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Unusual 6′-fatty acid esters of (24*S*)-24-ethylcholesta-5,25-dien-3β-yl β-D-glucopyranoside from *Teucrium fruticans*

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Abstract

An unusual (24*S*)-24-ethylcholesta-5,25-dien-3β-yl β-D-glucopyranoside possessing a mixture of fatty acid esters at the C-6′ position of the sugar moiety has been isolated from *Teucrium fruticans*. Spectroscopic studies and chemical transformations allowed the characterization of the sterol and sugar parts and palmitic, stearic, oleic, linoleic and linolenic acids as the main constituents of the 6′-*O*-acyl moiety. © 1998 Elsevier Science Ltd. All rights reserved.

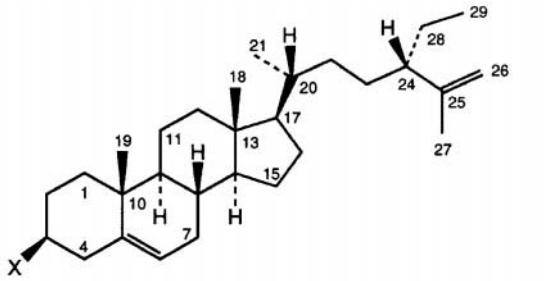
Keywords: *Teucrium fruticans*; Labiatae; Sterol glycoside; 6′-Fatty acid esters; (24*S*)-24-Ethylcholesta-5,25-dien-3β-yl 6′-*O*-acyl-β-D-glucopyranoside

1. Introduction

In previous studies, we reported the isolation of several *neo*-clerodane diterpenoids from *Teucrium fruticans* (Savona et al., 1978a,b; Bruno, de la Torre, Savona, Piozzi, & Rodríguez, 1990; Bruno et al., 1992). An investigation of the more polar chromatographic fractions of an acetone extract of the aerial parts of this plant has now allowed the isolation of a steryl glucoside esterified at the C-6′ position of the sugar part with a mixture of fatty acids. We report here the structural elucidation of this metabolite.

2. Results and discussion

The ^{1}H NMR spectrum of the natural compound (**1**, see Section 3) suggested that it was a steryl glycoside possessing fatty acid ester groups and not acetoxyl functions (absence of singlet signals between δ 2.3 and 1.8). Treatment of **1** with acetic anhydride–pyridine yielded a triacetyl derivative (**2**, singlets at δ 2.04, 2.01 and 2.00, 3H each) whose ^{1}H and ^{13}C NMR spectra, as well as COSY, TOCSY, HMQC and HMBC experiments, revealed the presence of a 2,3,4,6-tetra-*O*-acyl-β-glucopyranosyl moiety in a $^4C_1$ conformation [δ_H 4.58 d, J = 7.9 Hz (H-1′), 4.95 dd, J = 7.9, 9.5 Hz (H-2′), 5.20 t, J = 9.5 Hz (H-3′), 5.04 td, J = 9.7, 2.3 Hz (H-4′, long-range coupled with the H_B-6′ proton), 3.67 ddd, J = 9.7, 5.3, 2.5 Hz (H-5′), 4.12 dd, J = 12.1, 2.5 Hz (H_A-6′), 4.22 ddd, J = 12.1, 5.3, 2.3 (H_B-6′); δ_C 99.6 d (C-1′), 71.5 d (C-2′), 72.9 d (C-3′), 68.7 d (C-4′), 71.7 d (C-5′), 62.0 t (C-6′)] attached to the C-3β position ($\delta_{H\text{-}3\alpha}$ 3.47 m, $\delta_{C\text{-}3}$ 80.1 d) of 24-ethylcholesta-5,25-dien-3β-yl part [δ_H 5.35, 1H, br d, J = 5.4 Hz



