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STEROLS FROM *TEUCRIUM ABUTILOIDES* AND *T. BETONICUM*

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Key Word Index—*Teucrium abutiloides*; *T. betonicum*; Labiatae; phytosterols; 24β-ethylcholestane derivatives; (24*S*)-24-ethylcholesta-5,22(*E*),25-trien-3β-ol; clerosterol; poriferasterol.

Abstract—The sterol composition of two *Teucrium* species (*abutiloides* and *betonicum*) was studied. Both plants were shown to contain three 24-ethylcholestane derivatives, all of them having the 24β-configuration. These were identified as (24*S*)-24-ethylcholesta-5,22(*E*),25-trien-3β-ol, (24*S*)-24-ethylcholesta-5,25-dien-3β-ol (clerosterol) and (24*R*)-24-ethylcholesta-5,22(*E*)-dien-3β-ol (poriferasterol) by spectroscopic analysis of their acetates and by comparison of their physical data (mp, $[\alpha]_D$) with those reported in the literature for these compounds. Some disagreements in the previously published data of these sterols are clarified and chemotaxonomic aspects are briefly discussed. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

The species belonging to the genus *Teucrium* (Labiatae) have afforded a great number of neo-clerodane and 19-nor-neo-clerodane diterpenoids [1–4], but the sterol composition of these plants has received little attention [5]. Recently, Ulubelen *et al.* [6] reported the isolation of 24α-ethylcholesta-5,25-dien-3β-ol‡, sitosterol and other steroids from *T. chamaedrys* subsp. *chamaedrys*. The perusal of this communication [6], together with the confusion existing in the literature about the configuration at C-24 in clerosterol {for which both (24*R*) = 24α [6, 8–11] and (24*S*) = 24β [12] 24-ethylcholesta-5,25-dien-3β-ol structures have been suggested}, prompted us to report our results on the sterol composition of *T. abutiloides* and *T. betonicum*, two species endemic to Madeira Island.

RESULTS AND DISCUSSION

GC analysis of the sterol mixture isolated from *T. abutiloides* and *T. betonicum* showed that it was the same in both plants (see Experimental) and was constituted by three components. The most abundant sterol of *T. abutiloides* (**1**, *ca* 93%) was purified by successive crystallizations from methanol, whereas all the three constituents were separated by chromatography of their acetates on silver nitrate impregnated silica gel, obtaining compounds **1a**, **2a** and **3a**.

Compound **1** was identical in all respects (mp. $[\alpha]_D$, IR, ^{1}H NMR and mass spectrum) to (24*S*)-24-ethylcholesta-5,22(*E*),25-trien-3β-ol, previously isolated from *Clerodendrum campbellii* (Verbenaceae) and whose structure and 24β-configuration have been rigorously established [13]. However, the only datum reported for the acetyl derivative **1a** (mp 141° [13]) did not entirely agree with our results (**1a**: mp 151–153°), but this may be due to polymorphism or impurities, because the ^{1}H NMR spectra of **1** and its acetate (**1a**) showed the chemical shifts and coupling values of the H-22 and H-23 olefinic protons (see Experimental) identical with those reported for (24*S*)-3β-acetoxy-24-ethyl-5α-cholesta-7,22(*E*),25-trienes and slightly different from those observed for the corresponding (24*R*) = 24α epimers [14]. In particular, the difference between the chemical shifts of the H-22 and H-23 protons in **1** and **1a** (0.08 and 0.06 ppm, respectively) is identical to that observed in the (24*S*) = 24β derivatives (0.08 ppm [14]), whereas in the C-24 epimers this difference is 0.11 ppm [14]. Compound **1** has recently been isolated from *T. oliverianum* [15] and, together with **2**, from *T. royleanum* [16].

