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DITERPENES FROM THE FLOWERS OF *SALVIA CANARIENSIS*

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Key Word Index—*Salvia canariensis*; Labiatae; flowers; diterpenes.

Abstract—The new diterpenes, 11,12-dimethoxy-abieta-6,8,11,13-tetraen-20-oic acid methyl ester, rosmanoyl carnosate, 7-oxocarnosic acid, 6-oxo-7β-hydroxycarnosic acid and 6-oxo-7α-hydroxycarnosic acid, together with the already known rosmanol, galdosol, isorol, carnosol and carnosic acid were isolated from the flowers of *Salvia canariensis*.

INTRODUCTION

We have shown that *Salvia canariensis* L., a plant endemic to the Canary Islands, contains the diterpenes galdosol [1] and arucatriol [2] in its aerial parts, deoxocarnosol-12-methyl ether, salvicanol and 6α-hydroxydemethylcryptojaponol in its roots [3] and rosmanol in its flowers [4]. Five new diterpenes have now been isolated in acetate form from the flowers of this plant.

RESULTS AND DISCUSSION

The least polar of the new natural compounds was identified as 11,12-dimethoxy-abieta-6,8,11,13-tetraen-20-oic acid methyl ester (**9**) in accordance with the following data. Its IR spectrum showed absorptions for ester, aromatic and ether groupings while in its ^{1}H NMR spectrum there were signals typical of an aromatic isopropyl, two methoxyls, a carbomethoxy, two angular methyls, an aromatic proton (δ6.67) and a double bond as an ABX system. The chemical shift of the A and B protons (δ6.01 and 6.35) indicated a C-6(C-7) double bond allylic to the aromatic ring with the X part of the system assigned to the H-5 proton on the basis of a double resonance experiment. These data, plus the molecular ion at *m/z* 372, were in accordance with structure **9** which had been assigned earlier to a minor product from carnosol treated with dimethylsulphate in acetone [5]. This would appear to be the first time that this product has been obtained from a natural source.

The second new compound was assigned structure **10** on the basis of the following considerations. On TLC it showed one spot only and was homogeneous by GC. Its molecular ion was at *m/z* 828 and its IR showed bands characteristic of an aromatic group, a γ-lactone (1770 cm^{-1}), aromatic esters (1750 cm^{-1}) and an aliphatic ester. The ^{1}H NMR data indicated a 1:1 mixture of two aromatic diterpenes with signals for four angular methyls, two of which (δ0.41 and 0.70) displayed an unusually high chemical shift, two aromatic isopropyl groups, four aromatic acetates and two aromatic protons. A noteable feature was the presence in the spectrum of a pair of doublets (J = 3.2 Hz) centred at δ4.23 and 5.97. The form and chemical shift of these signals were very similar to those of the H-6 and H-7 protons, respectively, in the ^{1}H NMR spectrum of rosmanol triacetate (**1**) [4], in which the chemical shift of the H-6 signal was slightly lower. Another interesting feature was the absence of the signal corresponding to the methyl of the aliphatic acetate group of rosmanol triacetate (**1**) and of any other signal assignable to a $-CH_2-\overset{O}{\overset{\|}{C}}-O-$ grouping, indicating that in **10** the aliphatic alcohol group of rosmanol was esterified with the carbonyl group attached to a bridge-head carbon which, given the duplication of signals in the ^{1}H NMR of **10**, must also belong to a diterpene acid of the abieta-8,11,13-triene series with two acetoxy aromatic groups. The MS of **10** and the chemical shift at δ6.88 of one of the aromatic protons suggested it was carnosic acid diacetate [6]. The ^{13}C NMR data of **10** were in accordance with the data set out above.

