Pergamon

PII: S0031-9422(96)00565-1

Phytochemistry, Vol. 44, No. 4, pp. 755–757, 1997
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0031-9422/97 \$17.00+0.00

# AN UNUSUAL DITERPENE AND OTHER CONSTITUENTS FROM *BACCHARIS OCHRACEA*

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(*Received* 1 *July* 1996)

**Key Word Index**—*Baccharis ochracea*; Asteraceae; α-spinasterol; chlorogenic acid; isochlorogenic acid; isoquercitrin; geranylgeraniol derivative.

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**Abstract**—*Baccharis ochracea* contains a new diterpene in addition to the known compounds chlorogenic acid, isochlorogenic acid, α-spinasterol and isoquercitrin. The diterpene is a geranylgeraniol derivative with a butenolide ring. Copyright © 1997 Elsevier Science Ltd

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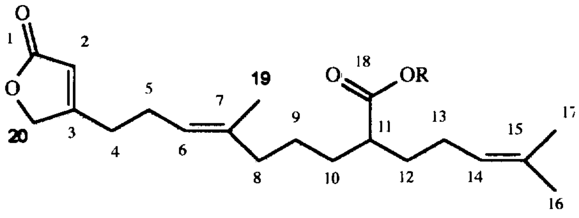
## INTRODUCTION

The genus *Baccharis* is native and almost exclusive to America, and encompasses almost 400 species. It is of pharmaceutical interest, with many species, such as *B. trimera*, *B. articulata* and *B. crispa* being used in folk medicine and also in pharmaceutical products. Most of the reputed medicinal *Baccharis* species have been chemically investigated, but there are no reports on *B. ochracea* Spreng, which is used in southern Brazil under the folk name 'erva-santa' for digestive disturbances [1].

Because the crude extract of its aerial parts showed significant effects on the reproduction of female rats [2], we initiated a chemical investigation. Herein, we report on the occurrence of the known compounds chlorogenic and isochlorogenic acid, α-spinasterol and isoquercitrin and the structural determination of a new diterpene, found to be in the main component of the methylene chloride extract.

## RESULTS AND DISCUSSION

The methylene chloride extract of the aerial parts yielded as main components compound **1** and the known compound α-spinasterol (7,22-stigmastadien-3β-ol) which has been reported from *B. genistelloides* [3], *B. ulicina*, and *B. pingreea* [4]. In the ethanol–water extract the known compounds chlorogenic acid, isochlorogenic acid and isoquercitrin could be identified by comparison with authentic samples.



**1** R = H
**2** R = Me

Compound **1** proved to be difficult to purify. However, after finding that it could be extracted with aqueous NaOH or $NaHCO_3$, a diazomethane derivative was prepared from the impure fractions and subsequently purified by column chromatography over silica gel, resulting in 27 mg of methylated product **2**.

The $^{13}C$ NMR spectra indicated the presence of two carbonyls, three other double bonded quaternary carbon atoms, four methine, eight methylene and four methyl groups. The mass spectra (GC/MS) indicated an $M_r$ of 348. Thus, the most likely molecular formula was $C_{21}H_{32}O_4$. The six double bond equivalents, together with the six olefinic carbons and two carbonyls suggested the presence of a ring. Evidence for a butenolide ring followed from the 1785 $cm^{-1}$ absorption band in the IR spectrum and from the characteristic NMR signals, i.e. a methylene signal at $\delta_C$ 73.1 and $\delta_H$ 4.73 (*d*, 2 Hz).

The structure of the chain attached to the ring was deduced by detailed investigation of the one and two dimensional NMR spectra. The 600 MHz $^1H$ NMR spectrum provided all the $^1H$–$^1H$ coupling constants.

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The two-dimensional COSY spectrum yielded the proton connectivities. The HSQC spectrum showed the direct connectivities between the protons and carbons, while the HMBC spectrum provided the long-range connectivities.

The HMBC spectrum proved particularly useful for the assignment of the $^{13}C$ and $^{1}H$ NMR spectra. The experiment was optimized for long-range $^{13}C$–$^{1}H$ coupling constants of about 8 Hz, providing a large number of connectivities. In fact, only four out of 75 two-bond and three-bond correlations were not detected: $^{2}J_{H6-C7}$, $^{2}J_{H14-C15}$, $^{3}J_{H20-C4}$ and $^{3}J_{H12a-C14}$. Two connectivities of low intensity were observed that must be attributed to a four-bond coupling, i.e. $^{4}J_{H4-C1}$ and $^{4}J_{H5-C8}$. In both cases the coupling path contains a double bond, which might explain such a correlation.

To our knowledge, this compound has never been described and geranylgeraniol derivatives with a butenolide end-group have been reported only for a few plants. The isolated compound resembles only the diterpene lactones reported from the genera *Conyza*, *Gutierrezia*, *Nardophyllum*, and *Solidago*, belonging to the same tribe Asteracea [5, 6 (and references cited therein)] and this restricted occurrence may be of chemotaxonomic relevance. It is interesting that compound **1** could be detected only in the plant materials collected during the flowering (first and third collection), but not in the material collected before flowering.

## EXPERIMENTAL

*General. NMR*: Bruker AMX-600 spectrometer, using a 5 mm triple resonance inverse probe for all experiments. Spectra were recorded in $CDCl_3$ at room temp. Chemical shifts are presented in $\delta$ values relative to TMS. $^{1}H$-detected heteronuclear single-quantum coherence (HSQC) was measured according to the method of ref. [7]; HMBC was performed according to the method of ref. [8]. A phase-sensitive double quantum filtered COSY spectrum was recorded according to the method of ref. [9].