Compound **2a** had a melting point and $[\alpha]_D$ value identical to those reported [10] for stigmasta-5,25-dien-3β-yl acetate [(24*R*) = 24α configuration] and identical melting point to that of (24*S*)-24-ethylcholesta-5,25-dien-3β-yl acetate found in the green alga *Codium fragile* [12] ($[\alpha]_D$ value not reported). The ^{1}H NMR spectra of these steryl acetates [10, 12] and **2a** were identical, thus suggesting that these three compounds are the same substance. Since the authors of ref. [12] established a (24*S*) = 24β-configuration for the sterol isolated from *C. fragile* on the basis of chemical

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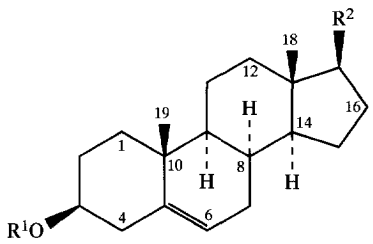
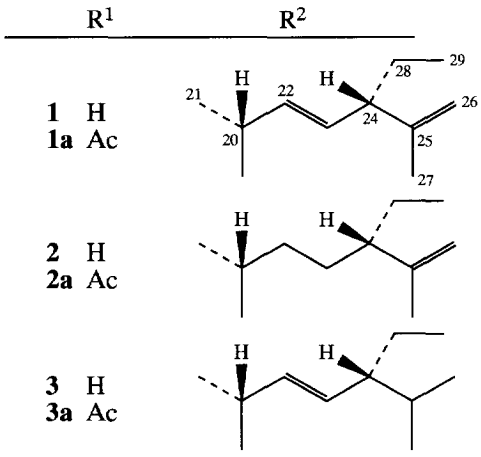
‡Throughout this paper we use the IUPAC recommended (*R*),(*S*) nomenclature. For a better understanding, however, we give in addition the more lucid α,β nomenclature [7].





correlation with (24*S*)-24-ethylcholest-5-en-3β-yl acetate and a careful comparison with the ^{1}H NMR spectra of both epimers at C-24, it seems to be evident that **2a** and the acetate of the sterol isolated and synthesized by Sucrow [10] possess a (24*S*) = 24β-configuration. Moreover, clerosterol [8, 9, 11] and the sterol recently isolated from *T. chamaedrys* [6], whose stereochemistry at the C-24 asymmetric centre was not rigorously investigated, are probably identical to (24*S*)-24-ethylcholesta-5,25-dien-3β-ol [12].

It is important to note that, in the ^{13}C NMR spectral data for this last sterol (**2**) [6, 9], there is an error in the chemical shift of the C-7 carbon atom (δ 33.8 and 33.7, respectively), because it is rigorously established [17] that the C-7 and C-8 carbons of Δ^5-sterols resonate at an identical field (δ 31.9).

Finally, **3a** was identified with (24*R*)-24-ethylcholesta-5,22(*E*)-dien-3β-yl acetate (poriferasterol acetate, with a 24β-configuration) by a careful comparison of its ^{1}H NMR spectrum with published data [18]. The lack of material precluded further identification.

The physical and spectroscopic data for **1**, **2a** and **3a** have already been reported. However, in order to facilitate the identification of these substances in the future, we include, in the Experimental section, the data obtained by us for these compounds, together with the unpublished data for **1a**.

From a chemotaxonomic point of view [19], it is important to indicate that all the sterols isolated from *T. abutiloides* and *T. betonicum* possess a 24β-configuration and that **1** and **2** have previously been reported as constituents of some species belonging to the family Verbenaceae [4, 8, 11]. This could support some papers on the necessary reclassification of Labiatae and Verbenaceae [20] although, apparently, development and plant anatomy may influence the sterol composition [21].

EXPERIMENTAL

Mps: uncorr. Plant materials of *T. abutiloides* and *T. betonicum* were collected in June 1988 and July 1993, respectively, near Porto Moniz (Madeira Island, Portugal) and voucher specimens were deposited in the Herbarium of the 'Instituto Superior de Agronomia', Lisbon (Portugal) and the 'Jardim Botanico da Ilha da Madeira' (Portugal), respectively.

