



DEHYDROABIETANE DITERPENES FROM *NEPETA TEYDEA*

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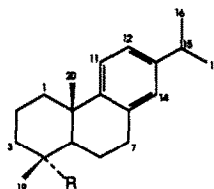
Abstract—The new diterpenes dehydroabietan-18-ol acetate, teideadiol 18-monoacetate, teideadiol 18-malonate and teidic acid have been isolated from the aerial parts of *Nepeta teydea*. The known diterpenes dehydroabietan-18-ol (pomiferin A) and teideadiol have also been obtained from this species. The ^{13}C NMR spectra of several dehydroabietane derivatives have been assigned.

INTRODUCTION

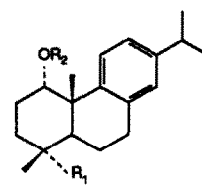
In a continuation of our investigation of the Canarian species of the Labiatae family, we have studied *Nepeta teydea* W.B., a species endemic to the island of Tenerife. In previous works, the diterpenes teideadiol (**5**) [1, 2], 13 α -isopropyl-8(14)-podocarpene-7 α , 18-diol [3, 4] and 13 α -isopropyl-7(8)-podocarpene-14 α , 18-diol [4, 5], the triterpenic acids oleanolic, ursolic, 2 α , 3 β -dihydroxy-ursolic and 2 α , 3 β , 19 α -trihydroxy-ursolic [2], and two spirostanic compounds [6] have been isolated from this species. The structure of **5** was subsequently confirmed by synthesis [7, 8]. Also, the essential oil of this species has been studied [9, 10]. In this work we describe the isolation of the new dehydroabietane diterpenes **2**, **7**, **9** and **12**.

RESULTS AND DISCUSSION

The high resolution MS of **1** was in accordance with the formula $\text{C}_{20}\text{H}_{30}\text{O}$. The oxygen of this molecule forms a part of a hydroxymethylene group because, in the ^1H NMR spectrum the characteristic double doublet of the AB system appears at δ 3.23 and 3.48 ($J=11$ Hz). This group must be equatorial and situated at C-4 taking into consideration these chemical shifts [11]. Other signals of this spectrum were those of four methyl groups and the aromatic system (δ 6.70 d, H-14; 6.99, dd, H-12; and 7.12, d, H-11). The ^{13}C NMR spectrum was also in accordance with structure **1**. Finally, reduction of dehydroabietic acid methyl ester (**4**) with LiAlH_4 afforded a compound which was identical with the natural alcohol.



- 1 R = CH_2OH
- 2 R = CH_2OAc
- 3 R = CO_2H
- 4 R = CO_2Me



- 5 $\text{R}_1 = \text{CH}_2\text{OH}$ $\text{R}_2 = \text{H}$
- 6 $\text{R}_1 = \text{CH}_2\text{OAc}$ $\text{R}_2 = \text{Ac}$
- 7 $\text{R}_1 = \text{CH}_2\text{OAc}$ $\text{R}_2 = \text{H}$
- 8 $\text{R}_1 = \text{CH}_2\text{OH}$ $\text{R}_2 = \text{Ac}$
- 9 $\text{R}_1 = \text{CH}_2\text{OMe}$ $\text{R}_2 = \text{H}$
- 10 $\text{R}_1 = \text{CH}_2\text{OMe}$ $\text{R}_2 = \text{Ac}$
- 11 $\text{R}_1 = \text{CO}_2\text{Me}$ $\text{R}_2 = \text{H}$
- 12 $\text{R}_1 = \text{CO}_2\text{H}$ $\text{R}_2 = \text{H}$
- 13 $\text{R}_1 = \text{CO}_2\text{Me}$ $\text{R}_2 = \text{Ac}$

Compound **1** has recently been isolated from *Salvia pomifera* and named pomiferin A [12]. Its 4-epimer, named dehydroabietinol, has been obtained from *Calceolaria ascendens* [13]. The mixture of both epimers has been found in *Juniperus phoenicea* [14].

Acetylation of **1** formed **2**, which was identical with a product now isolated from *N. teydea*. This is the first time that this substance has been found in nature. The corresponding C-4 epimer, dehydroabietinol acetate, has been obtained from *Pinus silvestris* [15].

