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NEO-CLERODANE DITERPENOIDS AND OTHER CONSTITUENTS FROM *BACCHARIS* SPECIES

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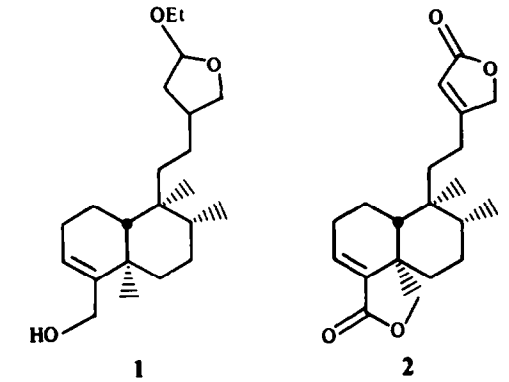
Key Word Index—*Baccharis pedicellata*; *Baccharis marginalis*; Compositae, Astereae; diterpenes; clerodanes.

Abstract—A new *neo*-clerodane dilactone, desoxyarticulin, and dihydrotucumanoic acid, together with other known compounds, were isolated from *Baccharis pedicellata* and *Baccharis marginalis*. The structures of the new compounds were elucidated by spectroscopic methods.

INTRODUCTION

In continuation of our chemical studies of the genus *Baccharis* [1] we analysed *B. marginalis* and *B. pedicellata* from northern and southern Chile. Besides the known diterpene (**1**) previously isolated from *B. rhomboidalis* [2], the aereal parts of *B. pedicellata* afforded scopoletin, oleanolic acid, erythrodiol, 3,7-dimethylkaempferol, genkwanin, apigenin and a new clerodane dilactone **2** which corresponded to desoxyarticulin. In addition to eriodictyol 7-methyl ether, a new *neo*-clerodane diterpenoid **3** was also isolated from *B. marginalis*. The known compounds were identified by direct comparison with authentic samples and the structures of the new compounds were established by spectroscopic methods.



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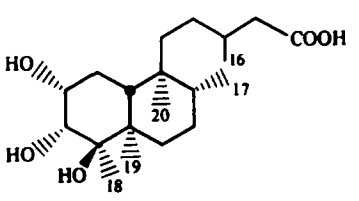

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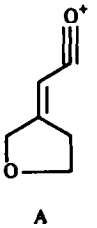

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4 $\Delta^{13,14}$

RESULTS AND DISCUSSION

The IR spectrum of desoxyarticulyn (**2**), $C_{20}H_{26}O_4$ (HRMS), indicated the presence of two unsaturated lactone groups and an olefinic double bond (see Experimental). Its ^{1}H NMR spectrum showed typical signals for a β-substituted γ-butenolide (a two-proton doublet at δ4.74 coupled to a triplet at 5.85) as well as signals corresponding to an olefinic β-proton of an α,β-unsaturated lactone group at 6.74, tertiary and a secondary methyl groups at 0.64 and 0.84, respectively, and an AB system at 3.92 and 4.28 which can be assigned to the oxymethylene protons of the remaining lactone group.

Comparison of this data with that of closely related clerodane dilactones previously isolated from other *Baccharis* species [2–6] suggested the structure **2** for this new diterpenoid.

The structure and relative configuration of **2** were ascertained by its ^{13}C NMR spectrum which exhibited chemical shifts for the tertiary and secondary methyl groups at C-9 and C-8, respectively, in agreement with the data of compounds having both of these substituents as alpha on a *trans*-clerodane skeleton [1, 2, 7, 8]. This was further confirmed by the *W*-coupling shown by the '*endo*' H-19 in **2** (δ3.92, *dd*, J = 8.1 and 1.8 Hz) indicating an α-axial configuration of the C-19 methylene group in agreement with *trans*-clerodanes having an axial methyl group at C-5 and an axial H-6 [1, 2, 9].

A distinct feature in the mass spectrum of **2** was the base peak at m/z 111, which corresponds to $C_6H_7O_2^+$ (A). The formation of this ion, which originated from the splitting of the side chain, is diagnostic of clerodane diterpenoids with a β-substituted γ-butenolide such as articulin B [6] or deoxymarrubialactone [10]. Compound **1**, a mixture of epimers, was identical to an acetal previously isolated from *B. rhomboidalis* [2] except that **1** possessed an ethoxy instead of a methoxy group at C-15. These compounds are artefacts from the extraction procedure.

