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THE 24α- AND 24β-EPIMERS OF 24-ETHYLCHOLESTA-5,22-DIEN-3β-OL IN TWO *CLERODENDRUM* SPECIES

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Key Word Index—*Clerodendrum fragrans*, *C infortunatum*, Verbenaceae, sterol, 24β-ethylcholesta-5,22*E*-dien-3β-ol, 24α-ethylcholesta-5,22*E*-dien-3β-ol, poriferasterol, stigmasterol

Abstract—^{13}C NMR spectroscopy of the 24-ethylcholesta-5,22*E*-dien-3β-ol isolated from the aerial parts of *Clerodendrum fragrans* and *C infortunatum* demonstrated the occurrence of a minor amount of the 24β-epimer, poriferasterol, in addition to the dominant 24α-epimer, stigmasterol. This was confirmed by 400 MHz ^{1}H NMR spectroscopy after enrichment of the 24β-epimer by repetitive reverse-phase HPLC. The ratios (24α·24β) of the C-24 epimeric mixtures were estimated to be 23:2 for the *C. fragrans* sterol and 19:1 for the *C. infortunatum* sterol

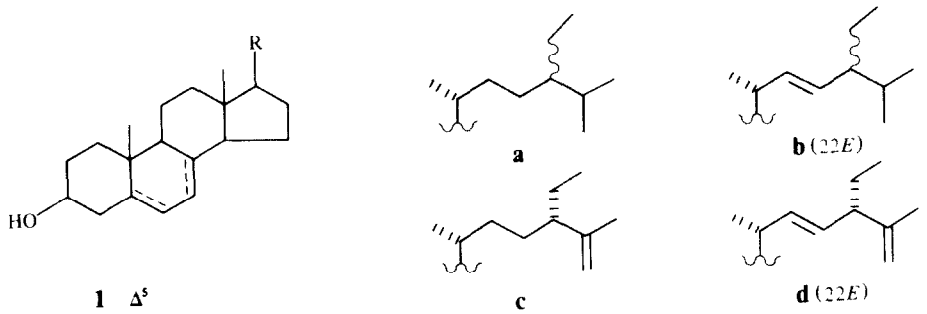
INTRODUCTION

Plant species belonging to the genus *Clerodendrum* of the family Verbenaceae are known to contain 24β-ethylsterols possessing a Δ^{25}-bond, i.e. 24β-ethylcholesta-5,25-dien-3β-ol (**1c**, clerosterol or 25-dehydrochlonasterol) and its 22*E*-dehydro derivative, 24β-ethylcholesta-5,22*E*,25-trien-3β-ol (**1d**, 22*E*-dehydroclerosterol or 25-dehydroporiferasterol), as the dominant sterols [1–8]. This is the characteristic feature for the *Clerodendrum* species since the great majority of higher plants contain 24α-alkylsterols (24*R*- if side chain saturated or Δ^{25}-unsaturated sterols, 24*S* if the Δ^{22}-derivative) which lack a Δ^{25}-bond as the major sterols such as 24α-ethylcholest-5-en-3β-ol (24α-**1a**, sitosterol) and 24α-ethylcholesta-5,22*E*-dien-3β-ol (24α-**1b**, stigmasterol) [9]. Our recent studies on the sterol constituents of two *Clerodendrum* species, *C fragrans* [10] and *C infortunatum* [11], have shown that these plants contain **1c** and **1d** as the major sterols in agreement with the previous observations on various *Clerodendrum* plants [1–8] In addition, we have demonstrated the occurrence of cholest-5-en-3β-ol (cholesterol), 24-methylcholesterol (mixture of the C-24 epimers), 24α-**1a** and 24α-**1b** as the minor sterol constituents in *C fragrans* [10] and *C infortunatum* [11], and moreover, two very rare sterols, 24β-methylcholesta-5,22*E*,25-trien-3β-ol and 24α-ethyl-5α-cholest-22*E*-en-3β-ol, in *C. fragrans* [10]. Although the stereochemistry at C-24 of the above 24-alkylsterols from the two *Clerodendrum* species [10, 11] has been determined based on the high-resolution ^{1}H NMR data (250 or 400 MHz), our attempted measurement of the ^{13}C NMR spectra for 24-ethylcholesta-5,22*E*-dien-3β-ol (**1b**) from both plants has suggested the occurrence of the 24β-epimer, poriferasterol (24β-**1b**), in addition to the dominant 24α-epimer, stigmasterol (24α-**1b**) This paper describes our demonstration of the co-occurrence of 24α- and 24β-**1b** in *C. fragrans* and *C. infortunatum*

