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CLERODANE DITERPENOIDS, LONG CHAIN ESTERS OF COUMARIC ACID AND OTHER COMPOUNDS FROM *BACCHARIS MYRSINITES*

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Abstract—A new clerodane diterpenoid and two new long chain esters of *trans*- and *cis*-coumaric acid, in addition to known triterpenoids and one known clerodane diterpenoid, have been isolated and characterized from *Baccharis myrsinites*. The structures were determined by spectroscopic techniques. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

More than 62 species from the large genus *Baccharis* have been chemically investigated. The most widespread compounds reported were clerodane and labdane diterpenoids and oleanolic triterpenoids. In addition, kaurane terpenoids, cinnamic acid esters and coumarin derivatives have been previously detected [1, 2]. As a continuation of our investigations of the chemical constituents of the Asteraceae [3–5], we now report the isolation and structure elucidation of a new clerodane diterpenoid and two novel long chain esters of coumaric acid from *B. myrsinites* (Lam.) Persoon, a species endemic to the Dominican Republic. A known diterpenoid **4** [6] and the triterpenoids oleanolic acid [7], maniladiol [8] and logipinogenin [9] were also isolated.

RESULTS AND DISCUSSION

Silica gel chromatography and RP-18 HPLC of the CH_2Cl_2–MeOH extract of the aerial parts of *Baccharis myrsinites* afforded, in addition to the known compounds, three new compounds **1**, **2** and **3**.

Compound **1**, a minor constituent, was isolated as a colourless powder, with a molecular formula of $C_{22}H_{38}O_4$, based on NMR and mass spectral data. Both ^{1}H NMR and ^{13}C NMR spectra showed that **1** was a mixture of two epimers. Comparison of its ^{1}H NMR and ^{13}C NMR data (Table 1) with those of **4** as well as with literature data [6, 10, 11] indicated that **1** had a clerodane skeleton. Both the ^{1}H NMR and ^{13}C NMR spectra had signals for an ethoxy group, instead of a methoxy group as in **4**; this finding was confirmed by NMR COSY and HETCOR experiments. The ^{1}H NMR spectrum of acetylated **1** showed two acetyl groups, indicating **1** had two free hydroxyl groups, which were assigned to C-18 and C-19 giving structure **1**, which was not previously reported.

The absolute configuration of compounds **1** and **4** was not totally established, but the X-ray diffraction experiments did confirmed the partial structure of the A- and B-rings in compound **4** [W. H. Watson, pers. comm.]. The X-ray analysis failed to establish the absolute structure of the side chain. The configuration shown in the formula is in accord with their negative rotation values, which were also compared to related neoclerodane diterpenoids of known absolute configuration [6, 12].

Compound **2** was obtained as a colourless amorphous powder from ethyl acetate. A molecular formula of $C_{43}H_{76}O_3$ was assigned based on NMR and CI-mass spectrometry data; the latter spectrum showed a molecular ion peak at m/z 639 $[M-1]^+$. The compound gave a positive phenol test (green-brown with $FeCl_3$), and the IR spectrum exhibited absorption bands at 3380 (free OH), 1700 (C=O), 1675, 1515 and 1470 (aromatic) cm^{-1}. The ^{1}H NMR spectrum had a multiplet signal at δ 1.20 for 62 protons, and the ^{13}C NMR also exhibited overlapping signals at high field (δ 29.6). A typical AA′BB′ system observed in the ^{1}H

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Table 1. ^{1}H NMR and ^{13}C NMR data* of compounds **1** and **4**

	1 (δ_C)	**1** (δ_H)	**4** (δ_C)	**4** (δ_H)
1	17.24		17.26	
2	26.82		26.82	
3	130.01	5.68, *t* (3.6)	129.43	5.70, *t* (3.4)
4	145.11		145.15	
5	39.37		39.25	
6	31.05		31.07	
7	26.37		26.40	
8	36.27		36.31	
9	38.59		38.63	
10	46.16		46.16	
11	37.18		37.16	
12	27.23		27.26	
13	37.55		37.45	
14	42.92		42.93	
15	103.82	5.07, *dd* (6.0, 3.0)	105.16	4.97, *dd* (6.0, 3.0)
	104.24	5.05, *d* (4.5)	105.59	4.94, *d* (4.8)
16	72.60	3.35–3.43, *m*	72.63	3.80–3.90, *m*
	71.84	4.0, *t* (7.5)	71.82	4.01, *t* (7.8)
17	15.83	0.76, *d* (6.0)	15.82	0.78, *d* (6.3)
18	64.37	4.12, 3.74, *d* (11.5)	64.25	4.16, 3.80, *d* (11.4)
19	65.65	3.91, 3.54, *d* (10.5)	64.99	3.94, 3.60, *d* (10.8)
20	18.75	0.69, *s*	18.91	0.71, *s*
OMe			54.92	3.31, *s*
			54.50	3.29, *s*
OEt	62.58	3.66, m; 3.40, *m*		
	62.79	3.66, m; 3.40, *m*		
	15.17	1.15, *t* (6.9)		
	15.23	1.14, *t* (6.9)		

