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CO-OCCURRENCE OF C-24 EPIMERIC 24-ETHYL-Δ^7-STEROLS IN THE ROOTS OF *TRICHOSANTHES JAPONICA*

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Key Word Index—*Trichosanthes japonica*; Cucurbitaceae; roots; 24 - ethyl - Δ^7 - sterols; configuration at C-24; ^{13}C NMR spectroscopy; 22-dihydrospinasterol; 22-dihydrochondrillasterol; spinasterol; chondrillasterol; 24 - ethyl - 5α - cholesta - 7,24(25) - dien - 3β - ol.

Abstract—^{13}C NMR spectroscopy has demonstrated that the major components (~80%) of 24 - ethyl - 5α - cholest - 7 - en - 3β - ol and 24 - ethyl - 5α - cholesta - 7,*trans* - 22 - dien - 3β - ol isolated from the roots of *Trichosanthes japonica* are the 24α-epimers, 22-dihydrospinasterol and spinasterol, accompanied by minor amounts (~20%) of their 24β-epimers, 22-dihydrochondrillasterol and chondrillasterol, respectively. The possible biosynthetic pathway leading to these sterols is discussed. This seems to be the first instance of the detection of 22-dihydrochondrillasterol in a higher plant.

INTRODUCTION

The major sterols of the seeds of some Cucurbitaceae species are 24β-ethylsterols, 24β - ethylcholesta - 7,25(27) - dienol (**3**) [1, 2], 25(27)-dehydrochondrillasterol (**4**) [1–4] and chondrillasterol (**2b**) [5, 6], of which the last sterol is occasionally accompanied [2] or replaced [1] by its 24α-epimer, spinasterol (**2a**). Conversely, only 24α-ethylsterols, **2a** and 22-dihydrospinasterol (**1a**) are present in considerable amounts in mature tissues of Cucurbitaceae plants, as has been demonstrated with the pericarp and leaves of *Cucurbita pepo* [7]. Thus there is a sharp contrast between the C-24 configuration of the sterols in seeds and in the mature tissues of Cucurbitaceae. We have undertaken an investigation of the mature tissues of another plant in this family, the tuberous roots of *Trichosanthes japonica*, and determined the C-24 configuration of the isolated 24-ethylsterols by ^{13}C NMR spectroscopy.

Nomenclature: 22-Dihydrospinasterol (**1a**) = 24α - ethyl - 5α - cholest - 7 - en - 3β - ol; 22 - dihydrochondrillasterol (**1b**) = 24β - ethyl - 5α - cholest - 7 - en - 3β - ol; spinasterol (**2a**) = 24α - ethyl - 5α - cholesta - 7,*trans* - 22 - dien - 3β - ol; chondrillasterol (**2b**) = 24β - ethyl - 5α - cholesta - 7,*trans* - 22 - dien - 3β - ol; 24β - ethylcholesta - 7,25–27) - dienol (**3**) = 24β - ethyl - 5α - cholestra - 7,25(27) - dien - 3β - ol; 25(27) - dehydrochondrillasterol (**4**) = 24β - ethyl - 5α - cholesta - 7,*trans* - 22,25(27) - trien - 3β - ol; avenasterol (**5**) = 24 - ethyl - 5α - cholesta - 7,*trans* - 24(28) - dien - 3β - ol; 24 - ethylcholesta - 7,24(25) - dienol (**6**) = 24 - ethyl - 5α - cholesta - 7,24(25) - dien - 3β - ol; cholesterol = cholest - 5 - en - 3β - ol; citrostadienol = 4α - methyl - 24 - ethyl - 5α - cholesta - 7,*trans* - 24(28) - dien - 3β - ol; 24 - ethyl - 24(25) - dehydrolophenol = 4α - methyl - 24 - ethyl - 5α - cholesta - 7,24(25) - dien - 3β - ol.

