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Pterocarpans from *Bituminaria morisiana* and *Bituminaria bituminosa*

Luisa Pistelli[a,*], Cecilia Noccioli[a], Giovanni Appendino[b,*], Federica Bianchi[b], Olov Sterner[c], Mauro Ballero[d]

[a]*Dipartimento di Chimica Bioorganica e Biofarmacia, 56126 Pisa, Italy*
[b]*Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Viale Ferrucci 33, 28100 Novara, Italy*
[c]*Department of Organic and Bioorganic Chemistry, Lund University, PO Box 124, 221 00 Lund, Sweden*
[d]*Dipartimento di Scienze Botaniche, Viale San Ignazio 13, 09123 Cagliari, Italy*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

The aerial parts of Mediterranean papilionaceous plants *Bituminaria morisiana* and *B. bituminosa* afforded, along with known phenolics, the prenylated pterocarpans bitucarpin A and B, whose structure was elucidated by spectroscopic techniques. A known isoflavonoid (8-prenyldaidzein) was also obtained for the first time as a genuine plant constituent. The accumulation of pterocarpans at the expense of biogenetically more primitive shikimate metabolites like furanocoumarins or isoflavonoids supports the inclusion of this plant, once part of the genus *Psoralea*, into the distinct genus *Bituminaria*.

Keywords: *Bituminaria morisiana*; *B. bituminosa*; Fabaceae; Pterocarpans; Isoflavonoids; Chemotaxonomy

1. Introduction

The large (over 120 species), polymorphous, and cosmopolitan genus *Psoralea* L. (=*Bituminaria* Heister ex Fabr., family Fabaceae, subfamily Papilionatae) is firmly entrenched in phytochemistry as a source of furanocoumarins, whose linear archetypal compound (furo[3,2-*g*][1]benzopyran-7-one) was named psoralen after it. Paradoxically, the occurrence of furanocoumarins is not a main feature of the genus, whose pattern of secondary metabolites is characterized by other shikimates, especially isoflavonoids, and meroterpenoids (Gordaliza et al., 1999). The taxonomy of *Psoralea* is controversial, and modern investigations have suggested its fractionation into at least 10 distinct genera (Engler, 1964; Takhtajan, 1997), two of which, *Bituminaria* and *Cullen*, occur in Europe (Yeo et al., 1972). As part of a study on Mediterranean papilionaceous plants, we have investigated the two *Bituminaria* (=*Psoralea*) species growing in Italy (Pignatti, 1982), discovering a phytochemical pattern so far unreported within the genus *Psoralea*.

B. morisiana (Pignatti & Metlesics) Greuter (=*P. morisiana* Pignatti & Metlesics) is endemic to the inner parts of Sardinia, while *B. bituminosa* (L.) Stirton (=*P. bituminosa* L.) is distributed all over the Mediterranean coastal area. *B. bituminosa* shows a marked polymorphism, but the two plants are sufficiently distinct in morphology and distribution to be considered separate species. Current interest in these xerophytic shrubs centres on the potential of *B. bituminosa* to protect coastal soil from erosion (Andrei et al., 1995), a use which might lead to extensive plantations and the availability of large amount of biomass to be investigated for useful applications. *B. morisiana* lacks the strong bituminous smell typical of *B. bituminosa* and, presumably because of its limited distribution and rarity, has not been investigated before. *B. bituminosa* is instead known to contain uncharacterized antibacterial compounds and its use as a hair restorer has been ethnopharmacologically documented (Rivera and Obón, 1995), somewhat echoing a similar claim from the patent

* Corresponding authors. Tel.: +39-050-23600 (L. Pistelli); +39-0321-657652 (G. Appendino); fax: +39-050-43321 (L. Pistelli); +39-0321-657621 (G. Appendino).
E-mail addresses: luipi@gamma.farm.unipi.it (L. Pistelli), appendino@pharm.unipmn.it (G. Appendino).

