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THE OCCURRENCE OF C_{29} STEROLS WITH DIFFERENT CONFIGURATIONS AT C-24 IN *CUCURBITA PEPO* AS SHOWN BY 270 MHz NMR

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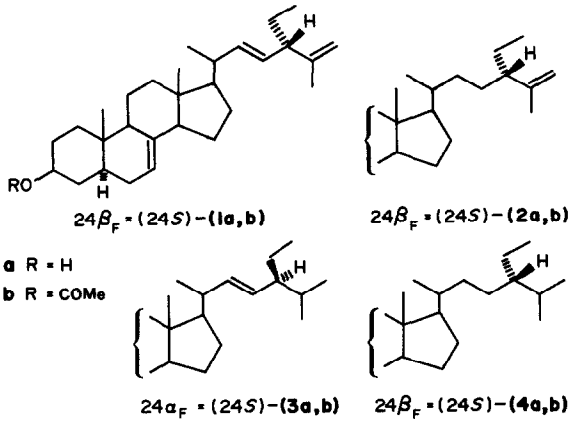
Key Word Index—*Cucurbita pepo*; Cucurbitaceae; pumpkin; configurations at C-24; 270 MHz NMR spectra; (24*S*)-24-ethyl-5α-cholesta-7,22,25-trien-3β-ol; (24*S*)-24-ethyl-5α-cholesta-7,25-dien-3β-ol; α-spinasterol.

Abstract—The 270 MHz NMR spectra of the major sterols of pumpkin seeds show that the configuration at C-24 of 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol and 24-ethyl-5α-cholesta-7,25-dien-3β-ol is $24\beta_F = (24S)$ whereas the α-spinasterol has the $24\alpha_F = (24S)$ configuration.

INTRODUCTION

The major sterols present in the seeds of *Cucurbita pepo* are 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (**1a**), 24-ethyl-5α-cholesta-7,25-dien-3β-ol (**2a**) and 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**3a**) [1–3]. A stereospecific synthesis of the two C-24 epimers of **1a** showed the pumpkin sterol to possess the $24\beta_F = (24S)$ configuration‡ [4,5], unusual in higher plants [6].

The synthesis of **2a** was not stereospecific [7] and **3a** was referred to as α-spinasterol at the time for convenience even though its configuration at C-24 was not known. It was also shown that **1a** and **2a** are biochemically closely related whereas **3a** seemed to lie on a different biosynthetic pathway [8].



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‡ Throughout this paper we use the IUPAC recommended (*R*),(*S*) nomenclature. For a better understanding, however, we give in addition at some important points the more lucid α_F,β_F nomenclature.

Differentiation of 24-ethylcholesterol derivatives which are epimeric at C-24 is difficult by determination of their melting points, optical rotations or chromatographic behavior [10]. It has been recently shown, however, that this differentiation is possible by high field NMR spectroscopy [11–14]. We therefore used this method to determine the configurations of the pumpkin seed sterols.

RESULTS AND DISCUSSION

Sterol preparations

The acetates of the (24*R*)-and (24*S*)-epimers of **1** were synthesized [4,5] and selectively hydrogenated over a soluble rhodium catalyst to yield α-spinasteryl (24*S*)-(**3b**) and chondrillasteryl (24*R*)-(**3b**) acetates respectively. The latter, in turn, was then hydrogenated over Raney nickel [9] to give (24*S*)-(**4b**). This, together with its $24\alpha_F$-epimer schottenyl acetate (24*R*)-(**4b**) [9], was now compared to the product obtained by the hydrogenation of the C-25 double bond of the acetate of naturally occurring **2a**.

3β-Acetoxy-24-ethyl-5α-cholesta-7,22,25-trienes (**1b**)

Slight differences in the shifts of the C-21 and C-29 methyl groups between the (24*R*)- and (24*S*)-epimers of **1b** had already been observed in their 100 MHz spectra [4]. These differences can be seen more readily in the 270 MHz spectra of the two compounds (Table 1). The difference of 0.01 ppm between the two methyl resonances appears as 2.7 mm on the chart of the 270 MHz spectrum and is threfore quite readily observed. The difference between the two epimers is even greater in the olefinic region. The distance between the C-22 and C-23 proton signals is 0.03 ppm (5.4 mm on the chart) greater for the (24*R*)- than for the (24*S*)-epimer.