The major dehydroabietane diterpene isolated from *N. teydea* was **5**, a compound already shown to occur in this plant [1, 2]. We have now obtained two new esters of this diol, the 18-monoacetate (**17**) and the 18-malonate (**9**) of teideadiol, respectively. The ^1H NMR spectrum of **7** was very similar to that of **5**, the difference observed being due

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Table 1. ^{13}C NMR data of 1, 2, 4–9, 11 and 13

C	1	2	4	5	6	7	8	9	11	13
1	38.4	38.2	38.0	71.7	73.7	71.4	74.0	71.4	71.0	73.2
2	18.6 ^a	18.5 ^a	18.5	25.7	22.2	23.6	22.4	23.6	23.6	23.5
3	35.1	35.5	36.6	27.8	29.1	28.4	28.6	28.4	30.5	30.0
4	37.8	36.7	47.6	37.5	36.3	36.5	37.4	36.6	47.4	47.1
5	43.9	44.0	44.8	37.4	39.2	38.0	38.6	38.0	38.5	39.6
6	18.8 ^a	18.9 ^a	21.7	18.4	18.9	18.6	18.6	18.7	21.4	21.4
7	30.1	30.2	30.0	30.6	30.3	30.7	30.2	30.6	29.7	30.0
8	134.7	134.2	134.6	137.2	135.0	137.1	135.1	137.0	137.1	135.1
9	147.3	147.1	146.9	146.2	145.6	146.3	145.6	146.4	146.5	145.7
10	37.3	37.4	36.9	43.4	41.7	43.5	41.7	43.5	43.0	41.3
11	124.2	124.3	124.1	124.5	124.0	124.7	123.9	124.7	124.7	123.9
12	123.7	123.7	123.9	123.7	123.9	123.7	123.9	123.7	123.5	123.6
13	145.5	145.6	145.7	142.1	142.3	141.9	142.5	141.8	141.6	142.1
14	126.3	126.9	126.8	127.9	127.0	127.9	127.0	127.9	128.0	127.0
15	33.4	33.4	33.4	33.5	33.3	33.5	33.4	33.5	33.5	33.5
16	24.0	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9
17	23.0	23.9	23.9	23.9	23.6	23.9	23.9	23.7	23.9	23.9
18	72.2	72.4	179.1	72.0	72.6	72.5	72.0	73.5	71.0	178.7
19	17.4	17.5	16.5	17.5	17.4	17.4	17.3	17.3	16.4	16.4
20	25.2	25.3	25.1	25.9	25.8	25.9	25.8	25.9	25.6	25.5

^aThese values can be interchanged.

to the presence of the acetyl group and the shielding at high field (δ 3.75 and 4.01, $J=11$ Hz) of the double doublet of the methylene group at C-18. In the case of 7, the resonance of this pair of doublets is at δ 3.84 and 4.07, and that of the methylene of the malonate ester 9 is seen as a singlet at δ 3.37. The ^{13}C NMR spectra of 7 and 9 have been assigned (Table 1). In the spectrum of this last product the resonance of the ester appears at δ 166.5, 41.7 and 166.7. The natural monoacetate 7 was identical with the major monoacetate obtained on partial acetylation of 5.

Compound 11 was obtained by methylation of the fractions containing the corresponding acid. Its structure was assigned on the basis of the following considerations. The high resolution MS was in accordance with the formula $\text{C}_{21}\text{H}_{30}\text{O}_3$. Two of the oxygen atoms of the molecule form part of a methyl ester and the third of a secondary alcohol. The ^1H NMR spectrum contains signals for two angular methyls, an isopropyl group, a methoxyl group, a proton geminal to a secondary alcohol and three aromatic protons. The resonance patterns of these last hydrogens were identical with those of the other dehydroabietane diterpenes described above. The methoxyl group resonates at δ 3.69 and the geminal hydrogen to the secondary hydroxyl group appears as a broad singlet at δ 4.38. The resonance of this last proton pointed to the positions C-1 (α) or C-3 (α) for the alcoholic group. The presence in the plant of 5 indicated that this compound must be the C-18 methyl ester of the corresponding acid, with the hydroxyl at C-1. This hypothesis was confirmed, first by assignment of the ^{13}C NMR spectrum of 11 (Table 1), and then, chemically by reduction of this compound with LiAlH_4 , which gave 5. We have named the corresponding natural acid teidic acid 12.

In Table 1 we have given the ^{13}C NMR spectra of several dehydroabietane diterpenes. The signals in the spectrum of dehydroabietic acid methyl ester (4) were assigned by application of 2D NMR experiments, COSY, HMQC [16], HMBC [18] and by comparison with those of teidic acid methyl ester (11). This study confirmed the assignments previously reported for 4 [18, 19].

We have also isolated dehydroabietan-18-ol (1), 5, sitosterol and a mixture of ursolic and oleanolic acid from a CH_2Cl_2 extract obtained from 16-day-old plantlets of *N. teydea* grown *in vitro*.

