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# STUDIES ON THE C-24 CONFIGURATIONS OF $\Delta^7$-STEROLS IN THE SEEDS OF *CUCURBITA MAXIMA*

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**Key Word Index**—*Cucurbita maxima*; Cucurbitaceae; $\Delta^7$-sterols; avenasterol; spinasterol; dihydrospinasterol; 24ξ-methyllathosterol; 25(27)-dehydrofungisterol; 24β-ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol; 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol; 24*Z*-ethylidene-5α-cholest-7-en-3β-ol; 24α-ethyl-5α-cholesta-7,22-dien-3β-ol; 24α-ethyl-5α-cholest-7-en-3β-ol; 24ξ-methyl-5α-cholest-7-en-3β-ol; 24β-methyl-5α-cholesta-7,25(27)-dien-3β-ol.

**Abstract**—Uncertainties surrounding the structures of the $\Delta^7$-sterols in the seeds of *Cucurbita maxima* have been resolved. Seven components were found by TLC, GLC, HPLC, mass spectrometry and $^1$H NMR. They were 24β-ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol, 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol, avenasterol, spinasterol, 24-dihydrospinasterol, 24ξ-methyllathosterol and 25(27)-dehydrofungisterol. The $^1$H NMR spectra indicated that the sterols with an ethyl substituent at C-24 occurred in the absence of their C-24 epimers. This seems to be the first instance of the detection of 25(27)-dehydrofungisterol in a higher plant.

## INTRODUCTION

The major sterols of the seeds of the family Cucurbitaceae are 24-ethyl-$\Delta^7$-sterols with one or two additional double bonds, viz. 24-ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol* (**1**), 24-ethyl-5α-cholesta-7,25(27)-dien-3β-ol (**2a**) and 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**3a**) [1–7]. In *Cucurbita pepo* (pumpkin) the configuration at C-24 of **1** and **2a**, which contain $\Delta^{25(27)}$-bonds, has been shown by $^1$H NMR spectroscopy and other means to be 24β, whereas in **3a**, which lacks a $\Delta^{25(27)}$-bond, it was demonstrated to be 24α [8]. This observation was consistent with reports of the co-occurrence of 24α- and 24β-ethyl-sterols of the $\Delta^5$-series in other higher plants; the 24β-ethyl stereochemistry seemed always to be associated with the presence of a $\Delta^{25(27)}$-bond [9–11], while the $\Delta^{25(27)}$-bond has never been observed with 24α-ethyl-sterols. In other words, whenever a 24-ethyl-sterol lacking a $\Delta^{25(27)}$-bond was isolated from a tracheophyte and adequate identification was made, the configuration at C-24 was always α. In contrast, more recent work by Iida *et al.* [12] has demonstrated the presence of the 24β-ethyl configuration in a Cucurbitaceae sterol which lacks the $\Delta^{25(27)}$-bond. These authors made a direct comparison of the high-resolution $^1$H NMR spectra of both epimers. They found that the 24-ethyl-$\Delta^{7,22}$-sterol isolated from gourd and sponge cucumber seed oils, which was previously identified as spinasterol [5, 13], was in fact its 24β-epimer, i.e. chondrillasterol. In several other genera of Cucurbitaceae this finding was further substantiated recently by $^{13}$C NMR studies in which chondrillasterol was shown to co-occur with spinasterol [14, 15]. The 22(23)-dihydro derivatives (**4**) of chondrillasterol and spinasterol are also now known to co-occur in a cucurbit [15].