RESULTS AND DISCUSSION

The sterol fraction that was separated from the unsaponifiable lipid of *T. japonica* root oil was acetylated, and the resulting acetate fraction (0.9 g) was separated into four bands (referred to as Bands 1–4 in order of polarity, beginning with the least polar) by argentation TLC. Bulky Bands 1 and 2 gave 24 - ethyl - 5α - cholest - 7 - en - 3β - yl (**1**) acetate (270 mg) and 24 - ethyl - 5α - cholesta - 7,*trans* - 22 - dien - 3β - yl (**2**) acetate (150 mg), respectively. Faint Band 3 (21 mg) contained an unknown steryl acetate (RR_t 2.31) accompanied by several minor components. The mass spectrum of this steryl acetate showed that it was an acetate of a C_{29}-sterol with two double bonds (*m/z* 454, M^+, $C_{31}H_{50}O_2$). The fragment ion at *m/z* 313 (base peak, $M^+ - C_{10}H_{19}[SC] - 2H$) showed the presence of a C_{10} side chain with one double bond, and the ion indicated moreover that the other double bond was probably located at C-7 since it is characteristic for the sterols with the Δ^7-bond in addition to a double bond in the side chain [8, 9]. A prominent ion at *m/z* 356 ($M^+ - C_7H_{14}$), which might arise as a result of McLafferty rearrangement, showed that the side chain double bond was located either at the 24(28)- or at the 24(25)-position [8]. The following GLC correlation provided evidence for the 24(25)-position of the double bond. The separation factor between the steryl acetate vs avenasteryl (**5**) acetate was calculated as 1.07, which was consistent with the 24 - ethyl - $\Delta^{24(25)}$/24 - ethyl - *trans* - $\Delta^{24(28)}$ side chain separation factor calculated from the retention data of the acetates of 24 - ethyl - 24(25) - dehydrolophenol (RR_t 2.60) vs citrostadienol (RR_t 2.41). Hence the sterol was considered to have the structure 24 - ethylcholesta - 7,24(25) - dien - 3β - ol (**6**). Only the mature tissue of *C. pepo* [7] and the seeds of *Helianthus annuus* [10] have so far been shown to contain this sterol. The most polar Band 4 (39 mg) contained the acetates of 24 - ethylcholesta -

7,25(27) - dienol (**3**) and **5** accompanied by other minor components.

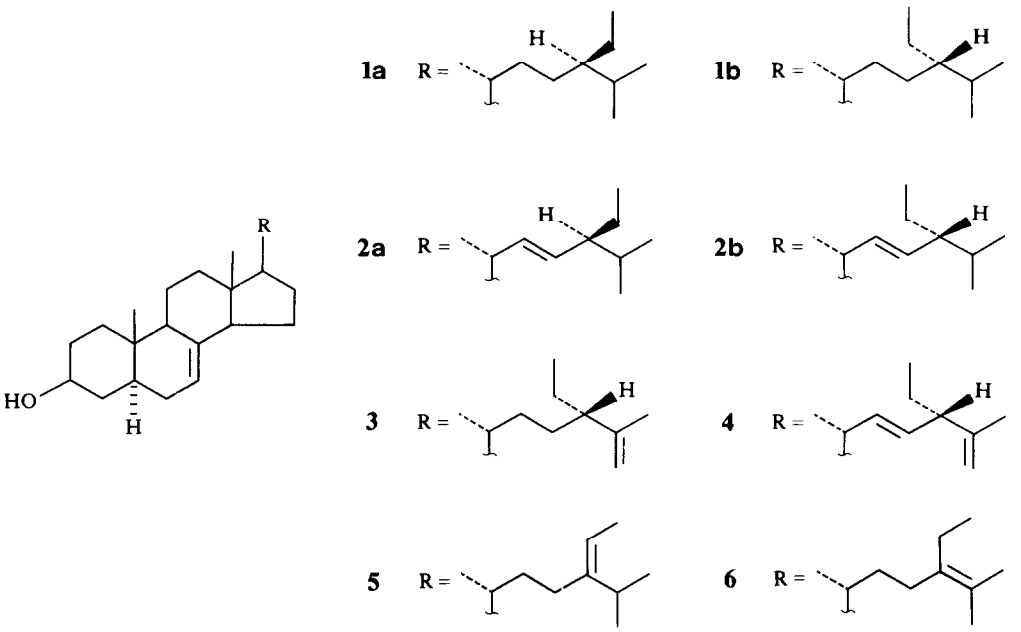
In order to determine the configuration at C-24, the above two steryl acetates, **1**- and **2**-acetates, were submitted to ^{13}C NMR spectroscopy. Table 1 shows the ^{13}C NMR spectra of these steryl acetates, in which the signals were assigned by direct comparison with the published data of the acetates of **1a**, 22-dihydrochondrillasterol (**1b**), **2a** and **2b** [2]. In the spectra of both the acetates of **1** and **2**, the signals arising from the 24α-epimers, **1a** and **2a**, predominated, but the weak resonances due to their 24β-counterparts, **1b** and **2b**, were also observed. The approximate ratio of the 24α- and 24β-epimers was estimated to be 5:1 for **1** and 4:1 for **2** from the relative intensities of the signals by which the epimers were distinguished from each other.