The spectra also showed that the synthetic (24*R*)- and (24*S*)-(**1b**) were each free of the other epimer. As expected from previous evidence [4,5], the spectrum of the acetate

Table 1. 270 MHz NMR data* of (24*R*)- and (24*S*)-3β-acetoxy-24-ethyl-5α-cholesta-7,22,25-trienes

	C-18	C-19	C-29	C-21	C-27	C-23	C-22
3β-Acetoxy-24-ethyl-5α-cholesta-7,22,25-triene							
24α_F = (24*R*)-(**1b**)	*s* 0.54	*s* 0.81	*t* 0.83 (8.0)	*d* 1.03 (6.8)	*s* 1.65 (broad)	*dd* 5.16 (7.5 and 15.5)	*dd* 5.27 (8.0 and 15.5)
24β_F = (24*S*)-(**1b**)	*s* 0.54	*s* 0.81	*t* 0.84 (7.5)	*d* 1.02 (6.7)	*s* 1.65 (broad)	*dd* 5.18 (*ca* 7 and 15.7)	*dd* 5.26 (*ca* 8 and 15.7)

* Chemical shifts in δ (ppm) in $CDCl_3$ with TMS as an internal standard; *s* = singlet, *d* = doublet, *dd* = double doublet, *t* = triplet, the figures in parentheses give the coupling constants, *J*, in Hz.

Table 2. 270 MHz NMR data* of α-spinasteryl and chondrillasteryl acetates

	C-18	C-19	C-29	C-26, C-27	C-21
α-Spinasteryl acetate					
24α_F = (24*S*)-(**3b**)	*s* 0.55	*s* 0.81	*t* 0.81 (6.5)	*d* 0.80, *d* 0.85 (*ca* 7, 6.5)	*d* 1.03 (6.6)
Chondrillasteryl acetate					
24β_F = (24*R*)-(**3b**)	*s* 0.54	*s* 0.81	*t* 0.81 (7.2)	*d* 0.79, *d* 0.84 (*ca* 7, 6.5)	*d* 1.03 (6.5)

* For conditions and signs see Table 1.

Table 3. Melting points and optical rotations of α-spinasterol and chondrillasterol and their acetates

	from *Cucurbita pepo*		lit. [15–17]		synthetic	
	mp	$[\alpha]_D$	mp	$[\alpha]_D$	mp	$[\alpha]_D$
α-Spinasterol						
24α_F = (24*S*)-(**3a**)	171–173°	−2.7°	172–175°	−3°	172–173°	−2.5°
-acetate (24*S*)-(**3b**)	186–187°	−4.8°	185–187°	−5°	187–189°	−5.2°
Chondrillasterol						
24β_F = (24*R*)-(**3a**)			168–169°	−2°	172–174°	−2.0°
-acetate (24*R*)-(**3b**)			175–176°	−1.0°	182–184°	−4.5°

Table 4. 270 MHz NMR data* of schottenyl and dihydrochondrillasteryl acetates

	C-18	C-19	C-26, C-27	C-29	C-21
Schottenyl acetate					
24α_F = (24*R*)-(**4b**)	*s* 0.53	*s* 0.81	*d* 0.81, *d* 0.84 (7.1, 6.6)	*t* 0.85 (6.5)	*d* 0.92 (6.1)
Dihydrochondrillasteryl acetate 24β_F = (24*S*)-(**4b**)	*s* 0.53	*s* 0.81	*d* 0.81, *d* 0.83 (6.8, 7.0)	*t* 0.85 (7.4)	*d* 0.93 (6.2)

* For conditions and signs see Table 1.

Table 5. Melting points and optical rotations of schottenol, dihydrochondrillasterol and their acetates

	free alcohols		acetates	
	mp	$[\alpha]_D$	mp	$[\alpha]_D$
Schottenol [1,9]	151–152°	8.0°	160°	7.4°
Dihydrochondrillasterol				
from (24*R*)-(**3**)	151–152°	5.8°	166–168°	5.5°
from (**2**)	152–153°	5.8°	166–167°	5.6°

of the naturally occurring triene (**1b**) was identical to that of the synthetic 24β_F = (24*S*)-(**1b**).

3β-Acetoxy-24-ethyl-5α-cholesta-7,22-dienes (**3b**)

α-Spinasteryl acetate 24α_F = (24*S*)-(**3b**) prepared from stigmasterol [9] and the α-spinasteryl acetate from *Cucurbita pepo* seeds had identical 270 MHz NMR spectra. As with the $\Delta^{5,22}$ epimers, stigmasteryl acetate and poriferasteryl acetate [13], the NMR spectra of the $\Delta^{7,22}$ epimers α-spinasteryl acetate and chondrillasteryl acetate 24β_F = (24*R*)-(**3b**) show slight differences in the positions of the C-26 and C-27 doublets (Table 2). When compared to the invariant C-29 triplets, these give rise to significantly different peak patterns.

In contrast to the NMR spectra, the melting points and optical rotations of α-spinasterol and chondrillasterol and their acetates are not significantly different (Table 3). The reported data for chondrillasteryl acetate [15] should be revised.

