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biochemical
systematics
and ecology

Biochemical Systematics and Ecology 31 (2003) 417–422

www.elsevier.com/locate/biochemsysseco

Coumarins and a kaurane from *Gochnatia polymorpha* ssp. *polymorpha* from Paraguay

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Received 18 April 2002; accepted 1 June 2002

Keywords: *Gochnatia polymorpha* ssp.; *polymorpha*; *Gochnatia haumaniana*; Gochnatiinae; Mutisieae; Asteraceae; Coumarins; Kaurane; Triterpenes

1. Subject and source

Aerial parts of *Gochnatia polymorpha* (Less.) Cabrera ssp. *polymorpha* were collected on December 14, 1997 in Paraguay, Departamento Concepcion, at Yby-Jau at the intersection of highways 3 and 5. A voucher specimen is on deposit in the herbarium at the Instituto Miguel Lillo, Tucumán (leg. A. Schinini and M. Demathesis No. 33692, LIL 552576). Aerial parts of *Gochnatia haumaniana* Cabr. were collected at the flowering stage on December 14, 1997 in the Departamento Amambay, Paraguay. A voucher specimen (leg. A. Schinini and M. Dematheis No. 33437) is on deposit in the herbarium of the Instituto de Miguel Lillo (LIL 355735).

2. Previous work

Gochnatia polymorpha (Less.) Cabrera is one of 69 species of a mainly neotropical genus within Mutisieae (Cabrera, 1935, 1950, 1971; Sancho, 1999) traditionally

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placed in a subtribe Gochnatiinae which has more recently been absorbed within subtribe Mutisiinae (Brewer, 1994). Three subspecies, ssp. *polymorpha*, *ceanthifolia*, and *floccosa*, have been recognized (Cabrera, 1971). Previous articles on the chemistry of subspecies *polymorpha* whose distribution is limited to southeastern Brazil and Paraguay have reported isolation of the sesquiterpene lactones costunolide, dehydrocostunolide, a series of dimeric guaianolides and various bisabolenes from a Paraguay collection (Bohlmann et al., 1983, 1986), dehydrocostunolide and baurenyl acetate from a Brazilian collection under an old name *Moquinia polymorpha* (Less.) DC. (Tomassini and Gilbert 1972, Monteiro de Farias et al., 1984) and the eudesmanolide santamarin, two new polyfunctionalized kaurane diterpenoids of a type more commonly found in Astereae as well as various triterpenes and flavones from a collection near São Paulo, Brazil (Sacilotto et al., 1997). *Gochnatia haumaniana* Cabr., from the Sierra de Amambay, Paraguay and parts of southeastern Brazil (Cabrera, 1971) has not been studied previously.

3. Present work

3.1. General procedures

For HPLC, three columns 10 x 250 mm each (5 μ packing) were used: column A, Beckman Ultrasphere C18; column B, Beckman Ultrasphere C8 and column C, Phenomenex Luna Phenyl-hexyl. GC-EIMS were recorded using a Hewlett-Packard 6890 Series II gas chromatograph equipped with a capillary column HP-5MS (5% phenylmethylsiloxane, 30 m x 0.25 mm i.d. x 0.25 μ m film thickness) coupled to a HP 5973 (70 ev) mass spectrometer detector, He carrier gas. ^1H NMR spectra were recorded in CDCl_3 at 500 MHz on a Varian Inova spectrometer. Mass spectra were run on a Finnigan MAT 90 instrument.

