

DITERPENES FROM *SIDERITIS CANARIENSIS*

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(Received 28 February 1991)

Key Word Index—*Sideritis canariensis* var. *pannosa*; Lamiaceae; diterpenes; trachinol acetate; ribenol formate; trachinodiol 7 β -acetate-18-formate; *ent*-2-oxo-13-*epi*-manoyl oxide; trachinodiol 18-palmitate; *ent*-3 β -hydroxy-pimara-8(14),15-diene; canadiol; sicanadiol.

Abstract—The new diterpenes trachinol acetate, ribenol formate, trachinodiol 7 β -acetate-18-formate, *ent*-2-oxo-13-*epi*-manoyl oxide, trachinodiol 18-palmitate, *ent*-3 β -hydroxy-pimara-8(14),15-diene, canadiol and sicanadiol have been isolated from *Sideritis canariensis* var. *pannosa*.

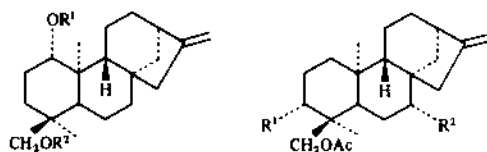
INTRODUCTION

For the past few years we have been interested in the phytochemical study of species of the genus *Sideritis*, endemic to the Canary Islands. In continuation of this work we have now studied the aerial parts of *Sideritis canariensis* var. *pannosa* [1-5].

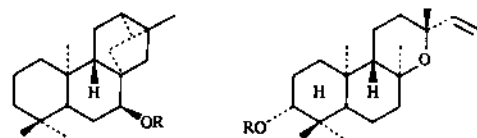
RESULTS AND DISCUSSION

One of the new products isolated from *S. canariensis* var. *pannosa* was obtained by acetylation and subsequent chromatography. Its HR mass spectrum was in accordance with the molecular formula $C_{24}H_{36}O_4$. Its 1H NMR spectrum showed signals characteristic of two angular methyls, an exocyclic double bond, a $-CH_2OAc$ group and a geminal proton to a secondary acetyl group. All of these data are in accordance with the structure of a kaur-16-ene diterpene, probably of the *enantio* series like other compounds of this type isolated from *Sideritis*. This compound must have an equatorial acetoxyethylene group at C-4 because a doublet at δ 3.59 and 3.85 ($J = 11$ Hz) appears in the 1H NMR spectrum, which is characteristic of the resonance of the two hydrogens at this position [6]. The geminal proton to the other acetoxy group appears in this spectrum as a doublet at δ 4.57 ($J = 9$ and 5 Hz), typical of an equatorial substituent at C-1, C-3 or C-7. Positions 3 or 7 were excluded because our compound **1** was different from *ent*-3 β ,18-diacetoxy-kaur-16-ene (**3**) [7] and candicandiol diacetate (**4**) [8-10], respectively. The secondary acetoxy group was therefore assigned to C-1. This position was also confirmed by the study of the ^{13}C NMR spectrum (Table 1). Thus, the novel natural alcohol corresponding to this diacetate **2** was assigned the structure **1** and named canadiol.

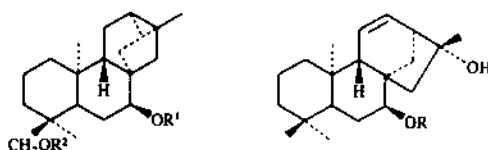
A further two new compounds isolated from this species were trachinol acetate (**5**) and ribenol formate (**7**), which were identical with synthetic samples, the former obtained previously by acetylation of trachinol (**6**) [1] and the latter prepared in this work by treatment of ribenol (**8**) [4] with formic acid.



- 1** $R^1 = R^2 = H$ **3** $R^1 = OAc, R^2 = H$
2 $R^1 = R^2 = Ac$ **4** $R^1 = H, R^2 = OAc$



- 5** $R = Ac$ **7** $R = CHO$
6 $R = H$ **8** $R = H$
9 $R = Ac$



- 10** $R^1 = R^2 = H$ **15** $R = Ac$
11 $R^1 = H, R^2 = Palm$ **16** $R = H$
12 $R^1 = Ac, R^2 = CHO$
13 $R^1 = R^2 = Ac$
14 $R^1 = Ac, R^2 = H$

Two new trachinodiol esters were also obtained and characterized as trachinodiol 18-palmitate (**11**) and trachinodiol 7 β -acetate-18-formate (**12**). The HR mass spectrum of **11** shows two ions at m/z 524 and 286 formed from the molecular ion by loss of water and palmitic acid, respectively. Its 1H NMR spectrum showed signals for three angular methyls, an esterified hydroxymethylene