The structural elucidation of the diterpenic acid **3** isolated from *B. marginalis* was initially hampered by conflicting mass spectral data which showed ions at either m/z 338, 321, 320 and/or 303 as likely $[M]^+$ values in several EIMS determinations. Eventually the composition of **3** as $C_{20}H_{36}O_5$ (m/z 356) was deduced from

CIMS (methane and/or ammonia) which showed strong ions at m/z 374 $[M+18]^+$, 357 $[M+1]^+$, 338, 321 and 303 indicating the presence of at least three hydroxyl groups.

The ^{13}C NMR spectrum of compound **3** showed the presence of two secondary and one tertiary hydroxyl groups at δ68.4, 77.9 and 76.2, respectively. A signal at δ174.7 assigned to an unconjugated carboxyl group, together with the remaining 16 carbon signals, confirmed the molecular composition.

The ^1H NMR spectrum of compound **3** showed signals for two secondary and three tertiary methyl groups of a clerodane skeleton at δ0.76, 0.96, 0.71, 1.04 and 1.22, respectively. The two protons geminal to the secondary hydroxyl groups absorbed at δ3.98 (m, $W_{1/2}$ = 17 Hz) and 3.50 (d, J = 3.5 Hz). In addition, the ^1H NMR spectrum of **3** showed two one-proton double doublets at δ2.26 (J = 14.8 and 6.1 Hz) and 2.09 (J = 14.8 and 7.8 Hz) which can be assigned to the C-14 methylene protons.

Comparison of the combined spectroscopic data of compound **3** with that of tucumanoic acid (**4**), a clerodane terpenoid previously isolated from *Baccharis tucumanensis* [11], showed significant differences only for the signals corresponding to the side chain. Based on this close similarity of the ^1H and ^{13}C NMR spectral data, we conclude that compound **3** represents a clerodane diterpenoid with configurations at C-2/C-5 and C-8/C-10 as in tucumanoic acid. Therefore, we propose stereostructure **3** for this new diterpenoid, namely dihydrotucmanoic acid. The configuration at C-13 was not determined with the available data.

The absolute configuration of compounds **2** and **3** was not established but it very probably corresponds to that shown in the formulae in keeping with their negative molecular rotation values as also shown by related neoclerodanes of known absolute configuration isolated so far from *Baccharis* [2, 4, 9].

Two flavanones, sakuranetin [12] and eriodictyol 7-methylether [13] were also isolated from *B. marginalis* and their structures were confirmed by comparison of their spectroscopic data with those reported in the literature including the hitherto unreported ^{13}C NMR data of eriodictyol 7-methylether. This last compound, which until recently was reported as a 'rare' flavanona [14–16], has in fact been detected in several genera of the Compositae [Wollenweber, E., personal communication].

EXPERIMENTAL

Mps: uncorr; ^1H NMR: 500 MHz in $CDCl_3$ with TMS as int. standard; ^{13}C NMR: 100 MHz, $CDCl_3$ with TMS as int. standard; IR: KBr pellets; MS: inlet, 70 eV.

Baccharis pedicellata DC and *B. marginalis* DC were collected in December in Liquiñe (Valdivia) and in Fray Jorge (La Serena), respectively. Voucher specimens are kept at the Herbarium (M.M.), Facultad de Ciencias. Universidad de Chile.

The aereal parts of *B. pedicellata* (3 kg) were percolated at room temp. with 95% EtOH (3 × 15 l.) for 48 hr. The EtOH extract (525 g) was first partitioned between $CHCl_3$ and MeOH–H_2O (1:9), then the $CHCl_3$ solubles were partitioned between petrol and MeOH–H_2O (9:1) to give 20 g aq. MeOH solubles. This extract was first fractionated by flash CC on silica gel eluted with mixtures of increasing polarity of petrol and EtOAc.

The aereal parts of *B. marginalis* (3 kg) were extracted at room temp. with CH_2Cl_2 for 12 hr, affording 390 g of a clear syrup. This crude material (100 g) was chromatographed on a silica gel column (400 g) and eluted with mixtures of petrol and EtOAc of increasing polarity. Fractions of 100 ml were taken and combined based upon TLC monitoring. Repeated CC on silica gel of suitable fractions afforded pure compounds.