* The assignment is based on comparison with literature reports and COSY and HETCOR evidence. Protons at positions 1, 2, 5–8 and 10–14 are heavily overlapped and, thus, could not be definitely assigned.

NMR spectrum (2H, *d*, $J = 8.2$ Hz) at δ 7.34 and 6.67 indicated the presence of four aromatic protons corresponding to H-2,6 and H-3,5, respectively. The coupling constants for the double bond protons (15.9 Hz) indicated a *trans*-double bond. These ^{1}H NMR signals are characteristic for an ester of coumaric acid and a long chain hydrocarbon alcohol. In addition to the molecular ion, the mass spectrum of **2** exhibited major fragment peaks at *m/z* 119 and 147, which are characteristic of a coumarate moiety [13]. The UV spectrum in methanol [λ 228 (log ε 4.04) and 312 (log ε 2.01)] was almost identical to that of erythrinasinate, a coumaric acid ester previously isolated for *Erythrina senegalensis* [14]. The ^{13}C NMR spectrum of **2** confirmed the structure (see Experimental). The spectrum had a peak at δ 168.0 (C-3′) in accord with a carbonyl group of an ester function and peaks at δ 145.0 (C-1′) and 114.5 (C-2′) for the coumarate double bond. The signals for the methylene protons in the ^{1}H NMR spectrum at δ 1.20 (62H), 1.63 (2H, *m*) and δ 4.10 (2H, *m*) showed that the hydrocarbon chain is triacontyl. Compound **2** is therefore the novel ester *n*-triacontyl coumarate.

Compound **3** was also obtained as a white amorphous solid from $CHCl_3$. Its molecular formula was deduced as $C_{39}H_{68}O_3$ by CI-mass spectrometry, which exhibited a molecular ion at *m/z* 583 $[M\text{-}1]^+$. The ^{1}H NMR and ^{13}C NMR, UV and IR spectra for **3** (see Experimental) were similar to those of **2**, while the UV spectrum was different for band I, that is, 285 nm instead of 312 nm suggesting a *cis*-double bond. These findings indicated a second ester of coumaric acid. Again, the ^{1}H NMR of **3** exhibited a typical AA′BB′ system (2H, *d*, $J = 8.6$ Hz) at δ 7.58 and 6.67 for H-2,6 and H-3.5, respectively, as well as two coupled *cis*-olefinic protons (1H, *d*, $J = 12.7$ Hz) at δ 6.78 and 6.56 [15, 16]. The methylene protons on the alcohol carbon atom forming the ester bond appeared at δ 4.09 (2H, *t*, $J = 6.6$ Hz), while the adjacent methylene protons were at δ 1.60 (2H, *m*). A broad signal at δ 1.20 integrating for 54 protons was assigned to the other methylene protons in the straight chain while the terminal methyl group gave a triplet at δ 0.90 ($J = 6.5$ Hz). Therefore, **3** is a *cis*-coumaric acid ester on an *n*-C_{30} alcohol; *trans*-isomer, was previously reported [17, 18].

EXPERIMENTAL

General. Mps: uncorr. IR: KBr; ^{1}H NMR and ^{13}C NMR, $CDCl_3$ as solvent and reference, Varian 300 MHz (^{1}H) and 75 MHz (^{13}C), respectively; chroma-

tography: silica gel 60–120 mesh; Sephadex LH-20; spots visualized under UV light enhanced by vaniline; the identity of each known compound was confirmed by IR, MS and ^{1}H NMR.

Plant material. Baccharis myrsinites aerial parts were collected in Santo Domingo, Dominican Republic in October, 1995. Herbarium specimens documenting the collection are deposited at the Jardin Botanico Nacional, Santo Domingo.