3.2. Extraction and isolation of constituents

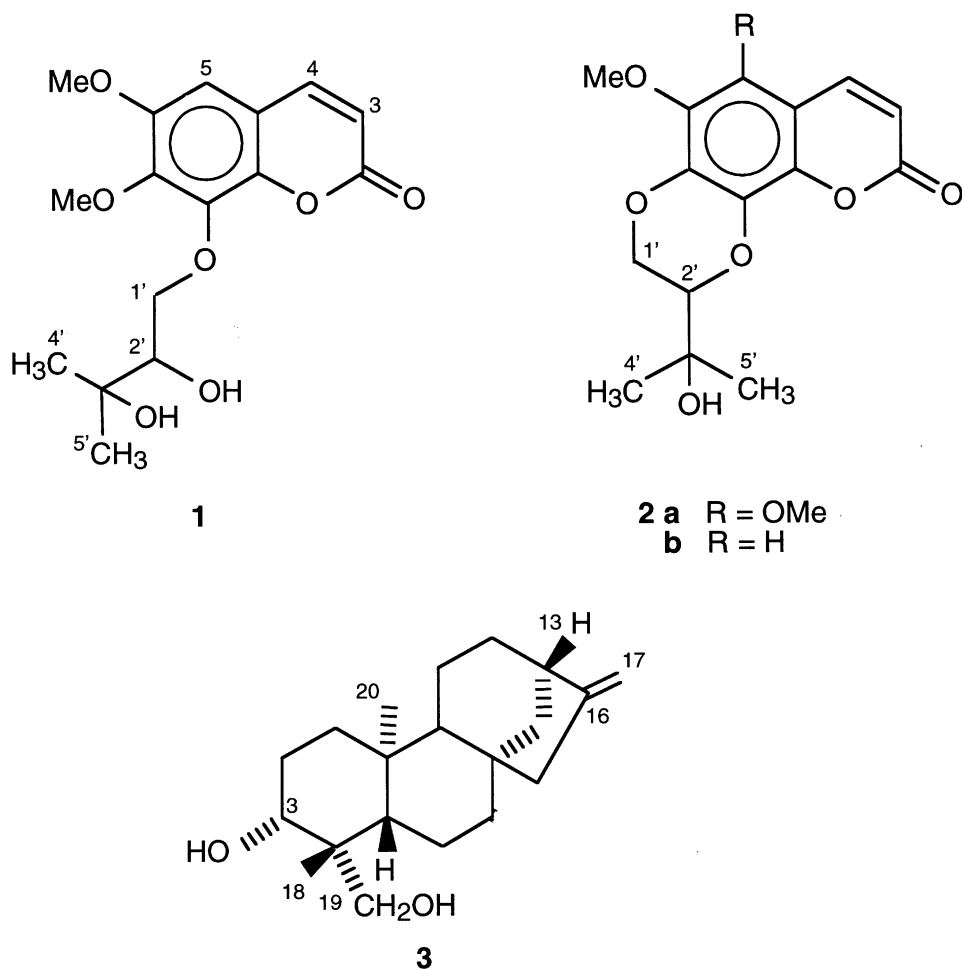
A. Aerial parts (700 g, mainly leaves) of *G. polymorpha* ssp. *polymorpha* were extracted with CHCl_3 (2 x 5 l) at rt for 7 days to give 29 g (4.1%) of crude extract which was suspended in EtOH (300 ml) at 55°, diluted with H_2O (225 ml) and extracted successively with hexane (3 x 250 ml) and CHCl_3 (3 x 300 ml). Evaporation of the hexane extract gave a residue (16.2 g) which was not studied further. The CHCl_3 extract on evaporation at red press furnished a residue which was subjected to CC (Si gel Merck 70–230 mesh) using CHCl_3 with increasing amounts of EtOAc (0–20%, frs 1–80) and finally CHCl_3 with increasing amounts of MeOH, (2–10%, frs 81–125). Frs 1–38 (1.77 g) were united and repeatedly chromatographed over Si gel using CHCl_3 -EtOAc mixtures to give two frs (25 mg and 15 mg) containing a mixture of alkanols $\text{C}_n\text{H}_{2n-1}\text{OH}$ ($n = 18$ –22), and three frs (385 mg, 15 mg, and 57 mg) of oleanolic acid containing very small amounts of different impurities. Frs 55–69 (193 mg) were reunited; a portion (81 mg) on HPLC (column A, MeOH- H_2O 1:1, 2 ml min^{-1}) gave 4 mg of loliolide (R_t 10.5 min), 3.5 mg of **1** (R_t 12 min), 4