In the present investigation, we have undertaken a detailed examination of the configuration at C-24 of the $\Delta^7$-sterols in seeds of *Cucurbita maxima*. This species has been extensively used for studies of the biogenesis of $\Delta^7$-sterols as well as for an examination of the C-24 alkylation mechanisms in the 24α- and 24β-pathways [16–18], and a knowledge of the correct C-24 stereochemistry is important for an understanding of biosynthetic work. Although the sterols of *C. maxima* seeds have been reported in an earlier publication [19], the establishment of the C-24 stereochemistry of some of the sterols was not entirely certain. For example, the sterol believed to be dihydrospinasterol was reported to have a $^1$H NMR spectrum identical to that described in the literature [8] for its 24β-epimer, dihydrochondrillasterol, and was also supposedly distinctly different from the literature spectrum [8] of dihydrospinasterol. Thus, this sterol should have been identified [19] as dihydrochondrillasterol and not as dihydrospinasterol. Unfortunately, the earlier investigators [19] apparently did not have authentic samples of both epimers for comparison. In order to clarify this problem, we have carefully compared the high-resolution $^1$H NMR spectra of both of the 24-ethyl-sterols ($\Delta^7$ and $\Delta^{7,22}$) lacking a $\Delta^{25(27)}$-bond (**4** and **3a**) with those of their authentic 24α- and 24β-epimers, and have unequivocally established their configurations. We have also re-investigated the structures of the other $\Delta^7$-sterols of *C. maxima* confirming earlier assignments [19]. Evidence was also obtained for a new minor sterol not previously found in higher plants. In addition, as described in another paper [20], we isolated and characterized a number of $\Delta^5$-sterols from seeds of this species.

## RESULTS AND DISCUSSION

The seeds of *C. maxima* contained predominately $\Delta^7$-sterols (82% of the total 4-desmethyl fraction), as

*Throughout this work, all $\Delta^{25}$-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 [11, 32, 34] and [2-$^{14}$C]MVA does not label the methylene carbon atom in the case examined [10].

expected for a cucurbit. However, a substantial amount (*ca* 18%) of $\Delta^5$-sterols was also found [20].

After removal of $\Delta^5$-sterols, the $\Delta^7$-fraction was separated into four components (referred to as components **1** to **4** in order of polarity, beginning with the least polar) by preparative HPLC. GLC and analytical HPLC of component **1** showed the presence of only one peak with an $RR_t$ of 1.56 and an $\alpha_c$ of 0.71. The mass spectrum of this component gave major peaks at *m/z* (fragment, relative intensity in %): 410 $[M]^+$ (23), 395 $[M-Me]^+$ (11), 381 $[M-C_2H_5]^+$ (11), 377 $[M-Me-H_2O]^+$ (6), 363 $[M-C_2H_5-H_2O]^+$ (5), 326 $[M-C_6H_{12}]^+$ (5), 300 $[M-C_7H_{10}O]^+$ (15), 285 $[M-125]^+$ (8), 273 $[M-SC]^+$ (26), 272 $[M-SC-H]^+$ (30), 271 $[M-SC-2H]^+$ (100), 255 $[M-SC-H_2O]^+$ (49), 253 $[M-SC-H_2O-2H]^+$ (15), 246 $[M-164]^+$ (10), 231 $[M-SC-C_3H_6]^+$ (21), 229 $[M-SC-C_3H_8]^+$ (40), 213 $[M-SC-C_3H_6-H_2O]^+$ (30), and 211 $[M-SC-C_3H_8-H_2O]^+$ (13) (SC = side chain). A peak for the molecular ion at *m/z* 410 indicated it was a $C_{29}$-sterol with three double bonds, and was identified on this basis as 24ξ-ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol. The peaks at *m/z* 271 and 273 corresponded to the loss of a di-unsaturated side chain with and without the loss of two hydrogens from the nucleus, respectively. In addition, the observed fragmentation pattern was consistent with that reported in the literature for this sterol isolated earlier from pumpkin [3] and cucumber [21].