Table 1. ^{13}C NMR data (50.3 MHz) of compounds 2, 7, 9, 12, 20, 22 and 23

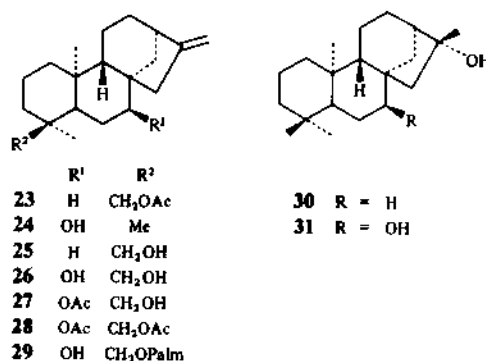
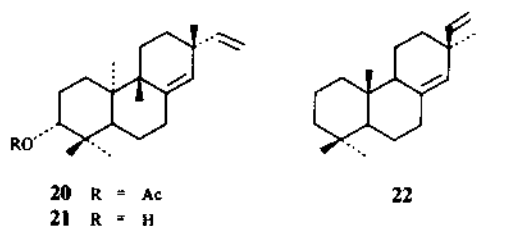
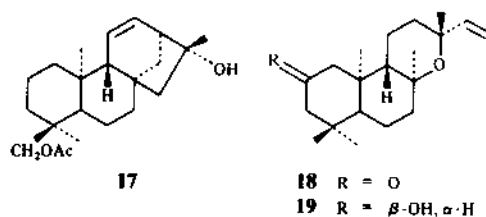
| C | 2 | 7 | 9 | 12 | 20 | 22 ^a | 23 ^b |
|----|--------|--------|--------|-------|--------|-----------------|-----------------|
| 1 | 83.81 | 37.39 | 37.32 | 38.51 | 36.94 | 39.70 | 39.9 |
| 2 | 24.76 | 23.88 | 23.57 | 17.39 | 24.18 | 19.40 | 17.4 |
| 3 | 33.48 | 80.95 | 80.65 | 35.84 | 81.21 | 42.50 | 35.9 |
| 4 | 36.45 | 37.80 | 37.74 | 36.08 | 38.05 | 33.50 | 36.5 |
| 5 | 55.55 | 55.47 | 55.38 | 41.75 | 54.37 | 55.20 | 50.2 |
| 6 | 19.89 | 19.45 | 19.39 | 25.19 | 22.15 | 22.90 | 20.2 |
| 7 | 40.42 | 43.03 | 42.93 | 78.06 | 35.79 | 36.30 | 39.9 |
| 8 | 44.88 | 75.35 | 75.53 | 44.03 | 137.93 | 138.80 | 44.1 |
| 9 | 48.92 | 58.22 | 58.15 | 48.58 | 51.25 | 51.80 | 56.0 |
| 10 | 43.68 | 36.55 | 36.46 | 38.26 | 38.78 | 38.80 | 39.2 |
| 11 | 19.83 | 16.11 | 16.03 | 19.46 | 19.25 | 19.10 | 18.2 |
| 12 | 33.41 | 34.91 | 34.80 | 20.47 | 35.69 | 36.30 | 33.3 |
| 13 | 43.95 | 73.40 | 73.30 | 23.94 | 38.18 | 38.80 | 44.0 |
| 14 | 41.45 | 147.65 | 147.58 | 32.63 | 128.43 | 128.10 | 40.8 |
| 15 | 48.78 | 109.59 | 109.53 | 45.25 | 147.45 | 147.70 | 49.2 |
| 16 | 155.70 | 32.62 | 32.59 | 22.99 | 111.95 | 112.90 | 155.6 |
| 17 | 103.16 | 23.88 | 23.63 | 21.42 | 29.57 | 29.80 | 103.0 |
| 18 | 72.24 | 27.89 | 27.92 | 71.69 | 28.56 | 34.50 | 73.1 |
| 19 | 17.75 | 16.24 | 16.24 | 17.39 | 17.00 | 22.50 | 17.9 |
| 20 | 14.91 | 15.87 | 15.88 | 15.00 | 14.94 | 14.90 | 18.0 |

^{a,b}Data from refs [12] and [20], respectively.

group at C-18, a geminal hydrogen to the alcoholic function at C-7 (δ 3.51, *br s*), and a linear chain of methylene groups (δ 1.25, *s*). The HR mass spectrum of **12** was consistent with the molecular formula $\text{C}_{23}\text{H}_{34}\text{O}_4$. Its ^1H NMR spectrum was similar to that of trachinodiol diacetate (**13**) [1, 4], also isolated from this species, except that one of the acetates has been substituted by another ester, which was identified as formate in accordance with the resonance of a hydrogen at δ 8.05 [11]. Another difference between both NMR spectra was that in the case of diacetate **13**, the two hydrogens of the primary ester appeared as a singlet, whilst in compound **12** the resonance was a double doublet, thus indicating that the formate is at C-18. Finally, the structure of **12** was established by preparing it by formolysis of trachinodiol 7 β -acetate (**14**) [4].

