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CODISTEROL AND OTHER Δ^5-STEROLS IN THE SEEDS OF *CUCURBITA MAXIMA*

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Key Word Index—*Cucurbita maxima*; Cucurbitaceae; Δ^5-sterols; 25(27)-dehydroporiferasterol; clerosterol; isofucosterol; stigmasterol; sitosterol; campesterol; codisterol; 24β-ethylcholesta-5,22,25(27)-trien-3β-ol; 24β-ethylcholesta-5,25(27)-dien-3β-ol; 24*Z*-ethylidenecholest-5-en-3β-ol; 24α-ethylcholesta-5,22-dien-3β-ol; 24α-ethylcholest-5-en-3β-ol; 24α-methylcholest-5-en-3β-ol; 24β-methylcholesta-5,25(27)-dien-3β-ol.

Abstract—A substantial amount (*ca* 18%) of the sterol found in the seeds of *Cucurbita maxima* had a Δ^5-bond and consisted of seven components. They were identified as 25(27)-dehydroporiferasterol, clerosterol, isofucosterol, stigmasterol, sitosterol, campesterol and codisterol. The C-24 configuration of each of the sterols was unequivocally established by a ^{1}H NMR spectral comparison with authentic standards. This is the first time codisterol has been found in a higher plant and also the first time the structures and configurations of the Δ^5-sterols from a Cucurbitaceae species have been clearly characterized.

INTRODUCTION

Sterols bearing a Δ^5-bond, e.g. cholesterol and sitosterol, are believed to be the most common biological types and seem to be either required or preferred by the great majority of eukaryotes. This may be associated with a superior biological role which the Δ^5-structure allows [1, 2]. However, the existence of some higher plant species which contain predominately Δ^7-sterols is well documented [1–7]. Among these are members of the family Cucurbitaceae in which the occurrence of Δ^5- along with the Δ^7-sterols is assumed to be rare [4, 8–11]. Only a few investigators have reported the presence of Δ^5-sterols in Cucurbitaceae plants [12–14] (in seeds and seedlings), and in most cases, a sufficient amount of sterol was not isolated to allow proper identification. However, in one publication, a significant level (*ca* 25%) of Δ^5-sterols was reported to occur in the seed oil of the cucurbit *Trichosanthes kirilowii* [14]. The structures of the various Δ^5-components were determined by UV, IR and mass spectrometry as well as by GLC on a variety of liquid phases [14]. Although the configuration at C-24 was not determined, based on a pattern in which a homologous series at C-24 (H, CH_3, C_2H_5) occurred together with the Δ^{22}-derivative of the 24-ethyl-sterol, the components probably were cholesterol, campesterol, 22-dihydrobrassicasterol, sitosterol and stigmasterol, reflecting a 'mainline' distribution in which the 24-ethyl components are 24α and the 24-methyl analogues 24α and 24β [9, 15]. In addition, a $\Delta^{5,25(27)}$-sterol* was present. Since no 24α-ethyl-$\Delta^{25(27)}$-sterol has yet been isolated from a plant, the $\Delta^{25(27)}$-compound from *T. kirilowii* was probably 24β, i.e. clerosterol previously isolated from *Clerodendrum infortunatum* [16] and *Kalanchoe diagremontiana* [9].

In view of the existence of Δ^5-sterols in the cucurbits as a family, we made an exceptionally careful study of the sterols of *Cucurbita maxima* seeds to ascertain whether Δ^5-sterols could be found in this species. A substantial amount of Δ^5-sterols was actually found distributed among seven components. In this paper we report the identification of each of these by the use of GLC, HPLC, mass spectrometry and ^{1}H NMR techniques.

