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CO-OCCURRENCE OF CHONDRILLASTEROL AND SPINASTEROL IN TWO CUCURBITACEAE SEEDS AS SHOWN BY ^{13}C NMR

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Key Word Index—*Lagenaria leucantha* var. *gourda*; *Citrullus battich*; Cucurbitaceae; seeds; configuration at C-24; ^{13}C NMR spectroscopy; chondrillasterol; spinasterol; 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol; 24β-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-ol.

Abstract—^{13}C NMR spectroscopy of the sterols isolated from seeds of bottle gourd (*Lagenaria leucantha* var. *gourda*) and water melon (*Citrullus battich*) has demonstrated the co-occurrence of the C-24 epimers spinasterol and chondrillasterol.

24-Ethyl-5α-cholesta-7,*trans*-22-dien-3β-ol (**1**), 24-ethyl-5α-cholesta-7,25(27)-dien-3β-ol (**2**) and 24-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-ol (**3**) are the major sterols in the seeds of some Curcurbitaceae [1–6]. The configuration at C-24 of **2** and **3** isolated from the seeds of pumpkin (*Cucurbita pepo*) was established as 24β by 270 MHz ^1H NMR spectroscopy [7] and further by stereospecific synthesis of the two C-24 epimers of **3** [8, 9], whereas **1** isolated from the seeds was shown to be the 24α-epimer, i.e. spinasterol (**1b**, 24α-ethyl-5α-cholesta-7,*trans*-22-dien-3β-ol), by ^1H NMR spectroscopy [7]. On the other hand, **1** isolated from the seeds of the other two Cucurbitaceae, gourd (*Lagenaria siceraria*) and sponge cucumber (*Luffa cylindrica*), was quite recently indicated to be chondrillasterol (**1a**, 24β-ethyl-5α-cholesta-7,*trans*-22-dien-3β-ol) by 270 MHz ^1H NMR spectroscopy [10] and by ^{13}C NMR spectroscopy in the case of the gourd sterol [11]. In this study, the configurations at C-24 of the sterols, **1**, **2** and **3**, isolated from the seeds of a further two species of Cucurbitaceae, bottle gourd (*Lagenaria leucantha* var. *gourda*) and water melon (*Citrullus battich*), were determined and compound **1** from both the plants was shown to be a mixture of **1a** and **1b**, whereas **2** and **3** from both the plants were, as demonstrated with the pumpkin seeds [7–9], the 24β-ethyl epimers, i.e. 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol and 24β-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-ol, respectively.

Table 1 shows the ^{13}C NMR spectra of the acetates of **1**, **1b**, **2**, **3**, **4a** (22-dihydrochondrillasterol, 24β-ethyl-5α-cholest-7-en-3β-ol) and **4b** (22-dihydrospinasterol, 24α-ethyl-5α-cholest-7-en-3β-ol). Signals arising from the ring system carbons, C-1 to C-19, were assigned by