The final confirmation of the structure of component **1** was provided by high-resolution $^1$H NMR. The spectrum showed signals at δ 0.545 (3H, *s*, H-18), 0.797 (3H, *s*, H-19), 0.837 (3H, *t*, $J$ = 7.4 Hz, H-29), 1.017 (3H, *d*, $J$ = 6.6 Hz, H-21), 1.651 (3H, *s*, H-26), 2.421 (1H, *q*, H-24), 3.597 (1H, *m*, H-3), 4.704 (2H, *s*, H-27), 5.154 (1H, *br s*, H-7), 5.182 (1H, *dd*, $J$ = *ca* 7.4 Hz, H-22 or 23) and 5.260 (1H, *dd*, $J$ = *ca* 7.4 Hz, H-22 or 23). As expected, these signals were consistent with a 24-ethyl, $\Delta^{7,22,25(27)}$ structural deduction [2, 8, 22]. The configuration at C-24 was deduced on the basis of the work by Sucrow *et al.* [8], who examined the $^1$H NMR spectra of both the 24α- and 24β-epimers of this compound. The diagnostic features establishing the C-24 configuration were the chemical shifts of the methyl protons at H-21 and H-29 and of the olefinic protons at H-22 and H-23. When compared with the spectra of the two epimers, the signals for component **1** were practically identical to those of the 24β-epimer, but were significantly different from the 24α-epimer. This component was therefore identified as 24β-ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol.

In addition to this main component, the $^1$H NMR spectrum in the H-18 methyl region also indicated the presence of one other component in minute quantity (*ca* 1%). The H-18 signal for this component was distinctly upfield from that of the major component, at δ 0.527 (3H, *s*), indicating the absence of a $\Delta^{22}$-bond [11, 23, 24]. Since no other $^1$H NMR signals were recognized for this component, it was tentatively identified on the basis of the mobility on the preparative HPLC as 24ξ-methyl-5α-cholesta-7,25(27)-dien-3β-ol. A small mass spectral peak for the molecular ion at *m/z* 398 $[M]^+$ was consistent with this identification. Since no 24α-alkyl-$\Delta^{25(27)}$-sterol is known in higher plants, the C-24 configuration of this sterol was probably 24β, i.e. 25(27)-dehydrofungisterol. This assignment was further supported by the presence of large quantities of an analogous sterol in the $\Delta^5$-fraction (24-methyl-$\Delta^{5,25(27)}$-sterol), in which the C-24 configuration was unequivocally established to be 24β (codisterol), and its 24α-epimer(24-epicodisterol) was not present at all [20]. This seems to be the first record of the occurrence of 25(27)-dehydrofungisterol in a higher plant. Previously, a C-24 epimeric mixture of 24-methyl-$\Delta^{7,25(27)}$-sterols was shown to occur in the sponge *Verongia cauliformis* [25].

GLC and analytical HPLC of component **2** indicated that it was a mixture of two sterols, which were identified by their mobilities [26, 27] as 24ξ-ethyl-5α-cholesta-7,25(27)-dien-3β-ol (**2a**, 62.4%; $RR_t$ 1.75, $\alpha_c$ 0.91), and 24-ethylidene-5α-cholest-7-en-3β-ol (**2b**, 37.6%; $RR_t$ 1.87, $\alpha_c$ 0.93). The mass spectral analysis of the mixture indicated that, as expected, both sterols produced very similar fragmentation. The major peaks were *m/z* (fragment, relative intensity in %): 412 $[M]^+$ (11), 397 $[M-Me]^+$ (9), 379 $[M-Me-H_2O]^+$ (3), 314 $[M-C_7H_{14}]^+$ (17), 299 $[M-C_7H_{14}-Me]^+$ (7), 285 $[M-127]^+$ (5), 273 $[M-SC]^+$ (7), 272 $[M-SC-H]^+$ (23), 271 $[M-SC-2H]^+$ (100), 255 $[M-SC-H_2O]^+$ (17), 253 $[M-SC-H_2O-2H]^+$ (5), 246 $[M-166]^+$ (12), 231 $[M-SC-C_3H_6]^+$ (13), 229 $[M-SC-C_3H_8]^+$ (11), 213 $[M-SC-C_3H_6-H_2O]^+$ (12) and 211 $[M-SC-C_3H_8-H_2O]^+$ (7). A single molecular ion peak for a $C_{29}$-sterol at *m/z* 412 and a strong peak at *m/z* 271 were consistent with the presence of two double bonds, one being in the nucleus and one in the side chain of each of the components. A prominent peak at *m/z* 314 was consistent with both the $\Delta^{24(28)}$- [3, 11] and $\Delta^{25(27)}$- [3, 11] structures indicated earlier by the chromatographic mobilities.