	R^1	R^2
1	H	Fatty acid esters
2	Ac	Fatty acid esters
3	H	H
4	Ac	Ac

5 X=OAc

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(H-6), 0.66, 3H, s (Me-18), 0.98, 3H, s (Me-19), 0.89, 3H, d, $J = 6.6$ Hz (Me-21), 4.64, 1H, dq, $J_{26A,26B} = 2.5$ Hz, $J_{26A,27} = 0.7$ Hz (H_A-26), 4.72, 1H, sext, $J_{26B,26A} = 2.5$ Hz, $J_{26B,27} = J_{26B,24} = 1.3$ Hz (H_B-26), 1.56, 3H, dd, $J = 2.5$, 1.3 Hz (Me-27), 0.80, 3H, t, $J = 7.5$ Hz (Me-29); (for ^{13}C NMR data see Section 3) (Rubinstein & Goad, 1974; Gaspar, Brito Palma, de la Torre, & Rodríguez, 1996; Ahmad, Aliya, Perveen, & Shameel, 1992; Tori, Yoshimura, Arita, & Tomita, 1977). In addition, the ^1H and ^{13}C NMR spectra of **2** showed typical signals for a mixture of fatty acid esters [δ_H 5.35 m (olefinic), 2.80 t, $J = 6.1$ Hz (allylic), 2.32 t, $J = 7.4$ Hz (α-methylene protons), 1.60 m (β-methylene protons), 1.25 br s [$(CH_2)_n$], 0.86 t, $J = 6.8$ Hz (ω-Me); δ_C 173.4 s (C-1), 34.1 t, 34.0 t (C-2), 14.2 q, 14.1 q (ω-Me), 131.9–127.1 (10 signals, d, olefinic), 25.5 t, 25.3 t (allylic) and other signals at δ 22.7 t, 29.1–29.7 t, 30.1 t, 31.8 t] (Teixeira et al., 1997; Razdan, Kachroo, Qurishi, Kalla, & Waight, 1996).

The HMBC spectrum of **2** showed connectivities between the carboxyl carbon of the fatty acid esters (δ 173.4 s) and both the C-6 methylene protons of the glucopyranoside part (δ 4.12 and 4.22), whereas the carboxyl carbons of the three acetates (δ 170.3 s, 169.33 s and 169.27 s) were correlated with the sugar protons at δ 5.20 (H-3′), 5.04 (H-4′) and 4.95 (H-2′), respectively, thus establishing that the fatty acid esters were attached to the C-6 position of the glucopyranoside. The HMBC spectrum of **2** also displayed three bonds coupling between the C-1′ carbon of the glucose (δ 99.6 d) and the H-3α proton (δ 3.47 m) of the sterol, and between the axial H-1′ proton of the sugar (δ 4.58 d, $J = 7.9$ Hz) and the C-3 steryl carbon (δ 80.1 d). Moreover, the C-2, C-4 and C-5 carbons of the steryl moiety were upfield shifted ($\Delta\delta$ −2.1, −3.2 and −0.5 ppm, respectively) with respect to those reported in the literature (Wright et al., 1978) for Δ^5-sterols, thus confirming (Tori et al., 1977; Kasai, Suzuo, Asakawa, & Tanaka, 1977; Faghih et al., 1985) that **2** was a Δ^5-ster-3β-yl 2,3,4,6-tetra-*O*-acyl-β-D-glucopyranoside.

From all the above data it was evident that the natural compound and its peracetyl derivative possessed the structures depicted in **1** and **2**, respectively.

Alkaline hydrolysis of **1** gave **3** (which without characterization was transformed into its tetraacetyl derivative **4**) and an alkali soluble fraction which, after methylation and GC-MS analysis, allowed the identification of the methyl ester of palmitic (34.35%), stearic (5.38%), oleic (7.98%), linoleic (11.12%) and linolenic (37.50%) acids together with minor quantities of the *n*-alkanoic acid C_{14} (0.40%), C_{15} (0.46%), C_{17} (1.12%), C_{20} (0.79%) and C_{22} (0.64%) methyl esters.

It is of interest to indicate that the H-4′ and H_B-6′ glucopyranosyl protons of **1** and **2** showed a long-range coupling ($^4J = 3.5$ and 2.3 Hz, respectively, see Section 3 and above), and this peculiarity was not observed in the tetra-*O*-acetyl derivative **4** (see Section 3). This different behavior can be explained considering that in the case of **1** and **2** the presence of long-chain esters at the C-6′ position causes a restriction of the rotation around the C-5′, C-6′ bond, thus favoring a spatial arrangement in which such protons are long-range coupled.