RESULTS AND DISCUSSION

Comparison of the 400 MHz ^{1}H NMR data (Table 1) with the acetates of authentic stigmasterol (24α-**1b**) and poriferasterol (24β-**1b**) showed apparently that the acetyl derivatives of 24-ethylcholesta-5,22*E*-dien-3β-ol (**1b**) isolated from the aerial parts of *Clerodendrum fragrans* and *C. infortunatum* were the 24α-epimers in agreement with our recent observations [10, 11] However, the ^{13}C NMR spectra of the **1b**-acetates from both plants displayed the weak but distinctive signals due to the carbons C-16, C-21 and C-29 for the 24β-epimer in addition to the signals for the dominant 24α-epimer (Table 2). As is evident from the ^{13}C NMR data for the acetates of authentic 24α- and 24β-**1b**, the 24β-epimer afforded the C-16 (24β·δ28 80; 24α:δ28 91) and C-21 (24β:δ20 97; 24α.δ21.02) signals at slightly higher field, while the C-29 (24β·δ12.42; 24α δ12.25) signal was at somewhat lower field than the corresponding signal of the 24α-epimer These signals are the useful ones chosen to discriminate between the C-24 epimeric sterols possessing a 24-ethyl-Δ^{22} side chain [12–14] Thus, the ^{13}C NMR spectral data suggested the presence of the 24β-epimer in addition to the dominant 24α-epimer in **1b** isolated from *C fragrans* and *C infortunatum*. The ratios 24α·24β of the epimeric mixtures of **1b** were estimated to be 23:2 for the *C fragrans* sterol and 19 1 for the *C infortunatum* sterol based on the comparison of peak heights of the relevant signals (C-16, C-21 and C-29).

In order to confirm the co-occurrence of both C-24 epimers of **1b** from the two *Clerodendrum* plants, these were further investigated by reversephase HPLC and ^{1}H NMR spectroscopy HPLC on an Ultrasphere ODS column showed that the acetates of authentic 24α- and 24β-**1b** possess slightly different retention properties (24α-**1b**-acetate, RR_t = 1 06, 24β-**1b**-acetate, RR_t = 1 09). Taking this into account, the **1b**-acetates from *C. fragrans* and *C infortunatum* were subjected to HPLC fraction-



1 Δ^5
2 5α-H Δ^7

Table 1 ^{1}H NMR chemical shifts (δ) of 24-ethyl-$\Delta^{5,22}$-sterol (**1b**) acetates (400 MHz, $CDCl_3$, TMS as internal standard)*

	Authentic sterols		Acetate *Clerodendrum fragrans*			*C. infortunatum*		
				1b†			**1b**‡	
^{1}H species	24α-**1b**	24β-**1b**	**1b**	(24α)	(24β)	**1b**	(24α)	(24β)
18-H_3 (*s*)	0.697	0.695	0.698	0.697		0.696	0.697	
19-H_3 (*s*)	1.021	1.021	1.022	1.021		1.021	1.022	
21-H_3 (*d*)	1.021	1.025	1.022	1.024		1.021	1.025	
	(6.6)	(6.6)	(6.6)	(6.6)		(6.1)	(6.6)	
26-H_3 (*d*)	0.846	0.844	0.848	0.845		0.847	0.847	
	(6.6)	(6.0)	(6.6)	(5.5)		(6.7)	(5.5)	
27-H_3 (*d*)	0.796	0.792	0.798	0.796	0.792	0.796	0.798	0.793
	(7.1)	(6.6)	(7.1)	(7.1)	(6.6)	(6.7)	(7.1)	(6.6)
29-H_3 (*t*)	0.805	0.812	0.807	0.805	0.812	0.805	0.807	0.812
	(7.4)	(7.4)	(7.1)	(7.4)	(7.4)	(7.0)	(7.1)	(7.4)
23-H (*dd*)§	5.017	5.022	5.017	5.016	5.022	5.016	5.017	5.022
	(8.5, 15.1)	(8.8, 14.8)	(8.8, 15.4)	(8.8, 15.4)	(8.8, 15.4)	(8.5, 15.3)	(8.8, 15.4)	(8.2, 15.4)
22-H (*dd*)§	5.157	5.164	5.157	5.157	5.164	5.157	5.157	5.165
	(8.5, 15.1)	(8.5, 15.1)	(8.8, 15.4)	(8.8, 14.8)	(8.8, 15.4)	(8.6, 15.3)	(8.8, 15.4)	(8.2, 14.8)