Extraction and isolation. Air dried and powdered plant material (840 g) of *Baccharis myrsinites* was extracted with CH_2Cl_2–MeOH (1:1) for 48 hr. The concentrate was suspended in H_2O, and this aq. mixt. was extracted successively with hexane (800 ml), CH_2Cl_2 (1000 ml) and EtOAc (600 ml). The residue (122 g) from the CH_2Cl_2 extract was flash chromatographed over a silica gel column, eluting with hexane with an increasing amount of CH_2Cl_2. Frs of hexane–CH_2Cl_2 (2:1, 800 ml), (1:1, 1500 ml) and (1:2, 3000 ml) afforded residue A (3.1 g), B (16.8 g) and C (79 g). Residue A was subjected to a silica gel column, eluted with hexane–EtOAc (150 ml frs). Fr. 2 (hexane–EtOAc, 10:1) gave mixture D (23 mg), fr. 4 (hexane–EtOAc 10:2) afforded compound **2** (50 mg); Fr. 8 (hexane–EtOAc 10:3) gave crystals of maniladiol (43 mg). Mixture D afforded compound **3** (9 mg) after silica gel CC using hexane–Et_2O (10:1) as eluent. Sephadex LH-20 column chromatography (hexane–EtOAc 1:1) of residue B afforded oleanolic acid (13 mg). Residue C was chromatographed on a silica gel column, which was washed with hexane with an increasing amount of EtOAc. The material eluted from the column was further purified over Sephadex columns, using EtOAc as eluent to yield two colourless crystalline residues: logispinogenin (12 mg) and the diterpenoid mixture (20 mg). This solid mixture was further sepd by RP-18 HPLC (eluent MeOH–H_2O, 5:1), affording compound **1** (6 mg) and compound **4** (12 mg).

Compound **1**. White powder. IR ν_{max}: 3130–3150 (br), 1670, 1450, 1380, 1050, 1000, 900, 835, 785 and 750 cm^{-1}. ^{1}H and ^{13}C NMR data, see Table 1. CI-MS *m/z*: 366 ([M]$^+$, 2.3), 351 ([M − CH_3]$^+$, 2.9), 303 (100), 285 (71), 255 (45), 223 (48).

Compound **2**. White powder; IR ν_{max}: 3380, 1710, 1675, 1605, 1515, 1470, 1275, 1370, 980, 830 cm^{-1}. UV λ_{max}^{MeOH} (log ε): 228 (4.04), 312 (2.01). ^{1}H NMR ($CDCl_3$): δ 0.83 (3H, *t*, J = 6.5 Hz), 1.20 (62H *br*, $(CH_2)_{31}$), 1.63 (2H, *m*, $OCH_2\underline{CH_2}R$), 4.10 (2H, *t*, J = 6.6 Hz, $O\underline{CH_2}CH_2R$), 6.21 (1H, *d*, J = 15.9 Hz, H-2′) 6.77 (2H, *d*, J = 8.2 Hz, H-3,5), 7.34 (2H, *d*, J = 8.2 Hz, H-2,6), 7.55 (1H, *d*, J = 15.9 Hz, H-1′). ^{13}C NMR ($CDCl_3$): δ 126.0 (C-1), 130.0 (C-2 and C-6), 116.0 (C-3 and C-5), 159.0 (C-4), 145.0 (C-1′), 114.5 (C-2′), 168.0 (C-3′), 64.5 (C-1″), 25.9 (C-2″), 28.7 (C-3″), 29.2 (C-4″), 29.6 (C-5″ → C-30″), 29.1 (C-31″), 31.9 (C-32″), 22.6 (C-33″), 14.0 (C-34″). CI-MS *m/z* (rel. int.): 639 [M − 1]$^+$ (7.3), 611 (4.0), 583 (6.0), 555 (7.2), 527 (5.0), 499 (5.5), 147 (15.9), 119 (100).