A more detailed analysis of the ^{1}H NMR of **10** confirmed the above and showed that the conformation of the molecule in solution was as proposed; hence the chemical shifts of the C-18 and C-19 methyl groups moved from δ0.90 and 0.97, respectively, in rosmanol triacetate (**1**) to δ0.70 and 0.41 in **10**. The H-5 proton appeared at δ2.30 in **1** and at δ1.92 in **10** and the H-6 proton which had been at δ4.61 in **1** appeared at δ4.22 in **10**. The higher chemical shifts of these protons in the ^{1}H NMR of **10** may be attributed to the shielding of the carnosic acid portion of the molecule by the aromatic ring.

On the other hand, the C-18′ methyl group showed practically the same chemical shift in **10** as in carnosic acid diacetate (**3**) while C-19′ methyl group was higher (δ0.77) in **10** than the corresponding C-19 methyl group (δ0.85) in **3** which is due to the greater shielding of the C-19′ methyl group by the aromatic ring of the rosmanol portion of the molecule.

The lower resonance of the H-14 proton in **10** compared to rosmanol acetate **1** is also compatible with the greater influence of the carbonyl group of the ester bridge in that conformation.

Methylation and rechromatography of several frac-

tions containing carnosic acid diacetate (3) as the major component afforded three more new diterpenes as diacetate methyl esters. The third new diterpene, with molecular formula $C_{25}H_{32}O_7$, showed IR absorptions characteristic of an aromatic function and of carbonyl groups, one of which was conjugated (1680 cm^{-1}). The ^1H NMR spectrum had signals for two aromatic acetates, two angular methyls, a carbomethoxy and an isopropyl group, which had to be attached to an aromatic ring because a multiplet at δ2.93 could be assigned to the hydrogen of the isopropyl function. Another signal in the spectrum was that of an aromatic hydrogen at δ8.06 (*s*). The chemical shift of this proton was typical for H-14 when there is a carbonyl group on C-7. All these data are in accord with the structure (4) assigned to this compound.

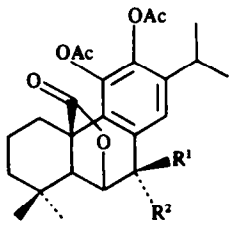
Chemical proof of structure 4 was provided by the fact that oxidation of carnosic acid diacetate methyl ester (3) with chromic anhydride in acetic acid afforded 4.

The fourth and fifth new diterpenes were isolated as a mixture of diacetate methyl esters which gave one spot only on TLC and showed bands in the IR characteristic of a hydroxyl, an aromatic function and aromatic and aliphatic ester groups.

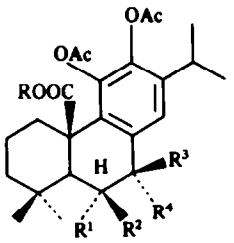
The ^1H NMR spectrum clearly showed the mixture to be formed by two aromatic diterpenes of the abieta-8,11,13-triene series, in not quite equal amounts. Signals corresponding to angular methyls, isopropyl, aromatic acetate and carbomethoxy groups and to aromatic protons were very close in both products, indicating two very similar structures, the most important difference being the chemical shifts of two protons geminal to secondary alcohol groups, one of which appeared at δ4.89 in one compound and at δ5.06 in the other.

The chemical shift of the H-14 aromatic proton which was at δ7.27 in one of the diterpenes and δ7.31 in the other indicated a hydroxyl group at C-7 in both.

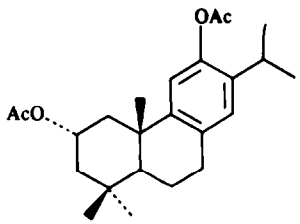
On the other hand, the low chemical shifts and the form of the signals corresponding to the geminal protons to these alcohol groups and the chemical shift of the H-5 protons which appear superimposed as a broad singlet at δ2.53 were indicative of the presence in both compounds



