H-21), 0.814 and 0.837 (each 3H, *d*, *J* = 6.5 Hz, H-26 or 27), 0.845 (3H, *t*, *J* = 7.2 Hz, H-29).

24-Methylenecholesterol (**9a**). *RR*$_t$ 1.28, α_c 0.77; MS *m/z* (rel. int.): 398 [M]$^+$ (6), 383 (11), 380 (2), 365 (3), 314 (100), 299 (23), 273 (5), 271 (46), 253 (7), 231 (21), 213 (22). Since **9a** was present in a trace amount only, its identity could not be confirmed by ^{1}H NMR.

25(27)-Dehydrofungisterol (**1b**). This sterol was identified on the basis of the mass spectral peak for the molecular ion at *m/z* 398 [M]$^+$, and on the basis of the H-18 NMR signal at δ0.527 (3H, *s*), as described earlier [20].

25(27)-Dehydrochondrillasterol (**2b**). *RR*$_t$ 1.58, α_c 0.72; MS *m/z* (rel. int.): 410 [M]$^+$ (31), 395 (8), 392 (4), 381 (5), 300 (14), 273 (30), 272 (30), 271 (100), 255 (14), 253 (5), 246 (10), 231 (10), 229 (10), 213 (14); ^{1}H NMR: δ0.545, 0.797 and 1.653 (each 3H, *s*, H-18, 19 and 26), 0.834 (3H, *t*, *J* = 7.3 Hz, H-29), 1.019 (3H, *d*, *J* = 6.5 Hz, H-21), 4.705 (2H, *s*, H-27), 5.221 (2H, *m*, H-22, 23), 3.599 (1H, *m*, H-3), 5.159 (1H, *br s*, H-7)

24β-Ethyl-25(27)-dehydrolathosterol (**3b**). *RR*$_t$ 1.75, α_c 0.90; MS *m/z* (rel. int.): 412 [M]$^+$ (21), 397 (10), 394 (4), 379 (5), 371 (3), 369 (15), 351 (5), 301 (5) 300 (20), 299 (11), 273 (25), 272 (19), 271 (100), 255 (50), 253 (9), 246 (21), 231 (22), 213 (47); ^{1}H NMR: δ0.526, 0.795 and 1.566 (each 3H, *s*, H-18, 19 and 26), 0.800 (3H, *t*, *J* = 7.2 Hz, H-29), 0.909 (3H, *d*, *J* = 6.5 Hz, H-21), 4.692 (2H, *br s*, H-27), the H-3 and H-7 signals were the same as those of **2b**.

Avenasterol (**4b**). *RR*$_t$ 1.88, α_c 0.92; the MS data was more or less the same as those of **3b**; ^{1}H NMR: δ0.537 and 0.795 (each 3H, *s*, H-18 and 19), 0.949 (3H, *d*, *J* = 6.4 Hz, H-21), 0.976 (6H, 2*d*, *J* = 6.7 Hz, H-26 and 27), 1.588 (3H, *d*, *J* = 6.5 Hz, H-29), 2.830 (1H, *m*, H-25), 5.106 (1H, *m*, H-28).

Spinasterol (**5b**). *RR*$_t$ 1.61, α_c 1.09; MS *m/z* (rel. int.): 412 [M]$^+$ (40), 397 (15), 369 (21), 301 (8), 300 (26), 299 (18), 273 (35), 272 (28), 271 (100), 255 (41), 253 (5), 247 (11), 246 (31), 231 (20), 213 (100); ^{1}H NMR: δ0.551 and 0.800 (each 3H, *s*, H-18 and 19), 1.025 (3H, *d*, *J* = 6.5 Hz, H-21), 0.799 and 0.849 (each 3H, *d*, *J* = 6.2 Hz, H-26 or 27), 0.805 (3H, *t*, *J* = 7.1 Hz, H-29), 5.027 and 5.163 (each 1H, *dd*, *J* = *ca* 7.5 Hz, H-22 or 23).

24ξ-Methyllathosterol (**6b**). *RR*$_t$ 1.50, α_c 1.12; this sterol was identified on the basis of the MS peak for the molecular ion at *m/z* 400 [M]$^+$, and on the basis of the H-18 NMR signal at δ0.537 (3H, *s*), as described earlier [20].

22-Dihydrospinasterol (**8b**). *RR*$_t$ 1.81, α_c 1.30; MS *m/z* (rel. int.): 414 [M]$^+$ (100), 399 (32), 381 (5), 273 (31), 256 (20), 255 (66), 247 (5), 246 (19), 231 (25), 229 (24), 213 (20); ^{1}H NMR: δ0.536 and 0.795 (each 3H, *s*, H-18 and 19), 0.924 (3H, *d*, *J* = 6.5 Hz, H-21), 0.814 and 0.836 (each 3H, *d*, *J* = 6.5 Hz, H-26 or 27), 0.845 (3H, *t*, *J* = 7.4 Hz, H-29).

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