3β-Acetoxy-24-ethyl-5α-cholest-7-enes (**4b**)

The differences that can be seen in the 270 MHz NMR spectra of schottenyl acetate (24*R*)-(**4b**) and its epimer (24*S*)-(**4b**) are similar to those observed in the spectra of sitosteryl and clionasteryl acetates [13,14]. In (24*S*)-(**4b**) the C-21 doublet is 0.01 ppm downfield and one of the C-26,C-27 doublets is 0.01 ppm upfield from those of the (24*R*)-(**4b**) epimer and the C-29 coupling constant is larger in the former. In addition, the coupling constants of the C-26,C-27 doublets differ in the two epimers (Table 4) and this, in conjunction with the above observations, gives significantly different patterns in the Me-region. The NMR spectrum of the hydrogenation product (**4b**) of 3β-acetoxy-24-ethyl-5α-cholesta-7,25-diene (**2b**) was identical to that of dihydrochondrillasteryl acetate 24β_F = (24*S*)-(**4b**) and different from that of schottenyl acetate 24α_F = (24*R*)-(**4b**).

The corresponding melting points and optical rotations are given in Table 5.

Conclusion

As shown by the 270 MHz NMR spectra of their acetates, 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (**1a**) and 24-ethyl-5α-cholesta-7,25-dien-3β-ol (**2a**) from *Cucurbita pepo* seeds both belong to the 24β_F series which is unusual in higher plants, whereas the third sterol from these seeds, 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**3a**), is α-spinasterol and belongs to the 24α_F series.

EXPERIMENTAL

General. The NMR spectra were recorded on a Bruker WH 270 under conditions given in the footnote of Table 1. The mps are corrected, the optical rotations were taken in $CHCl_3$ at 22° and c = 1.00. The natural sterols and their acetates were isolated according to [1]. All of the compounds gave a single peak on GLC.

α-Spinasteryl acetate (24S)-(**3b**) *from* (24R)-(**1b**). 200 mg (24*R*)-(**1b**) in 40 ml C_6H_6 and 40 ml EtOH were hydrogenated with 80 mg $(Ph_3P)_3RhCl$ for 30 min and the residue chromatographed on $AgNO_3$–Si gel. Crystals (151 mg) from heptane, data see Table 3. NMR see Table 2; in addition δ 2,03 (3H, *s*, MeCO), 4.6–4.8 (1H, *m*, C-3), 5.1–5.2 (1H, *m*, C-7). Identical in all respects with (24*S*)-(**3b**) from stigmasterol [9].

α-Spinasterol. From (24*S*)-(**3b**) by alkaline hydrolysis; crystals from heptane, data see Table 3.

Chondrillasteryl acetate from (24S)-(**1b**). 200 mg (24*S*)-(**1b**) were hydrogenated and chromatographed as above; crystals (165 mg) from heptane, data see Table 3. NMR see Table 2, in addition signals as above.

Chondrillasterol. From (24*R*)-(**3b**) by alkaline hydrolysis, data see Table 3.

(24S)-*3β-Acetoxy-24-ethyl-5α-cholest-7-ene* (24S)-(**4b**) *from* (24R)-(**3b**). 50 mg (24*R*)-(**3b**) in 25 ml EtOAc were hydrogenated for 1 day with 0.2 ml triethylamine and 1 g Raney nickel. The crude product was ozonized for 5 min in CH_2Cl_2 at 0° to destroy unreacted (*ca* 30%) chondrillasteryl acetate. Chromatography with petrol–Et_2O (99:1) on Si gel gave pure (GLC) (24*S*)-(**4b**). Crystals (18 mg) from heptane, data see Table 5. NMR see Table 4, in addition δ 2.03 (3H, *s*, MeCO), 4.6–4.8 (1H, *m*, C-3), 5.1–5.2 (1H, *m*, C-7).

(24S)-*24-Ethyl-5α-cholest-7-en-3β-ol.* From (24*S*)-(**4b**) by alkaline hydrolysis, data see Table 5.

(24S)-(**4b**) *from* (24S)-(**2b**). 150 mg natural (24*S*)-(**2b**), with some 3β-acetoxy-5α-stigmasta-7,24(28)-diene as impurity were hydrogenated in 10 ml C_6H_6 and 10 ml EtOH with 30 mg $(Ph_3P)_3RhCl$ for 1 hr. Separation from the unreacted impurity was achieved by chromatography on $AgNO_3$–Si gel with petrol–Et_2O (98:2). Crystals (50 mg) of pure (GLC) (24*S*)-(**4b**) from heptane, data see Table 5. NMR exactly as that of (24*S*)-(**4b**) from (24*R*)-(**3b**). Alkaline hydrolysis gave (24*S*)-(**4a**), identical in all respects with that above.

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