1 R^1 = H, R^2 = OAc
2 R^1, R^2 = O



3 R^1 = R^2 = R^3 = R^4 = H, R = H
4 R^1 = R^2 = H, R^3, R^4 = O, R = Me
5 R^1, R^2 = O, R^3 = OH, R^4 = H, R = Me
6 R^1, R^2 = O, R^3 = H, R^4 = OH, R = Me



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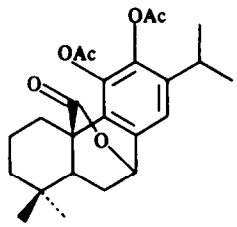

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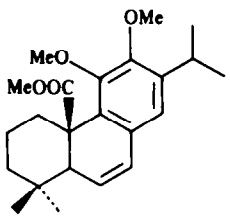

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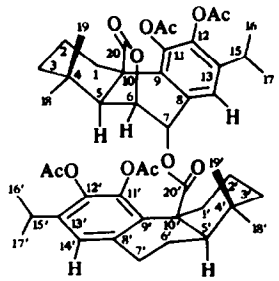

10

of a carbonyl group at C-6. This was supported by the three-bond coupling through a carbonyl group [7] between H-5 and H-7, demonstrated by a double resonance experiment.

The MS of the mixture showed, in addition to the same relevant fragments as the MS of **4**, fragments corresponding to the loss of 16 mass units from each compound.

All the above data agree with the epimeric structures **5** and **6** for the diacetate methyl esters of the two new natural diterpenes, 6-oxo-7β-hydroxycarnosic acid and 6-oxo-7α-hydroxycarnosic acid, respectively.

A noteable feature in the ^{1}H NMR of this mixture of diterpenes was a broad singlet at δ 7.91, interchangeable with deuterium oxide, which was attributed to the formation of a hydrogen bonding between the H-7 protons and the C-6 carbonyl group. As can be seen on Dreiding models, whether the C-7 hydroxyl group is axial or equatorial, the molecule can adopt the appropriate conformation to form such a hydrogen bonding. This feature explains the difficulty in acetylating the hydroxyl groups and the failure of 6-oxo-7β-hydroxycarnosic acid to form lactones.

The signals in the ^{1}H NMR spectrum were more intense in the compound which showed the higher chemical shift for the H-14 aromatic proton, indicating that the influence of the C-7 hydroxyl group thereon is weaker. On a Dreiding model this compound corresponds to **5** which is therefore the major diterpene in the mixture.

Five more compounds isolated in acetate form from the flowers of *Salvia canariensis* were identified with rosmanol (**1**) [4], galdosol (**2**) [1], isorol (**7**) [2], carnosol (**8**) [8] and carnosic acid (**3**) [6].

EXPERIMENTAL

Mps are uncorr.; ^{1}H and ^{13}C NMR: $CDCl_3$, 200 MHz, unless otherwise stated; IR: $CHCl_3$; Dry CC: silica gel (0.05–0.2 mm); Prep. TLC: pre-coated Scheleicher and Schüll plates. Voucher plant specimens were lodged with the Herbarium of the Department of Botany, Faculty of Biology, University of La Laguna.

The finely cut flowers of *S. canariensis* (4 kg) were extracted with Me_2CO (5 l) at room temp. for 4 weeks. Filtration and evapn of the solvent gave a reddish-brown extract (158 g). Dry CC of this extract was carried out using petrol–EtOAc mixtures as eluents, and 500 ml fractions were collected. Only fractions 158–194 (37 g) were studied and on TLC proved to be a mixture of compounds with very similar R_fs. The ^{1}H NMR spectrum was examined to check that there were no acetoxy groups in the compounds forming the mixture. The mixture was then acetylated and chromatographed, affording the new diterpenes as acetates.