*Figures in parentheses denote *J* values (Hz). Other signals observed for all of the **1b**-acetates were δ 2.030 (3H, *s*, 3β-OAc), 2.31 and 2.33 (each 1H and *m*, 7-H_2), 4.60 (1H, *m*, $W_{1/2}$ = 24 Hz, 3α-H), 5.37 (1H, *m*, $W_{1/2}$ = 10 Hz, 6-H).

†24β-Epimer enriched sample (3 times of the HPLC enrichment, 24α:24β = 45:55).

‡24β-Epimer enriched sample (4 times of the HPLC enrichment, 24α:24β = 49:51).

§Assignment made by 2D ^{1}H–^{1}H COSY NMR technique. Thus, the assignment of the corresponding signals made for 24-ethyl-$\Delta^{8,22}$-sterol in our recent paper (ref. [18]) must be revised.

ation. The HPLC elution peak of **1b**-acetate was divided into two portions. One was the first half (fraction A) and the other was the latter half (fraction B) of the elution peak, in which fraction B was expected to contain more 24β-epimer than in fraction A. Fraction B was subjected again to HPLC with collection of the latter half of the elution peak. After this repeated enrichment procedure by HPLC for the 24β-epimer, the **1b**-acetates from *C. fragrans* (after 3 HPLC enrichments) and *C. infortunatum* (4 times) were subjected to ^{1}H NMR spectroscopy (measurement of the ^{13}C NMR spectra was abandoned due to the minute quantities of the **1b**-acetates eventually obtained). As shown by the ^{1}H NMR data in Table 1, the **1b**-acetates which had undergone the HPLC enrichment were the mixtures of the 24α- and 24β-epimers which confirmed the presence of the 24β-**1b** (poriferasterol) in addition to the 24α-epimer (stigmasterol) in the two *Clerodendrum* plants. The 24α:24β epimeric ratios of the **1b**-acetates which had undergone HPLC enrichment were estimated to be 45:55 for *C. fragrans* sterol and 49:51 for *C. infortunatum* sterol on the basis of the peak heights of the 22-H and 23-H olefinic signals. Although the side chain methyl signals are the usual criteria for differentiating the C-24 epimers of 24-ethyl-Δ^{22}-sterol [15, 17], the 22-H and 23-H olefinic signals might be more useful for the stereochemical assignment in the ^{1}H NMR spectrum at 400 MHz since a clear difference was observed in the chemical shifts of the olefinic proton double doublets between the C-24 epimers of **1b**-acetate, i.e. 24α-epimer (authentic compound), δ 5.017 and 5.157 (each 1H), 24β-epimer, δ 5.022 and 5.164 (each 1H), which was observed also for the C-24 epimers of 24-ethyl-5α-cholesta-8,22*E*-dien-3β-ol acetate [18].