RESULTS AND DISCUSSION

A significant level of Δ^5-sterols (accounting for *ca* 18% of the total 4-desmethyl fraction) was isolated from the seeds of *C. maxima* by TLC separation. These Δ^5-sterols were further separated by preparative HPLC into four fractions, referred to as fractions **1** to **4** in order of polarity beginning with the least polar. Each one of these fractions was separately analysed by GLC, analytical HPLC, mass spectrometry, and high resolution ^{1}H NMR. GLC and analytical HPLC of fraction **1** indicated that it was a mixture of two sterols: a major sterol, **1a** (RR_t 1.28, α_c 0.69), accounting for 90% of the mixture, and a minor sterol, **1b** (RR_t 1.40, α_c 0.72), accounting for only 10% of the mixture. Components **1a** and **1b** were identified on the basis of their mobilities [17, 18] as 24ξ-methylcholesta-5,25(27)-dien-3β-ol (codisterol or 24-epicodisterol) and 24ξ-ethylcholesta-5,22,25(27)-trien-3β-ol, respectively. The mass spectrum of the major component (**1a**) gave peaks at *m/z* (fragment, relative intensity in %): 398 $[M]^+$ (69), 383 $[M-Me]^+$ (31), 380 $[M-H_2O]^+$ (21), 365 $[M-Me-H_2O]^+$ (13), 314 $[M-C_6H_{12}]^+$ (46), 300 $[M-C_6H_{10}O]^+$ (31), 299 $[M-C_6H_{12}-Me]^+$ (38), 273 $[M-SC]^+$ (13), 272 $[M-SC-H]^+$ (33), 271 $[M-SC-2H]^+$ (92), 255 $[M-SC-H_2O]^+$ (28), 253 $[M-SC-H_2O-2H]^+$ (31), 231 $[M-SC-C_3H_6]^+$ (33), 229 $[M-SC-C_3H_8]^+$ (41), 213 $[M-SC-C_3H_6-H_2O]^+$

*Throughout this work, all Δ^{25}-sterols are considered to have the 25(27)-designation rather than 25(26), because C-26 is taken to be derived from C-2 of MVA [1, 2, 9] and [2-^{14}C]MVA does not label the methylene carbon atom in the case examined [23].

(100) and 211 $[M - SC - C_3H_8 - H_2O]^+$ (33) (SC = side chain). The molecular ion peak at m/z 398 and other peaks at m/z 314, 273, 271 and 255 indicate a di-unsaturated C_{28}-sterol, which is consistent with the above structural deduction of **1a** [19–22].

The final confirmation of the structure of sterol **1a** was provided by ^{1}H NMR analysis. The spectrum showed signals at δ0.672 (3H, *s*, H-18), 0.912 (3H, *d*, J = 6.4 Hz, H-21), 0.992 (3H, *d*, J = 6.5 Hz, H-28), 1.006 (3H, *s*, H-19), 1.637 (3H, *s*, H-26), 3.500 (1H, *m*, H-3), 4.662 (2H, *br s*, H-27), 5.359 (1H, *br s*, H-6). These signals are consistent with a 24-methyl, $\Delta^{5,25(27)}$-sterol structure, and were observed with this sterol when isolated from marine invertebrates [20, 21] or the siphonous marine algae of the genus *Codium* [19, 22]. The configuration at C-24 was established by comparing the spectrum of **1a** with those of the two possible epimers, codisterol (24β) and 24-epicodisterol (24α). Both these epimers are known to occur in the sponge *Verongia cauliformis*, and their ^{1}H NMR spectra have been documented [20]. The ^{1}H NMR spectrum in the present study did not show the olefinic methyl signal (H-26) at δ1.651 which corresponds to 24-epicodisterol [20]. Instead, the signal appeared at 1.637, which is characteristic of codisterol (24β-epimer) [19–22]. Thus, sterol **1a** was pure 24β-methylcholesta-5,25(27)-dien-3β-ol (codisterol) without any of its 24α-epimer (24-epicodisterol). Codisterol is a rare sterol in nature, and to our knowledge this work represents the first instance of the detection of this sterol in a higher plant.

