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STIGMASTA-7,*E*-24(28)-DIEN-3β-OL FROM *BRYONIA DIOICA* ROOTS

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Abstract—Stigmasta-7,*E*-24(28)-dien 3β-ol was isolated from the roots of *Bryonia dioica*; it has been previously synthesised, but never found in the plant kingdom. The stereochemistry of the 24(28) double bond was unambiguously proved by high resolution (250 MHz) ^{1}H NMR.

INTRODUCTION

The roots of *Bryonia dioica* are characterised by the presence of cucurbitacins, oxygenated tetracyclic triterpenes which possess a wide range of biological activities [1] and pronounced antagonistic activity towards giberellin action [2]. The occurrence of stigmast-7-en-3β-ol [3], stigmasta-7,16-dien-3β-ol and other unidentified sterols has also been reported [4].

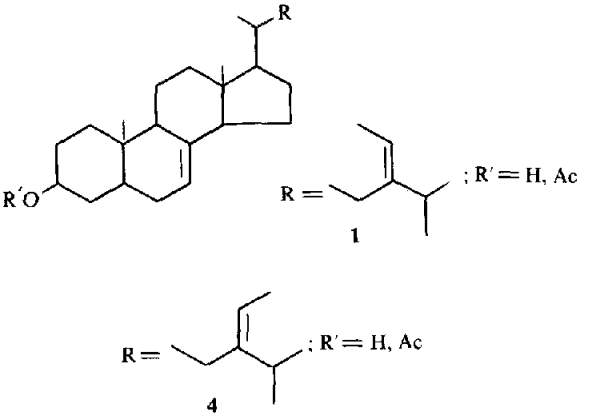
In the course of research on the structure of some cucurbitacins present in *Bryonia dioica* roots [5], we reinvestigated the sterol fraction. We now report here the isolation and the structure elucidation of stigmasta-7,*E*-24(28)-dien-3β-ol (**1**, R′ = H) which has previously been synthesised [6] and detected in small amount in the starfish *Asterias rubens* [7], but never found in the plant kingdom.

RESULTS AND DISCUSSION

Purification of sterol acetates by PLC on $AgNO_3$ impregnated silica gel (system b) enabled the mixture to be separated into three components analysed by TLC, GLC, MS, IR and high resolution (250 MHz) ^{1}H NMR. The two less polar compounds were identified as (24*R*)-24-ethyl-5α-cholest-7-en-3β-yl acetate (**2**) and (24*S*)-24-ethyl-5α-cholesta-7,22-dien-3β-yl acetate (**3**).

In the ^{1}H NMR spectra of (**2**) and (**3**) the chemical shift and the coupling constant of the C-29 methyl triplet indicated that **2** and **3** possessed the 24α_F stereochemistry* [8].

The MS fragmentation pattern of the more polar substance (**1**, R′ = Ac) indicated a C_{29} steryl acetate, having M^+ at *m/e* 454 and a mono-unsaturated side chain. The presence of a strong molecular ion and of a base peak at *m/e* 356, arising from a McLafferty rearrangement, indicated a steryl acetate with a Δ^7 double bond



and a 24-ethylidene group [9, 10]. This was confirmed by the IR band at 890 cm^{-1} (>CH=CH—CH$_3$) and by the ^{1}H NMR signals at δ 5.15 (C-7, multiplet) and 5.12 (C-28, complex quadruplet).

The steryl acetate (**1**, R′ = Ac) was allowed to react with OsO_4 in pyridine in the standard conditions and the crude reaction mixture treated with sodium periodate and reacetylated giving the 24-oxo-cholest-7-en-3β-yl acetate, also obtained from stigmasta-7,*Z*-24(28)-dien-3β-yl acetate (**4**, R′ = Ac) [11].

The *E* configuration of **1** (R′ = Ac) at the C-24(28) double bond was assigned on the basis of the ^{1}H NMR signal of the C-25 proton which appears at a significantly higher field than in **4** (R′ = Ac)† [12], in accordance with the published data for fucosterol and isofucosterol [13, 14] (Table 1).

Comparison between the ^{1}H NMR spectra of **1** (R′ = Ac) and **4** (R′ = Ac) revealed also appreciable differences in the chemical shift of the C-28 olefinic proton and C-29 methyl group. Furthermore, the C-29 methyl group coupling constant was lower for **1** (R′ = Ac) than for **4** (R′ = Ac) and this, in conjunction with the above observations, gives significantly different patterns in the methyl and olefinic region. Consequently high resolution ^{1}H NMR spectroscopy could profitably be used to distinguish between *E* and *Z* C-24(28) isomers (Table 1).

* Throughout this paper we use the IUPAC recommended (*R*), (*S*) nomenclature. For a better understanding, however, we give in addition the more lucid α_F, β_F nomenclature as recommended by other authors [8].

† Stigmasta-7,*Z*-24(28)-dien-3β-ol was isolated from *Cucurbita maxima* seedlings (unpublished results).

Table 1. 250 MHz ^{1}H NMR data* of 24-ethylidene steryl acetates

Compound	C-18	C-19	C-26,27	C-21	C-29	C-25	C-24 (28)	C-3	C-7
1 (R′ = Ac)	0.54 *s*	0.81 *s*	0.98 *d* (7)	0.99 *d* (6)	1.57 *d* (6.3)	2.20 *sp* (7)	5.17 *m*	4.69 *m*	5.14 *m*
4 (R′ = Ac)	0.54 *s*	0.81 *s*	0.98 *d* (6.9)	0.98 *d* (6.9)	1.59 *d* (7)	2.83 *sp* (7)	5.12 *m*	4.69 *m*	5.15 *m*

* Chemical shifts in δ (ppm) in $CDCl_3$ with TMS as internal standard. The figures in parentheses give the coupling constant *J* in Hz.