Finally, acid hydrolysis of **1** yielded the aglycone which, after treatment with acetic anhydride–pyridine, was rigorously characterized as (24*S*)-24-ethylcholesta-5,25-dien-3β-yl acetate (**5**) (Rubinstein & Goad, 1974; Gaspar et al., 1996; Sucrow, Slopianka, & Kircher, 1976) by its melting point, $[\alpha]_D$, ^1H NMR and mass spectra and by comparison (mixed mp, TLC on silver nitrate impregnated silica gel plates) with an authentic sample (Gaspar et al., 1996).

3. Experimental

3.1. General

Mps: uncorr. Plant materials were collected in June 1996 near Palermo, Sicily, and voucher specimens were deposited in the Herbarium of the Botanic Garden of Palermo.

3.2. Extraction and isolation of *1*

Dried and powdered *Teucrium fruticans* L. aerial parts (2 kg) were extracted (3×) with Me_2CO (10 l) at room temp. for 5 days. The extract (60 g) was subjected to CC (Si gel, deactivated with 15% H_2O, w/v, 600 g) eluting with a petrol–EtOAc gradient from 10–100% EtOAc, yielding the *neo*-clerodanes previously reported as constituents of this species (Savona et al., 1978a,b; Bruno et al., 1992). Final elution with CH_2Cl_2–MeOH (49:1) gave **1** (500 mg): soft substance; $[\alpha]_D^{18}$ −51.5° ($CHCl_3$; c 0.163); IR ν_{max}^{NaCl} cm^{-1}: 3400 br (OH), 3080, 1645, 890 (terminal methylene), 1740 (esters), 2940, 2860, 1470, 1380, 1170, 1080, 1060, 1025, 840, 800, 720; ^1H NMR (400 MHz, $CDCl_3$): δ 5.35, 1H, br d, $J = 5.1$ Hz (H-6), 4.71, 1H, sext, $J = 2.5$, 1.3 Hz (H_B-26), 4.63, 1H, dq, $J = 2.5$, 0.6 Hz (H_A-26), 4.46, 1H, ddd, $J = 12.2$, 4.6, 3.5 Hz (H_B-6′), 4.37, 1H, d, $J = 7.8$ Hz (H-1′), 4.25, 1H, dd, $J = 12.2$, 1.8 Hz (H_A-6′), 3.56, 2H, m (H-3′, H-4′), 3.45, 1H, m (H-3α), 3.43, 1H, ddd, $J = 9.6$, 4.6, 1.8 Hz (H-5′), 3.36, 1H, dd, $J = 9.0$, 7.8 Hz (H-2′), 1.56, 3H, dd, $J = 2.5$, 0.6 Hz (Me-27), 0.99, 3H, s (Me-19), 0.89, 3H, d, $J = 6.6$ Hz (Me-21), 0.79, 3H, t, $J = 7.5$ Hz (Me-29), 0.66, 3H, s (Me-18); fatty acid esters: δ 5.36 m (olefinic), 2.79 *t*, $J = 6.0$ Hz (allylic), 2.34 t, $J = 7.2$ Hz (α-methylenes), 1.62 m (β-methylenes), 1.25 br s [$(CH_2)_n$], 0.87 t, $J = 6.9$ Hz (ω-Me).

3.3. Triacetate **2**

Treatment of **1** (100 mg) with Ac_2O–pyridine (1:1, 10 ml) at room temp. for 48 h yielded **2**: mp 118–120°C (from EtOH); $[\alpha]_D^{18}$ −20.8° ($CHCl_3$; c 0.721); IR ν_{max}^{KBr} cm^{-1}: 3080, 1645, 890 (terminal methylene), 1750 br (esters), 1240 (OAc), 2940, 2860, 1470, 1380, 1370, 1170, 1080, 1060, 1040, 910, 840, 800, 720, 690; ^1H NMR (400 MHz, $CDCl_3$): see text; ^{13}C NMR (100 MHz, $CDCl_3$): for the sugar and fatty acid esters moieties see text, aglycone: δ 37.2 t (C-1), 29.5 t (C-2), 80.1 d (C-3), 39.0 t (C-4), 140.3 s (C-5), 122.1 d (C-6), 31.8 t (C-7), 31.9 d (C-8), 50.1 d (C-9), 36.7 s (C-10), 21.0 t (C-11), 39.7 t (C-12), 42.3 s (C-13), 56.7 d (C-14), 24.3 t (C-15), 28.1 t (C-16), 56.0 d (C-17), 12.0 q (C-18), 19.3 q (C-19), 36.7 d (C-20), 18.6 q (C-21), 35.5 t (C-22), 29.2 t (C-23), 49.5 d (C-24), 147.5 s (C-25), 111.3 t (C-26), 17.8 q (C-27), 26.5 t (C-28), 11.8 q (C-29).

