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2β-HYDROXYHAUTRIWAIC ACID, A CLERODANE TYPE DITERPENOID AND OTHER TERPENOIDS FROM THREE *BACCHARIS* SPECIES

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Key Word Index—*Baccharis sp.*; Compositae; leaf resin; hautriwaic acid; 2β-hydroxyhautriwaic acid; maniladiol; oleanolic acid.

Abstract—The terpenoid composition of the leaf resin of three *Baccharis* species has been studied. *Baccharis heterophylla* afforded the trivial compound oleanolic acid and the rare triterpene maniladiol. *Baccharis sarothroides* yielded oleanolic acid, the diterpene hautriwaic acid and its derivative 2β-hydroxyhautriwaic acid which is a novel natural product. *Baccharis vaccinoides* produces oleanolic acid, maniladiol and hautriwaic acid. The structures of these terpenoids have been established by spectroscopic methods and by comparison with the data reported for related compounds.

INTRODUCTION

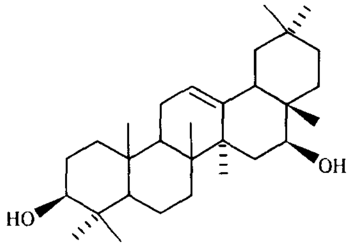
Of the approximately 400 species of the American genus *Baccharis* (Compositae, Asteraceae) only some 10% have been studied phytochemically until now. Most reports concern diterpenoids [1–4] and flavonoid aglycones [5–7]. No previous studies are known for *B. heterophylla* H.B.K. and *B. vaccinoides* H.B.K. but there is one report of the identification of two flavonoids from *B. sarothroides* A. Gray [8]. A detailed investigation on the composition of the leaf resin of seven *Baccharis* species [9] deals in particular with flavonoid aglycones. We report now the major terpenoids found in the three species cited, namely oleanolic acid, maniladiol (**1**), hautriwaic acid (**2**) and 2β-hydroxyhautriwaic acid (**3**).

RESULTS AND DISCUSSION

Oleanolic acid, maniladiol (**1**) and hautriwaic acid (**2**) were identified on the basis of their physical and spectral properties. For the ^{1}H NMR data of maniladiol see the Experimental, for the ^{1}H NMR and ^{13}C NMR data of hautriwaic acid and its acetate (**2a**) see Tables 1 and 2, respectively.

Elemental analysis of compound **3** provided the molecular formula $C_{20}H_{28}O_5$. IR bands at 3400–2500, 1705 and 880 cm^{-1} indicated a carboxyl group and a furan ring. The β-substituted furan moiety was confirmed by the typical resonance pattern arising from three aromatic protons at δ6.26, 7.20 and 7.34 (Table 1). In addition, the mass spectrum exhibited the expected peaks for a β-ethyl side chain at *m*/*z* 95 and 81 (base peak).

Two methyls at δ0.88 (*d*) and 0.76 (*s*) were assigned to Me-17 and Me-20, respectively. The grouping –C–CH$_2$OH was observed as two doublets at δ3.78 and 4.22 with the characteristic geminal coupling constant between the methylene protons (11 Hz). The one-proton multiplet at δ4.40 was resolved as a triplet (δ4.67, 3.5 Hz) when the spectrum was recorded using pyridine-*d*$_5$ as solvent.



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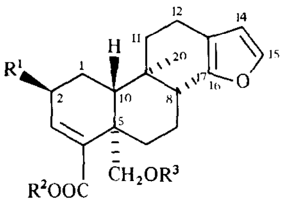

	R¹	R²	R³
2	H	H	H
2a	H	H	Ac
3	OH	H	H
3a	OAc	H	Ac
3b	OAc	Me	Ac

A signal at δ6.60 (1H, *d*, *J* = 3.5 Hz) was assigned to a β-olefinic proton conjugated with a carboxylic acid. Spin decoupling experiments on irradiation at δ6.60 and 4.40 confirmed that the second hydroxyl group must be

Table 1. ^{1}H NMR chemical shifts of hautriwaic acid derivatives

	2a	**3**		**3a**	**3b**
	($CDCl_3$)	($CDCl_3$)	(pyridine-d_5)	($CDCl_3$)	($CDCl_3$)
H-2	—	4.40 *m*	4.67 *t*	5.44 *dt*	5.45 *t*
H-3	6.77 *dd*	6.60 *d*	7.21 *d*	6.84 *d*	6.85 *d*
H-14	6.26 *m*	6.26 *m*	6.48 *dd*	6.19 *dd*	6.22 *m*
H-15	7.37 *m*	7.34 *m*	7.55 *t*	7.32 *t*	7.35 *m*
H-16	7.22 *m*	7.20 *br s*	7.48 *br s*	7.16 *br s*	7.19 *br s*
Me-17	0.86 *d*	0.88 *d*	0.78 *d*	0.86 *d*	0.86 *d*
H-19	4.59 *d*	4.22 *d*	4.50 *d*	4.55 *d*	4.60 *d*
H-19′	4.32 *d*	3.78 *d*	4.16 *d*	4.31 *d*	4.40 *d*
Me-20	0.80 *s*	0.76 *s*	0.87 *s*	0.79 *s*	0.81 *s*
OAC	2.02 *s*			2.04 *s*	2.05 *s*
				2.02 *s*	2.01 *s*
OMe					3.72 *s*

