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NEO-CLERODANE DITERPENOIDS FROM *BACCHARIS MACRAEI*

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Key Word Index—*Baccharis macraei*; Compositae; diterpenes; neo-clerodanes.

Abstract—Two new neo-clerodane diterpenes, hautriwaic acid acetate and 4β-hydroxyisobacchasmacranone, were isolated from the aerial parts of *Baccharis macraei*. The structures of the new compounds were elucidated by spectroscopic methods. The structure of hautriwaic acid acetate was confirmed by correlation with its known deacetyl derivative, hautriwaic acid.

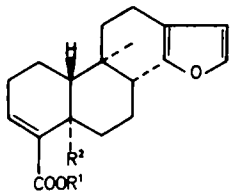
INTRODUCTION

A previous paper [1] described the characterizations of bacchasmacranone (**2**) and 2β-hydroxybacchasmacranone (**4a**), as well as the identification of other known neo-clerodane-type diterpenoids (**1a** and **5b**) from the dichloromethane extract of *Baccharis macraei*. The present paper describes the isolation and structure elucidation of two additional diterpenoids, hautriwaic acid acetate (**5a**) and 4β-hydroxyisobacchasmacranone (**3**), from *B. macraei*.

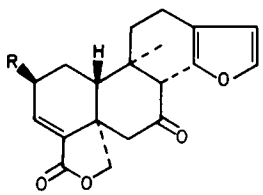
RESULTS AND DISCUSSION

The dichloromethane extract of the aerial parts of *B. macraei* was subjected to column chromatography on silica gel using increasing proportions of ethyl acetate in petrol to afford (−)-hardwickiic acid (**1a**) [1], bacchasmacranone (**2**) [1], hautriwaic acid acetate (**5a**), 4β-hydroxyisobacchasmacranone (**3**), 2β-hydroxybacchasmacranone (**4a**) [1] and (−)-hautriwaic acid (**5b**) [1].

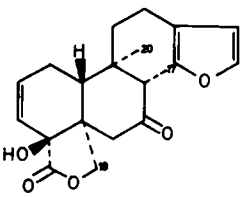
The new diterpenoid **5a** had the molecular formula $C_{22}H_{30}O_5$ by mass spectrometry ([M]$^+$ at *m/z* 374) and ^{13}C NMR, and its IR spectrum showed carboxylic, acetoxy and furanic group absorptions. The ^{1}H NMR spectrum indicated the presence of a β-substituted furan [δ7.35 *t* (*J* = 1.6 Hz), 7.20 *br s* and 6.24 *dd* (*J* = 1.0, 1.6 Hz)], a one-proton double doublet (*J* = 4.5 and 5.0 Hz) centred at δ7.07, which suggested an olefinic β-proton (H-3) of an α,β-unsaturated carboxyl group, and also showed a secondary and a tertiary methyl group at δ0.87 (*J* = 6.7 Hz) and 0.83, respectively, which are typical of clerodane diterpenes. Furthermore, a characteristic primary acetoxyl group [δ4.60 *d* (*J* = 11.5 Hz), 4.36 *d* (*J* = 11.5 Hz) AB system, and 2.00 *s* (3H)] was tentatively assigned to C-19 of **5a**. The assignments of the ^{13}C NMR spectral signals of **5a** (Table 1) were made on the basis of the observed multiplicities and comparison with reported ^{13}C NMR spectral data of similar derivatives **1a** [2] and **5b** [3]. The differences observed in chemical shift could be rationalized by considering the effects of the acetoxyl group. Therefore, placing the acetoxyl group at C-19, **5a** is shown to be hautriwaic acid acetate. In order to confirm the structure **5a** proposed for hautriwaic acid acetate, compound **5b** was converted into its acetyl derivative. The spectral and physical data of this compound were in full agreement with those of compound **5a**. Hautriwaic acid acetate (**5a**) was suggested to be present as the free acid in *Conyza scabrida*, since its methyl ester was found in an extract fraction after treatment with diazomethane [4]. However, this compound had not previously been isolated as a natural product.