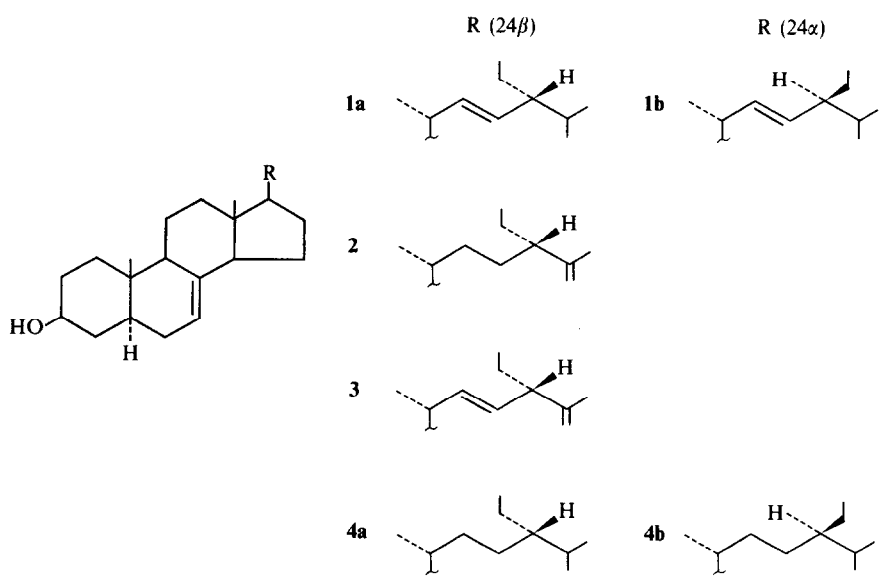

Table 1. ^{13}C NMR chemical shifts (δ) of 24-ethyl-Δ^7-steryl acetates

Carbon No.	Steryl acetates					
	1* (1a)	(1b)	1b†	2*	3*	4a‡	4b§
C-1	36.8		36.9	36.8	36.8	36.8	36.8
C-2	27.5		27.5	27.5	27.5	27.5	27.5
C-3	73.5		73.4	73.4	73.4	73.5	73.5
C-4	33.8		33.8	33.8	33.8	33.8	33.8
C-5	40.1		40.0	40.1	40.1	40.1	40.1
C-6	29.5		29.5	29.5	29.5	29.5	29.5
C-7	117.3		117.3	117.3	117.3	117.2	117.2
C-8	139.5		139.4	139.5	139.4	139.5	139.5
C-9	49.3		49.2	49.3	49.3	49.3	49.3
C-10	34.2		34.2	34.2	34.2	34.2	34.2
C-11	21.5		21.5	21.4	21.4	21.5	21.5
C-12	39.4		39.3	39.5	39.4	39.5	39.5
C-13	43.3		43.2	43.3	43.3	43.3	43.4
C-14	55.1		55.1	55.0	55.1	55.0	55.0
C-15	23.0		23.0	23.0	23.0	23.0	23.0
C-16	28.4	28.5	28.5	27.9	28.3	27.9	28.0
C-17	55.9		55.9	56.0	55.8	56.0	56.1
C-18	12.1		12.1	12.1	12.1	11.9	11.9
C-19	13.0		12.9	13.0	13.0	13.0	13.0
$\underline{C}H_3CO$	21.5		21.5	21.4	21.4	21.5	21.5
$MeC\underline{O}$	170.7		170.6	170.5	170.6	170.7	170.7
C-20	40.8		40.8	36.0	40.5	36.3	36.6
C-21	21.0	21.1	21.1	18.8	21.0	18.9	18.9
C-22	138.1		138.1	33.6	137.0	33.9	33.8
C-23	129.4		129.4	29.5	130.2	26.5	26.2
C-24	51.2		51.2	49.5	52.0	46.0	45.9
C-25	31.9		31.8	147.4	148.5	28.9	29.1
C-26	19.0	21.5	21.5	17.7	20.2	19.0	19.8
C-27	21.5	19.0	19.0	111.4	109.6	19.6	19.1
C-28	25.4		25.4	26.5	25.7	23.0	23.0
C-29	12.5	12.3	12.3	11.8	12.2	12.3	12.3

* Isolated from bottle gourd and water melon seeds. † Isolated from spinach seeds. ‡ Prepared from **2** and **3** by hydrogenation. § Prepared from **1b** by hydrogenation.

comparison with those of the literature data for 5α-cholest-7-en-3β-ol acetate [12]. Side-chain signals of **1** and **4** acetates were assigned by comparison and correlation with the published data on related sterols [13]. Moreover, off-resonance decoupling experiments and the comparison with the model olefin data [14] enabled the side-chain signals to be assigned. The 25(27) designation rather than 25(26) for the Δ^{25}-double bond in **2** and **3** was made in this study according to the consideration proposed by Nes *et al.* [15].