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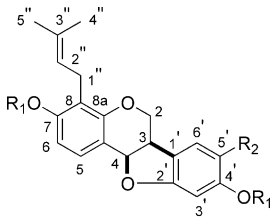
literature regarding *P. corylifolia* L. (Sung et al., 2000). However, apart from a study on the occurrence of furanocoumarins in hydrolytically treated samples of *B. bituminosa* (Innocenti et al., 1997), no chemical investigation had been carried out on these plants.

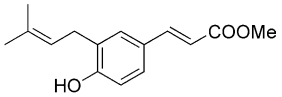
2. Results and discussion

The flower aerial part of *B. morisiana* afforded large amounts of the antimicrobial prenylated pterocarpan erybraedin C (**1a**) (Mitscher et al., 1988) as the major constituents (0.11% of dried material), accompanied by lower amounts (0.0029%) of its 5′-deprenyl dimethyl derivative (**1b**), a new compound. The structure elucidation of **1b** ($C_{22}H_{24}O_4$, HRMS) was straightforward, and mainly guided by comparison with **1a**. Thus, only the signal of one prenyl group could be detected in the ^{1}H NMR spectra of **1b**. These, when compared to **1a**, also showed the signals of two methoxyls (δ 3.62 s and 3.70 *s*) and the replacement of the AB spin system of ring B by an ABX system (δ 6.99 *d* (J = 8.8 Hz); δ 6.31 *dd* (J = 8.8, 2.4 Hz); and δ 6.33 *d* (J = 2.4 Hz); H-6′, H-5′ and H-3′, respectively). The location of the prenyl group was secured by HMBC measurements, which correlated the resonance of the isochronous allylic methylene protons (δ 3.25 *d*) with two deshielded oxygenated aromatic carbons (δ 159.0 *s*, C-7 and δ 154.7 *s*, C-8a). The location of the AB system at C-5 and C-6 of ring A was also in accordance with the detection of a NOE-correlation between the oxymethine at C-4 (δ 5.39) and the AB doublet centred at δ 7.23 (H-5). Compounds **1a** and **1b** showed a similar splitting pattern of the ring C aliphatic protons (H-2a,b, H-3, H-4), diagnostic of a *cis* relationship between H-3 and H-4 ($J_{3,4}$ = 6.4 Hz), and displayed a negative optical rotation, strongly suggesting an identical configuration (3*R*, 4*R*) at the two stereogenic centres. Thus, **1b** is 5′-deprenyl dimethylerybraedin C, for which we suggest the name bitucarpin A. Apart from widespread steroids (β-sitosterol and its glucoside), relatively large amounts (0.018%) of the chemopreventive (Menon et al., 1999) prenylated cinnamate plicatin B (**2**) were also present in *B. morisiana*, as well as the isoflavonoid 8-prenyldaidzein (**3**) (0.066%), previously obtained in trace amounts from copper chloride-treated stems of kudzu (*Pueraria lobata* Ohwi) (Hakamatsuka et al., 1991). The presence of the abiotic elicitor is essential for the formation of **3** in kudzu, and the compound had never been isolated before from "natural" plant tissues.

Compared to *B. morisiana*, *B. bituminosa* showed a similar phytochemical profile. Apart from β-sitosterol, its glucoside, plicatin B (**2**, 0.070%), **1a** (0.245%), and **1b** (0.053%), an additional new pterocarpan was isolated (**1c,** 0.00065%). Like **1b**, **1c** also was mono-prenylated, but the spin system for the pterocarpan core was



	R_1	R_2
1a	H	Prenyl
1b	Me	H
1c	H	OH



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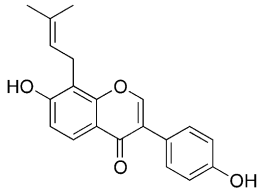

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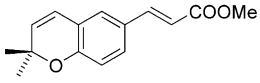