The new diterpene sicanadiol (**16**) was isolated as its acetate (**15**) by acetylation and chromatography of several fractions of the extract. The ^1H NMR spectrum presents signals of four methyl groups, one of which must be on a carbon united to an oxygen function. This spectrum also shows a pair of double doublets at δ 5.51 and 5.90 characteristic of two adjacent vinyl hydrogens, each of which is coupled to a methine hydrogen, and of a geminal proton to an axial hydroxyl group. With these spectral data, and taking into consideration those of sidendrodiol acetate (**17**) [6], we assigned to this substance the structure **15** and to the natural compound the structure **16**.

A new *ent*-13-*epi*-manoyl oxide derivative (**18**) has also been isolated from this species. With the molecular formula $\text{C}_{20}\text{H}_{32}\text{O}_2$, the IR spectrum presented absorptions typical of a carbonyl and of a vinyl group. Its ^1H NMR spectrum showed signals of five angular methyls and of a vinyl group on a fully substituted carbon atom. Taking into consideration these data and after searching the literature, we identified this compound as a product



obtained by chromic oxidation of *ent*-2 α -hydroxy-13-*epi*-manoyl oxide (**19**) [12]. Thus, **18** is *ent*-2-oxo-13-*epi*-manoyl oxide, obtained from nature for the first time. We also found in the literature that Bohlmann and Zdero

isolated from *Printzia laxa*, a substance with a ^1H NMR spectrum identical with that of our compound, a similar optical rotation but with an opposite sign. Thus, the substance obtained by the German authors must be the corresponding 13-*epi*-manoyl oxide derivative, an enantiomer of our compound, and not that described in their publication [13].

The structure **20** was assigned to another novel diterpene obtained from several acetylated fractions of the extract on the basis of the following considerations: Its molecular formula was $\text{C}_{22}\text{H}_{34}\text{O}_2$. The ^1H NMR spectrum shows resonances of the four methyl groups, of the geminal hydrogen to the acetoxyl group at C-3 (δ 4.52, *dd*, $J = 11$ and 4 Hz), of the vinyl group at C-13 (4.90, 4.94 and 5.71, $J = 17$, 11 and 2 Hz), of the vinylic hydrogen at C-14 (δ 5.15, *br s*). The position of the acetate group at C-3, and not at C-1, C-7 or C-12, was assigned in accordance with the ^{13}C NMR data (Table 1). This spectrum in comparison with that of pimaradiene (**22**) [14] was used to assign the stereochemistry at C-13. Finally, the absolute configuration of this substance was provisionally given as being of the *entio* series because all the diterpenes isolated from this species possess this configuration. Thus, the structure of this diterpene was determined as **20**, and that of the natural alcohol as *ent*-3 β -acetoxypimara-8(14),15-diene (**21**). An analogous diterpene of the *normal* series and epimeric at C-13 has been isolated from *Xylia dolabriformis* [15].

Other known substances isolated from this species were the diterpenes *ent*-13-*epi*-manoyl oxide, *ent*-3-oxo-13-*epi*-manoyl oxide, ribenol acetate (**9**), trachinodiol diacetate (**13**) [16], ribenol (**8**) [4], kauranol (**30**), trachinol (**6**) [1], candol A (**24**) [17], candol B (**25**) [17], epicandicandiol 18-palmitate (**29**) [6], epicandicandiol 7 β -acetate (**27**) [17], trachinodiol 7 β -acetate (**14**) [4], trachinodiol (**10**) [1], epicandicandiol (**26**) [9, 10], powerol (**31**) [3], and episinferral [18], the triterpenes squalene, glutinol, α -amyrin, β -amyrin, and β -sitosterol, and the lignán (+)-sesamin.

EXPERIMENTAL

Mps: uncorr; NMR: CDCl_3 ; MS 70 eV (probe). The ^{13}C NMR spectra were run at 50.32 MHz. CC was performed on silica gel 0.063–0.2 mm. The substances were crystallized from petrol-EtOAc except where otherwise indicated.