11,12-Dimethoxy-abieta-6,8,11,13-tetraen-20-oic acid methyl ester (**9**). Obtained as a gum. IR ν_{max} cm^{-1}: 3000, 2960, 2930, 2870, 1710, 1460, 1450, 1445, 1430, 1410, 1390, 1375, 1330, 1300, 1220, 1140, 1115, 1090, 1070, 1050, 1000, 990, 940, 880; ^{1}H NMR: δ0.86, 1.00 (each 3H, *s*, –CH($\underline{CH}_3$)$_2$), 1.23, 1.26 (each 3H, *d*, J = 7Hz, –CH($C\underline{H}_3$)$_2$, 2.54 (1H, *t*, J = 3 Hz, H-5), 3.23 (1H, *m*, $W_{1/2}$ = 14.8 Hz, –$\underline{C}$H(CH$_3$)$_2$,), 3.52 (3H, *s*, $C\underline{H}_3$O–CO–), 3.74, 3.77 (each 3H, *s*, O$C\underline{H}_3$) 6.01 (1H, *dd*, J_1 = 9.70, J_2 = 3 Hz, H-6), 6.60 (1H, *dd*, J_1 = 9.70, J_2 = 3 Hz, H-7), 6.67 (1H, *s*, H-14); EIMS *m/z* (rel. int.): 372 [M]$^+$ (60), 357(2), 327 (6), 313 (94), 312 (24), 290 (11), 271 (75), 270 (14), 256 (35), 243 (100), 228 (36), 226 (13), 213 (13), 201 (50), 183 (11), 165 (12), 141 (11).

Rosmanoyl-carnosate tetraacetate (**10**). IR ν_{max} cm^{-1}: 3010, 2960, 2910, 2860, 1770, 1750, 1720, 1460, 1365, 1195, 1180, 1140, 1110, 1070, 1040, 1020, 970, 875; ^{1}H NMR: δ0.41, 0.70 (each 3H, *s*, $C\underline{H}_3$-18, C$\underline{H}$-19), 0.77, 0.92 (each 3H, *s*, $C\underline{H}_3$-18', $C\underline{H}_3$-19'), 1.08 (3H, *d*, J = 7Hz, –CH($\underline{CH}_3$)$_2$), 1.15 (3H, *d*, J = 7 Hz, –C$\underline{H}$(C$\underline{H}_3$)$_2$), 1.16, 1.16 (each 3H, *d*, J = 7 Hz, –C'H($C\underline{H}_3$)$_2$), 1.92 (1H, *s*, H-5'), 2.26, 2.27 (each 3H, *s*, Ar-O$\underline{A}$c), 2.29, 2.31 (each 3H, *s*, Ar'-O$\underline{A}$C), 2.85 (2H, *m*, $W_{1/2}$ = 15.4 Hz, 2-C$\underline{H}$(CH$_3$)$_2$), 4.23 (1H, *d*, J = 3.2 Hz, H-6'), 5.97 (1H, *d*, J = 3.2 Hz, H-7') 6.88 (1H, *s*, H-14) and 7.33 (1H, *s*, H-14'); ^{13}C NMR (50 MHz): δ 18.86 (*t*), 19.08 (*t*), 20.05 (*t*), 20.54 (*q*), 20.88 (*q*), 21.01 (*q*), 21.78 (*q*), 22.18 (*q*), 22.40 (*q*), 22.82 (*q*), 23.25 (*q*), 26.67 (*d*), 27.51 (*q*), 27.95 (*q*), 29.87 (*q*), 31.27 (*q*), 31.74 (*d*), 32.44 (*t*), 34.43 (*s*), 35.66 (*t*), 37.97 (*t*), 41.47 (*t*), 46.80 (*s*), 46.98 (*s*), 49.14 (*s*), 51.44 (*d*), 54.25 (*d*), 69.62 (*d*), 75.08 (*d*), 125.81 (*d*), 128.47 (*d*), 128.99 (*s*), 131.06 (*s*), 131.52 (*s*), 131.80 (*s*), 136.85 (*s*), 140.35 (*s*), 140.68 (*s*), 141.51 (*s*), 141.70 (*s*), 142.39 (*s*), 168.32 (*s*), 168.63 (*s*), 168.70 (*s*), 174.99 (*s*), 176.23 (*s*), 178.06 (*s*); EIMS *m/z* (rel. int.): 828 [M]$^+$, 787 (1), 768 (2), 744 (4), 726 (2), 388 (5), 346 (5), 342 (8), 329 (15), 328 (35), 326 (12), 313 (6), 300 (25), 284 (76), 279 (67), 271 (22), 270 (13), 269 (34), 261 (7), 243 (21), 239 (7), 230 (38), 228 (32), 218 (20), 215 (50), 213 (17), 204 (15), 203 (11), 185 (9), 167 (85), 150 (53), 149 (100), 121 (14), 115 (12), 112 (31).