J (Hz): **2a** $3,2\alpha = 3$; $3,2\beta = 7$; $17,8 = 6$; $19\alpha,19\beta = 11$; **3** ($CDCl_3$) $3,2\alpha = 3.5$; $17,8 = 6$; $19\alpha,19\beta = 11$; **3** (pyridine-d_5) $2\alpha,1\beta = 3.5$ Hz; $2\alpha,3 = 3.5$; $14,15 = 1.6$; $14,16 = 0.7$; $15,16 = 1.6$; $19\alpha,19\beta = 11$; $17,8 = 6$; **3a**: $2\alpha,1\alpha = 1$; $2\alpha,1\beta = 3.5$; $2\alpha,3 = 3.5$; $14,15 = 1.7$; $14,16 = 0.8$; $15,16 = 1.7$; $17,8 = 6$; $19\alpha,19\beta = 11.5$; **3b**: $2\alpha,1\beta = 4.5$; $2\alpha,3 = 4.5$; $17,8 = 6$; $19\alpha,19\beta = 11.5$.

Table 2. ^{13}C NMR chemical shifts of hautriwaic acid and derivatives [δ (ppm) in $CDCl_3$]

Carbon	**2**	**2a**	**3a**	**3b**
1	17.0	17.6	24.9	25.1
2	26.9*	27.9*	66.9	67.0
3	139.4	135.5	136.0	141.6
4	141.0	138.8	141.2	140.8
5	38.8	37.9	38.6	38.5
6	31.4	34.6	33.1	33.3
7	26.8*	27.7*	27.4	27.3
8	36.3	36.6	36.3	36.2
9	42.1	41.2	41.1	40.9
10	46.4	48.2	42.5	42.4
11	38.6	38.9	36.2	35.8
12	18.3	19.7	17.6	18.4
13	125.3	125.0	125.1	125.0
14	110.9	110.8	110.7	110.7
15	142.8	142.9	142.9	143.4
16	138.5	138.5	138.4	136.0
17	15.9	15.6	15.6	15.6
18	172.9	169.2	170.4	167.0
19	65.5	71.8	70.1	70.1
20	18.6	18.1	17.8	17.8
$O\underline{C}OMe$		170.7	170.8	170.5
			170.7	170.0
$OCO\underline{Me}$		21.0	21.0	21.1
			20.8	20.8
OMe				51.6

*Interchangeable assignments.

attached to C-2 and its *β*-axial configuration was established on the basis of the absence of a $J_{\text{ax-ax}}$ coupling constant for H-2 as can be observed from the Dreiding models.

The mass spectrum afforded additional proof of the structure since the highest peak at m/z 330 is caused by water loss from the molecular ion (M^+ 348, not observed). Further water loss gave rise to the ion at m/z 312.

Acetylation of **3** in the usual manner gave a diacetate **3a** (singlets at δ2.02 and 2.04). The two doublets corresponding to the C-19 methylene were shifted to 4.16 and 4.50, and H-2 was likewise deshielded to 5.44. On treatment with diazomethane compound **3a** afforded **3b**, which showed a –COOMe group (singlet, δ3.72). Comparison of the ^{1}H NMR data for compound **3** and its derivatives as well as with the literature data [10] showed good agreement with the proposed structure.

Finally, a ^{13}C NMR study of compounds **3a** and **3b** was made (Table 2). Assignment of the carbon multiplicities was made through DEPT subspectra [11] while differentiation among the signals with the same multiplicity was made by comparison with data reported for related diterpenes [12] and with calculated values [13].

The ^{1}H NMR and other data of maniladiol (**1**) agree with those reported for this compound in the literature [14] (see Experimental).

Hautriwaic acid (**2**) and its acetate (**2a**) were identified by comparison with the data reported for hautriwaic acid in pyridine-d_5 [15]. The shifts observed for C-5, C-6 and C-19 in **2a** must be ascribed to the acetyl group attached to C-19. Additional differences between some carbons in **2** may be due to the solvent effect ($CDCl_3$–pyridine-d_5).

Comparison of the spectrum of **3a** with that of **2a** was useful to confirm the structure proposed for **3a**. According to this comparison the deshielding (7.3 ppm) observed for C-1 is due to the presence of an acetyl group attached to C-2, which appears at low field (δ66.9). The *β*-axial orientation of this substituent is confirmed by the shielding effect of almost 5 ppm from the *peri* carbon C-10. The remaining signals are unchanged and the stereochemistry of **3a** can be assumed to be the same as for hautriwaic acid and so is that of **3b**, differing only at the –COOMe group. All these data confirm the structure of 2*β*-hydroxyhautriwaic acid for compound **3**.