The mass spectrum of **1b**, the minor component, showed a molecular ion peak at m/z 410 and other characteristic peaks at m/z 381 $[M - C_2H_5]^+$ and 300 $[M - C_7H_{10}O]^+$. All the other fragments were the same as those of the major component. This fragmentation pattern was consistent with that reported for this sterol in the literature [9, 23]. The ^{1}H NMR analysis showed signals distinctly different from those of the major component at δ0.693 (3H, *s*, H-18), 0.833 (3H, *t*, J = 7.3 Hz, H-29) and 5.214 (2H, *m*, H-22, 23). The H-18, H-22 and H-23 signals agree with the presence of a Δ^{22}-bond, and the H-29 triplet with the presence of a 24-ethyl structure [15, 24]. In addition to the H-18 and H-29 signals, the signals for H-21 (3H, *d*), H-26 (3H, *s*) and H-27 (2H, *s*) at δ1.02, 1.64 and 4.70, respectively, were also observed as shoulders on the major component signals. All the other signals were completely masked by those of the major component and thus were apparently at the same positions for both sterols. These observations are consistent with a 24-ethyl, $\Delta^{5,22,25(27)}$-structural deduction [9, 23, 25, 26]. The configuration at C-24 was deduced on the basis of the H-29 (*t*) signals, which showed a chemical shift identical to that of an authentic 24β-epimer isolated earlier in this laboratory from *Clerodendrum splendens* [26]. Thus, sterol **1b** was 24β-ethylcholesta-5,22,25(27)-trien-3β-ol, i.e. 25(27)-dehydroporiferasterol. As with codisterol (**1a**), 25(27)-dehydroporiferasterol (**1b**) is also a rare sterol in higher plants, and in addition to the present report in *C. maxima* **1b** has so far been reported only in some *Clerodendrum* species [23, 25, 26], *Kalanchoe diagremontiana* [9] and *Enhydra fluctuans* [27].

GLC and analytical HPLC of fraction **2** as with fraction **1** also indicated the presence of two sterols, which were identified by their mobilities as 24ξ-ethylcholesta-5,25(27)-dien-3β-ol (**2a**, 72.8%; RR_t 1.57, α_c 0.88) and 24-ethylidenecholest-5-en-3β-ol (**2b**, 27.2%; RR_t 1.67, α_c 0.90). The mass spectral analysis of the mixture gave major peaks at m/z (fragment, relative intensity in %): 412 $[M]^+$ (49), 397 $[M - Me]^+$ (11), 394 $[M - H_2O]^+$ (9), 379 $[M - Me - H_2O]^+$ (18), 314 $[M - C_7H_{14}]^+$ (62), 300 $[M - C_7H_{12}O]^+$ (20), 299 $[M - C_7H_{14} - Me]^+$ (38), 273 $[M - SC]^+$ (14), 271 $[M - SC - 2H]^+$ (100), 255 $[M - SC - H_2O]^+$ (17), 253 $[M - SC - H_2O - 2H]^+$ (25), 231 $[M - SC - C_3H_6]^+$ (29), 229 $[M - SC - C_3H_8]^+$ (54), 213 $[M - SC - C_3H_6 - H_2O]^+$ (66) and 211 $[M - SC - C_3H_8 - H_2O]^+$ (42). As expected, both **2a** and **2b** produced a very similar fragmentation pattern, which is in agreement with the literature [9, 13, 28, 29]. On the other hand, the ^{1}H NMR signals for each of the two sterols were quite distinct and could be assigned to the respective sterols on the basis of their relative amounts. The signals for sterol **2a** appeared at δ0.671 (3H, *s*, H-18), 0.800 (3H, *t*, J = 7.3 Hz, H-29), 0.905 (3H, *d*, J = 6.6 Hz, H-21), 1.007 (3H, *s*, H-19), 1.566 (3H, *s*, H-26), 3.495 (1H, *m*, H-3), 4.643 and 4.724 (2H, *s*, H-27, terminal methylene protons resonating at two distinct δvalues) and 5.356 (1H, *br s*, H-6). These signals are consistent with the structural deduction of **2a** and were also obtained with this sterol previously isolated from *Kalanchoe daigremontiana* in our laboratory [9]. The C-24 configuration of the *K. daigremontiana* sterol was demonstrated to be 24β-ethyl by comparing the ^{1}H NMR spectrum of its 25(27)-dihydro derivative with those of the corresponding 24α- (sitosterol) and 24β- (clionasterol) epimers [9]. Since the ^{1}H NMR spectrum of **2a** from the present work was identical to that of the above-mentioned *K. daigremontiana* sterol, the C-24 configuration of **2a** was also deduced to be 24β-ethyl. Thus, sterol **2a** was identified as 24β-ethylcholesta-5,25(27)-dien-3β-ol (clerosterol). This is a rare sterol in higher plants, and to our knowledge, in addition to *K. daigremontiana* [9], *C. maxima* is only the second higher plant species in which the occurrence of **2a** has been supported by NMR (for configuration). However, a 24-ethyl-$\Delta^{5,25(27)}$-sterol, the C-24β configuration of which is yet to be established, has been found also in *Clerodendrum infortunatum* [16], *Calendula officinalis* [30], *Enhydra fluctuans* [31] and *Brassica napus* [29].

