



TERPENOID CONSTITUENTS OF *VIGUIERA TUCUMANENSIS*

KARINA M. MERAGELMAN,* LUIS ARIZA ESPINAR,* VIRGINIA E. SOSA,*† MARÍA L. URIBURU‡ and JUANA R. DE LA FUENTE‡

*Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Instituto Multidisciplinario de Biología Vegetal (IMBIV) CONICET-UNC, C.C. 61-Suc. 16-5016 Córdoba, Argentina; ‡Consejo de Investigaciones-Universidad Nacional de Salta, 4400 Salta, Argentina

(Received in revised form 8 June 1995)

Key Word Index—*Viguiera tucumanensis*; Asteraceae—Heliantheae; sesquiterpene lactones; diterpenes; sesquiterpenes; coumarin; chemotaxonomy.

Abstract—Two varieties of *Viguiera tucumanensis*, collected in two different geographical regions of Argentina, were shown to have a very close chemical constitution. They all gave the new diterpene clerod-14-ene-3 α ,4 β ,13 ξ -triol. The taxonomic implications are discussed briefly.

INTRODUCTION

The genus *Viguiera* includes ca 150 species which are exclusive to warm and tropical America [1]. Sesquiterpene lactones, mainly germacranolides, heliangolides, 2(3H)-furanoheliangolides and diterpenes have been found to be the major constituents in the genus.

According to Cabrera [2], three varieties are recognized for *Viguiera tucumanensis* (Hook. et Arn.) Griseb. based on leaf morphology; however, he indicates that in some cases the limits between taxa are not clear thus making precise identification difficult.

We report here on the isolation and identification of the constituents of *Viguiera tucumanensis* var. *discoidea* (Griseb.) Cabr. and *V. tucumanensis* var. *oligodonta* (Blake) Cabr. In both cases, two populations from different geographical regions of Argentina were studied.

RESULTS AND DISCUSSION

The samples taken for analysis each contained three common constituents; the new diterpene, clerod-14-ene-3 α ,4 β ,13 ξ -triol (**1a**), annulide A (**6**) [3] and 17,18-dihydroleptocarpin (**4a**) [4]. The samples from the Salta and Jujuy Provinces yielded acetyl-17,18-dihydroleptocarpin (**4b**) [5], tomentosin (**8**) [6] and dihydroniveusin B (**5b**) [7, 8]. The Jujuy material also provided roseostachenone (**2**) [9], and the one from Córdoba gave leptocarpin (**4c**) [4], eupatolide (**7a**) [10], dihydroniveusin A (**5a**) [7], niveusin A (**5c**) [11], oplopanone (**9**) [12], scoparone and the diterpene 12 β -ethoxy-ent-kaur-9(11), 16-dien-19-oic acid (**3**) [13].

Known compounds were identified by comparison of their spectroscopic properties with those reported in the literature.

The ¹H NMR spectrum of **1a** (Table 1) showed the typical signals for the five methyl groups of a clerodane type diterpene, i.e. a doublet at δ 0.76 and four singlets at δ 1.28, 1.24, 1.15 and 0.74 (3H each). Signals at δ 5.87 (*dd*, *J* = 17.4 and 10.7 Hz), 5.17 (*dd*, *J* = 17.4, 1.3 Hz) and 5.03 (*dd*, *J* = 10.7, 1.3 Hz) together with a three proton singlet at δ 1.28 revealed the presence of a side chain at C-9 as in roseostachenone (**2**) [9]. This was confirmed by the ¹³C NMR spectrum (Table 2) which showed the expected signals at δ 145.0 (*t*), 111.8 (*t*), 73.5 (*s*) and 18.5 (*s*) [9]. A triplet at δ_{H} 3.56 (*J* = 2.8 Hz) indicated a hydroxyl group on a secondary carbon atom, C-2 or C-3. Both the multiplicity and the COSY 45 H-H spectrum confirmed the substitution was at C-3, while the value of the coupling constant (2.8 Hz) established that the hydroxyl group was axial. Three proton singlets at δ 1.24 had to be on a carbon atom attached to a carbon atom bearing an hydroxyl group: this was confirmed by a singlet at δ 76.4 in the ¹³C NMR spectrum (Table 2). The mass spectrum data supported the gross structure of **1a** (Experimental). The only remaining question about the clerodane **1a** was the stereochemistry of the ring junction. In both 5 α ,10 β -*trans*- and *cis*-5 β ,10 β -*cis*-clerodanes the C-4 methyl group adopts an axial orientation, that is *trans* to H-10 [14]. The stereochemistry of **1a** was determined by comparing the ¹H NMR chemical shifts of the methyl groups with the data of a large number of clerodanes. Thus, the shifts of the methyl groups at C-8 and C-9 were in agreement with the reported data for compounds having α -substituents on a *trans*-clerodane skeleton [15]. On the other hand, the data for the C-4 and C-5 methyl

