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BENZOPYRAN DERIVATIVES FROM *WERNERIA NUBIGENA*

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Key Word Index—*Werneria nubigena*; Asteraceae; *p*-hydroxyacetophenone derivatives; benzopyrans; quinic acid derivatives; pyrrolizidine alkaloids.

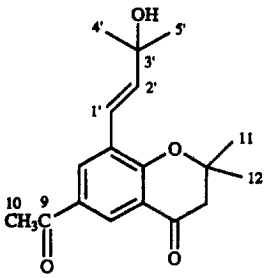
Abstract—Investigation of the aerial parts of *Werneria nubigena* afforded, in addition to pyrrolizidine alkaloids, *p*-hydroxyacetophenone and quinic acid derivatives, the rare, 2,2-dimethyl-6-acetyl-8-(3′-hydroxy-3′-methyl-*trans*-but-1′-enyl)-chrom-3-ene, and the new, 2,2-dimethyl-6-acetyl-8-(3′-hydroxy-3′-methyl-*trans*-but-1′-enyl)-chroman-4-one. Structures were elucidated by spectroscopic methods. The chemotaxonomic implications are discussed briefly. © 1997 Elsevier Science Ltd

INTRODUCTION

As a part of a systematic phytochemical investigation of the genus *Werneria*, we have reported on the isolation of four *ent*-13-epi-manoyloxides from *W. dactylophylla* [1], as well as benzofurans, *p*-hydroxyacetophenones [2], *ent*-manoyloxide and *ent*-kaurane derivatives from *W. ciliolata* [3]. Roeder and co-workers reported on the isolation of the pyrrolizidine alkaloids (PAs), retrorsine, retrorsine-*N*-oxide, senecionine and integerrimine from *W. nubigena* [4], which is well known in South-American folk medicine for its anti-rheumatic, anti-hypertensive and digestive uses [5]. PAs have received considerable attention over the last 30 years, largely on account of their biological activities, which include hepatotoxic, mutagenic and anti-cancer properties [6, 7].

Since Roeder and co-workers directed their studies towards the isolation of PAs, continuing our studies on the *Werneria* species, we have undertaken a systematic investigation of *W. nubigena* to verify the occurrence of other classes of metabolites, in particular *p*-hydroxyacetophenone, benzopyran and benzofuran derivatives, which represent useful taxonomic markers at the tribal and generic level of the Asteraceae [2].

The present paper deals with the isolation of the new, 2,2-dimethyl-6-acetyl-8-(3′-hydroxy-3′-methyl-*trans*-but-1′-enyl)-chroman-4-one (**1**) and the rare, 2,2-dimethyl-6-acetyl-8-(3′-hydroxy-3′-methyl-*trans*-but-1′-enyl)-chrom-3-ene (**2**), together with *p*-hydroxyacetophenone (**3**), *p*-hydroxyacetophenone-*O*-β-D-glucopyranoside (**4**), 3,5 di-*O*-caffeoylquinic acid (**5**), 3-*O*-caffeoylquinic acid (**6**), retrorsine (**7**), retrorsine-*N*-oxide (**8**) and rosmarinine-*N*-oxide (**9**).



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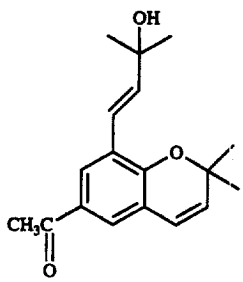

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RESULTS AND DISCUSSION

The chloroform extract of *W. nubigena* yielded compounds **1** and **2** after sequential silica column chromatography and semi-prep. RP-HPLC. The molecular formula $C_{18}H_{22}O_4$ of **1** was determined by ^{13}C NMR and DEPT ^{13}C NMR analysis, and by FAB-MS (negative ion mode), which gave a quasi-molecular anion $[M-H]^-$ at *m/z* 301. The ^{13}C NMR of **1** exhibited 18 signals, which were divided by the analysis of DEPT ^{13}C NMR into four sp^2 CH (δ 119.9, 127.1, 132.7 and 142.0), four quaternary sp^2 carbons (δ 120.7, 128.2, 130.7 and 161.5), of which the last one was hydroxylated, two C=O groups (δ 193.6 and 198.6), a CH_2 (49.0), two quaternary hydroxylated