The air-dried aerial parts of the plant, collected at Teno Alto (Tenerife Island) in May, were used for this work. A voucher specimen has been deposited at the Herbarium of the 'Centro de Investigación y Tecnología Agraria' (ORT 29.291).

A general description of the procedure to isolate the terpenoid substances of species of the genus *Sideritis* has been published previously [19]. Thus, squalene (16 mg), trachinol acetate (**5**) (100 mg), *ent*-13-*epi*-manoyl oxide (100 mg), ribenol formate (**7**) (13 mg), *ent*-3-oxo-13-*epi*-manoyl oxide (1.1 g), *ent*-2-oxo-13-*epi*-manoyl oxide (**18**) (4 mg), ribenol acetate (**9**) (12 mg), trachinodiol 7 β -formate-18-acetate (**12**) (7 mg), trachinodiol diacetate (**13**) (11 mg), ribenol (**8**) (600 mg), kauranol (**30**) (3.1 g), trachinol (**6**) (270 mg), candol A (**24**) (8 mg), candol B (**25**) (14 mg), glutinol (50 mg), α - and β -amyrin (17 mg), β -sitosterol, *ent*-3 β -hydroxypimara-8(14)-diene (**21**) (6 mg), trachinodiol 18-palmitate (**11**) (2 mg), epicandicandiol 18-palmitate (**29**) (3 mg), epicandicandiol 7 β -acetate (**27**) (60 mg), trachinodiol 7 β -acetate (**14**) (50 mg), (+)-sesamin (22 mg), *ent*-1 β ,18-dihydroxy-kaur-16-ene (canadiol) (**1**) (6 mg), trachinodiol (**10**) (1.1 g), epicandicandiol (**26**) (4.4 g),

powerol (**31**) (330 mg), sicanadiol (**16**) (7 mg), and episinferral (140 mg) were isolated from *S. canariensis*.

ent-1 β ,18-Diacetoxy-kaur-16-ene (canadiol diacetate) (**2**). This product was isolated by acetylation in the usual way, and subsequent chromatography. Mp 130–132°; $[\text{M}]^+$ at m/z 388.2612. $\text{C}_{24}\text{H}_{36}\text{O}_4$ requires 388.2611; ^1H NMR (200 MHz): δ 0.84 and 1.25 (each 3H, *s*), 2.02 and 2.06 (each 3H, *s*), 2.63 (1H, *br s*, H-13), 3.59 and 3.85 (1H, *d*, $J = 11$ Hz, H-18), 4.57 (1H, *dd*, $J = 9$ and 5 Hz, H-1), 4.71 and 4.77 (each 1H, *s*, H-17); EI-MS m/z (rel. int.): 388 $[\text{M}]^+$ (16), 328 (22), 268 (27), 255 (100), 239 (13), 227 (12), 225 (32), 211 (12), 199 (18).

ent-2-Oxo-13-*epi*-manoyl oxide (**18**). $[\alpha]_D^{25} -47^\circ$ (CHCl_3 ; c 0.26) (lit. [11], -61°); $[\text{M}]^+$ at m/z 304.2347. $\text{C}_{20}\text{H}_{32}\text{O}_2$ requires 304.2332; ^1H NMR (200 MHz): δ 0.74, 0.86, 1.05, 1.14 and 1.22 (each 3H, *s*), 4.93 (1H, *d*, $J = 18$, H-15), 4.98 (1H, *d*, $J = 11$ Hz, H-15); 6.00 (1H, *dd*, $J = 18$ and 11 Hz, H-14); EI-MS m/z (rel. int.): 304 $[\text{M}]^+$ (1), 289 (96), 271 (68), 259 (9), 243 (15), 234 (5), 227 (10), 219 (14), 206 (36), 191 (27).

Ribenol formate (**7**). Mp 159–161°; $[\text{M} - \text{Me}]^+$ at m/z 319.2285. $\text{C}_{20}\text{H}_{31}\text{O}_3$ requires 319.2273; ^1H NMR (200 MHz): δ 0.76, 0.84, 0.88, 1.13 and 1.22 (each 3H, *s*), 4.60 (1H, *t*, $J = 7$ Hz, H-3), 4.91 (1H, *dd*, $J = 11$ and 0.8 Hz, H-15), 4.97 (1H, *dd*, $J = 18$ and 0.8 Hz, H-15), 6.01 (1H, *dd*, 18 and 11 Hz, H-14), 8.11 (1H, *s*, H-CO); EI-MS m/z (rel. int.): 334 $[\text{M}]^+$ (1), 319 (100), 316 (1), 301 (1), 289 (2), 273 (13), 255 (90), 236 (15), 218 (7), 203 (15), 201 (17), 190 (16).