†Author to whom correspondence should be addressed.

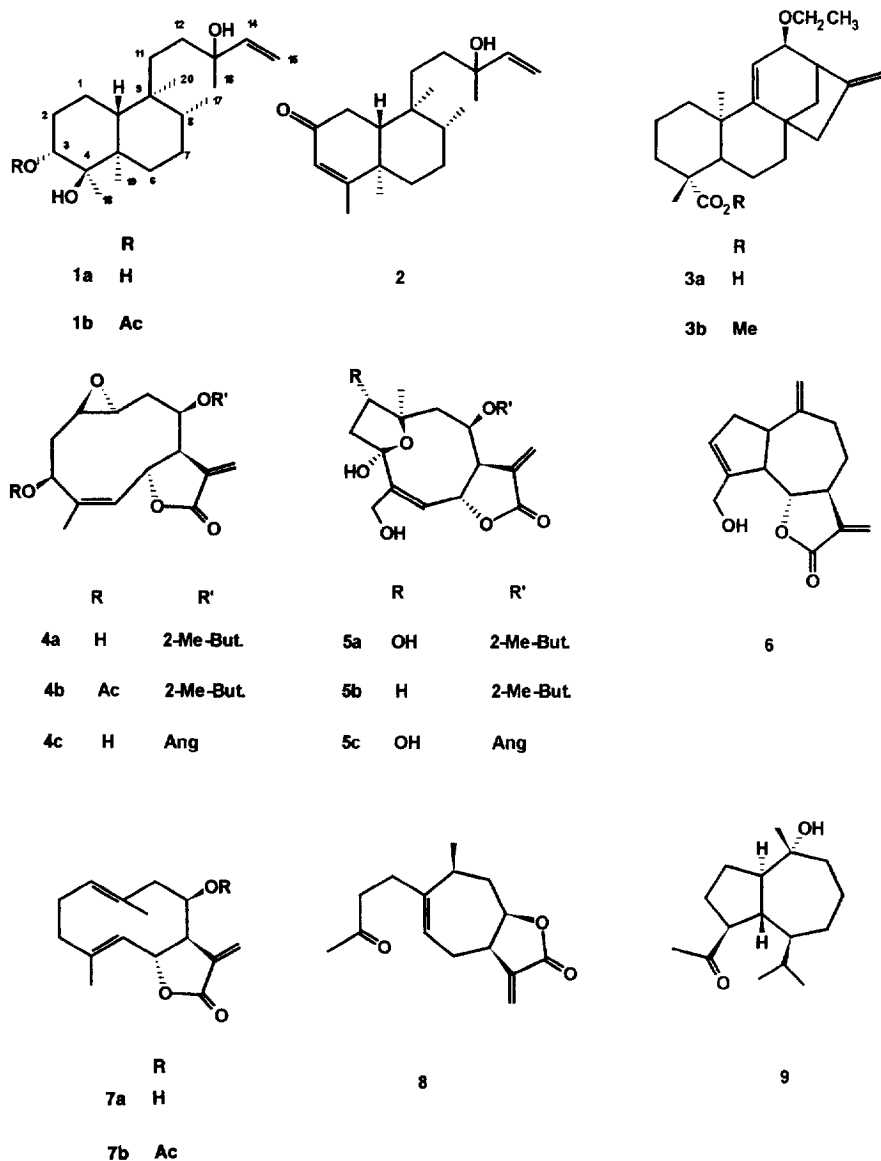


Table 1. ^1H NMR spectra of compounds **1a** and **1b** (200 MHz, CDCl_3 , TMS as int. st.)*