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carbons (δ 71.4 and 81.7) and five methyls (δ 26.2, 26.5 and 29.7, the last two ones, each for two carbons). In the ^{1}H NMR spectrum, in addition to two signals ascribable to methyls at oxygen-bearing carbons (δ 1.43, 6H, *s* and δ 1.54, 6H, *s*), two signals at δ 2.59 (3H, *s*) and 2.83 (2H, *s*) requiring, respectively, a methyl and a methylene group adjacent to carbonyl groups, were evident. Further features were two doublets (δ 6.61, 1H, $J = 16$ Hz and δ 6.94, 1H, $J = 16$ Hz), suggesting the occurrence of a *trans*-double bond and, in the aromatic region, two signals (δ 8.32, 1H, $J = 2$ Hz and δ 8.34, 1H, $J = 2$ Hz), ascribable to *meta*-coupled protons.

Analysis of the observed data were in good agreement with a 2,2-dimethylchroman-4-one substituted with an acetyl group and a five-membered side-chain, which was established as 3′-hydroxy-3′-methyl-*trans*-but-1′-enyl [8]. The positions of the acetyl group and the side-chain could be easily deduced taking as model, *m*-alchyl-*p*-hydroxyacetophenones [9]. HETCOR and COLOC experiments allowed the unambiguous assignment reported in Table 1, supporting the structure of **1** as 2,2-dimethyl-6-acetyl-8-(3′-hydroxy-3′-methyl-*trans*-but-1′-enyl)- chroman-4-one.

The NMR spectral pattern of **2** ($C_{18}H_{22}O_3$) showed a close similarity to that of **1**, the main differences being in the ^{1}H and ^{13}C NMR spectra were the absence of the signals for the carbonyl (δ 193.6) and methylene groups (δ 49.0 in the ^{13}C NMR spectrum and δ 2.83 in the ^{1}H NMR spectrum) of the chromanone skeleton, which were replaced by the signals at δ 5.83 (1H, *d*, $J = 10$ Hz) and 6.48 (1H, *d*, $J = 10$ Hz) in the ^{1}H NMR spectrum and at δ 132.3 and 122.7 in the ^{13}C NMR spectrum (Table 1), ascribable to the double bond of a chromene derivative [10]. Thus, **2** was established as 2,2-dimethyl-6-acetyl-8-(3′-hydroxy-3′-methyl-*trans*-but-1′-enyl)-chrom-3-ene, previously isolated from a *Stoebe* species and identified only on the basis of ^{1}H NMR data [11].

Table 1. NMR data of compounds **1** and **2** (CD_3OD)*

	1		**2**	
	δ_C	δ_H (J_{HH} Hz)	δ_C	δ_H (J_{HH} Hz)
2	81.7		78.4	
3	49.0	2.83 *s*	132.3	5.83 *d* (10)
4	193.6		122.7	6.48 *d* (10)
4a	120.7		119.0	
5	127.1	8.38 *d* (2)	126.7	8.00 *d* (2)
6	130.7		130.9	
7	132.7	8.32 *d* (2)	127.9	7.61 *d* (2)
8	128.2		128.0	
8a	161.5		159.6	
9	198.6		197.5	
10	26.2	2.59 *s*	26.2	2.53 *s*
11	26.5	1.54 *s*	28.4	1.50 *s*
12	26.5	1.54 *s*	28.4	1.50 *s*
1′	119.9	6.94 *d* (16)	120.5	6.89 *d* (16)
2′	142.0	6.61 *d* (16)	140.4	6.54 *d* (16)
3′	71.4		71.2	
4′	29.7	1.43 *s*	29.8	1.42 *s*
5′	29.7	1.43 *s*	29.8	1.42 *s*

* Assignments confirmed by HETCOR and COLOC experiments.