	R¹	R²
1a	H	Me
1b	Me	Me
5a	H	CH_2OAc
5b	H	CH_2OH



2 R = H
4a R = OH
4b R = OAc



3

The IR spectrum of compound **3**, $C_{20}H_{24}O_5$ ([M]$^+$ at *m/z* 344), indicated the presence of a furan ring, an olefinic double bond, a lactone, a ketone and a hydroxyl group, which was considered to be tertiary because the compound could not be acetylated under standard conditions. The ^{1}H NMR spectrum of **3** showed the characteristic signals of a β-substituted furan, and a secondary and a

Table 1. ^{13}C NMR spectral data of compounds **5a**, **3** and **4a** (22.15 MHz, $CDCl_3$, TMS as internal standard)

C	5a	3	4a*
1	17.4	29.7	27.0
2	27.5	123.6	62.6
3	136.9	132.7	134.2
4	142.8	74.4	138.8
5	40.8	44.8	47.9
6	33.6	50.2	49.9
7	27.2	211.2	210.6
8	36.3	50.6	51.3
9	38.8	44.3	43.0
10	46.8	38.0	39.0
11	38.9	37.5	38.1
12	18.2	18.4	17.7
13	125.3	124.1	124.3
14	110.9	110.6	110.6
15	143.1	143.1	142.7
16	138.5	138.6	138.5
17	15.9	7.5	7.5
18	172.4	176.5	168.7
19	67.7	69.5	70.6
20	18.1	23.6	19.0
MeCO	170.7		
MeCO	20.9		

*These data had not been reported in ref. [1].

tertiary methyl group, typical of a clerodane-type diterpenoid (see Experimental). Furthermore, a doublet of a double doublet (J = 9.9, 5.5 and 2.2 Hz) centred at δ6.23 (1H) and a double triplet (J = 2.2 and 9.9 Hz) at δ5.67 (1H) were assigned to the C-2 and C-3 protons, respectively. These assignments are in agreement with those reported for teucrin F, a diterpene with an identical A-ring to that of compound **3** [5]. In fact, irradiation at δ6.23 removed the 9.9 Hz coupling from the signal at 5.67. On the other hand, a pair of doublets at δ4.05 and 4.13 (J = 11.5 Hz) indicated an oxygen-bearing methylene group, most likely part of a saturated 18,19-γ-lactone. The ^{1}H NMR spectrum also showed two one-proton doublets at δ2.96 (J = 13.0 Hz) and 2.27 (J = 13.0 Hz) and a one-proton quartet at δ2.80 (J = 6.6 Hz), which were attributed to the C-6β, C-6α and C-8β protons, respectively, on the basis of spin-decoupling experiments and by comparison with the corresponding values (^{1}H NMR and ^{13}C NMR) in the compounds **2**, **4a** and **4b**, diterpenoids with an identical B-ring to that of compound **3** [1]. Finally, the assignments of the remaining signals in the ^{13}C NMR spectrum of **3** (Table 1), especially those of the A-ring, were made by comparison with reported ^{13}C NMR spectral data of teucrin F [5], including the C-4β position of the tertiary hydroxyl group. The C-10 position for this group was unambiguously eliminated because no γ-effects were observed on C-8 and C-6 (see Dreidig molecular model). The observed values are completely in accord with the proposed structure, and compound **3** was thus identified as 4β-hydroxyisobacchasmacranone.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR: 400 MHz in $CDCl_3$ with TMS as internal standard. ^{13}C NMR: 22.15 MHz. Assignments of ^{13}C NMR chemical shifts were made with the aid of SFORD. IR: $CHCl_3$. MS: direct inlet, 70 eV.

Baccharis macraei Hook et Arn., collected in Concón, Viña del Mar, Chile in November 1985, was identified by Dr. Otto Zoellner, Universidad Católica de Valparaíso. A voucher specimen has been deposited at Universidad Federico Santa María.

The aerial parts of *B. macraei* (2.0 kg) were extracted at room temp., with CH_2Cl_2 for 6 hr, affording 125 g of a clear syrup. This crude material (40 g) was chromatographed on a silica gel column (1.0 kg) and eluted with mixtures of petrol and EtOAc of increasing polarity. Fractions of 250 ml were taken and combined based upon TLC monitoring, yielding the following compounds in order of elution: (−)-hardwickiic acid (**1a**, 650 mg), a mixture of oleanolic acid and bacchasmacranone (**2**), a mixture of hautriwaic acid acetate (**5a**) and 4β-hydroxyisobacchasmacranone (**3**), 2β-hydroxybacchasmacranone (**4a**, 2.4 g) and (−)-hautriwaic acid (**5b**, 320 mg). A portion of the mixture of oleanolic acid and 2β-hydroxybacchasmacranone (**2**) (500 mg), after treatment with CH_2N_2, was rechromatographed on a silica gel column (60 g) and eluted with petrol–EtOAc (3:1), yielding oleanolic acid methyl ester (115 mg) and **2** (220 mg). Finally, the mixture of **5a** and **3** (380 mg) was rechromatographed on a silica gel column (50 g) and eluted with petrol–EtOAc (7:3), yielding pure **5b** (80 mg) and **3** (40 mg). The known compounds (**1a**, **2**, **4a** and **5b**) were identified by direct comparison (TLC, $[\alpha]_D^{25}$, ^{1}H NMR, MS) with authentic samples.