T. japonica roots thus contain the 24α-ethylsterols, **1a** and **2a**, as the major sterol constituents. This is consistent with the observation in the mature tissue, the pericarp and leaves of *C. pepo* [7], and again indicated the existence of a marked ontogenetic change in the sterol composition in Cucurbitaceae, since the seeds are known to contain 24β-ethylsterols as the major sterols [1–4]. However, our study also demonstrated the occurrence of the two 24β-ethylsterols, **1b** and **2b**, though in minor amounts, accompanied by much smaller quantities of **3**, **5** and **6**, in the roots. Although no experimental proof was available, the 24β-ethyl configuration is highly probable for the 24 - ethyl - $\Delta^{7,25(27)}$ - sterol, **3**, from biosynthetic considerations of 24-alkylsterols [7, 11].

Two pathways have been proposed for the biosynthesis of 24-ethylsterols from 24-methylenesterols in tracheophytes [7]. One of them yields the 24α-ethyl end-product through a $\Delta^{24(25)}$-sterol, i.e. **5**→**6**→**1a**→**2a**, and the other one gives the 24β - ethyl - Δ^{22} - sterol through a $\Delta^{25(27)}$-sterol, i.e. **3**→**4**→**2b**. Although *T. japonica* roots did not contain **4** in detectable amount, the co-occurrence of the 24α-ethylsterols, **1a**

Table 1. ^{13}C NMR chemical shifts (δ) of the acetates of 24 - ethyl - Δ^7 - sterols isolated from *Trichosanthes japonica*

Carbon	24-Ethyl-5α-cholest-7-en-3β-yl (**1**) acetate		24-Ethyl-5α-cholesta-7,*trans* 22-dien-3β-yl (**2**) acetate	
	1a	**1b**	**2a**	**2b**
C-1	36.8		36.8	
C-2	27.4		27.4	
C-3	73.4		73.4	
C-4	33.8		33.8	
C-5	40.0		40.0	
C-6	29.5		29.5	
C-7	117.2		117.3	
C-8	139.5		139.4	
C-9	49.2		49.2	
C-10	34.2		34.2	
C-11	21.4		21.4	
C-12	39.5		39.5	
C-13	43.3		43.2	
C-14	54.9		55.0	
C-15	23.0		23.0	
C-16	27.9		28.5	28.4
C-17	56.0	55.8	55.8	
C-18	11.8		12.0	
C-19	12.9		12.9	
*Me*CO	21.4		21.4	
Me*C*O	170.6		170.6	
C-20	36.6	36.4	40.8	
C-21	18.9		21.1	20.9
C-22	33.8		138.1	
C-23	26.1	26.4	129.4	
C-24	45.8	46.0	51.2	
C-25	29.1	28.9	31.8	
C-26	19.8	19.0	21.4	19.0
C-27	19.0	19.6	19.0	21.4
C-28	23.0		25.4	
C-29	11.9	12.3	12.2	12.4
Approximate ratio	5 :	1	4 :	1



and **2a**, and the 24β-ethylsterol, **2b**, along with **3** (probably having a 24β-ethyl configuration), **5** and **6**, may reflect the co-existence of the two pathways of 24-ethylsterol biosynthesis and may operate in the roots, as also has been proposed recently for the seeds of other Cucurbitaceae, *Lagenaria leucantha* var. *gourda* and *Citrullus battich* [2]. However, the existence of a further 24β-ethylsterol, **1b**, together with the apparent absence of **4**, may suggest the presence of an alternative pathway to the 24β - ethyl - Δ^{22} - sterol in the roots, i.e. **3**→**1b**→**2b**, which is one of the possible sequences to 24β-ethylsterols in algae [12]. Sterol **1b** has so far been detected only in green algae [13–15].