2*β*-Hydroxyhautriwaic acid in described here for the first time as a natural product. We found it in the leaf resin of two populations of *Baccharis sarothroides*. Hautriwaic acid itself, which we found in *B. sarothroides* and in *B. vaccinoides*, has been reported previously from two further *Baccharis* species [16, 17]. Oleanolic acid and baccharis oxide are the most widely distributed triterpenoids in the genus. The unusual triterpene maniladiol, now found in *B. heterophylla* and in *B. vaccinoides*, was first isolated from *Manila elemi* [18] and more recently from *Gymnosporia trilocularis* [14]. It has as yet not been reported for any species of the genus *Baccharis*.

EXPERIMENTAL

Plant material. *Baccharis heterophylla* was collected in May 1983 in Mexico, Edo. Oaxaca (Yatskievych and Wollenweber 83-152; voucher at ARIZ). *Baccharis sarothroides* was collected in May 1982 in Pima Co., AZ, U.S.A. (Yatskievych s.n., voucher in the private herbarium of E.W.). *Baccharis vaccinoides* was collected in May 1983 in Mexico, Edo. Hidalgo (Yatskievych and Wollenweber 83-129A, voucher at ARIZ). Aerial parts of these plants were air-dried in paper sacks.

Product isolation. The leaf and stem resins were obtained from the air-dried plant materials by rinsing with Me_2CO and evaporation *in vacuo*. The yields were 8 % dry wt in *B. heterophylla*, 7 % dry wt in *B. sarothroides* and 14 % dry wt in *B. vaccinoides*. Portions of these materials were divided into terpenoids and flavonoid aglycones by a passage over Sephadex LH-20, eluted with MeOH. Individual terpenoids were isolated by CC on silica gel and crystallization from relevant fractions.

Maniladiol (**1**), isolated from *B. heterophylla*, forms colourless crystals, mp 198°. Its MS and ^{1}H NMR data were in accordance with those reported for maniladiol from *Gymnosporia trilocularis* [14]. ^{1}H NMR (CDCl$_3$): δ1.22, 1.00, 0.99, 0.94, 0.91, 0.89, 0.80, 0.80 (*s*, Me), 3.22 (*dd*, $J = 9.5$ and 5.5 Hz; H-3$_{ax}$), 4.19 (*dd*, $J = 11.5$ and 5 Hz; H-16 in CH–OH), 5.25 (*t*, 3.5 Hz; H-12 in olefinic double bond); (pyridine-d_5): δ1.34, 1.23, 1.16, 1.07, 1.05, 0.98, 0.95, 0.92 (all *s*, 8 × Me).

Hautriwaic acid (**2**), isolated from *B. sarothroides*, was crystallized from C$_6$H$_6$–EtOH, mp 178–179°. Its IR, MS and NMR data agreed with those reported previously for this product [10]. For ^{13}C NMR data see Table 2.

Compound **2** (25 mg) in pyridine–Ac$_2$O (1 : 1) (1 ml) kept at room temp. overnight yielded 25 mg of **2a**, colourless oil. IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 3400–2500, 1740, 1705, 1245 and 880. The MS was in agreement with the reported data for this compound [15]. For ^{1}H NMR data see Table 1; ^{13}C NMR data are compiled in Table 2.

2β-Hydroxyhautriwaic acid (**3**) crystallized from C$_6$H$_6$, mp 188–189°. IR data see Results section. ^{1}H NMR data are in Table 1, ^{13}C NMR data are in Table 2. MS *m/z* (rel. int.): 348 $[M]^+$ (—), 330 $[M - H_2O]^+$ (13), 312 $[M - 2H_2O]^+$ (2), 235 [M −$_{C_2H_4}$]$^+$ (6), 205 (16), 149 (20), 135 (18), 95 [^{C_2H_4}]$^+$, (72), 81 []$^+$ (100). (Found: C, 68.74; H, 8.29. Calc. for $C_{20}H_{28}O_5$: C, 68.92; H, 8.10%.)

Compound **3** (40 mg) in pyridine–Ac$_2$O (1 : 1) (1 ml) kept at room temp. overnight, afforded 35 mg of **3a**, colourless oil; IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 3400–2500, 1740, 1705, 1245 and 880. MS *m/z* (rel. int.): 432 $[M]^+$ (—), 372 $[M - AcOH]^+$ (13), 330 (7), 312 $[M - 2\,AcOH]^+$ (24), 299 (51), 281 (53), 217 (44), 95 $[C_6H_7O]^+$ (44), 81 $[C_5H_5O]^+$ (60) and 43 (100). For ^{1}H NMR and ^{13}C NMR see Tables 1 and 2.

To 30 mg of **3a** in Et$_2$O, CH$_2$N$_2$ was added and after evaporation 30 mg of **3b** were obtained as a colourless oil. IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 1740, 1715, 1240 and 880. For ^{1}H NMR and ^{13}C NMR see Tables 1 and 2.

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