In the ^{13}C NMR spectrum of **1** acetate of bottle gourd seeds, the signals for C-16, C-21 and C-29 appeared as pairs with components in the approximate ratio 1:1. The chemical shifts of one counterpart agreed with those of **1b** acetate of spinach seeds, whereas the remaining ones coincided with the reported data of **1a** [11]. Therefore **1** of bottle gourd seeds was regarded as a mixture of **1a** and **1b** in approximately equal proportions. Both **2** and **3** acetates of the seeds afforded 24-ethyl-Δ^7-monoene steryl acetate upon hydrogenation. The chemical shifts of the side-chain signals for the steryl acetate were in accordance with those of a 24β-ethyl sterol [13], but most of which differed slightly from those of **4b** acetate, obtained from **1b** acetate by hydrogenation. Thus the sterol was considered to be a 24β-ethyl sterol, i.e. **4a**, and consequently both **2** and **3** of bottle gourd seeds were established to have the 24β configuration. The ^{13}C NMR evidence further showed that **1** from water melon seeds was also a mixture of **1a** and **1b** in almost equal proportions, whereas both **2** and **3** from the seeds had the 24β configuration.

The co-occurrence of chondrillasterol (**1a**) and spinasterol (**1b**) together with the two 24β-ethyl-$\Delta^{25(27)}$-sterols, **2** and **3**, in the seeds of bottle gourd and water melon may reflect the co-existence of two pathways of sterol biosynthesis, a 24β-pathway to **1a** and a 24α-pathway to **1b**, which have already been discussed [15] and as shown in Scheme 1 may operate in the two Cucurbitaceae seeds. Though only one of the C-24

epimers of **1** has been detected in the seeds of pumpkin [7], and gourd and sponge cucumber [10, 11], the possibility exists for the presence of the other epimer as a minor companion, and the occurrence of **1** as a C-24 epimeric mixture in other Cucurbitaceae seeds is highly probable.

EXPERIMENTAL

Recrystallizations were performed in Me_2CO–MeOH. Mps were taken on a heat block and are uncorr. ^{13}C FT NMR spectra were recorded on a JNM FX-100 spectrometer operating at 25.05 MHz using 0.15 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 k Hz, pulse width: 6 μsec, acquisition time: 2.5 sec, and a number of data points: 8192. MS (70 eV, > *m/e* 200) were taken with a direct inlet system. GLC on an OV-17 SCOT glass capillary column was under the conditions already described [16] and the RR_t was given relative to cholesterol acetate.

The seeds of bottle gourd (*Lagenaria leucantha* var. *gourda*) and water melon (*Citrullus battich*) were courteously supplied by Sakata Seeds Co., Yokohama, and the seeds of spinach (*Spinacia oleracea*) were purchased locally. Sterols **1**, **2** and **3** from the two Cucurbitaceae seeds and **1b** from spinach seeds were isolated as described previously [3, 17]. Hydrogenation of **1b**, **2** and **3** acetates in Et_2O at room temp. was carried out over PtO_2 and the residue chromatographed on $AgNO_3$–Si gel to remove the slightly less polar $\Delta^{8(14)}$-compound, an isomerization by-product. Each of the acetylated sterols showed a single peak on GLC and the ^{13}C NMR spectra recorded for the sterol acetates described below are shown in Table 1.

Sterols of the seeds of two Cucurbitaceae. Sterols of bottle gourd seeds: **1**-Acetate (mixture of **1a** and **1b**-acetates): mp 180–183°. RR_t 1.69, MS *m/e* (rel. int.): 454 (M^+, 33), 439 (14), 411 (25), 379 (5), 351 (14), 342 (21), 315 (22), 313 (100), 288 (16), 273 (10), 255 (48), 229 (22), 213 (20). **2**-Acetate: mp 155–158° (lit. [1] mp 154–157°), RR_t 1.94, MS *m/e* (rel. int.): 454 (M^+, 30), 439 (25), 394 (9), 379 (11), 341 (9), 313 (100), 299 (7), 288 (11), 273 (8), 255 (30), 229 (11), 228 (9), 227 (10), 213 (22). **3**-Acetate: mp 175–178° (lit. [1] mp 166–171°), RR_t 1.80, MS *m/e* (rel. int.): 452 (M^+, 20), 437 (7), 423 (9), 392 (7), 363 (8), 342 (20), 313 (100), 299 (8), 288 (6), 255 (48), 229 (17), 227 (10), 213 (19). **4a**-Acetate prepared from **2**-acetate by hydrogenation: mp 165–168° (lit. [7] mp 166–167°), RR_t 1.92, MS *m/e* (rel. int.): 456 (M^+, 100), 441 (22), 396 (18), 381 (16), 315 (10), 288 (10), 273 (14), 255 (79), 229 (30), 213 (38). Hydrogenation of **3**-acetate also gave **4a**-acetate, the mp, RR_t and MS data of which were in fair agreement with those recorded for **4a**-acetate obtained from **2**-acetate by hydrogenation. Sterols of water melon seeds: acetates of **1**, **2** and **3** of water melon seeds, and **4a**-acetate prepared from **2** and **3**-acetates by hydrogenation showed the identical mp, RR_t and MS data with those of the corresponding steryl acetates of bottle gourd seeds, respectively.