Trachinodiol 18-palmitate (**11**). Obtained as a gum, $[\text{M} - \text{H}_2\text{O}]^+$ at m/z 524.4584. $\text{C}_{36}\text{H}_{60}\text{O}_2$ requires 524.4593; ^1H NMR (200 MHz): δ 0.79, 0.95 and 1.15 (each 3H, *s*), 1.25 (*s*, $n\text{CH}_2$), 3.48 and 4.03 (each 1H, *d*, $J = 11$ Hz, H-18), 3.51 (1H, *br s*, H-7); EI-MS m/z (rel. int.): 524 $[\text{M} - \text{H}_2\text{O}]^+$ (16), 496 (1), 328 (2), 286 (5), 270 (10), 268 (100), 255 (44), 253 (49), 239 (35), 226 (29).

Trachinodiol 7 β -acetate-18-formate (**12**). Mp 154–156°; $[\text{M}]^+$ at m/z 374.2448. $\text{C}_{23}\text{H}_{34}\text{O}_4$ requires 374.2457; ^1H NMR (200 MHz): δ 0.80, 0.98 and 1.12 (each 3H, *s*), 2.02 (3H, *s*), 3.67 and 3.85 (1H, *d*, $J = 11$ Hz, H-18), 4.72 (1H, *br s*, H-7), 8.05 (1H, *s*, H-CO); EI-MS m/z (rel. int.): 374 $[\text{M}]^+$ (5), 314 (100), 299 (16), 268 (11), 255 (30), 239 (16), 227 (4), 225 (6), 211 (9), 199 (14).

Sicanadiol 7 β -acetate (**15**). Mp 166–168°; $[\text{M} - \text{C}_2\text{H}_4\text{O}_2]^+$ at m/z 286.2280. $\text{C}_{20}\text{H}_{30}\text{O}$ requires 286.2296; ^1H NMR (200 MHz): δ 0.78 (6H, *s*), 0.94 and 1.33 (each 3H, *s*), 2.04 (3H, *s*), 2.22 (1H, *dd*, $J = 7$ and 4 Hz, H-13), 4.89 (1H, *br s*, H-7), 5.51 (1H, *dd*, $J = 10$ and 4 Hz, H-12), 5.90 (1H, *dd*, $J = 10$ and 7 Hz, H-11); EI-MS m/z (rel. int.): 286 $[\text{M} - \text{C}_2\text{H}_4\text{O}_2]^+$ (56), 270 (7), 255 (6), 231 (6), 228 (100), 213 (40), 199 (6).

Trachinol acetate (**5**). Mp 141–145°; $[\text{M}]^+$ at m/z 330.2540. $\text{C}_{22}\text{H}_{34}\text{O}_2$ requires 330.2558; ^1H NMR (200 MHz): δ 0.74, 0.76, 0.93 and 1.12 (each 3H, *s*), 2.03 (3H, *s*), 4.71 (1H, *br s*, H-7); EI-MS m/z (rel. int.): 330 $[\text{M}]^+$ (2), 270 (28), 255 (11), 242 (1), 227 (3), 214 (3), 199 (6).

ent-3 β -Acetoxy-pimara-8(14),15-diene (**20**). Obtained as a gum, $[\text{M}]^+$ at m/z 330.2544. $\text{C}_{22}\text{H}_{34}\text{O}_2$ requires 330.2559; ^1H NMR (200 MHz): δ 0.76 (3H, *s*), 0.89 (6H, *s*), 0.99 (3H, *s*), 2.05 (3H, *s*), 4.52 (1H, *dd*, $J = 11$ and 4 Hz, H-3), 4.90 (1H, *dd*, $J = 17$ and 2 Hz, H-16), 4.94 (1H, *dd*, 11 and 2 Hz, H-16), 5.15 (1H, *br s*, H-14), 5.71 (1H, *dd*, 17 and 11 Hz, H-15); EI-MS m/z (rel. int.): 330 $[\text{M}]^+$ (19), 315 (12), 270 (23), 255 (30), 227 (9), 213 (2), 199 (3).

Formylation of ribenol. A soln of **8** (10 mg) in CHCl_3 (2 ml) was treated with HCO_2H (3 ml) and left with stirring for 24 hr. Usual work-up and chromatography of the residue afforded **7** (8 mg), identical with the natural compound. The same procedure was used for the formylation of trachinodiol 7 β -monoacetate (**14**).

Acknowledgements—We thank M. Fernández Galván (C.I.T.A., Tenerife) for classifying and gathering the plant material, and the CICYT (Madrid) for financial support to the project PB87-0255.

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