The structures of components **2a** and **2b** were unequivocally established by the $^1$H NMR spectrum of the mixture. The signals for each of the two sterols were quite distinct and could be assigned to one or the other of the sterols on the basis of relative amounts. The signals for sterol **2a** appeared at δ 0.526 (3H, *s*, H-18), 0.794 (3H, *s*, H-19), 0.801 (3H, *t*, $J$ = 7.2 Hz, H-29), 0.909 (3H, *d*, $J$ = 6.4 Hz, H-21), 1.565 (3H, *s*, H-26), 3.597 (1H, *m*, H-3), 4.658 and 4.735 (2H, *s*, H-27, terminal methylene protons resonating at two distinct δ values) and 5.153 (1H, *br s*, H-7). These signals were consistent with those reported for the same sterol in the literature [2, 8, 19], and a comparison showed that, as in all earlier reports, the configuration at C-24 was 24β-ethyl. This deduction was based on earlier work [8] in which a product obtained by the hydrogenation of the C-25 double bond of naturally occurring **2a** was compared with the corresponding 24α-(dihydrospinasterol) and 24β- (dihydrochondrillasterol) epimers. It was found that the $^1$H NMR signals and other properties of the reduction product were consistent with a 24β-configuration [8]. Since the $^1$H NMR spectrum of **2a** from the present work was practically identical to that of the sterol [1, 2] which was confirmed [8] as the 24β-epimer, the C-24 configuration of **2a** probably was 24β-ethyl. Thus, sterol **2a** was identified as 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol. The $^1$H NMR signals for sterol **2b** appeared at δ 0.538 (3H, *s*, H-18), 0.794 (3H, *s*, H-19), 0.951 (3H, *d*, $J$ = 6.4 Hz, H-21), 0.976 (6H, 2*d*, $J$ = 6.8 Hz, H-26 and H-27), 1.591 (3H, *d*, $J$ = 6.6 Hz, H-29), 2.830 (1H, *m*, H-25), 3.597 (1H, *m*, H-3), 5.104 (1H, *m*, H-28) and 5.153 (1H, *br s*, H-7). These values, which are in accord with the literature [28–30], were also obtained with an authentic sample of avenasterol. However, a difference was noted from the previously published data of Cattel *et al.* [19]. These authors reported an H-21 doublet at δ 0.98, while we observed it at δ 0.951 as found in all other reports [28–30]. The configuration of the

$\Delta^{24(28)}$-bond was assigned on the basis of the signal at $\delta$ 2.83 for the H-25 multiplet. This is the position for the *Z*-isomer [28, 29]. In the case of the *E*-isomer, this signal appears at $\delta$ 2.20 [28, 29]. Thus, sterol **2b** was 24*Z*-ethylidene-5α-cholest-7-en-3β-ol (avenasterol).

As with component **2**, GLC and analytical HPLC of component **3** also indicated the presence of two sterols: a major sterol, **3a** ($RR_t$ 1.60, $\alpha_c$ 1.11), accounting for 97% of the mixture, and a minor sterol, **3b** ($RR_t$ 1.48, $\alpha_c$ 1.12), accounting for the rest. Compounds **3a** and **3b** were identified by their mobilities as 24ξ-ethyl-5α-cholesta-7,22-dien-3β-ol (spinasterol or chondrillasterol) and 24ξ-methyl-5α-cholest-7-en-3β-ol (24ξ-methyllathosterol), respectively. The mass spectral analysis indicated that the fragmentation pattern of **3a** (the major component) was very similar to those of authentic spinasterol and chondrillasterol, and agreed with the literature [3, 11, 30]. The major peaks were *m/z* (fragment, relative intensity in %): 412 $[M]^+$ (23), 397 $[M-Me]^+$ (8), 379 $[M-Me-H_2O]^+$ (5), 369 $[M-C_3H_7]^+$ (10), 351 $[M-C_3H_7-H_2O]^+$ (5), 327 $[M-85]^+$ (7), 301 $[M-C_7H_{11}O]^+$ (15), 300 $[M-C_7H_{12}O]^+$ (13), 273 $[M-SC]^+$ (30), 272 $[M-SC-H]^+$ (28), 271 $[M-SC-2H]^+$ (100), 255 $[M-SC-H_2O]^+$ (47), 253 $[M-SC-H_2O-2H]^+$ (8), 246 $[M-166]^+$ (27), 231 $[M-SC-C_3H_6]^+$ (30), 229 $[M-SC-C_3H_8]^+$ (32), and 213 $[M-SC-C_3H_6-H_2O]^+$ (40). The final proof of the structure of **3a**, and in particular of its C-24 configuration, was again provided by high-resolution $^1H$ NMR analysis. The data (Table 1) clearly show that the spectrum of **3a** is consistent with that of authentic spinasterol and is markedly different from that of authentic chondrillasterol. The chemical shifts of the epimeric standards were distinctly different for the methyl protons at H-26, H-27 and H-29, and agreed with the previous reports [8, 11, 24, 30]. Thus, component **3a** isolated from *C. maxima* seeds is 24α-ethyl-5α-cholesta-7,22-dien-3β-ol (spinasterol), and not its 24β-epimer (chondrillasterol) or an epimeric mixture as observed in some other cucurbits [12, 14, 15].