Sterols of spinach seeds. **1b**-Acetate, the 24α configuration of which, isolated from spinach, has already been established [15, 18]: mp 180–184° (lit. [7] mp 187–189, [17] mp 182–183°). **4b**-Acetate obtained from **1b**-acetate by hydrogenation: mp 160–162° (lit. [7] mp 166–167°, [17] mp 158.5–159.5°). The RR_t and MS data of **1b** and **4b**-acetates were indistinguishable from those of **1** and **4a**-acetates respectively, described above.

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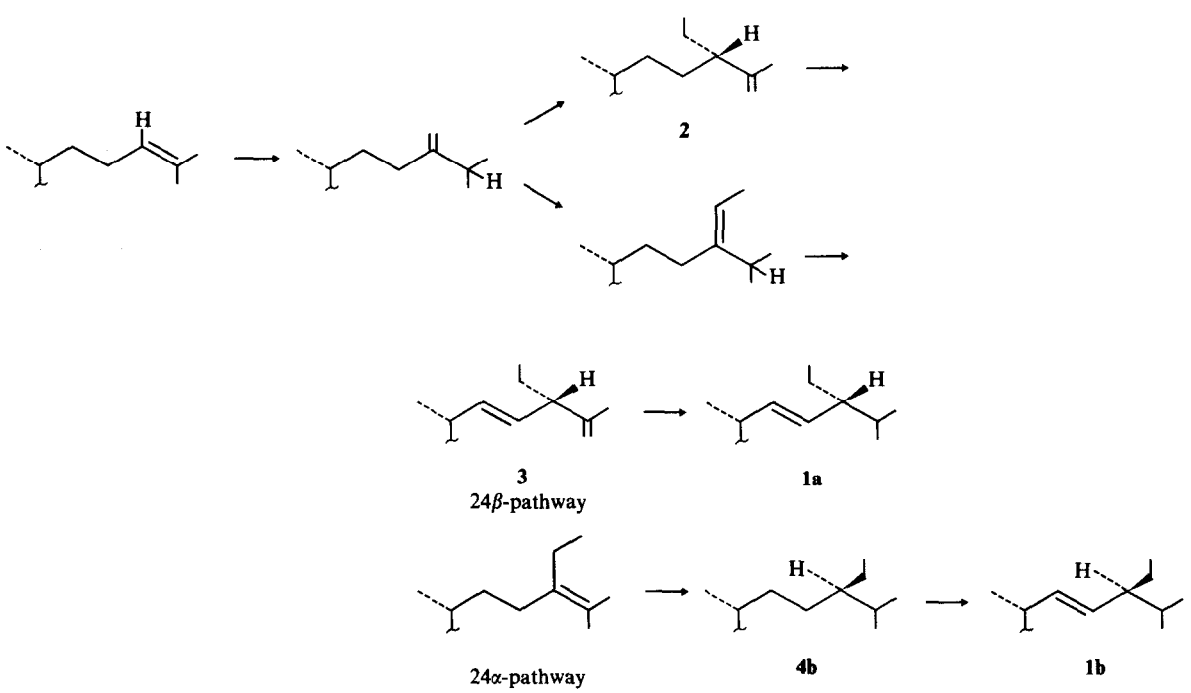

Scheme 1. Possible pathway to chondrillasterol (**1a**) and spinasterol (**1b**) in Cucurbitaceae seeds (cf. ref. [15]).

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