Compound **1**. Oil (1 g). IR ν_{max}^{film} cm^{-1}: 3400, 2925, 1650, 1440, 1370. ^1H NMR (60 MHz, $CDCl_3$): δ0.70 (s, H-20), 0.77 (d, J = 5 Hz, H-17), 1.07 (s, H-19), 1.1 (t, J = 8 Hz, –OCH$_2$Me), 3.25–3.99 (m, 4 H), 4.0 (*br s* H-18), 5.09 and 5.16 (*br*, *s* H-15), 5.56 (m, H-3). ^{13}C NMR (62.5 MHz, $CDCl_3$, multiplicities obtained by DEPT method): δ147.9 (s, C-4), 122.0 (d, C-3), 104.4 and 104.0 (d, C-15), 72.7 and 72.0 (t, C-16), 63.2 and 62.9 (t, –O<u>C</u>H$_2$Me), 62.7 (t, C-18), 46.2 (d, C-10), 39.4 (t, C-14), 38.6 (s, C-9), 37.7 (t, C-11), 37.3 (s, C-5), 37.1 (t, C-6), 36.3 (d, C-13), 36.2 (d, C-8), 27.3 (t, C-12), 26.6 (t, C-2), 26.3 (t, C-7), 21.4 (q, C-19), 18.3 (t, C-1), 18.2 (q, C-17), 16.0 and 15.9 (q, C-20), 15.4 and 15.3 (q, O–CH$_2$–<u>M</u>e). EIMS (70 eV) m/z (rel. int.): 332 $[M-H_2O]^+$ (0.6), 304 $[M-EtOH]^+$ (3), 286 $[M-H_2O-EOH]^+$ (15), 189 (100).

Desoxyarticulin (**2**). 0.75 g, mp 130–132° (Et_2O). IR ν_{max}^{KBr} cm^{-1}: 2930, 1765, 1735, 1620, 990, $[\alpha]_D^{25}$-146.4°, $[\alpha]_{546}$-177.3°, $[\alpha]_{436}$-322.6°, $[\alpha]_{365}$-608.7°. ($CHCl_3$, c = 1.1), UV λ_{max}^{MeOH} (ε) 218 nm (11 817). ^1H NMR (500 MHz, $CDCl_3$): δ0.64 (s, H-20), 0.84 (d, J = 6.1 Hz, H-17), 3.92 (dd, J = 8.1 Hz, H-19), 4.28 (d, J = 8.1 Hz, H-19'), 4.74 (d, J = 1.6 Hz, H-16), 5.85 (t, J = 1.6 Hz, H-14), 6.74 (dd, J = 7.3, 2.0 Hz, H-3). ^{13}C NMR (100 MHz, $CDCl_3$, multiplicities obtained by DEPT method): δ173.3 (s, C-15), 170.0 (s, C-13), 169.1 (s, C-18), 138.4 (s, C-4), 135.4 (d, C-3), 115.2 (d, C-14), 73.0 (t, C-19), 71.6 (t, C-16), 48.0 (d, C-10), 45.4 (s, C-5), 38.7 (s, C-9), 36.5 (d, C-8), 34.4 (t, C-11), 34.3 (t, C-6), 27.6 (t, C-7), 27.6 (t, C-2), 22.0 (t, C-12), 19.6 (t, C-1), 17.5 (q, C-20), 15.5 (q, C-17). EIMS (70 eV) m/z (rel. int.): 330.1849 $[M]^+$ (calc. for $C_{20}H_{26}O_4$: 330.1830) (19), 300 $[M-CH_2O]^+$ (51), 220.1466 (calc. for $C_{14}H_{20}O_2$: 220.1462) (30), 219 (19), 189 (16), 187.1145 (calc. for $C_{13}H_{15}O$: 187.1122) (45), 161 (34), 111.0469 (calc. for $C_6H_7O_2$: 111.0446) (100), 105 (60).

Scopoletin, oleanolic acid and erythrodiol were identified by comparison with authentic samples. 3,7-Dimethylkaempferol, 3-methylkaempferol, genkwanin, apigenin were identified by comparison of their spectroscopic properties with those reported in the lit.