EXPERIMENTAL

NMR: CDCl_3 ; MS: 70 eV (probe); CC: silica gel 0.063–0.2 mm. A commercial sample of dehydroabietic acid (American Drugs, California, U.S.A.) has been used in this work.

Plant material was collected in June at the highest part of 'Barranco de Erques' near the Teide National park (Tenerife) and a voucher specimen has been deposited in the Herbarium of the Department of Botany, University of La Laguna (Tenerife).

Extraction and isolation of the chemical constituents. The finely cut aerial parts (5 kg) were extracted with EtOH at room temp. under vacuum and the residue (320 g) was chromatographed on silica gel, eluting with petrol followed by increasing proportions of EtOAc in petrol, to give several mixts of substances which were rechromatographed on silica gel dry columns to afford in polarity order the following products.

Dehydroabietan-18-ol acetate (2). ^1H NMR (200 MHz): δ 0.96 (3H, s), 1.23–1.26 (9H), 2.04 (3H, s), 2.88 (3H, m, H-7

and H-15), 3.71 and 4.00 (1H, *d*, *J* = 11 Hz, H-18), 6.92 (1H, *br s*, H-14), 7.02 (1H, *br d*, *J* = 8 Hz, H-12), 7.21 (1H, *d*, *J* = 8 Hz, H-11); EIMS *m/z* (rel. int.): 328 [M]⁺ (11), 211 (7), 197 (4), 185 (5), 173 (9), 159 (6).

Dehydroabietan-18-ol (1). [M]⁺ at *m/z* 286.2301. C₂₀H₃₀O requires 286.2297; ¹H NMR (200 MHz): δ 1.20–1.23 (9H), 2.80 (3H, *m*, H-7 and H-15), 3.23 and 3.48 (1H, *d*, *J* = 11 Hz, H-18), 6.70 (1H, *d*, *J* = 1.8 Hz, H-14), 6.99 (1H, *dd*, *J* = 8 and 1.8 Hz, H-12), 7.18 (1H, *d*, *J* = 8 Hz, H-11); EIMS *m/z* (rel. int.): 286 [M]⁺ (48), 271 (98), 253 (100), 239 (4), 225 (7), 211 (23), 173 (62), 159 (51), 143 (24).

Teideadiol 18-monoacetate (7). ¹H NMR (200 MHz): δ 0.96 and 1.25 (each 3H, *s*), 1.23 (6H, *d*), 2.06 (3H, *s*), 2.62 (3H, *m*, H-7 and H-15), 3.75 and 4.01 (1H, *d*, *J* = 11 Hz, H-18), 4.37 (1H, *br s*, H-1), 6.94 (1H, *d*, *J* = 1.5 Hz, H-14), 7.08 (1H, *dd*, *J* = 8 and 1.5 Hz, H-12), 7.25 (1H, *d*, *J* = 8 Hz, H-11); EIMS *m/z* (rel. int.): 344 [M]⁺ (19), 326 (5), 284 (7), 271 (23), 267 (18), 253 (98), 186 (100), 155 (26), 143 (60), 129 (48).

Teideadiol 18-malonate (9). ¹H NMR (200 MHz): δ 0.96 (3H, *s*), 1.20–1.25 (9H), 2.83 (3H, H-7 and H-15), 3.37 (2H, *s*, malonate), 3.84 and 4.07 (each 1H, *d*, *J* = 11 Hz, H-18), 4.36 (1H, *br s*, H-1), 6.93 (1H, *br s*, H-14), 7.08 (1H, *br d*, *J* = 8 Hz, H-12), 7.24 (1H, *d*, *J* = 8 Hz, H-11); EIMS *m/z* (rel. int.): 284 [M–C₃H₄O₄]⁺ (11), 266 (47), 253 (100), 251 (27), 227 (17), 225 (19), 223 (15), 211 (17), 209 (18), 199 (13), 197 (20), 195 (10), 186 (87), 171 (40). Acetate (9): ¹H NMR (200 MHz): δ 0.99 and 1.20 (each 3H, *s*), 1.23 and 1.27 (each 3H, *d*), 1.83 (3H, *s*, OAc), 2.82 (3H, *m*, H-7 and H-15), 3.38 (2H, *s*, malonate), 3.87 and 4.10 (each 1H, *d*, *J* = 11 Hz, H-18), 5.52 (1H, *br s*, H-1), 6.88 (1H, *br s*, H-14), 6.95 (2H, *br s*, H-11 and H-12).