The structure of component **3b** (the minor component) could not be confirmed due to an insufficient quantity. However, an H-18 signal at $\delta$ 0.537 (3H, *s*), distinctly upfield from that of the major component, indicated the absence of a $\Delta^{22}$-bond [11, 23, 24], and supported the tentative identification (made from chromatographic mobilities, see above) as 24ξ-methyllathosterol. A mass spectral peak for the molecular ion at *m/z* 400 $[M]^+$ was also consistent with this identification.

Component **4**, the most polar component of the $\Delta^7$-mixture, was shown to contain only one sterol by GLC and analytical HPLC, with an $RR_t$ of 1.83 and an $\alpha_c$ of 1.29. This component was identified as 24ξ-ethyl-5α-cholest-7-en-3β-ol (dihydrospinasterol or dihydrochondrillasterol). The mass spectrum of this component gave major peaks at *m/z* (fragment, relative intensity in %): 414 $[M]^+$ (100), 399 $[M-Me]^+$ (25), 396 $[M-H_2O]^+$ (7), 368 (25), 337 (25), 329 (5), 315 (21), 303 $[M-C_7H_{11}O]^+$ (5), 300 (29), 273 $[M-SC]^+$ (36), 272 $[M-SC-H]^+$ (18), 255 $[M-SC-H_2O]^+$ (68), 231 $[M-SC-C_3H_6]^+$ (32), 229 $[M-SC-C_3H_8]^+$ (32) and 213 $[M-SC-C_3H_6-H_2O]^+$ (36). This fragmentation pattern was consistent with that of either of the authentic epimeric samples, and was also in agreement with the literature [3, 11, 30]. The $^1H$ NMR analysis (Table 2) showed that as with **3a** the spectrum of **4** was consistent with that of its authentic 24α-epimer (dihydrospinasterol), but was markedly different from that of the 24β-epimer (dihydrochondrillasterol). The signals for protons at H-21, H-27 and H-29 were diagnostic for the two epimers, as previously shown in the literature [8, 11, 30]. Based on this, component **4** was unequivocally identified as pure 24α-ethyl-5α-cholest-7-en-3β-ol (dihydrospinasterol).

Based on the analytical data, a total of seven 4-desmethylsterols were identified in the $\Delta^7$-sterol mixture from the seeds of *C. maxima*. The absolute amounts and the per cent compositions of each of these sterols are given in Table 3. The sterol composition is more or less similar to that reported by the earlier investigators [19]. However, we found that spinasterol (**3a**) was the most abundant sterol, whereas in the earlier work dihydrospinasterol (**4**) was found to be most abundant. The C-24 configuration of these sterols from *C. maxima* showed a pattern similar to most of the other higher plant sterols, in which the 24β-ethyl stereochemistry is associated with the presence of a $\Delta^{25(27)}$-bond, while sterols lacking a $\Delta^{25(27)}$-bond have a 24α-ethyl stereochemistry [8–11]. Unlike some other Cucurbitaceae species [12, 14, 15], there was no evidence for a 24β-ethyl-sterol in which the $\Delta^{25(27)}$-