*Plant material.* Aerial parts of *B. ochracea* Spreng were collected in March 1989 (235 g), during flowering, in Porto Alegre (Voucher no. ICN 95036). A second collection was made in November 1990 (1600 g, before flowering) and a third one in March 1991 during flowering (430 g) (Voucher no. Leg. Sobral 6817). Voucher specimens are deposited in the Botany Department Herbarium of Rio Grande do Sul Federal University. The plants were identified by Marcos Sobral, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul.

*Extraction and isolation.* The crude plant materials were extracted by maceration with $CH_2Cl_2$–MeOH (9:1) to give 7 g, 50 g and 13 g of crude extracts, respectively. These crude extracts were partitioned with MeOH (90%) and petrol. A more polar extract was prepared from the first collection (235 g of aerial parts) by maceration with EtOH–$H_2O$ (2:3). The crude extract obtained (7.2 g) was submitted to prep. TLC on cellulose with BuOH–HOAc–$H_2O$ (4:1:5) to separate the main fluorescent compounds. These were recovered from the TLC plates with water and characterized by chromatographic and UV comparison with authentic samples (Sigma products) as chlorogenic acid, isochlorogenic acid and isoquercitrin. The chromatographic comparison was performed by 2D-TLC on cellulose, using water as the first developing solvent and HOAC (10%) as the second.

The MeOH fr. (4 g) from the second collection was fractionated by flash CC on silica gel (0.063–0.2 mm, Merck), eluting gradually with $CH_2Cl_2$ and $CH_2Cl_2$–MeOH (99:1, 98:2, 97:3, 96:4, 95:5) to give 50 frs of 100 ml. Frs 40–50, with a common main component, were combined and submitted to repeated CC ($CH_2Cl_2$; petrol–EtOAc, 4:1) to yield 37 mg of $\alpha$-spinasterol: structure secured by IR (3 400 and 1 040 $cm^{-1}$), EIMS of the peracetylated derivative [*m/z* (rel. int.): 454 [M] (1), 314 (31), 255 (34), 213 (20)], and $^{1}H$- and $^{13}C$ NMR data [3].

Compound **1** was obtained from the MeOH fraction by silica gel CC ($CHCl_3$–MeOH, 19:1) as the main component from the first and third plant material (flowering plant), but not from the second collection. The frs containing compound **1** proved to be difficult to purify; after repeated silica gel CC with different mixts of petrol–EtOAc, methylation with $CH_2N_2$ was used to obtain a pure compound, resulting in 60 mg of a more apolar fr. This was purified over silica gel with the eluent petrol–EtOAc (4:1), resulting in 27 mg methylated product **2** as a viscous oil. IR $\nu^{KBr}$ $cm^{-1}$: 1 785 ($\gamma$-lactone), 1 740 (ester): GC–MS (70 eV) *m/z* (rel. int.) 348 $[M]^+$ (2), 317 $[M-CH_3O]^+$ (5), 316 $[M-MeOH]^+$ (5), 266 (10), 235 (18), 219 (20), 109 (32), 98 (100), 82 (95); $^{1}H$ NMR (600 MHz, $CDCl_3$): Table 1; $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 176.7 (C-18), 174.0 (C-1), 170.1 (C-3), 137.2 (C-7),

Table 1. $^{1}H$ NMR data of compound **2**

| H | $\delta$ |
|---|---|
| 2 | 5.84 *tt* (2.0; 1.5) |
| 4 | 2.45 *t* (7.2) (2H) |
| 5 | 2.29 *td* (7.2; 6.5) (2H) |
| 6 | 5.08 *ttq* (6.5; 1.3; 1.2) |
| 8 | 1.97 *t* (7.0) (2H) |
| 9 | 1.35 *dtd* (8.5; 7.0; 7.0) (2H) |
| 10a | 1.55 *dddd* (13.7; 9.0; 8.5; 7.0) |
| 10b | 1.40 *dddd* (13.7; 8.5; 7.0; 5.0) |
| 11 | 2.36 *tt* (9.0; 5.0) |
| 12a | 1.66 *dddd* (13.7; 9.0; 8.5; 7.0) |
| 12b | 1.46 *dddd* (13.7; 8.5; 7.0; 5.0) |
| 13 | 1.95 *ddd* (8.5; 7.0; 6.5) (2H) |
| 14 | 5.06 *tqq* (6.5; 1.4; 1.2) |
| 16 | 1.68 *d* (1.4) (3H) |
| 17 | 1.58 *d* (1.2) (3H) |
| 19 | 1.59 *d* (1.2) (3H) |
| 20 | 4.73 *d* (2.0) (2H) |
| $COOCH_3$ | 3.67 *s* (3H) |

132.2 (C-15), 123.6 (C-14), 122.0 (C-6), 115.6 (C-2), 73.1 (C-20), 51.3 ($COOCH_3$), 45.0 (C-11), 39.3 (C-8), 32.5 (C-12), 31.9 (C-10), 28.7 (C-4), 25.9 (C-13), 25.7 (C-16), 25.6 (C-5), 25.5 (C-9), 17.6 (C-17), 16.0 (C-19).

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