Dihydrotucumanoic acid (**3**). 5.5 g, mp 190–191° (Me_2CO). $[\alpha]_{589}^{25}$-16.0°, $[\alpha]_{578}$-17.0°, $[\alpha]_{546}$-19.0°, $[\alpha]_{436}$-12.0°, $[\alpha]_{365}$-13.0° (MeOH; c 0.1). IR ν_{max}^{KBr} cm^{-1}: 3500, 3300, 3000–2850, 1700, 1150. ^1H NMR (500 MHz, $CDCl_3$–DMSO-d_6): δ0.71 (s, H-20), 0.76 (d, J = 5.7 Hz, H-17), 0.96 (d, J = 7.6 Hz, H-16), 1.04 (s, H-19), 1.22 (s, H-18), 1.55 (*br t*, 1 H), 1.70–1.85 (m, 2 H), 2.10 (dd, J = 14.8, 7.8 Hz, H-14), 2.25 (dd, J = 14.8, 6.0 Hz, H-14'), 3.50 (d, J = 3.5 Hz, H-3), 3.94 (m, $W_{1/2}$ = 17 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$–DMSO-d_6, multiplicities obtained by DEPT method): δ174.7 (s, C-15), 77.9 (d, C-3), 76.2 (s, C-4), 68.4 (d, C-2), 41.3 (t, C-14), 40.5 (s, C-5), 37.9 (s, C-9), 37.9 (d, C-10), 35.7 (d, C-8), 35.6 (t, C-11), 31.4 (t, C-12), 30.5 (d, C-13), 29.0 (t, C-6), 26.2 (t, C-1), 25.3 (t, C-7), 21.1 (q, C-18), 19.6 (q, C-19), 18.1 (q, C-16), 16.7 (a, C-20) and 15.9 (q, C-17). EIMS m/z (rel. int.): 338 $[M-18]^+$ (13), 320 (11), 303 (8), 241 (18), 223 (31), 205 (44), 137 (68), 123 (100), 109 (43). CIMS (ammonia): 374 $[M+18]^+$ (100), 357 $[M+1]^+$ (32), 338 $[M-H_2O]^+$ (36), 320 (28), 303 (31), 258 (18), 223 (21), 123 (18).

Eriodictyol 7-methylether. 8.5 g ^{13}C NMR (100 MHz, $CDCl_3$, multiplicities obtained by DEPT method: δ196.2 (C-4), 167.9 (C-8a), 167.8 (C-7), 164.0 (C-5), 145.3 (C-3'), 145.3 (C-4'), 129.9 (C-1'), 118.1 (C-6'), 115.5 (C-5'), 115.4 (C-2'), 103.0 (C-4a), 94.8 (C-8), 94.0 (C-6), 79.2 (C-2), 55.6 (OMe), 42.0 (C-3). Eriodictyol 7-methylether was further identified by direct comparison with an authentic sample (TLC, mp, UV, ^1H NMR and MS [13]).

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REFERENCES

1. Givovich, A, San-Martín, A. and Castillo, M. (1986) *Phytochemistry* **25**, 2829.
2. San-Martín, A., Rovirosa, J., Labbé, C., Givovich, A., Mahú, M. and Castillo, M. (1986) *Phytochemistry* **25**, 1893.
3. Bohlmann, F., Kramp, W., Grenz, M., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 1907.
4. Herz, W., Pilotti, A. M., Söderholm, A. C., Shuhama, I. K. and Vichnewski, W. (1977) *J. Org. Chem.* **42**, 3913.
5. Bohlmann, F., Kramp, W., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 1011.
6. Stapel, G., Menssen, H. G. and Snatzke, G. (1980) *Planta Med.* **38**, 366.
7. Luteijn, J. M., Van Veldhuizen, A. y de Groot, A. (1982) *Org. Magn Reson.* **19**, 95.
8. Sharma, S. C., Tandon, J. S., Porter, B., Raju, M. S. and Wenkert, E. (1984) *Phytochemistry* **23**, 1194.
9. Gambaro, V., Chamy, M. C., Garbarino, J. A., San-Martın, A. and Castillo, M. (1986) *Phytochemistry* **25**, 2175.
10. Popa, D. P., Salei, L. A. (1976) *Khim. Prir. Soedin.* **3**, 393; chem. abstracts (1976) **85** 124188s.
11. Rossomando, P. C., Giordano, O. S., Espiñeira, J. and Joseph-Nathan, P. (1985) *Phytochemistry* **24**, 787.
12. Assumpcao, R. M. V., Silva, K. S. M. and Gotlieb, O. R. (1968) *An. Acad. Brasil. Cienc.* **40**, 297.
13. Giordano, O. S. y Guerreiro, E. (1973) *An. Asoc. Quim. Argent.* **61**, 161.
14. Wollenweber, E. (1981) *Z. Naturforsch.* **36**, 604.
15. Proksch, P., Budzikiewicz, H., Tanowitz, B. D. and Smith, D. (1984) *Phytochemistry* **23**, 679.
16. Charle, W. D. and Wollenweber, E. (1985) *Phytochemistry* **24**, 1122.