This study has demonstrated unambiguously that '24-

Table 2 ^{13}C NMR chemical shifts (δ) of 24-ethyl-$\Delta^{5,22}$-sterol (**1b**) acetates (62.9 MHz, $CDCl_3$, TMS as internal standard)

	Authentic sterols		Acetate *Clerodendrum fragrans*		*C. infortunatum*	
C	24α-**1b**	24β-**1b**	**1b*** (24α)	(24β)	**1b**† (24α)	(24β)
1	36.99	36.99	37.01		36.99	
2	27.78	27.78	27.78		27.78	
3	73.92	73.95	73.94		73.95	
4	38.12	38.12	38.12		38.12	
5	139.57	139.58	139.60		139.60	
6	122.58	122.59	122.58		122.59	
7	31.86	31.86	31.88		31.88	
8	31.86	31.86	31.88		31.88	
9	50.05	50.05	50.06		50.05	
10	36.59	36.61	36.61		36.61	
11	21.02	21.02	21.02		21.02	
12	36.99	36.99	36.93		36.99	
13	42.19	42.21	42.21		42.21	
14	56.77	56.76	56.77		56.77	
15	24.36	24.34	24.36		24.36	
16	28.91	28.80	28.89	28.78	28.91	28.81
17	55.92	55.90	55.94		55.92	
18	12.05	12.07	12.05		12.05	
19	19.31	19.31	19.31		19.31	
MeCO	21.40	21.43	21.42		21.43	
MeCO	170.37	170.40	170.42		170.46	
20	40.49	40.44	40.47		40.50	
21	21.02	20.97	21.02	20.96	21.02	20.98
22	138.26	138.21	138.25		138.26	
23	129.23	129.29	129.26		129.24	
24	51.24	51.21	51.22		51.22	
25	31.86	31.86	31.88		31.88	
26	21.23	18.94	21.22‡		21.22‡	
27	18.99	21.99	18.99‡		18.99‡	
28	25.41	25.40	25.40		25.41	
29	12.25	12.42	12.23	12.43	12.26	12.45

*24α 24β = 23.2

†24α 24β = 19.1

‡Assignment in each column interchanges as for the 24β-epimer

ethylcholesta-5,22*E*-dien-3β-ol' isolated from two *Clerodendrum* species (*C. fragrans* and *C. infortunatum*) consists of the dominant 24α-epimer, stigmasterol, together with a minor amount of the 24β-epimer, poriferasterol. The occurrence of a 24β-ethylsterol, which lacks a Δ^{25}-bond in the side chain, is very rare in higher plants. Only some plants of the family Cucurbitaceae have so far been demonstrated to contain the 24β-ethylsterols such as 24β-ethyl-5α-cholest-7-en-3β-ol (24β-**2a**, 22-dihydrochondrillasterol) and 24β-ethyl-5α-cholesta-7,22*E*-dien-3β-ol (24β-**2b**, chondrillasterol) [14, 18]. It seems probable that both the C-24 epimers of 24-ethylsterols lacking a Δ^{25}-bond (e.g., side chains **a** and **b**) will occur in other higher plants, which contain 24β-ethylsterols possessing a Δ^{25}-bond (e.g. side chains **c** and **d**) as the dominant sterol components.

EXPERIMENTAL

Mps: uncorr. HPLC was carried out on an Ultrasphere ODS 5 μ column (Altex, 25 cm × 10 mm i.d.) with MeOH as a mobile phase (flow rate, 4 ml/min) which was monitored by a RI detector. *RR*, on HPLC of the sterol acetates were expressed relative to cholesterol acetate. ^1H NMR (400 MHz) and ^{13}C NMR (62.9 MHz) spectra were determined in $CDCl_3$ with TMS as internal standard. Isolation of 24-ethylcholesta-5,22*E*-dien-3β-ol (**1b**) from the aerial parts of *Clerodendrum fragrans* (Vent.) R. Br. [10] (rel. abundance of **1b** in the sterol mixture was 6.4%) and *C. infortunatum* L. [11] (relative abundance of **1b** in the sterol mixture was 3.3%) was as described previously [10, 11]. The authentic sample of stigmasterol (24α-**1b**; acetate, mp 143.0–144.5°) was isolated from Cucurbitaceae plants [14, 18], while that of poriferasterol (24β-**1b**, acetate, mp 142.0–144.5°)

was prepared from 22*E*-dehydroclerosterol (**1d**) by partial hydrogenation over pre-reduced PtO_2 in EtOH at atmospheric pressure and temp for 6 hr followed by HPLC purification

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