3.4. Alkaline hydrolysis of **1**

To a solution of **1** (40 mg) in EtOH (2 ml) was added a solution of KOH in EtOH (10%, w/v, 10 ml) and the reaction mixture was left at room temp. for 3 days. After usual work-up, the acids and **3** were separately recovered. The acid fraction (≈5 mg) was dissolved in Et_2O (5 ml) and treated with an excess of an ethereal solution of CH_2N_2 for 2 h at room temp. The solvent was evaporated and the residue subjected to GC-MS analysis under standard conditions, by using a Hewlett Packard 5890 gas chromatograph coupled to a HP 5971A mass detector. The result of this analysis is reported in Section 2.

Crude **3** (12 mg), without characterization, was treated with Ac_2O–pyridine as usual yielding **4** [10 mg, after CC on silica gel, petrol–EtOAc (3:1) as eluent]: mp 170–172°C (EtOH); $[\alpha]_D^{19}$ −30.0° ($CHCl_3$; c 0.311); IR ν_{max}^{KBr} cm^{-1}: 3080, 1640, 910 (terminal methylene), 1750, 1740, 1260, 1230 (OAc), 2950, 2870, 1450, 1380, 1370, 1160, 1105, 1070, 1060, 1040, 880, 800, 700; ^1H NMR (400 MHz, $CDCl_3$): δ 5.34, 1H, br d, J = 5.1 Hz (H-6), 5.19, 1H, t, J = 9.4 Hz (H-3′), 5.06, 1H, t, J = 9.7 Hz (H-4′), 4.94, 1H, dd, J = 9.6, 8.0 Hz (H-2′), 4.71, 1H, sext, J = 2.5, 1.3 Hz (H_B-26), 4.62, 1H, dq, J = 2.5, 0.7 Hz (H_A-26), 4.58, 1H, d, J = 8.0 Hz (H-1′), 4.24, 1H, dd, J = 12.2, 4.9 Hz (H_B-6′), 4.10, 1H, dd, J = 12.2, 2.5 Hz (H_A-6′), 3.66, 1H, ddd, J = 9.7, 4.9, 2.5 Hz (H-5′), 3.47 m (H-3α), 2.06, 2.03, 2.00 and 1.99, 3H each, s (4 × OAc), 1.55, 3H, dd, J = 2.5, 0.7 Hz (Me-27), 0.97, 3H, s (Me-19), 0.89, 3H, d, J = 6.4 Hz (Me-21), 0.79, 3H, t, J = 7.5 Hz (Me-29), 0.65, 3H, s (Me-18); positive FAB-MS: $[MH]^+$ at m/z 743. (Found: C, 69.47; H, 8.83%. $C_{43}H_{66}O_{10}$ requires: C, 69.51; H, 8.95%).

3.5. Acid hydrolysis of **1**

Compound **1** (30 mg) was refluxed with 2 N HCl in aqueous MeOH (50%, 30 ml). After 4 h the MeOH was evaporated under reduced pressure, diluted with H_2O (15 ml) and the hydrolysate was then extracted with EtOAc (3 × 20 ml). The extract (12 mg) was treated with Ac_2O–pyridine for 24 h at room temp. The acetylated compound (**5**, 10 mg after crystallization from MeOH) showed mp (127–128°C), $[\alpha]_D^{20}$ [−48.9° ($CHCl_3$; c 0.318)], ^1H NMR and MS identical to those reported previously (Rubinstein & Goad, 1974; Gaspar et al., 1996; Sucrow et al., 1976) for the acetate of (24*S*)-24-ethylcholesta-5,25-dien-3β-ol. Comparison [mp, 126–128°C, TLC on $AgNO_3$ impregnated silica gel plates, petrol–EtOAc (49:1) as eluent] with an authentic sample confirmed the identity.

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