The ^{1}H NMR signals for sterol **2b** appeared at δ0.682 (3H, *s*, H-18), 0.947 (3H, *d*, J = 6.3 Hz, H-21), 0.976 (6H, 2*d*, J = 6.9 Hz, H-26 and H-27), 1.007 (3H, *s*, H-19), 2.819 (1H, *m*, H-25), 3.495 (1H, *m*, H-3), 5.115 (1H, *m*, H-28) and 5.356 (1H, *br s*, H-6). The H-29 signal (*d* at *ca* 1.58) was not clearly detected as it was masked by the H-26 signal for the major component (**2a**). These signals were consistent with the literature [28, 32], and matched those of an authentic sample of isofucosterol. The configuration of the $\Delta^{24(28)}$-bond was assigned on the basis of the signal at δ2.819 for the H-25 multiplet. This is the position for the *Z*-isomer [28, 32], whereas in the case of *E*-isomer this signal appears distinctly upfield at δ2.22 [32]. Thus, sterol **2b** was 24*Z*-ethylidenecholest-7-en-3β-ol (isofucosterol).

As with the first two fractions, GLC and analytical HPLC of fraction **3** indicated that two sterols were present. These were identified as 24ξ-ethylcholesta-5,22-dien-3β-ol (**3a**, stigmasterol or poriferasterol, 65.65%; RR_t 1.42, α_c 1.09) and 24ξ-methylcholest-5-en-3β-ol (**3b**, campesterol or 22-dihydrobrassicasterol, 34.35%; RR_t 1.30, α_c 1.11). The major component (**3a**) upon mass spectrometry showed peaks at m/z (fragment, relative intensity in %): 412 $[M]^+$ (42), 394 $[M - H_2O]^+$ (8), 379 $[M - Me - H_2O]^+$ (25), 369 $[M - C_3H_7]^+$ (8), 327 $[M - 85]^+$ (8), 314 $[M - 98]^+$ (31), 300 $[M - C_7H_{12}O]^+$ (39), 299 $[M - 113]^+$ (22), 273 $[M - SC]^+$ (39), 272

$[M-SC-H]^+$ (32), 271 $[M-SC-2H]^+$ (56), 255 $[M-SC-H_2O]^+$ (95), 253 $[M-SC-H_2O-2H]^+$ (24), 231 $[M-SC-C_3H_6]^+$ (51), 229 $[M-SC-C_3H_8]^+$ (37), 213 $[M-SC-C_3H_6-H_2O]^+$ (100) and 211 $[M-SC-C_3H_8-H_2O]^+$ (22). The peaks corresponding to **3b** were at *m/z*: 400 $[M]^+$ (51), 385 $[M-Me]^+$ (17), 382 $[M-H_2O]^+$ (25), 367 $[M-Me-H_2O]^+$ (24), 315 $[M-85]^+$ (29) and 289 $[M-C_7H_{11}O]^+$ (34). The base peak and all other peaks for **3b** were the same as those for **3a**. These fragmentation patterns are in agreement with those described for **3a** and **3b** in the literature [9].

The final confirmation of the structures of sterols **3a** and **3b** was provided by ^{1}H NMR analysis. In each case, the spectrum of the natural sterol was compared with those of the authentic samples of the two possible C-24 epimers (24α and 24β). The data (Table 1) clearly show that the spectrum of **3a** is practically identical to that of authentic stigmasterol (24α-epimer) and is markedly different from that of authentic poriferasterol (24β-epimer). The chemical shifts of the epimeric standards were distinctly different in the H-29 methyl proton region and agree with those reported in the literature [15, 24]. Component **3a** was therefore identified as 24α-ethylcholesta-5,22-dien-3β-ol (stigmasterol). The signals for component **3b** (Table 2) are also identical to those of its corresponding 24α-epimer (campesterol) and are significantly different from those of the corresponding 24β-epimer (22-dihydrobrassicasterol) [9, 15, 24]. However, a slight shoulder distinctly downfield (*ca* 0.01 ppm) from that of the 24α-epimer in the H-21 methyl proton region, indicated that the 24β-epimer may be present as a very minor component. Thus, sterol **3b** was mostly 24α-methylcholest-5-en-3β-ol (campesterol), with the co-presence of a very small amount of 24β-epimer (22-dihydrobrassicasterol).