The isolation of **1** and **2** as natural products from *W. nubigena*, together with *p*-hydroxyphenone derivatives, is interesting from a chemotaxonomic and biogenetical point of view, because *p*-hydroxy acetophenones have been proposed as the precursors of benzofurans and benzopyrans, which occur widely in the Asteraceae [2]. Benzopyran derivatives are reported to possess bacteriostatic, antitumoral and insecticidal activity [12]. On the other hand, the occurrence of PAs which exhibit hepatotoxic and mutagenic properties [6, 7] strongly limits the use of *W. nubigena* as a home remedy for inflammatory and gastrointestinal deseases. The co-occurrence of acetophenones, benzopyrans and PAs, although not observed in *W. dactylophylla* and *W. ciliolata*, is typical of the Senecioneae, in particular, of the genus *Senecio* [13].

EXPERIMENTAL

FABMS spectra, DEPT, HETCOR and COLOC experiments were performed as described earlier [14].

Plant material. Werneria nubigena was collected at Yamobamba, Agallpampa, Otuzco Province, Departamento de la Libertad, Peru, at 2830 m above the sea level. A voucher sample is deposited at the Departamento de Quimica, Pontifica Universidad Catolica del Perù.

Extraction and isolation. Air-dried aerial parts (250 g) were defatted with petrol (40–70°) and successively extracted with $CHCl_3$ (14 g), $CHCl_3$–MeOH (9:1) (8 g) and MeOH (21 g). A portion of the $CHCl_3$–MeOH residue (2.5 g) was chromatographed on a Sephadex LH-20 column (80 × 2 cm). Frs (8 ml) were eluted with MeOH and checked by TLC on silica gel in $CHCl_3$–MeOH–H_2O (70:30:3) and *n*-BuOH–HOAc–H_2O (12:3:5). Frs 23–37 (650 mg), containing a crude alkaloid mixt., were further purified by HPLC on a C-18 μ-Bondapak column using MeOH–H_2O (3:7) (flow rate 2 ml min^{-1}) to yield pure retrorsine (**7**) (50 mg, R_t 50 min), retrorsine-*N*-oxide (**8**) (135 mg, R_t 20 min) and rosmarinine-*N*-oxide (**9**) (33 mg, R_t 30 min). Frs 38–41 (125 mg), submitted to RP-HPLC using MeOH–H_2O (1:4) (flow rate 2 ml min^{-1}), afforded *p*-hydroxyacetophenone-*O*-β-D-glucopyranoside (**4**) (29 mg). Frs 44–50 (305 mg), 60–62 (15 mg) and 68–72 (20 mg) were found to contain, respectively, pure *p*-hydroxyacetophenone (**3**), 3,5-dicaffeoylquinic acid (**5**) and 3-caffeoylquinic acid (**6**). A part of $CHCl_3$ extract (4.5 g), chromatographed on a silica gel column using $CHCl_3$ and increasing amounts of MeOH, gave together with frs containing **3** and **7–9** in a large amount, frs 81–96 (116 mg) which, when submitted to HPLC using MeOH–H_2O (7:3) (flow rate 2 ml min^{-1}) yielded pure **1** (33.6 mg, R_t 14 min.) and **2** (7.2 mg, R_t

22 min). The MeOH extract showed a TLC profile very similar to that of the $CHCl_3$–MeOH extract.

Compound **1**. Negative FABMS *m/z*: $[M-H]^-$ 301. ^1H and ^{13}C NMR: Table 1.

Compound **2**. Negative FABMS *m/z*: $[M-H]^-$ 285. ^1H and ^{13}C NMR: Table 1.

Compounds **3–9**. Identified by comparison of their spectral data with those reported in the lit. [2, 4, 7, 15, 16].

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