7-Oxo-carnosic acid diacetate (**4**). Purified as its Me ester. [M]$^+$ at *m/z* 444.2143 (calc. for $C_{25}H_{32}O_7$, 444.2141); IR ν_{max} cm^{-1}: 3000, 2920, 2840, 1770, 1760, 1720, 1680, 1365, 1240, 1200, 1040; ^{1}H NMR: δ0.80, 0.95 (each 3H, *s*, $C\underline{H}_3$-18, $C\underline{H}_3$-19), 1.17, 1.27 (each 3H, *d*, J = 7 Hz, –CH($C\underline{H}_3$)$_2$), 2.15 (1H, *dd*, J_1 = 15.1, J_2 = 3.6 Hz, H-6α), 2.29, 2.30 (each 3H, *s*, 2Ar-O$\underline{A}$c), 2.71 (1H, *dd*, J = 17.3, J_2 = 3.6 Hz, H-6β), 2.93 (1H, *m*, $W_{1/2}$ = 14.8 Hz, –C$\underline{H}$(CH$_3$)$_2$), 3.31 (1H, *dd*, $J_{1\beta}$ = 17.3 Hz, $J_{2\alpha}$ = 15.1 Hz), 3.54 (3H, *s*, COOC$\underline{H}_3$), 8.06 (1H, *s*, H-14); EIMS *m/z* (rel. int.): 444 [M]$^+$ (12), 402 (25), 360 (58), 300 (100), 231 (33), 149 (28), 43 (84).

Oxidation of carnosic acid diacetate methyl ester with CrO_3–HOAc. Carnosic acid diacetate (**3**), 58.1 mg dissolved in Et_2O, was treated with CH_2N_2 in excess to give 59 mg of carnosic acid diacetate methyl ester (after evapn of the solvent) mp, ^{1}H NMR and IR superimposable upon those given in ref. [6]. This was then dissolved in HOAc (1.2 ml) and treated with CrO_3 (25.2 mg). The mixture was stirred at room temp. for 24 hr, poured into H_2O and extracted with Et_2O as usual, to give a product identical to **4** (R_f, ^{1}H NMR, IR).

6-Oxo-7β-hydroxycarnosic acid diacetate (**5**). Isolated with **6** in a mixture, as a methyl ester. IR ν_{max} cm^{-1}: 3670, 3010, 2960, 2930, 2870, 1770, 1765, 1715, 1710, 1600, 1360, 1270, 1200, 1175, 1125, 1040, 1000, 970; ^{1}H NMR: δ0.76, 0.98 (each 3H, *s*, $C\underline{H}_3$-18, $C\underline{H}_3$-19), 1.14, 1.24 (each 3H, *d*, J = 7 Hz, –CH($C\underline{H}_3$)$_2$), 2.25, 2.26 (each 3H, *s*, ArO$\underline{A}$c), 2.53 (1H, *s*(*br*), H-5), 2.92 (1H, *m*, $W_{1/2}$ = 14.8 Hz, –C$\underline{H}$(CH$_3$)$_2$), 3.50 (3H, *s*, COOC$\underline{H}_3$), 5.06 (1H, (*br*), H-7), 7.27 (1H, *s*, H-14) and 7.91 (1H, *s*, OH-7); EIMS *m/z* (rel. int.): 444 [M—16]$^+$ (5), 402 (11), 386 (19), 360 (26), 344 (15), 327 (9), 300 (50), 285 (16), 278 (11), 231 (23), 215 (16), 194 (20), 149 (15).