In contrast to the first three fractions from the preparative HPLC, fraction **4** was shown to contain only one sterol by GLC and analytical HPLC, with an RR_t of 1.62 and α_c of 1.26. This component was identified as 24ξ-ethylcholest-5-en-3β-ol (sitosterol or clionasterol). The mass spectral analysis gave major peaks at *m/z* (fragment, relative intensity in %): 414 $[M]^+$ (78), 399 $[M-Me]^+$ (30), 396 $[M-H_2O]^+$ (36), 381 $[M-Me-H_2O]^+$ (26), 329 $[M-85]^+$ (45), 303 $[M-C_7H_{11}O]^+$ (55), 273 $[M-SC]^+$ (36), 272 $[M-SC-H]^+$ (5), 271

Table 1. ^{1}H NMR chemical shifts (δ) of C_{29}-$\Delta^{5,22}$-sterols*

Proton	Sterol **3a** (from *C. maxima* seeds)	Stigmasterol†	Poriferasterol‡
H-18 (3H, *s*)	0.698	0.698	0.697
H-19 (3H, *s*)	1.011	1.012	1.011
H-21 (3H, *d*)	1.022 (6.5)	1.022 (6.5)	1.024 (6.5)
H-26 or 27 (3H, *d*)	0.795 (6.6)	0.795 (6.6)	0.791 (6.7)
H-26 or 27 (3H, *d*)	0.846 (6.5)	0.846 (6.5)	0.843 (6.5)
H-29 (3H, *t*)	0.805 (7.1)	0.804 (7.1)	0.810 (7.2)
H-3 (1H, *m*)	3.504	3.505	3.505
H-6 (1H, *br s*)	5.359	5.357	5.356
H-22 or 23 (1H, *dd*)	5.012 (*ca* 7.5)	5.015 (*ca* 7.5)	5.016 (*ca* 7.5)
H-22 or 23 (1H, *dd*)	5.156 (*ca* 7.5)	5.159 (*ca* 7.5)	5.162 (*ca* 7.5)

*Values in parentheses are the coupling constants (*J*, in Hz).
†Obtained from a commercial source.
‡Isolated from *Chlorella ellipsoidea*.

Table 2. ^{1}H NMR chemical shifts (δ) of C_{28}-Δ^5-sterols*

Proton	Sterol **3b** (from *C. maxima* seeds)	Campesterol†	22-Dihydro-brassicasterol‡
H-18 (3H, *s*)	0.680	0.680	0.680
H-19 (3H, *s*)	1.011	1.011	1.010
H-21 (3H, *d*)	0.911 (6.1)	0.910 (6.1)	0.921 (6.2)
H-26 or 27 (3H, *d*)	0.800 (6.0)	0.800 (6.0)	0.784 (6.1)
H-26 or 27 (3H, *d*)	0.851 (6.0)	0.850 (6.1)	0.862 (6.1)
H-28 (3H, *d*)	0.771 (6.0)	0.771 (6.0)	0.774 (6.0)
H-3 (1H, *m*)	3.504	n.m.	n.m.
H-6 (1H, *br s*)	5.359	n.m.	n.m.

*Values in parentheses are the coupling constants (*J*, in Hz); the spectra of campesterol and 22-dihydrobrassicasterol were recorded at 220 MHz, and that of sterol **3b** at 360 MHz; n.m. = not measured.
†Obtained from a commercial source.
‡Prepared from ergosterol.