H	1a	1b
2	1.64 m	
2'	1.96 m	
3	3.56 t (2.8)	4.73 t (2.8)
14	5.78 dd (17.4, 10.7)	5.89 dd (17.4, 10.7)
15	5.20 dd (17.4, 1.2)	5.17 dd (17.4, 1.2)
15'	5.06 dd (10.7, 1.2)	5.05 dd (10.7, 1.2)
16	1.28 s†	1.28 s†
17	0.76 d† (7)	0.80 d (7)†
18	1.24 s†	1.11 s†
19	1.15 s†	1.08 s†
20	0.74 s†	0.74 s†
OCOCH_3		2.06 s†

*Numbers in parentheses are coupling constants in Hz.

†Intensity three protons.

groups, both in an α -configuration, were consistent with other *trans*-clerodanes [16–19]. Furthermore, cursory examination of the signal at δ 1.15 due to the Me group at C-5, revealed that the peak was broader and smaller than those signals of the other Me groups in the molecule. This was due to a 4σ -bond coupling of the Me protons and the axial hydrogen at C-6 as was evident in the COSY 45 HH spectrum. Such long-range couplings of the protons of angular Me groups are well established and are indicative of an axial Me group on a *trans*-fused decaline, since only in such situations are the stereochemical requirements for significant 4σ -bonds created [20]. Therefore, the Me group at C-5 was α -axial and that at C-4 was α -equatorial as in methyl tucumanate, a related diterpene acid obtained from *Baccharis tucumanensis* [15]. Acetylation of **1a** gave the derivative **1b** whose ^1H NMR spectrum showed that the signal of H-3 was deshielded from δ 3.56 to 4.73 while the ^{13}C NMR spectrum allowed

Table 2. ^{13}C NMR spectra of compounds **1a** and **1b** (50 MHz, CDCl_3 , TMS as int. st.)*

C	1a	1b
1	16.2 t	16.8 t
2	30.3 t	27.8 t
3	76.2 d	75.7 d
4	76.4 s	73.7 s
5	41.2 s	41.5 s
6	32.4 t†	32.3 t†
7	26.4 t	26.6 t
8	36.0 d	36.1 d
9	38.3 s	38.4 s
10	40.7 d	40.5 d
11	32.2 t†	32.2 t†
12	35.3 t	35.4 t
13	73.4 s	73.4 s
14	145.0 d	145.1 d
15	111.8 t	111.8 t
16	27.5 q	27.5 q
17	15.9 q	15.9 q
18	21.3 q	21.4 q
19	17.2 q	16.9 q
20	18.5 q	18.5 q
-OCOCH ₃		21.2 q
-OCOCH ₃		170.2 s

*Multiplicities by DEPT.

†Assignments may be interchangeable.

confirmation of the assignments of C-2 and C-4 (Table 2).

There are some noticeable features in the metabolites found in this species. To our knowledge, this is the first time that oplopanone has been found in the tribe. This skeletal type being common in Senecioneae [21] and Eupatorieae [22]. The ethoxy group in **3** might be an artifact due to the extraction procedure (Experimental), however it has been found previously in *Viguiera excelsa* (Willd.) Bent. et Hook. [13] and *Stevia eupatoria* (Spreng.) Willd. [23] where no ethanol was used in the extraction and purification methods. It is also noteworthy that the presence of tomentosin (**5**), a sesquiterpene lactone of the xanthanolide group, has not been previously found in the genus.

The co-occurrence of germacranolides, heliangolides and guaianolides is also characteristic in *Helianthus*, a genus that is closely related to *Viguiera* and our findings support this point of view. Thus, eupatolide (**8**) is the major constituent of *Helianthus argophyllus* Torr. et Gray [24], niveusin A (**5c**) has been isolated from *H. niveus* (Benth.) Brand. [11] and annulide A (**6**) from cultivar *H. annuus* L. var. *SH-222* [3].