mg of a 3:2 mixture (R_t 19 min) of purpurasolol (**2a**, Debenedetti et al., 1996) and purpurasol (**2b**, Debenedetti et al., 1992) identified by ^1H NMR spectrometry (500 MHz, CDCl_3); **2a** δ 8.00 (*d*, $J = 9.5$ Hz, H-4), 6.35 (*d*, $J = 10$ Hz, H-3), 4.64(*dd*, $J = 11.5$, 2.5 Hz, H-2a), 4.12(*dd*, $J = 11.5$, 9 Hz, H-2b), 3.96 (*dd*, $J = 9$, 2.5 Hz, H-3a), 3.88 (*s*, 3p, OMe), 1.442 and 1.35 (each *s* and 3p, H-4 and H-5); **2b**, δ 7.57 (*d*, $J = 9.5$ Hz, H-4), 6.49 *s*, H-5), 6.29 (*d*, $J = 9.5$ Hz, H-3), 4.61 (*dd*, $J = 11.5$, 2.5 Hz, H-2a), 4.10 (*dd*, $J = 11.5$, 9 Hz, H-2b), 3.97 (*dd*, $J = 9$, 2.5 Hz, H-3), 3.90 (*s*, 3p, OMe), 1.439 and 1.34 (each *s* and 3p, H-4 and H-5). Rechromatography (Si gel) afforded pure **2a**. Frs 70–81 (151 mg) were reunited; a portion (81 mg) on HPLC (column A, $\text{MeOH-H}_2\text{O}$ 98:2, 1 ml min^{-1}) yielded mixtures and 1.5 mg of slightly contaminated **3** (R_t 15.5 min) which exhibited ^1H NMR signals (CDCl_3) at δ 4.90 (*q*, $J = 1$ Hz, H-17a), 4.85 (*brs*, H-17b), 3.72 (*d*, $J = \text{MHz}$, H-19a), 3.57 (*d*, $J = 11$ Hz, H-19b), 3.12 (*dd*, $J = 12$, 5 Hz, H-3), 2.73 (*br*, H-13, coupled to H-17a,b at 2.18 *m* and 1.76c), 1.05 (*s*, 3p, H-20), 0.97 (*s*, 3p, H-18). Kaurane **3** has previously been reported from *Beyeria leschenaultii* (Euphorbiaceae) (Baddeley et al., 1964) and *Helichrysum dendroideum* (Asteraceae, Gnaphaliinae) (Lloyd and Fales, 1967).

Frs 82–107 (2.51 g) decomposed on standing and were not investigated further. Frs 108–114 (364 mg) on HPL gave only unidentifiable material while frs 115–125 (2.93) contained intractable tar.

6,7-Dimethoxy-8-(2,3-dihydroxy-3-methylbutoxy)-coumarin (**1**). Gum, slightly contaminated by an impurity; EI (rel int.) m/z 324 (M^+ , 15), 222 ($\text{M-C}_5\text{H}_{10}\text{O}_2$, 100), 194 (33), 59; ^1H NMR (CDCl_3 , 500 MHz) δ 7.59 (*d*, $J = 9$ Hz, H-4), 6.67 (*s*, H-5), 6.34 (*d*, $J = 9$ Hz, H-3), 4.52 (*dd*, $J = 11$, 2.5 Hz, H-1a), 4.07 (*s*, 3p, 7-OMe), 3.98 (*dd*, $J = 11$, 8 Hz, H-1b), 3.98 (*s*, 3p, 6-OMe), 3.65 (*dd*, $J = 8$, 2.5 Hz, H-2), 1.26 *s* and 1.20 *s* (each 3p, H-4 and H-5). The absence of a C-5 oxygen function was indicated by the chemical shift of H-4 and the presence of a substituent on C-8 was indicated by the absence of long-range coupling from the H-4 signal. Irradiation of the aromatic singlet (H-5) enhanced the OMe signal at δ 3.89 (6-OMe) and the H-4 signal. Irradiation of the H-1a signal enhanced the signal of H-1b and slightly the second OMe signal at δ 3.89 (6-OMe), thus locating the five carbon substituent on C-8.

B. Aerial parts (480 g, leaves and some flowers) of *G. haumaniana* were extracted with CHCl_3 (2 x 4 l) at r_t for 5 days to give 18.7 g (3.9%) of crude extract which was suspended in EtOH (192 ml) at 55°, diluted with H_2O (145 ml) and extracted successively with hexane (3 x 150 ml) and CHCl_3 (3 x 200 ml). Evaporation of the hexane extract gave a residue (12.4 g) which was not investigated. The CHCl_3 extract on evaporation at red press furnished a residue (4.8g) which was subjected to CC over Si gel using CHCl_3 with increasing amounts of EtOAc (0–100%), 120 frs being collected. Frs. 26–32 (230 mg) on rechromatography over Si gel with hexane–EtOAc (9:1) furnished 57 mg of α -amyrin identified by GC–MS and coinjection with an authentic sample. Frs. 58–62 (409 mg) from the mother column were reunited; HPLC (column b, $\text{MeOH-H}_2\text{O}$ 9:1, 2ml/min) of a portion gave oleanolic acid and a mixture of oleanolic and ursolic acids, which on rechromatography could be separated into its constituents.