Sterols with a 24-ethylidene group, obtained from several sources, appear to be important intermediates in the biogenesis of C-29 phytosterols [15]. The *Z*-24-ethylidene sterols have been mainly isolated from higher plants, whereas fucosterol (stigmasta-5,*E*-24(28)-dien-3β-ol) is predominant in the marine brown algae [15].

The high percentage of stigmasta-7,*E*-24(28)-dien-3β-ol in *Bryonia dioica* roots (see Experimental) may help to clarify the central role of the 24-ethylidene sterols in the biosynthesis of the phytosterol side chain in higher plants.

EXPERIMENTAL

General. ^{1}H NMR spectra were recorded at 250 MHz in $CDCl_3$ soln, the chemical shifts are given in δ with TMS as internal standard. PLC was performed on MERK HF 254 plates (2, 0.5, 0.25 mm) with visualisation by berberine hydrochloride. For argentation TLC, plates were immersed in 10% soln of $AgNO_3$ in EtOH (3:1), dried for 12 hr and activated 30 min at 110°. After spraying with 0.1% soln of berberine hydrochloride in EtOH, the products were observed under UV (340 nm). The following solvents were used: system a, cyclohexane–EtOAc (85:15); system b, EtOH free $CHCl_3$. GLC employed a glass column (1.5m × 3mm) packed with 1% SE-30 on chromosorb G AW-DMCS; the column temp. was 270°; RR_t's are referred to cholesteryl acetate (R_t = 1). The steryl acetates were crystallised from MeOH.

Isolation of sterols. 1 kg of sliced fresh roots of *Bryonia dioica* Jacq. were refluxed with 1 l. of 80% aq. EtOH. After 20 hr the alcoholic extract was coned *in vacuo*, then extracted with petrol. This extract was saponified for 90 min in 5% KOH in MeOH. The unsaponifiable lipids (1g) were separated on PLC (system a, 2 mm) into triterpenes (15 mg), 4α-methyl sterols (3 mg) and sterols (100 mg), which were acetylated at room temp. for 14 hr using a mixture of C_5H_5N and Ac_2O. Steryl acetates were separated by argentation TLC (system b, 0.25 mm) into three compounds. *Compounds* **1** (R′ = Ac). Mp 155°; R_f 0.45 (system *b*); RR_t 1.685; relative amount (GLC) 34% of the steryl acetate mixture; MS *m/e* (rel. int.): 454 (M^+, 20), 439 (11), 356 (100), 313 (95), 255 (20), 229 (7), 213 (7); IR ν_{max}^{KBr} cm^{-1}: 800, 820, 830, 900. *Compound* **2**. Mp 152–54°; R_f 0.90 (system *b*); RR_t 1.508; relative amount (GLC) 44% of the steryl acetate mixture; MS *m/e* (rel. int.): 456 (M^+, 100), 441 (14), 396 (5), 315 (14), 273 (14), 288 (11), 255 (80), 213 (30); ^{1}H NMR: δ 0.53 (C-18, *s*), 0.81 (C-19, *s*), 0.81 (C-26, *d*, *J* = 6.8 Hz), 0.83 (C-27, *d*, *J* = 6 Hz), 0.85 (C-29, *t*, *J* = 6.5 Hz), 5.15 (C-7, *m*). *Compound* **3**. Mp 168–170°; R_f 0.86; RR_t 1.701; relative amount (GLC) 22% of the steryl acetate mixture; MS *m/e* (rel. int.): 454 (M^+, 90), 439 (14), 394 (6), 379 (8), 343 (23), 411 (24), 351 (12), 313 (100), 288 (19), 255 (46), 213 (20); ^{1}H NMR: δ 0.55 (C-18, *s*), 0.81 (C-19, *s*), 0.79 (C-26, *d*, *J* = 6.8 Hz), 0.85 (C-27, *d*, *J* = 6.6 Hz), 0.81 (C-29, *t*, *J* = 6.6 Hz), 5.15 (C-7, *m*).

24-Oxo-5α-cholest-7-en-3β-yl acetate from **1** (R′ = Ac). **1** (R′ = Ac) (40 mg) was dissolved in C_6H_6 (1 ml) and a soln of OsO_4 (34 mg) in C_6H_6 (2 ml) was added with 2 drops of Py. The soln was left in the dark for 3 days at room temp. and then EtOH (15 ml) together with a soln of Na_2SO_3 (200 mg) in H_2O (4 ml) was added. The mixture was heated at reflux for 3 hr, filtrated, extracted with $CHCl_3$ and the dried (Na_2SO_4) solvent was removed *in vacuo*. The crude diol (27 mg) was dissolved in EtOH (15 ml) and a soln of $NaIO_4$ (40 mg) in H_2O (2 ml) added. It was stirred at room temp. for 48 hr, filtered and the solvent evapd to dryness. The residue was acetylated overnight (Py, Ac_2O) and then purified by PLC (system a, 0.25 mm) to give 24-oxo-5α-cholest-7-en-3β-yl acetate (6 mg): mp 99–100°; IR ν_{max}^{KBr} cm^{-1}: 1730, 1715, 1250, 900; MS *m/e* (rel. int.): 442 (M^+, 100), 382 (78), 368 (39), 356 (13), 313 (53), 255 (62), 213 (67); ^{1}H NMR: δ 0.52 (C-18, *s*), 0.80 (C-19, *s*), 0.91 (C-21, *d*, *J* = 6 Hz), 1.09 (C-26, 27, *d*, *J* = 6.8 Hz), 2.30–2.54 (C-23, *m*), 2.61 (C-25, *septet*, *J* = 7Hz), 5.14 (C-7, *m*).

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