The constituents isolated from the *V. tucumanensis* collections show close similarity and this leads us to conclude that there is no such delimitation in varieties for this species and that the slight differences in the compounds isolated from the various samples may be due to environmental reasons.

EXPERIMENTAL

General. Mps: uncorr.; CC: silica gel 60 (70–230 mesh) unless otherwise indicated; TLC: precoated Silica gel 60 F₂₅₄ plates (Merck). Detection was achieved by irradiation with UV light and spraying with conc. H_2SO_4 as reagent followed by heating.

General extraction procedure. Air-dried aerial parts were exhaustively extracted with CHCl_3 . The residue obtained after evapn of the solvent was dissolved in hot EtOH and a soln of 4% $\text{Pb}(\text{AcO})_2$ was added. After standing overnight, the ppt. was filtered off, the organic solvent evaporated and the aq. soln extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 , the solvent evaporated under reduced pressure yielding a gummy residue which was divided into several main frs by CC.

Plant material. *Viguiera tucumanensis* var. *oligodonta* was collected near Toledo, Córdoba Province in April and identified by Dr Luis Ariza Espinar. A voucher specimen is deposited in the Museo Botánico, Córdoba (CORD). Another sample of the same variety was collected in Salta Province. It was identified by Ing Lázaro Novara and a voucher specimen is deposited at the Museo of Universidad Nacional de Salta (TOLABA 145).

Viguiera tucumanensis var. *discoidea* was collected in both El Alfarcito (Salta Province) (NOVARA 10254) and El Volcán (Jujuy Province) and identified by Ing Lázaro Novara. Both samples are deposited in the Museo of the Universidad Nacional de Salta (NOVARA 10600).

Isolation and purification. 2.5 kg of the plant material from Córdoba and processed as described above yielded 185 g of a dark extract. CC of the residue (14 g) using hexane containing increasing amounts of EtOAc as eluant yielded frs A–F. Further purification of fr. A by CC and radial chromatography yielded 3.5 mg **3a**. Methylation with CH_2N_2 afforded **3b** whose IR, NMR, and MS were identical to those reported in lit. [13, 21].

From fr. B 162 mg of eupatolide (**7a**) [10] were obtained by recrystallization and was identified through its acetyl derivative (**7b**) [25].

CC of fr. C yielded 18 mg dihydroleptocarpin (**4a**) [4], 20 mg leptocarpin (**4c**) [4], 9 mg annulide A (**6**) [3] and scoparone, while fr. D gave 10 mg **1a**. CC ($\times 2$) of fr. E produced 8 mg dihydroniveusin A (**5a**) [7] and 5 mg niveusin A (**5c**) [11].

Viguiera tucumanensis var. *oligodonta*. 360 g of the Salta material were extracted as described above yielding 7 g of a residue that was separated into frs A, B and C by CC using benzene containing increasing amounts of EtOAc as eluant. Fr. A, after a second CC and prep. TLC, yielded 4 mg tomentosin (**8**) [6]. Crystallization from EtOAc of fr. B afforded 6 mg acetyl-dihydroleptocarpin (**4b**) [5] while the mother liquors were passed through Sephadex LH-20 to give 38 mg dihydroniveusin B (**5b**) [7]. CC of fr. C on Sephadex LH-20 yielded 11 mg dihydroleptocarpin (**4a**) [4] and 10 mg **1a**.

Viguiera tucumanensis var. *discoidea*. 500 g of the Salta materials were extracted as described yielding 6 g of

final residue. It was divided into frs A, B and C by CC. Further purification gave 5 mg tomentosin (**8**) [6] from fr. A; 59 mg acetyl-dihydroleptocarpin (**4b**) [5] and 38 mg dihydroniveusin B (**5b**) [7,8] from fr. B while fr. C yielded 160 mg dihydroleptocarpin (**4a**) [4] and 12 mg **1a**.

Viguiera tucumanensis. var. *discoidea*. 1.1 kg of the samples collected in Jujuy and processed as described above yielded the same compounds as those obtained from the Salta variety. Prep. TLC of fr. A gave 11 mg roseostachenone (**2**) [9] and 8 mg annulide A (**6**) [3].