4. Chemotaxonomic significance

Among the 15 *Gochnatia* taxa studied chemically so far, coumarins, specifically three which, like **1** and **2a, b**, are 6,7,8-trioxygenated — namely fraxetin, fraxidin and capensin, have so far been found only in *G. argentina* (Cabr.) Cabr. (Garcia and Guerrero, 1988). The latter, like *G. polymorpha* and *G. haumaniana*, is a member of section *Moquiniastrium* (Cabrera, 1977) whose distribution is limited to northern Argentina. However coumarins were absent from *G. hauminiana*, as shown in the present study, and from other members of the section which have been examined chemically, i.e. *G. blanchetiana* which like *G. haumaniana* contained only triterpenes (Bohlmann et al., 1981), *G. paniculata* which yielded flavonoids, bisabolenes, costunolide, β -cyclocostunolide and unusual guaianolide dimers from the roots and clerod-

anes of the aerial parts (Bohlmann et al., 1984) and *G. velutina* whose heartwood oil contained eudesmanolides (Tomassini and Gilbert, 1972). *G. glutinosa* (section Pentaphorus) from near Tucumán contained as a minor constituent the common coumarin scopoletin in addition to three interesting linear sesquiterpene and homosesquiterpene lactones and flavonoids (Ybarra et al., 1994) while another collection from near Mendoza furnished pimaradienes (Garcia et al., 1985), so far the only representatives of this group of terpenoids from Mutisieae. Two subspecies of *G. foliolosa*, one of two members of section Gochnatia which have been studied, gave costunolide, costunolide derivatives and a variety of flavonoids (Faini et al., 1984; Hoeneisen et al., 1985, Hoeneisen and Becker, 1986) while *G. vernonoides* (Bohlmann et al., 1984) contained a great variety of germacradienolides and their epoxides as well as a eudesmanolide and a rare dilactone of a type later also found in two collections of *G. palosanto* in section Hedraiophyllum (Garcia and Guerrero, 1988; Ybarra et al., 1990). On the other hand, the Mexican species *G. smithii* of this section furnished only two well-known guaianolides (Ortega and Maldonado, 1984) while *G. hypoleuca*, also from Mexico (Dominguez et al., 1978; Bohlmann et al., 1986; Maldonado et al., 1988), gave a series of guaianolide dimers similar to those from *G. paniculata* (Bohlmann et al., 1984) and C-14 functionalized germacranolides. *Gochnatia discoidea* (section Discoseris) from Brazil contained a series of C-15 oxygenated germacradienolides as well as a 1(10)-4,5-*cis,cis*-germacradienolide (Bohlmann et al., 1981) while *G. rusbyana* (Section Moquiniastrium) contained only common triterpenes and the guaianolide zaluzanin (Bohlmann and Zdero, 1979).

While kaurane **3** is somewhat similar to two polyfunctionalized kauranes found in a collection of *G. polymorpha* ssp. *polymorpha* from near São Paulo, Brazil (Sacilotto et al., 1997), the variety of secondary metabolites reported from different collections of *G. polymorpha* is surprising and so far no satisfactory explanation for this phenomenon can be offered. Chemical results for the genus as a whole are also disparate although sesquiterpene lactones of various types seem to be fairly common. In this respect the chemistry of the *Gochnatia* species studied so far overlaps few other groups within Bremer's paraphyletic Mutisinae *sensu lato*. The sesquiterpene lactone chemistry of some of these, like *Brachylaena*, *Cnicothamnus*, *Cyclolepis*, *Diaspananthus*, *Macrolinidium*, *Oldenburgia*, *Pleiotaxis* and some *Hyalis* species is on the whole similar to that of the Centaureinae, while other genera containing sesquiterpene lactones (*Ainslea*, *Pertya*, *Dicoma*, *Hyaloseris*) fall in no distinct category. Another group (*Aphyllocladus*, *Brachyclados*, *Gerbera*, *Leibnitzia*, *Lycoseris*, *Mutisia* and *Onoseris*) seems to specialize in 4- or 3,4-substituted-5-methyl coumarins of various types; *Onoseris* also characteristically produces lactones generated by oxidation of furanoeudesmanes characteristic of many Senecioninae which have so far been found in no other member of Mutisieae. Thus, it appears that other criteria will have to be looked for to establish relationships within *Gochnatia* and more generally within Mutisiinae.

Acknowledgements

The authors are grateful to Dr. Carmen L. Cristobal, Instituto de Botanica del Nordeste (IBONE), Corrientes, for her help in collection and identification of plant material. Work in Tucumán was financially supported by Consejo de Investigaciones Científicas y Tecnicas de la Republica Argentina (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT).

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