Pergamon

S0031-9422(96)00104-5

Phytochemistry, Vol. 42, No. 5, pp. 1367–1368, 1996

0031-9422/96 $15.00 + 0.00

CHEMOTAXONOMY OF THE ARGENTINIAN SPECIES OF *GAILLARDIA*

ELISA M. PETENATTI,* MAURICIO J. PESTCHANKER,† LUIS A. DEL VITTO* and EDUARDO GUERREIRO†‡

*Herbario Univ. Nac. San Luis,, Argentina; †Química Organica-INTEQUI (CONICET-U.N. de San Luis), Chacabuco y Pedernera, 5700-San Luis, Argentina; 5700-San Luis, Argentina

(*Received in revised form* 4 *January* 1996)

Key Word Index—*Gaillardia*; Asteraceae; chemotaxonomy; pseudoguaianolides; flavonoids.

Abstract—The biosystematic study of the Argentinian entities of the genus *Gaillardia* Foug. have confirmed the differentiation of three species, *Gaillardia cabrerae*, *G. tontalensis* and *G. megapotamica*, and the three varieties of the latter (var. *megapotamica*, var. *scabiosoides* and var. *radiata*). The chemotaxonomic study carried out on the above species and varieties has confirmed the morphological findings and resulted in the isolation of the biogenetic precursor, the pseudoguaianolide 2β-hydroxy-2,3-dihydrohelenalin from *G. megapotamica* var. *radiata*.

INTRODUCTION

The genus *Gaillardia*, endemic of the American continent, is characterized by its disjunct areas. It has been found in North America (between 57° and 22° N) and South America (Uruguay, and the Eastern and Central part of Argentina to the North of Patagonia). It comprises about 30 species distributed mainly in Mexico and the United States. Only three of them were found as natives in Argentina.

It belongs to the tribe *Helenieae* (sensu Bentham & Hooker) and it differs from the other tribe genus in its stiff receptacle and setaceous or fragile, weak setae; for this reason some authors place it in the subtribe *Gaillardiinae* [1, 2]. Biddulph [3] has accepted only *Gaillardia megapotamica* (Spr.) Bak. for Argentina. Later, Covas [4] described *G. cabrerae* and more recently *G. tontalensis* Hieron. has been rehabilitated [5]. On the other hand, *G. megapotamica* is a complex composed by three varieties, which are currently considered valids, as follows: *G. megapotamica* var. *megapotamica*, *G. megapotamica* var. *scabiosoides* (Arn.) Bak. and *G. megapotamica* var. *radiata* (Gris.) Bak. So, the authors have attempted to study the chemistry of all five taxa.

The sesquiterpene lactones are characteristic compounds among the numerous secondary metabolites found in the *Asteraceae* family and in the genus *Gaillardia* and they can be considered chemotaxonomic markers, while some flavonoids are probably typical for them.

RESULTS AND DISCUSSION

The five taxa were investigated for their sesquiterpene lactone and flavonoid contents. The chemical constituents of *G. megapotamica* var. *radiata*, *G. cabrerae* and *G. tontalensis* have not previously been reported. The aerial parts were extracted with methanol to give a greenish oil which was submitted to a combination of chromatographies on silica-gel and Sephadex LH-20 for the isolation of lactones and flavonoids, respectively. The complex *G. megapotamica* and *G. cabrerae* showed the presence of helenalin (**1**). In addition, *G. megapotamica* var. *radiata* contained two other pseudoguaianolides, mexicanin I (**2**) and 2β-hydroxy-2,3-dihydrohelenalin (**3**). Sesquiterpenoids were not isolated from *G. tontalensis*. Only two previous papers [6, 7] have reported data about South American entities; the second report [7] on *G. megapotamica* var. *scabiosoides* was not concordant with our data. Compound **3** was isolated from *G. megapotamica* var. *radiata* as an extremely minor constituent. Its molecular formula was deduced from the HR-mass spectrum as $C_{15}H_{20}O_5$. The presence of the sesquiterpene typical α,β-unsaturated-γ-lactone system, was inferred from the IR (1740 and 1660 cm^{-1}), UV (254 nm), ^1H NMR (δ 6.35, *d*, 1H and 5.81, *d*, 1H) and ^{13}C NMR [C-12, δ 169.32, C-11, 138.4 and C-13, 121.4] data. The absence of a second unsaturation, besides the Δ 11(13) double bond and the presence of two oxymethine protons (^1H NMR: δ 4.18 and 4.52; ^{13}C NMR: δ 65.7 and 76.5) were also inferred from spectral data. The presence of the two free HO-groups was explored by the preparation of the diacetate, but instead we found helenalin-6-acetate. This was confirmed by comparison with an authentic sample. The fast elimination of the acetate, leading to the formation of the Δ^2-double bond under the mild acetylation conditions, can occur when the leaving group is placed in the axial position. Besides this, the coupling constant of H-2 and H-3, in the ^1H NMR

‡Author to whom correspondence should be addressed.

spectrum showed no protons in the axial position of C-2. It is then concluded that the second HO-group is attached to the C-2 in a β-position. The co-occurrence of **1** and **3** is in agreement with the generally accepted biogenetic scheme, in which **3** may be the precursor of helenalin [9]. Nepetin, a flavone present in all taxa, was accompanied by luteolin in *G. megapotamica* var. *radiata*, and by jaceosidin and the flavanone eriodictyol in *G. tontalensis*. Our results do not agree with those reported in Ref. [8], which includes other flavones identified by means of paper chromatography in *G. megapotamica* (var.?) from Catamarca province (Argentina).