Compound **3**. White powder; IR ν_{max}: 3400, 1710, 1680, 1600, 1515, 1470, 1460, 1170, 830 and 720 cm^{-1}. UV λ_{max}^{MeOH} (log ε): 228 (4.20), 284 (2.21), 312 (2.01, sh). ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, *t*, J = 6.5 Hz), 1.20 (54H, *br*, $(CH_2)_{27}$), 1.60 (2H, *m*, $OCH_2\underline{CH_2}R$), 4.09 (2H,*t*, J = 6.6 Hz, $O\underline{CH_2}CH_2R$), 5.76 (1H, *d*, J = 12.7 Hz, H-2′), 6.76 (2H, *d*, J = 8.6 Hz, H-3,5), 6.78 (1H, *d*, J = 12.7 Hz, H-1′), 7.58 (2H, *d*, J = 8.6 Hz, H-2,6). ^{13}C NMR ($CDCl_3$): δ 129.8 (C-1), 132.3 (C-2 and C-6), 114.8 (C-3 and C-5), 157.3 (C-4), 143.3 (C-1′), 115.7 (C-2′), 166.7 (C-3′), 64.4 (C-1″), 25.9 (C-2″), 28.6 (C-3″), 29.3 (C-4″), 29.6 (C-5″ → C-26″), 29.2 (C-27″), 31.9 (C-28″), 22.6 (C-29″), 14.1 (C-30″). CI-MS *m/z* (rel. int.): 583 [M − 1]$^+$ (5.0), 569 (4.0), 583 (5.0), 555 (18.0), 499 (14.0), 219 (30), 106 (18.0).

Compound **4**. Colourless needles, mp 130–133° (EtOAc). IR ν_{max}: 3050–3450 (br), 1650, 1485, 1450, 1210, 1030 and 990 cm^{-1}. ^{1}H and ^{13}C NMR data: Table 1. EI-MS *m/z* (rel. int.) 335 [M − OH]$^+$ (3.0), 319 (3.2), 303 (28.6), 154 (100).

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REFERENCES

1. Zdero, C., Bohlmann, F., King, R. M. and Robison, H., *Phytochemistry*, 1986, **25**, 2481.
2. Martin, A. S., Rovirosa, J. and Castillo, M., *Phytochemistry*, 1983, **22**, 1461.
3. Ahmed, A. A., Ahmed, M., William, H., Scott, I. A. and Mabry, T. J., *Journal of Natural Products*, 1993, **56**, 1276.
4. Ahmed A. A., Jakupovic, J. and Mabry, T. J., *Journal of Natural Products*, 1993, **56**, 1821.
5. Ahmed, A. A., Spring, O., El-Razek, M., Hussein, N. and Mabry, T. J. *Phytochemistry*, 1995, **39**, 1127.
6. San-Martin, A., Rovirosa, J., Labbe, C., Givovich, A., Mahu, M. and Castillo, M., *Phytochemistry*, 1986, **25**, 1393.
7. Martin, A. S., Rovirosa, J., Becker, R. and Castillo, M.,*Phytochemistry*, 1980, **19**, 1985.
8. Arriaga-Giner, F. J., Wollenweber, E., Schober, I., Dostal, P. and Braun, S., *Phytochemistry*, 1986, **25**, 719.
9. Morales, G. and McLaughlin, J. L., *Journal of Natural Products*, 1989, **52**, 381.
10. San-Martin, A., Givovich, A. and Castillo, M., *Phytochemistry*, 1989, **25**, 264.
11. Faini, F., Rivera, P., Mahu, M. and Castillo, M., *Phytochemistry*, 1987, **26**, 3281.
12. Gambaro, V., Chamy, M. C., Garbarino, J. A., San-Martin, A. and Castillo, M., *Phytochemistry*, 1986, **25**, 2175.

13. Achenback, H., Stocker, M. and Constenta, M. A., *Zeitschrift für Naturforschung*, 1986, **41C**, 164.
14. Fomum, Z. T., Ayafor, J. F., Wandji, J., Fomban, W. G. and Nkengfack, A. E. *Phytochemistry*, 1986, **25**, 757.
15. Li, C. J., Chen, D. H. and Xiao, P. G., *Acta Pharmaceutica Sinica*, 1994, **29**, 195.
16. Kumajima, H., Shibano, N., Baba, T., Takaishi, K. Inoue, K. and Shingo, T., *Phytochemistry*, 1996, **41**, 286.
17. Talapatra, B., Das, A. K. and Talapatra, S. K., *Phytochemistry*, 1989, **28**, 290.
18. Wandji, J., Nkengfack, A. E., Fomum. Z. T., Ubillas, R., Killday, K. B. and Tempesta, M. S., *Journal of Natural Products*, 1990, **53**, 1425.