6-Oxo-7α-hydroxycarnosic acid diacetate (**6**). Isolated together with **5** as a methyl ester. ^{1}H NMR: δ0.75, 1.02 (each 3H, *s*, $C\underline{H}_3$-18, $C\underline{H}_3$-19), 1.16, 1.24 (each 3H, *d*, J = 7 Hz, –CH($C\underline{H}_3$)$_2$), 2.25, 2.26 (each 3H, *s*, ArO$\underline{A}$c), 2.53 (1H, *s*(*br*), H-5), 2.92 (1H, *m*, $W_{1/2}$ = 14.8 Hz, –C$\underline{H}$(C$\underline{H}_3$)$_2$), 3.49 (3H, *s*, –COO$\underline{CH}_3$), 4.89 (1H, *s*,(*br*), H-7), 7.31 (1H, *s*, H-14), 7.91 (1H, *s*, OH-7).

Rosmanol triacetate (**1**). Mp 215–217°. [M]$^+$ at *m/z* 472.2082 (calc. for $C_{26}H_{32}O_8$, 472.2069); IR ν_{max} cm^{-1}: 3025, 2960, 2870, 1770, 1740, 1370, 1200, 1140, 1110, 1070, 1040, 1020, 970, 912, 880; ^{1}H NMR: δ0.90, 0.97 (each 3H, *s*, $C\underline{H}_3$-18, $C\underline{H}_3$-19), 1.15, 1.18 (each 3H, *d*, J = 7 Hz, –CH($C\underline{H}_3$)$_2$), 2.14 (3H, *s*, –OCO–$C\underline{H}_3$), 2.28, 2.1 (each 3H, *s*, 2ArO$\underline{A}$c), 2.86 2H, *m*, $W_{1/2}$ = 20.5 Hz), 4.61 (1H, *d*, J = 3.42 Hz, H-6), 5.95 (1H, *d*, J = 3.42 Hz, H-7), 7.16

(1H, *s*, H-14); EIMS *m/z* (rel. int.): 472 $[M]^+$ (4), 430 (2), 388 (49), 346 (42), 342 (19), 300 (34), 284 (17), 231 (12), 215 (17).

Carnosic acid diacetate (3). Mp 204–206°; $[M-(CH_2CO)-(HCOOH)]^+$ at 328.2027 (calc. for $C_{21}H_{28}O_3$, 328.2018); IR ν_{max} cm^{-1}: 3700 → 2750, 3010, 2960, 2920, 2870, 2840, 1770, 1760, 1725, 1690, 1600, 1370, 1200, 1190, 1180, 880; ^1H NMR: δ0.85, 0.95 (each 3H, *s*, C$\underline{H}_3$-18, C$\underline{H}_3$-19), 1.13, 1.20 (each 3H, *d*, J = 7 Hz, -CH(C$\underline{H}_3$)$_2$), 2.24, 2.26 (each 3H, *s*, ArO$\underline{Ac}$), 2.88 (2H, *m*, $W_{1/2}$ = 17.5 Hz), 3.20 (1H, *m*, $W_{1/2}$ = 22.5 Hz, H-1β), 6.95 (1H, *s*, H-14); EIMS *m/z* (rel. int.): 416 $[M]^+$ (0.8), 374 (0.21), 370 (1.3), 356 (5), 328 (66), 287 (39), 286 (100), 285 (8), 271 (15), 243 (21), 230 (40), 218 (25), 215 (17), 204 (17), 203 (7), 191 (7), 129 (7), 128 (7), 115 (8).

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