$[M-SC-2H]^+$ (4), 255 $[M-SC-H_2O]^+$ (46), 253 $[M-SC-H_2O-2H]^+$ (3), 231 $[M-SC-C_3H_6]^+$ (50), 229 $[M-SC-C_3H_8]^+$ (20), 213 $[M-SC-C_3H_6-H_2O]^+$ (100) and 211 $[M-SC-C_3H_8-H_2O]^+$ (6). This fragmentation was similar to those of authentic sitosterol and clionasterol, and agreed with the literature [9]. The final proof of the structure of **4** was again provided by ^1H NMR analysis. When compared with the spectra of the authentic epimers (Table 3), the spectrum of **4** was found to be identical to that of the corresponding 24α-epimer (sitosterol), but was markedly different from that of the 24β-epimer (clionasterol). The signals for protons at H-21 and H-29 were diagnostic for the two epimers [9, 15, 24]. Fraction **4** was therefore identified as 24α-ethylcholest-5-en-3β-ol (sitosterol).

In all, seven Δ^5-4-desmethylsterols were identified in the seeds of *C. maxima*. The absolute amounts and percentages of each of these sterols are given in Table 4. This work represents the first time the structures and configurations of the Δ^5-sterols from a Cucurbitaceae species have been clearly characterized. It is also the first report of the presence of Δ^5-sterols in *C. maxima*.

When compared with the Δ^7-fraction [33], it is interesting to note that the structures of all seven Δ^5-sterols were analogous to those of the Δ^7-sterols. This would indicate that structurally similar sterols are being synthesized in both the Δ^5- and Δ^7-series, and thus very similar biosynthetic pathways seem to exist. Although the biosynthetic route to Δ^5-sterols is usually believed to operate from Δ^7- to $\Delta^{5,7}$- to Δ^5-sterols [1, 2], in the present study HPLC of the 4-desmethyl mixture using a 282 nm monitor failed to reveal the presence of any $\Delta^{5,7}$-intermediates. In contrast to the structural similarity, the relative amounts of the various Δ^5-sterols (Table 4) were markedly different from those of their corresponding Δ^7-analogues [33]. The unusual 24β-methyl-$\Delta^{5,25(27)}$-sterol, codisterol, was the most abundant (35.3% of the total) Δ^5-sterol, whereas its Δ^7-analogue, 25(27)-dehydrofungisterol, was present in the Δ^7-fraction only in trace amounts [33]. Conversely, the Δ^7-analogue of the minor Δ^5-sterol, 25(27)-dehydroporiferasterol, was one of the major components in the Δ^7-series [33]. Furthermore, it is interesting to note that in the Δ^7-series [33], the 24-methyl-sterols (24ξ-methyllathosterol and 25(27)-dehydrofungisterol) constituted a much smaller part of the mixture than did the corresponding analogues (campesterol and codisterol) in the Δ^5-series. This difference has also been observed in other plants [1, 2] and thus seems to represent a general pattern. In addition, it has also been commonly noted in the literature that in the Δ^7-series the percentage of the Δ^{22}-24α-ethyl-sterol (spinasterol) is higher than is usually found for its Δ^5-analogue (stigmas-

Table 3. ^1H NMR chemical shifts (δ) of C_{29}-Δ^5-sterols*

Proton	Sterol **4** (from *C. maxima* seeds)	Sitosterol†	Clionasterol‡
H-18 (3H, *s*)	0.680	0.680	0.680
H-19 (3H, *s*)	1.009	1.010	1.011
H-21 (3H, *d*)	0.921 (6.6)	0.921 (6.5)	0.924 (6.5)
H-26 or 27 (3H, *d*)	0.813 (6.5)	0.814 (6.5)	0.810 (6.5)
H-26 or 27 (3H, *d*)	0.834 (6.5)	0.833 (6.5)	0.827 (6.5)
H-29 (3H, *t*)	0.845 (7.3)	0.845 (7.3)	0.855 (7.3)
H-3 (1H, *m*)	3.504	3.502	n.m.
H-6 (1H, *br s*)	5.358	5.358	n.m.

*Values in parentheses are the coupling constants (*J*, in Hz); the spectrum of clionasterol was recorded at 220 MHz, and the others at 360 MHz, n.m. = not measured.

†Obtained from a commercial source.

‡Isolated from *Chlorella ellipsoidea*.