Clerod-14-ene-3 α ,4 β ,13 ξ -triol (1a). White crystals (benzene), mp 118–120°. IR $\nu_{\text{cm}^{-1}}^{\text{KBr}}$: 3443, 1639, 1101, 1081, 978, 918; EIMS, m/z (rel. int.): 306 $[M - 18]^+$ (1.5), 225 $[M - C_6H_{11}O]^+$ (31), 207 $[M - C_6H_{11}O - 18]^+$ (20); NMR: Tables 1 and 2.

Acetylation of 1a. 7 mg of **1a** were dissolved in 1 ml pyridine and 2 ml Ac₂O. Usual work-up yielded 6 mg of **1b** as an amorphous solid. IR, $\nu_{\text{cm}^{-1}}^{\text{KBr}}$: 3473, 3427, 1743, 1650, 1249, 1110; Tables 1 and 2.

Acknowledgments—Work at Salta University was carried out with funds from Consejo de Investigaciones and that at Córdoba with funds from CONICOR and SECYT (UNC). K.M.M. thanks CONICET and SECYT (UNC) for doctoral fellowships.

REFERENCES

- Mabberley, D. J. (1990) *The Plant Book*, p. 607. Cambridge University Press, Cambridge.
- Cabrera, A. L. (1978) *Fl. Prov. Jujuy* **10**, 371.
- Macías, F., Varela, R., Torres, A. and Molinillo, J. (1993) *Phytochemistry* **34**, 669.
- Martínez, R., Ayamante, I., Núñez-Alarcón, J. and Romo de Vivar, A. (1979) *Phytochemistry* **18**, 1527.
- Bohlmann, F., Jakupovic, J., Ahmed, M., Grenz, M., Suding, H., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 113.
- Rodríguez, E., Yoshioka, H. and Mabry, T. J. (1971) *Phytochemistry* **10**, 1145.
- Gao, F. and Mabry, T. J. (1986) *Phytochemistry* **25**, 137.
- Guerreiro, E. (1986) *Phytochemistry* **25**, 748.
- Fazio, C., Passamanti, S., Paternostro, M. and Piozzi, F. (1992) *Phytochemistry* **31**, 3147.
- Dolejs, L. and Herout, V. (1962) *Coll. Czechoslov. Chem. Commun.* **27**, 2654.
- Ohno, N. and Mabry, T. J. (1980) *Phytochemistry* **19**, 609.
- Takeda, K., Minato, H. and Ishikawa, M. (1966) *Tetrahedron Suppl.* **7**, 219.
- Delgado, G., Cárdenas, H., Peláez, G. and Romo de Vivar, A. (1984) *J. Nat. Prod.* **47**, 1042.
- Forster, P. G., Ghisalberti, E. L. and Jefferies, P. R. (1985) *Phytochemistry* **24**, 2991.
- Rossomando, P. C., Giordano, O. S., Espiñeira, J. and Joseph-Nathan, P. (1985) *Phytochemistry* **24**, 787.
- McCrindle, R. and Nakamura, E. (1974) *Can. J. Chem.* **52**, 2029.
- McCrindle, R. and Nakamura, E. (1976) *J. Chem. Soc. Perkin Trans. 1* 1590.
- Bohlmann, F., Jakupovic, J., Dhar, A. K., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 843.
- Bohlmann, F., Bapuji, M., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 939.
- Bhacca, N. S. and Williams, D. H. (1964) *Applications of NMR Spectroscopy in Organic Chemistry, Illustrations from the Steroid Field*, pp. 116–121. Holden-Day, San Francisco.
- Joseph-Nathan, P., Villagómez, J. R., Román, L. U. and Hernández, J. D. (1990) *Phytochemistry* **29**, 977.
- Herz, W. and Watanabe, K. (1983) *Phytochemistry* **22**, 1457.
- Ortega, A., Morales, F. and Salmón, M. (1985) *Phytochemistry* **24**, 1850.
- Watanabe, K., Ohno, N., Yoshioka, H., Gershenzon, J. and Mabry, T. J. (1982) *Phytochemistry* **21**, 709.
- Doskotch, R. W. and El-Ferally, F. S. (1970) *J. Org. Chem.* **35**, 1928.