Table 1. $^1H$ NMR chemical shifts ($\delta$) of $C_{29}$-$\Delta^{7,22}$-sterols*

| Proton | Sterol **3a** (from *C. maxima* seeds) | Spinasterol† | Chondrillasterol‡ |
|---|---|---|---|
| H-18 (3H, *s*) | 0.550 | 0.552 | 0.550 |
| H-19 (3H, *s*) | 0.799 | 0.800 | 0.800 |
| H-21 (3H, *d*) | 1.027 (6.5) | 1.027 (6.5) | 1.030 (6.5) |
| H-26 or 27 (3H, *d*) | 0.799 (6.1) | 0.800 (6.2) | 0.825 (6.5) |
| H-26 or 27 (3H, *d*) | 0.850 (6.1) | 0.851 (6.1) | 0.845 (6.3) |
| H-29 (3H, *t*) | 0.805 (7.3) | 0.805 (7.3) | 0.799 (7.2) |
| H-3 (1H, *m*) | 3.598 | 3.598 | 3.597 |
| H-7 (1H, *br s*) | 5.173 | 5.172 | 5.172 |
| H-22 or 23 (1H, *dd*) | 5.027 (*ca* 7.5) | 5.027 (*ca* 7.5) | 5.029 (*ca* 7.5) |
| H-22 or 23 (1H, *dd*) | 5.163 (*ca* 7.5) | 5.162 (*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* species.

Table 2. $^1$H NMR chemical shifts ($\delta$) of $C_{29}$-$\Delta^7$-sterols*

| Proton | Sterol **4** (from *C. maxima* seeds) | Dihydrospinasterol† | Dihydrochondrillasterol‡ |
|---|---|---|---|
| H-18 (3H, *s*) | 0.536 | 0.536 | 0.535 |
| H-19 (3H, *s*) | 0.796 | 0.796 | 0.796 |
| H-21 (3H, *d*) | 0.926 (6.5) | 0.926 (6.5) | 0.931 (6.3) |
| H-26 or 27 (3H, *d*) | 0.813 (6.5) | 0.813 (6.5) | 0.812 (6.5) |
| H-26 or 27 (3H, *d*) | 0.835 (6.5) | 0.836 (6.5) | 0.832 (6.5) |
| H-29 (3H, *t*) | 0.846 (7.4) | 0.846 (7.3) | 0.855 (7.3) |
| H-3 (1H, *m*) | 3.600 | 3.601 | 3.601 |
| H-7 (1H, *br s*) | 5.166 | 5.165 | 5.167 |

*Values in parentheses are the coupling constants ($J$, in Hz).
†Isolated from *Spinacia oleracea*.
‡Isolated from *Chlorella* species.

Table 3. The $\Delta^7$-4-desmethylsterol composition of *C. maxima* seeds

| Sterol | Amount (mg/100 g of seeds) | % of total |
|---|---|---|
| 24$\beta$-Ethyl-5$\alpha$-cholesta-7,22,25(27)-trien-3$\beta$-ol (**1**) | 6.59 | 20.0 |
| 24$\beta$-Ethyl-5$\alpha$-cholesta-7,25(27)-dien-3$\beta$-ol (**2a**) | 6.96 | 21.1 |
| 24*Z*-Ethylidene-5$\alpha$-cholest-7-en-3$\beta$-ol (**2b**) (avenasterol) | 4.19 | 12.7 |
| 24$\alpha$-Ethyl-5$\alpha$-cholesta-7,22-dien-3$\beta$-ol (**3a**) (spinasterol) | 10.55 | 32.0 |
| 24$\alpha$-Ethyl-5$\alpha$-cholest-7-en-3$\beta$-ol (**4**) (dihydrospinasterol) | 4.29 | 13.0 |
| 24$\xi$-Methyl-5$\alpha$-cholest-7-en-3$\beta$-ol (**3b**) (24$\xi$-methyllathosterol) | 0.33 | 1.0 |
| 24$\beta$-Methyl-5$\alpha$-cholesta-7,25(27)-dien-3$\beta$-ol (25(27)-dehydrofungisterol) | 0.07 | 0.2 |