Table 4. The Δ^5-4-desmethylsterol composition of *C. maxima* seeds

Sterol	Amount (mg/100 g of seeds)	% of total
24β-Ethylcholesta-5,22,25(27)-trien-3β-ol (**1b**) (25(27)-dehydroporiferasterol)	0.31	4.3
24β-Ethylcholesta-5,25(27)-dien-3β-ol (**2a**) (clerosterol)	1.43	19.8
24*Z*-Ethylidenecholest-5-en-3β-ol (**2b**) (isofucosterol)	0.54	7.4
24α-Ethylcholesta-5,22-dien-3β-ol (**3a**) (stigmasterol)	0.79	10.9
24α-Ethylcholest-5-en-3β-ol (**4**) (sitosterol)	1.20	16.6
24α-Methylcholest-5-en-3β-ol (**3b**) (campesterol)*	0.41	5.7
24β-Methylcholesta-5,25(27)-dien-3β-ol (**1a**) (codisterol)	2.56	35.3

*A minute amount of the 24β-epimer, 22-dihydrobrassicasterol, was also present.

terol) [1, 2]. A similar pattern can be observed in *C. maxima*. In the Δ^7-series, spinasterol was the most abundant sterol (32.0% of the total) [33], while its Δ^5-analogue, stigmasterol, is considerably less in abundance (10.9% of the total; Table 4). Based on these observations, it seems likely that such distinct patterns in the relative ratios (of the various Δ^5-sterols) may be a reflection of their functional significance.

Finally, it should be pointed out that as with the Δ^7-sterols [33], the Δ^5-fraction of *C. maxima* also contains several unusual higher plant sterols which occur in addition to the usual ones of the 'main line' series, Of particular interest is the presence of a large amount of codisterol (**1a**); this has hitherto been found only in some marine invertebrates [20, 21] and the siphonous marine algae of the genus *Codium* [19, 22]. In fact this is the first time that such a high concentration of codisterol has been reported in any organism. While **1a** probably plays a definite role in *C. maxima*, the precise function must remain an open question. The other two unusual higher plant sterols were 25(27)-dehydroporiferasterol (**1b**) and clerosterol (**2a**). They have previously been reported to occur only in a few plant species [9, 23, 25–27, 29–31]. Thus, together with the Δ^7-sterols [33], the 4-desmethylsterols of *C. maxima* seeds represent an unusual array of sterols. This further strengthens the earlier suggestion [1, 2, 34] that the plants of the family Cucurbitaceae may show an evolutionary transition in terms of sterols.

EXPERIMENTAL

Squash (*Cucurbita maxima* cv True Hubbard) seeds were obtained from W. Atlee Burpee, Co., U.S.A. Specialized standards were obtained and purified from the following sources: stigmasterol, campesterol and sitosterol from Applied Science Laboratories; poriferasterol and clionasterol from *Chlorella ellipsoidea* (courtesy of G. W. Patterson); and 22-dihydrobrassicasterol was prepared from ergosterol [35].

The 4-desmethylsterols were extracted and separated from the neutral lipids as previously described [33]. The Δ^5- and Δ^7-mixtures were separated from each other by TLC. The plates (silica gel G coated; 0.25 and 1.00 mm thick) were developed 4 × in Et_2O–C_6H_6 (1:9) and the separated components were visualized under UV light after being sprayed with a 0.05% soln of rhodamine 6G in Me_2CO. Two bands, which were completely separated, were visualized, co-chromatographing with the standards containing authentic Δ^7- (lathosterol–spinasterol mixture) and Δ^5- (cholesterol–sitosterol mixture) sterols, respectively. The faster moving Δ^5-region was separated into glass tubes and the sterols were extracted with several washes of Et_2O. The sterol mixture thus obtained was analysed by GLC and HPLC, and finally separated into various components by prep. HPLC. The instrumentation and the techniques for the GLC and the HPLC have been described in ref. [33]. The term RR_t is used for GLC and α_c for HPLC.

Mass spectra were obtained by direct probe (EIMS, ionizing energy 70 eV) on a Finnigan Model 4000 instrument with a series 6000 data system. ^{1}H NMR spectroscopy was performed at 360 MHz at ambient temp. on a Bruker instrument, Model WH-360, in $CDCl_3$ with TMS as internal standard. The ^{1}H NMR spectroscopy of some of the standards was performed at 220 MHz.

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