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retained, suggesting the replacement of one prenyl with a hydroxyl. MS confirmed these findings, while the marked changes of the ring B proton- and carbon resonances located the catechol system in this moiety. The NOE- and HMBC experiments detailed for **1b** showed C-8 prenylation on ring A, and further confirmed the structural assignment. Thus, **1c** is 5′-deprenyl-5′-hydroxyerybraedin C, which we have named bitucarpin B. 8-Prenyldaidzein could not be isolated from *B. bituminosa*, which contained only trace amounts of its deprenyl derivative but afforded two further minor metabolites absent from *B. morisiana*, namely the chromene **4** (*E*-werneria chromene), previously isolated from *P. plicata* (Hamed et al., 1997) and the cyclitol *D*-pinitol. Remarkably, and contrary to a previous report (Innocenti et al., 1997), both species were devoid of furanocoumarins in their aerial parts, while the roots contained only widespread sterols (β-sitosterol, stigmasterol and their glucosides).

Biogenetically advanced flavonoids like coumestans have been isolated from plants belonging to the genus *Psoralea*. Nevertheless, the occurrence and accumulation of simple pterocarpans is so far unreported and therefore of relevance, pointing to a distinct chemical trait within plants belonging to one of the genera created from *Psoralea s.l.* Owing to the excellent isolation yield, *B. morisiana* and *B. bituminosa* qualify as novel and rich sources of pterocarpans, a relatively rare type of flavonoid with agricultural potential as pest control agents (Ingham, 1973). *Psoralea* is a difficult genus in taxonomic terms, but its current medicinal relevance (Sahrawat and Chand, 2001) provides ample rationale for undertaking a critical revision of the phytochemical data available and for their expansion to the many species still awaiting investigation.

3. Experimental

3.1. General

Silica gel 60 (70–230 mesh) was used for gravity CC. Optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter; IR spectra were recorded on a Shimadzu 8101 M apparatus; ^{1}H and ^{13}C NMR spectra were registered at 300 and 75 MHz, respectively on a Bruker AC300 instrument.

3.2. Plant material

B. morisiana was collected in Cagliari Province (Rio Picocca, Italy) in May, 2001, while *B. bituminosa* was collected in Elba Island (Italy) in April 1999. The plant material was authenticated by M.B. and S. Maccioni (University of Pisa, Dipartimento di Botanica), respectively. Voucher specimens are deposited at the Botanical Gardens of Cagliari (391/B) and Pisa (HHP-Nuove acquisizioni 3703/9).

3.3. Extraction and isolation of B. morisiana

Dried flowered aerial parts (410 g) were extracted with acetone (3×2.5 l), and the pooled extracts were evaporated, dissolved in EtOH (500 ml) and treated with equal volume of 4% lead acetate to remove pigments and waxes. After standing overnight, the suspension was filtered over Celite, concentrated to ca. half volume and extracted with EtOAc. After washing with brine and drying (Na_2SO_4), the solution was evaporated, leaving 30 g of a brownish thick oil, which was fractionated by open CC. Petroleum ether–ether 95:5 was used to pack the column and begin the elution, switching then to a gradient of petroleum ether–EtOAc. Fractions eluted with petroleum ether–ether 95:5 afforded **1b** (119 mg, 0.0029%), **2** (710 mg, 0.018%), and β-sitosterol (40 mg). Fractions eluted with petroleum ether–EtOAc 95:5 gave **1a** (4.46 g, 0.11%), and fractions eluted with petroleum ether–EtOAc 6:4 afforded β-sitosterol glucoside (39 mg) and **3** (272 mg, 0.066%).

Using the same conditions of extraction, the aerial parts of *B. bituminosa* (460 g) afforded **4** (8.3 mg, 0.0018%), **1b** (243 mg, 0.053%), **2** (322 mg, 0.070%) **2**, β-sitosterol (12.4 mg, 0.0027%), **1a** (1.127 g, 0.245%), **1c** (3 mg, 0.00065%), β-sitosterol glucoside (3 mg, 0.00065%) and *D*-pinitol (64 mg, 0.014%).