Hautriwaic acid acetate (**5a**). Gummy; $[\alpha]_D^{25}$ − 85.0° ($CHCl_3$; *c* 0.90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3030, 2980–2860, 1740, 1695, 1640, 1510, 1480, 1390, 1260, 1040, 920, 880. ^{1}H NMR: δ7.35 (1H, *t*, J = 1.6 Hz, H-15), 7.20 (1H, *br s*, H-16), 7.07 (1H, *dd*, J = 4.5, 5.0 Hz, H-3), 6.24 (1H, *dd*, J = 1.0, 2.0 Hz, H-14), 4.60 (1H, *d*, J = 11.5 Hz, H-19), 4.36 (1H, *d*, J = 11.5, H-19'), 2.00 (3H, *s*, MeCO), 0.87 (3H, *d*, J = 6.7 Hz, H-17), 0.83 (3H, *s*, H-20). ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 374 $[M]^+$ (9), 356 (19), 314 $[M - MeCOOH]$ (27), 301 (31), 283 (38), 279 $[M - C_6H_7O]^+$ (52), 219 $[M - HOAc - C_6H_7O]^+$ (87), 95 $[C_6H_7O]^+$ (71), 81 $[C_5H_5O]^+$ (62), 43 (100).

Acetylation of hautriwaic acid. Compound **5b** (50 mg) was treated with Ac_2O (2.0 ml) and pyridine (0.5 ml) at room temp. for 6 hr. After addition of EtOH, the mixture was evaporated to dryness and yielded hautriwaic acid acetate. The spectral and physical data (TLC, IR, ^{1}H NMR, MS) of this compound were in full agreement with those of **5a**.

4β-Hydroxyisobacchasmacranone (**3**). Mp 178–179° (petrol–EtOAc); $[\alpha]_D^{25}$ − 68.5° ($CHCl_3$; *c* 0.8). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3030, 2980, 2850, 1790, 1710, 1660, 1510, 1480, 1450, 1390, 1140, 1120, 1010, 880. ^{1}H NMR: δ7.36 (1H, *t*, J = 1.6 Hz, H-15), 7.24 (1H, *br s*, H-16), 6.27 (1H, *br s*, H-14), 6.23 (1H, *ddd*, J = 2.2, 5.5, 9.9 Hz, H-2), 5.67 (1H, *dt*, J = 2.2, 9.9 Hz, H-3), 4.13 (1H, *d*, J = 10.5 Hz, H-19), 4.05 (1H, *d*, J = 10.5 Hz, H-19'), 2.96 (1H, *d*, J = 13.0 Hz, H-6β), 2.80 (1H, *q*, J = 6.6 Hz, H-8β), 2.27 (1H, *d*, J = 13.0 Hz, H-6α), 1.02 (3H, *d*, J = 6.6 Hz, H-17), 0.67 (3H, *s*, H-20). ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 344 $[M]^+$ (65), 249 $[M - C_6H_7O]^+$ (100), 107 (47), 95 $[C_6H_7O]^+$ (94), 91 (55), 81 $[C_5H_5O]^+$ (88), 55 (59), 53 (51), 43 (65), 41 (72).

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REFERENCES

1. Gambaro, V., Chamy, M. C., Garbarino, J. A., San Martin, A. and Castillo, M. (1986) *Phytochemistry* **25**, 2175.

2. Sharma, S. C., Tandon, J. S., Porter, B., Raju, M. S. and Wenkert, E. (1984) *Phytochemistry* **23**, 1194.
3. Jolad, S. D., Hoffmann, J. J., Schram, K. H., Cole, J. R., Tempesta, M. S. and Bates, R. B. (1982) *J. Org. Chem.* **47**, 1356.
4. Bohlmann, F., Grenz, M., Wegner, P. and Jakupovic, J. (1983) *Justus Liebig's Ann. Chem.* 2008.
5. Rodriguez, M. C., Barluenga, J., Savona, G., Piozzi, F., Servettaz, O. and Rodriguez, B. (1984) *Phytochemistry* **23**, 1465.