EXPERIMENTAL

Recrystallizations were performed in Me_2CO–MeOH. Mps were taken on a heating block and are uncorr. IR spectra were recorded in KBr. MS (70 eV, > *m/z* 200) were taken with a GC/MS (2% OV-17, 2 m × 3 mm glass column). ^{13}C NMR spectra were recorded on a JNM FX-100 spectrometer operating at 25.05 MHz, for 0.2 M solns in $CDCl_3$. The chemical shifts (δ) are expressed in ppm relative to TMS and are estimated to be accurate ± 0.05 ppm. The probe temp. was *ca* 30°. FT NMR measurement conditions were as follows: spectral width: 5 kHz, pulse width: 6 μsec, acquisition time: 2.5 sec, and a number of data points: 8192. GLC was carried out on an OV-17 SCOT glass capillary column (30 m × 0.3 mm, 260°, split ratio 100:1) and RR_t is given relative to cholesteryl acetate. For TLC, $AgNO_3$–Si gel (1:4) plates (0.5 mm) were developed 4× with CH_2Cl_2–CCl_4 (1:5). Other techniques used in this study have been described previously [16]. The tuberous roots of *T. japonica* were collected at Fukushima (Japan). The acetates of 22-dihydrospinasterol (**1a**), 22-dihydrochondrillasterol (**1b**), spinasterol (**2a**), 24β - ethylcholesta - 7,25(27) - dienol (**3**), 25(27)-dehydrochondrillasterol (**4**), and a mixture of **2a** and chondrillasterol (**2b**) [2], avenasterol (**5**) [16], citrostadienol and 24 - ethyl - 24(25) - dehydrolophenol [17] were used as reference samples. The R_f on argentation TLC, the RR_t on GLC and the MS of the acetates of **1**, **2**, **3** and **5** described below were consistent with those of the authentic steryl acetates.

Sliced tuberous roots (10 kg) were dried, and after grinding, the roots (3.2 kg) were extracted in a Soxhlet apparatus with CH_2Cl_2 as solvent to give an oil (34 g). The unsaponifiable lipid (6.7 g) obtained from the oil by saponification, after Si gel TLC, yielded a sterol fraction (1 g). After acetylation, the acetate sterol fraction was separated into 4 bands by argentation TLC. The fraction from Band 1 (R_f 0.61) gave **1**-acetate (RR_t 1.94) after purification by repeated argentation TLC, mp 159–162°. IR ν_{max} cm^{-1}: 1725, 1240 (OAc), 840, 822, 795 (>C=CH–). MS *m/z* (rel. int.): 456 [M^+] (100), 441 (22), 396 (18), 381 (16), 315 (10), 288 (10), 273 (14), 255 (79), 229 (30), 213 (38). The ^{13}C NMR spectrum revealed that the steryl acetate was a C-24 epimer mixture of **1a**- and **1b**-acetates in the approximate ratio 5:1. The fraction from Band 2 (R_f 0.42) yielded **2**-acetate (RR_f 1.70) after purification by repeated argentation TLC, mp 182–184°. IR ν_{max} cm^{-1}: 1735, 1245 (OAc), 970 (*trans* –CH=CH–), 842, 825, 795 (> C=CH–). MS *m/z* (rel. int.): 454 $[M]^+$ (36), 439 (15), 411 (21), 379 (6), 351 (15), 342 (19), 315 (23), 313 (100), 288 (20), 273 (10), 255 (62), 229 (22), 213 (20). The **2**-acetate was indicated to be a mixture of **2a**- and **2b**-acetates in the approximate ratio 4:1 by ^{13}C NMR spectroscopy. Band 3 (R_f 0.23) gave 24 - ethylcholesta - 7,24(25) - dienyl (**6**) acetate (RR_t 2.31) accompanied by several unidentified components. The MS of **6**-acetate, *m/z* (rel. int.): 454 $[M]^+$ (4), 439 (7), 394 (2), 379 (4), 356 (33), 341 (6), 313 (100), 296 (6), 288 (7), 281 (6), 273 (5), 255 (13), 253 (13), 229 (6), 228 (9), 227 (8), 213 (19). The most polar Band 4 (R_f 0.08) afforded the acetates of **3** (RR_t 1.95) and **5** (RR_t 2.15) accompanied by several unidentified components. The MS of **3**-acetate, *m/z* (rel. int.): 454 $[M]^+$ (39), 439 (42), 394 (38), 379 (35), 343 (9), 341 (9), 313 (100), 283 (20), 273 (21), 255 (70), 253 (43), 229 (27), 227 (32), 213 (62). The MS of **5**-acetate, *m/z* (rel. int.): 454 $[M]^+$ (3), 439 (5), 379 (6), 356 (38), 341 (7), 327 (4), 313 (100), 296 (21), 288 (9), 281 (11), 273 (6), 255 (19), 253 (41), 228 (13), 227 (13), 213 (27), 201 (8). The presence of **4**-acetate (RR_t 1.80), which would occur in this fraction, has not been confirmed.

The approximate composition of the sterol fraction, as determined by combination of the data of argentation TLC, GLC and ^{13}C NMR is as follows: **1** (56%: **1a** 46%, **1b** 10%), **2** (26%: **2a** 20%, **2b** 6%), **3** (4%), **5** (2%) and **6** (1%). The other minor steryl acetates (11%) with RR_t 1.30, 1.35, 1.53, 1.56, 1.63, 1.74, 1.86 and 2.06 remain to be identified.

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