3.4. 5′-Deprenyldimethylerybraedin C (Bitucarpin A, 1b)

White powder, mp 98 °C; $[\alpha]_D^{25}$−112 (MeOH, *c* 0.8). HR-EIMS (70 eV), *m*/*z* $[M]^+$. Found 352.1687, calc. for $C_{22}H_{24}O_4$ 352.1675. IR (KBr) ς_{max} 1623, 1607, 1599, 1584, 1493, 1352, 1101, 841, 789 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.23 (1H, *d*, 8.8 Hz, H-5); 6.99 (1H, *d*, 8.8 Hz, H-6′); 6.51 (1H, *d*, 8.8 Hz, H-6); 6.33 (1H, *d*, 2.4 Hz, H-3′); 6.31 (1H, *dd*, J = 8.8, 2.4 Hz, H-5′); 5.39 (1H, *d*, 6.4 Hz, H-4); 5.07 (1H, *t*, 6.8 Hz, 2″); 4.16 (1H, *dd*, 10.8, 4.4 Hz, H-2α); 3.70 (3H, *s*, 7-OMe); 3.62 (3H, *s*, 4′-OMe); 3.49 (1H, *d*, 11.2 Hz, H-2β); 3.42 (1H, 1*m*, H-3); 3.25 (1H, *d*, 6.8 Hz, H-1″); 1.65 (3H, br *s*,H-4″); 1.54 (3H, br *s*, H-5″). ^{13}C NMR ($CDCl_3$): δ 161.8 (*s*, C-2′); 161.5 (*s*, C-4′); 159.0 (*s*, C-7); 154.7 (*s*, C-8a), 132.0 (*s*, C-3″); 129.4 (*d*, C-5); 125.4 (*d*, C-6′); 123.3 (*d*, C-2″); 120.1 (*s*, C-1′); 118.1 (*s*, C-8); 113.7 (*s*, C-4a); 106.9 (*s*, C-4′); 105.0 (*d*, C-6); 97.5 (*d*, C-3′); 80.0 (*d*, C-4); 67.4 (*t*, C-2); 56.5 (7-OMe); 56.2 (4′-OMe); 40.3 (*t*, C-2); 26.5 (*q*, C-4″); 23.2 (*t*, C-1″); 18.4 (*q*, C-5″).

3.5. 5′-Deprenyl-5′-hydroxyerybraedin C (Bitucarpin B, 1c)

Amorphous gum, $[\alpha]_D^{25}$−74 (MeOH, *c* 0.4). EIMS (70 eV), *m*/*z* $[M]^+$. (rel. int.): 338 (20), 281 (100), 269 (17), 189 (16), 147 (20), 115 (20), 91 (16), 43 (52). IR (liquid film) ς_{max} 3400, 1629, 1612, 1592, 1554, 1491, 1302, 1121, 860, 7995 cm^{-1}.^{1}H NMR (CD3OD): δ 7.12 (1H, *d*, 8.8 Hz, H-5); 6.99 (1H, *s*, H-6′); 6.49 (1H, *d*, 8.3 Hz, H-6); 6.26 (1H, *s*, H-3′); 5.41 (1H, *d*, J = 5.8 Hz, H-4); 5.17 (1H, *m*, H-2″); 4.27 (1H, *m*, H-2α); 3.44 (2H, *m*, H-2β, H-3); 3.21 (1H, *d*, J = 6.8 Hz, H-1″); 1.78 (3H, *s*, H-4″); 1.75 (3H, *s*, H-5″); ^{13}C NMR (CD_3OD): δ 160.5 (*s*, C-2′); 157.8 (*s*, C-7); 157.5 (*s*, C-4′); 155.9 (*s*, C-8a); 131.7 (*s*, C-3″); 130.0 (*d*, C-5); 128.2 (*d*, C-6′); 124.4 (*d*, C-2″); 119.6 (*d*, C-5′); 119.4 (*s*, C-1′); 117.5 (*s*, C-8); 113.3 (*s*, C-4a); 110.2 (*d*, C-6); 98.9 (*d*, C-3′); 80.7 (*d*, C-4); 68.1 (*t*, C-2); 41.3 (*d*, C-3); 26.1 (*d*, C-4″); 23.3 (*t*, C-1″); 18.1 (*q*, C-5″).

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