Extraction and isolation of the steroids. Dried and powdered *T. abutiloides* L'Hérit. aerial parts (1.46 kg) were extracted (3×) with Me_2CO (8 l) at room temp. for 5 days. The extract (37.5 g) was subjected to CC on silica gel (Merck, No. 7734, deactivated with 15% H_2O, 300 g) eluted with petrol and petrol–EtOAc mixts. Elution with petrol–EtOAc (4:1) yielded 450 mg of a mixt. of sterols from which pure **1** (^{1}H NMR at 500 MHz) was isolated by crystallization from MeOH (76 mg, after 10 successive crystallizations). The residue obtained by evapn of the solvent of crystallization was treated with Ac_2O–pyridine for 24 hr. The acetylated mixt. (390 mg) was subjected to CC on silica gel plus 8% $AgNO_3$. Elution with petrol–EtOAc (99:1) yielded the following compounds in order of increasing chromatographic polarity: **3a** (1 mg), **2a** (20 mg) and **1a** (300 mg).

Dried and powdered *T. betonicum* L'Hérit. aerial parts (195 g) were extracted as above. CC gave 32 mg of a mixt. of steroids. Acetylation and GC of the mixt. of steryl acetates showed 3 components, **1a** (77%), **2a** (19%) and **3a** (4%), with R_ts identical to those of compounds isolated from *T. abutiloides*. GC analysis was performed with a capillary column (SE-54, 25 m × 0.25 mm i.d.) and an oven temp. of 270°.

(24S)-24-*Ethylcholesta*-5,22(E),25-*trien*-3β-*ol* (**1**). Mp 146–148° (MeOH); $[\alpha]_D^{20}$ −40.6° ($CHCl_3$; *c* 0.94). IR ν_{max}^{KBr} cm^{-1}: 3440, 3320 (OH), 3080, 1645, 890 (terminal CH_2), 3030, 1660, 980, 970, 800 (olefinic double bonds), 2940, 2870, 1460, 1380, 1370, 1060, 1050. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.52 *septet*, 1H, $J_{3\alpha,2\beta} = J_{3\alpha,4\beta} = 9.6$ Hz, $J_{3\alpha,2\alpha} = J_{3\alpha,4\alpha} = 5.4$ Hz (H-3α); 5.35 *br d*, 1H, $J = 5.1$ Hz (H-6); 5.24 *dd*, 1H, $J = 15.3$, 7.9 Hz (H-22); 5.16 *dd*, 1H, $J = 15.3$, 6.4 Hz (H-23); 2.42 *br q*, 1H, $J = 6.4$ Hz (H-24); 4.69 *br s*, 2H (2H-26); 0.68 *s*, 3H (Me-18); 1.00 *s*, 3H (Me-19); 1.00 *d*, 3H, $J = 6.5$ Hz (Me-21); 1.64 *t*, 3H, $J = 0.9$ Hz (Me-27); 0.82 *t*, 3H, $J = 7.3$ Hz (Me-29). EIMS (70 eV, direct inlet) *m/z* (rel. int.): 410 $[M]^+$ (1), 395 (0.5), 392 (0.2), 271 (12), 255 (10), 159 (24), 147 (23), 138 (29),

137 (38), 121 (29), 109 (84), 107 (43), 105 (33), 95 (58), 93 (46), 91 (47), 81 (79), 79 (46), 67 (41), 55 (100), 41 (46). (Calc. for $C_{29}H_{46}O$: C, 84.81; H, 11.29%. Found: C, 84.42; H, 11.46%.)