Teideadiol (5) [1, 2]. ¹H NMR (200 MHz): δ 0.87 and 1.20 (each 3H, *s*), 1.24 (6H, *s*), 2.84 (3H, *m*, H-7 and H-15), 3.20 and 3.49 (each 1H, *d*, *J* = 11 Hz, H-18), 4.35 (1H, *br s*, H-1), 6.93 (1H, *br s*, H-14), 7.02 (1H, *br d*, *J* = 8 Hz, H-12), 7.19 (1H, *d*, *J* = 8 Hz, H-11); EIMS *m/z* (rel. int.): 302 [M]⁺ (2), 271 (6), 267 (3), 257 (4), 255 (4), 253 (9), 239 (4), 237 (7), 227 (8), 187 (54), 171 (34). Diacetate (6): ¹H NMR (200 MHz): δ 0.99, 1.19, 1.23 and 1.27 (each 3H, *s*), 1.80 and 2.08 (each 3H, *s*, OAc), 2.84 (3H, *m*, H-15 and H-7), 3.79 and 4.00 (each 1H, *d*, *J* = 11 Hz, H-18), 5.53 (1H, *br s*, H-1), 6.88 (1H, *s*, H-14), 6.97 (2H, *br s*, H-11 and H-12).

Teidic acid (12). Obtained in methyl ester form (11) by methylation of the frs containing the corresponding acid, [M]⁺ at *m/z* 330.2194. C₂₁H₃₀O₃ requires 330.2193; ¹H NMR (400 MHz): δ 1.21, 1.23, 1.24 and 1.29 (each 3H, *s*), 2.70 (1H, *dd*, *J* = 12 and 1.4 Hz, H-5), 2.83 (2H, *m*, H-7), 2.85 (1H, *m*, H-15), 3.69 (3H, *s*), 4.37 (1H, *br s*, H-1), 6.93 (1H, *br s*, H-14), 7.00 (*br d*, *J* = 8 Hz, H-12), 7.20 (1H, *d*, *J* = 8 Hz, H-11); EIMS *m/z* (rel. int.): 330 [M]⁺ (4), 315 (1), 271 (4), 253 (11), 239 (5), 237 (9), 186 (17), 171 (13), 144 (100), 129 (11). Acetate methyl ester 13: ¹H NMR (200 MHz): δ 1.19, 1.22, 1.27 and 1.31 (each 3H, *s*), 1.82 (3H, *s*), 2.66 (1H, *dd*, *J* = 12 and 1.6 Hz, H-5), 2.84 (3H, *m*, H-7 and H-15), 3.71 (3H, *s*), 5.52 (1H, *br s*, H-1), 6.88 (1H, *br s*, H-14), 6.95 (2H, *br s*, H-11 and H-12); EIMS *m/z* (rel. int.): 372 [M]⁺ (16), 357 (1), 312 (8), 297 (5), 271 (5), 253 (91), 237 (54), 209 (13), 195 (25), 186 (37), 171 (23), 155 (24), 143 (56), 128 (58).

Reduction of dehydroabietic acid methyl ester. A soln of **4** (24 mg) in dry Et₂O (5 ml) was added dropwise to a suspension of LiAlH₄ (mg) in the same solvent (10 ml). After refluxing for 6 hr the excess of reagent was destroyed by adding EtOAc and the mixt. washed with 10% aq. HCl and extracted with Et₂O. The organic layer was washed with NaHCO₃ and H₂O. The solvent was evapd to give **1** (mg), which proved to be identical with the natural product.

Reduction of teidic acid methyl ester. A soln of **11** (26 mg) was reduced with LiAlH₄ as described above for **4** giving **5** (21 mg), identical with the natural compound.

Growth of *N. teydea* in vitro. Sterile plants were established from surface-sterilized seeds with 5% (v/v) sodium hypochlorite soln for 20 min. Seeds were rinsed (× 3) in sterile distilled H₂O and germinated in Murashige and Skoog's medium [20] at 25° under 16 hr light/8 hr dark. Shoot-organ cultures were initiated from shoot nodes of the sterile plantlets by transferring 0.5 g of short nodes to 100 ml of MS medium containing 3% sucrose on gyratory shaker (100 rpm) for 16 days.

Extraction of the plantlets. Lyophilized shoots (9.5 g) were extracted in a Soxhlet with CH₂Cl₂ for 6 hr. The crude extract (480 mg) was chromatographed over silica gel and finally purified by HPLC (silica gel 5 μ, 10 mm × 25 cm) using different *n*-hexane–isoPrOH mixts as mobile phase. The amounts of compounds obtained were **1** (3.1 mg), **5** (3.6 mg), sitosterol (9 mg) and mixt. of ursolic and oleanolic acid (19 mg).

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