In particular, the chemical data have facilitated the differentiation of the three Argentine species of *Gaillardia* and also the three varieties of *G. megapotamica*. The anatomical, exomorphological, palynological and karyological results allowed us to reinforce the secession concepts between the taxonomical involucred entities [10, 11].

EXPERIMENTAL

Plant material. The aerial parts of all species and varieties mentioned were collected at the same phenological phase (full blooming period). *Gaillardia megapotamica* var. *scabiosoides* and *G. megapotamica* var. *radiata* were collected nearby San Luis city, in December 1989 (vouchers Del Vitto & Petenatti 4592 and 4594, respectively, Herbarium UNSL); *G. megapotamica* var. *megapotamica* from La Arenilla, San Luis province, in November, 1989 (voucher Del Vitto & Petenatti 4633, Herbarium UNSL); *G. cabrerae* from Lihuel-Calel National Park, La Pampa province, in March, 1991 (voucher Del Vitto & Petenatti 5820, Herbarium UNSL) and *G. tontalensis* from Los Paramillos, Mendoza province (voucher Del Vitto & Petenatti 5169, Herbarium UNSL).

Isolation and identification of the compounds. The aerial parts of each plant material were air-dried, finely ground and extracted at room temp with MeOH (3 × 48 hr) (*G. megapotamica* var. *megapotamica* 1.7 kg, *G. megapotamica* var. *scabiosoides* 2.5 kg, *G. megapotamica* var. *radiata* 1.9 kg, *G. cabrerae* 1.1 kg and *G. tontalensis* 0.13 kg). The crude extract obtained by evaporation under red. pres. were dissolved in MeOH–H_2O (8:2) and kept overnight in a refrigerator. After filtering to remove the ppt. the aq. soln was concd. The crude residue was adsorbed on silica gel and chromatographed over the same adsorbent packed in *n*-hexane. The elution was done with mixtures of increasing polarity of EtOAc and *n*-hexane affording: helenalin and nepetin from *G. megapotamica* var. *megapotamica* (20.62 g and 1.210 g), *G. megapotamica* var. *scabiosoides* (11.06 g and 2.03 g), *G. cabrerae* (2.41 g and 0.515 g), pseudoguaianolides, flavonoids mixtures from *G. megapotamica* var. *radiata* and flavonoids mixtures from *G. tontalensis*. Rechromatography of the flavonoids mixtures over Sephadex LH-20 (MeOH) from *G. megapotamica* var. *radiata* furnished nepetin (0.647 g) and luteolin (0.486 g), *G. tontalensis* furnished erodictyol (0.056 g), nepetin (0.017 g) and jaceosidin (0.021 g). Rechromatography of the pseudoguaianolides mixture over silica gel (gradient of EtOAc and *n*-hexane) furnished helenalin (8.20 g), mexicanin I (1.12 g) and 2β-hydroxy-2,3-dihydrohelenalin (0.012 g).

Known compounds were identified by comparison with authentic samples. The 2β-hydroxy-2,3-dihydrohelenalin (**3**) data are as follows: $[\alpha]_D$ +82.3, IR ν_{max}^{KBr} cm^{-1} 1740, 1660; UV λ_{max}^{MeOH} nm: 254; MS *m/z* (rel. int.): 280 $[M]^+$ (1), 262 $[M - H_2O]^+$ (**13**), 247 $[262 - CH_3]^+$ (1), 244 $[262 - H_2O]^+$ (6), 234 $[262 - CO]^+$ (9), 229 $[244 - CH_3]^+$ (5), 216 $[244 - CO]^+$ (3), 201 $[216 - CH_3]^+$ (2), 124 (100); ^1H NMR (200 Mhz, $CDCl_3$): δ 1.01 (3H, *s*, H-15), 1.18 (3H, *d*, $J = 7$ Hz, H-14), 3.45 (1 H, *m*, H-7), 4.18 (1 H, *d*, $J_{6,7} = 2.4$ Hz, H-6), 4.52 (1 H, *ddd*, $J_{1,2} = 2.7$ Hz, $J_{2,3\beta} = 2.1$ Hz, $J_{2,3\alpha} = 6.2$ Hz, H-2), 4.85 (1 H, *ddd*, $J_{8,7} = 7.0$ Hz, $J_{8,9\alpha} = 2.1$ Hz, $J_{8,9\beta} = 8.4$ Hz, H-8), 5.77 (1 H, *d*, $J_{7,13} = 2.4$ Hz, H-13), 6.29 (1 H, *d*, $J_{7,13'} =$ 2.6 Hz, H-13'); ^{13}C NMR (50.33): δ 16.2 (C-14), 19.0 (C-15), 22.8 (C-10), 37.7 (C-9), 46.5 (C-3), =2.7 48.6, 49.0 (C-1, C-7), 53.8 (C-5), 65.7 (C-2), 76.5 (C-6), 78.3 (C-8), 121.4 (C-13), 138.4 (C-11), 169.3 (C-12), 218.5 (C-5).

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