(24S)-24-*Ethylcholesta*-5,22(E),25-*trien*-3β-*yl acetate* (**1a**). Mp 151–153° (MeOH); $[\alpha]_D^{20}$ −41.7° ($CHCl_3$; *c* 0.641) IR ν_{max}^{KBr} cm^{-1}: 3080, 1645, 890 (terminal CH_2), 3030, 1660, 970, 960, 800 (olefinic double bonds), 1730, 1260 (OAc), 2950, 2860, 1460, 1385, 1375, 1040, 1020. ^1H NMR (500 MHz, $CDCl_3$): δ 4.59 *m*, 1H (H-3α); 5.36 *br d*, 1H, $J = 4.6$ Hz (H-6); 5.23 *dd*, 1H, $J = 15.3$, 8.2 Hz (H-22); 5.17 *dd*, 1H, $J = 15.3$, 7.4 Hz (H-23); 2.41 *br q*, 1H, $J = 7.4$ Hz (H-24); 4.68, 4.69 both *br s*, 1H each (2H-26); 0.68 *s*, 3H (Me-18); 1.01 *s*, 3H (Me-19); 1.00 *d*, 3H, $J = 7$ Hz (Me-21); 1.64 *br s*, 3H (Me-27); 0.82 *t*, 3H, $J = 7.3$ Hz (Me-29); 2.01 *s*, 3H (OAc). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ (C) 37.0 *t* (1), 27.8 *t* (2), 74.0 *d* (3), 38.1 *t* (4), 139.7 *s* (5), 122.6 *d* (6), 31.9 *t* (7), 31.9 *d* (8), 50.1 *d* (9), 36.6 *s* (10), 21.0 *t* (11), 39.6 *t* (12), 42.3 *s* (13), 56.8 *d* (14), 24.3 *t* (15), 28.7 *t* (16), 55.9 *d* (17), 12.0 *q* (18), 19.3 *q* (19), 40.2 *d* (20), 21.4 *q* (21), 137.2 *d* (22), 130.1 *d* (23), 52.0 *d* (24), 148.6 *s* (25), 109.5 *t* (26), 20.2 *q* (27), 25.7 *t* (28), 12.1 *q* (29), 170.5 *s*, 20.8 *q* (OAc). EIMS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 392 $[M - HOAc]^+$ (10), 255 (8), 253 (5), 213 (3), 159 (11), 147 (14), 145 (18), 137 (22), 121 (23), 109 (39), 107 (29), 105 (32), 95 (55), 93 (37), 91 (43), 81 (100), 79 (37), 69 (34), 67 (49), 55 (83), 43 (58), 41 (27). (Calc. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69%. Found: C, 81.95; H, 10.90%.)

(24S)-24-*Ethylcholesta*-5,25-*dien*-3β-*yl acetate* (**2a**). Mp 127–128° (from MeOH or 96% EtOH); $[\alpha]_D^{20}$ −49.6° ($CHCl_3$; *c* 0.119). ^1H NMR (300 MHz, $CDCl_3$): δ 4.59 *m*, 1H (H-3α); 5.36 *br dd*, 1H, $J = 5.3$, 0.7 Hz (H-6); 4.63 *dq*, 1H, $J = 2.6$, 0.5 Hz (H_A-26); 4.71 *sextet*, 1H, $J = 2.6$, 1.3 Hz (H_B-26); 0.66 *s*, 3H (Me-18); 1.00 *s*, 3H (Me-19); 0.89 *d*, 3H, $J = 6.5$ Hz (Me-21); 1.55 *br s*, 3H (Me-27); 0.79 *t*, 3H, $J = 7.4$ Hz (Me-29); 2.02 *s*, 3H (OAc). EIMS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 394 $[M - HOAc]^+$ (22), 281 (2), 255 (4), 253 (5), 213 (9), 173 (8), 159 (17), 147 (24), 145 (35), 133 (18), 121 (26), 107 (31), 105 (31), 95 (30), 93 (30), 91 (35), 83 (35), 81 (58), 79 (32), 69 (56), 67 (39), 57 (24), 55 (100), 43 (47), 41 (30). (Calc. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08%. Found: C, 81.69; H, 11.26%.)

(24R)-24-*Ethylcholesta*-5,22(E)-*dien*-3β-*yl acetate* (**3a**). ^1H NMR (300 MHz, $CDCl_3$): δ 4.60 *m*, 1H (H-3α); 5.36 *br dd*, 1H, $J = 5.3$, 0.7 Hz (H-6); 0.69 *s*, 3H (Me-18); 1.02 *s*, 3H (Me-19); 1.03 *d*, 3H, $J = 6.5$ Hz (Me-21); 0.84, 0.79 both *d*, 3H each, $J = 6.9$ Hz (Me-26, Me-27); 0.81 *t*, 3H, $J = 7.3$ Hz (Me-29); 2.02 *s*, 3H (OAc).

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