bond is reduced. In conclusion, the 24-ethyl-$\Delta^7$-sterols of *C. maxima* seeds belong to two separate series, viz. the 24$\beta$-ethyl series with a $\Delta^{25(27)}$-bond (**1** and **2a**), and the 24$\alpha$-ethyl series without a $\Delta^{25(27)}$-bond (**3a** and **4**). The presence of two distinct configurational series implies the existence of two biosynthetic pathways, one leading to 24$\alpha$- and the other to 24$\beta$-ethyl-sterols, respectively [11, 31, 32]. Avenasterol (**2b**) is most probably the precursor to the 24$\alpha$-ethyl-sterols, whereas 25(27)-dehydrofungisterol could be involved in a 24$\beta$-methyl pathway [11, 31].

## 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: spinasterol from Applied Science; chondrillasterol and dihydrochondrillasterol from *Chlorella* species (kindly supplied by G. W. Patterson); and dihydrospinasterol from *Spinacia oleracea*.

*Extraction, isolation and identification of sterols.* Seeds (1 Kg) were ground in a Polytron and extracted in a Soxhlet apparatus with $Me_2CO$ for 24 hr. The extracted material was evapd to near dryness and saponified in 10% KOH in 95% EtOH under reflux for 2 hr. The cooled mixture was diluted with $H_2O$ and extracted with $Et_2O$ to yield the neutral lipids. After evapn to dryness, the neutral lipids were chromatographed on alumina ($Al_2O_3$) containing 3% $H_2O$ in a system of $Et_2O$ graded into hexane. The 4-desmethylsterols eluted with $Et_2O$ were recrystallized and were further subjected to chromatography on thin layer plates (0.25 and 1.00 mm thick) coated with silica gel G. The plates were developed at least 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 6 G in $Me_2CO$. The 4-desmethylsterols were separated into two bands co-chromatographing with the standards containing authentic $\Delta^7$-(lathosterol–spinasterol mixture) and $\Delta$5-(cholesterol–sitosterol mixture) sterols, respectively. The $\Delta^7$- (slower moving) and $\Delta^5$-regions were scraped separately into glass tubes, and the sterols were extracted from the silica gel with several washes of $Et_2O$. Traces of rhodamine were removed from the $Et_2O$ solns by filtration through a thin layer of $Al_2O_3$. The sterol mixtures thus obtained were analysed by GLC and HPLC, and were finally separated into various components by prep. HPLC.

GLC was performed on 0.75% SE-30 on Gas Chrom Q in glass columns (length 1.8 m, i.d. 2 mm) on a Hewlett–Packard (HP 5840 A) gas chromatograph equipped with a flame ionization detector. Operating conditions were: carrier gas (He) flow rate 25 ml/min, injection temp. 235°, column temp. 230° isothermal and detector temp. 250°. Cholesterol was the standard for determination of the $RR_t$. The instrumentation for HPLC was a modification of the modular system assembled as described earlier [33]. In the present work, a Perkin–Elmer series-I LC pump and a Spectro-Monitor III variable wavelength UV detector were used and the column was warmed in an oven. Analytical HPLC was performed at 35° on a Zorbax ODS ($C_{18}$) column (250 × 4.6 mm) from the DuPont Company with *i*-PrOH–acetonitrile (1:4) as solvent at 1 ml/min flow rate. The column had 19 316 plates (total) and an asymmetry factor of 0.12. For prep. HPLC, a Perkin–Elmer $C_{18}$ column (250 × 22 mm) was used at 30° with *i*-PrOH–acetonitrile (1:4) as solvent at 10 ml/min flow rate. The column had 56 000 plates per m and an asymmetry factor < 1.2. Sample peaks were detected at 205 nm

and the rate of movement is described as $\alpha_c$ which is $k$ for the test sterol/$k$ for cholesterol [33]. Throughout the text of this paper, the term $RR_t$ is used for GLC and $\alpha_c$ 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.

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