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ISOQUINOLINE ALKALOIDS FROM *BERBERIS VULGARIS* SUBSP. *AUSTRALIS*

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Key Word Index—*Berberis vulgaris* subsp. *australis*; Berberidaceae; root bark; isoquinoline Alkaloids; *bis*benzylisoquinolines; *seco-bis*benzylisoquinolines; tejedine; protoberberines.

Abstract—Sixteen isoquinoline alkaloids were isolated from *Berberis vulgaris* subsp. *australis*. In addition to quaternary protoberberines and bisbenzylisoquinolines, a new *seco-bis*benzylisoquinoline, (-)-tejedine, is reported. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Berberis vulgaris is the most significant European representative of the Berberidaceae [1]. Root bark extracts of this species are used as an antirheumatic in folk medicine on account of their antiinflammatory properties, which has been attributed to their alkaloid composition [2]. The alcoholic extract of the European plant, *B. vulgaris* subsp. *vulgaris*, has been shown to contain quaternary protoberberines and *bis*benzylisoquinoline alkaloids [2, 3]. The Mediterranean endemic *B. vulgaris* subsp. *australis* (Boiss.) [syn. *B. hispanica* (Boiss. & Reuter, Pugill)]- differs from subsp. *vulgaris* in the small size of its honey-leaves, its almost black berries and the dark purple colour of one-year-old shoots. The present paper reports the isolation and characterization of 16 alkaloids from *B. vulgaris* subsp. *australis* including (−)-tejedine (**1**), a new *seco-bis*benzylisoquinoline.

RESULTS AND DISCUSSION

The alkaloids were extracted from the aqueous phase at acid and alkaline pH values. The fraction of tertiary bases was found to contain bisbenzylisoquinoline alkaloids. In addition to berbamine (**2**), isotetrandrine (**3**) and oxyacanthine (**4**), previously encountered in the subsp. *vulgaris*, obaberine (**5**), aromoline (**6**), obamegine (**7**) and thaligrisine (**8**) were isolated and fully characterized [4–7].

The non-basic alkaloids thalifoline (**9**), 8-oxyberberine (**10**) and chilenine (**11**) were isolated by extraction at acid pH. The structure of **10** compared positively with a synthetic sample obtained by ferricyanide oxidation of berberine (**12**) [8]. The structure of **11** was conclusively established by careful comparison of spectroscopic data with those for the isomeric prechilenine [9].

A minor component (**1**) of the above fraction was optically active and analyzed for $C_{38}H_{40}N_2O_9$, in agreement with a parent peak $[M + H]^+$ at *m/z* 669, and with a *bis*benzylisoquinoline-type structure. Two carbonyl carbon atoms resonating at δ 166.5 and 164.5 in the ^{13}C NMR spectrum, correlated with the carbonyl bands at 1710 and 1640 cm^{-1} in the IR spectrum, assigned to an ester and a lactam, respectively. The ^1H NMR spectrum revealed the presence of two *N*-methyl groups (one amine and one lactam) at δ 2.30 and 3.03 respectively, and included four methoxyl signals. Three of them corresponded to the isoquinoline nucleus, as deduced from the EI mass spectrum, with a base peak at *m/z* 411 that analyzed for $C_{23}H_{27}N_2O_5$ and reflected the upper part of a *seco-bis*benzylisoquinoline. The lower part must bear a phenol function *para* to the carbonyl ester, consistent with the bathochromic shift in the UV spectrum upon addition of base. The aromatic part of the ^1H NMR spectrum was crucial in order to establish the structure of **1**. A low-field singlet *peri* to the carbonyl lactam revealed a 7 to 8′ linkage (the NOE observed between H-8 and the high field methoxyl group at C-7′ confirm this assumption). Significant enough,

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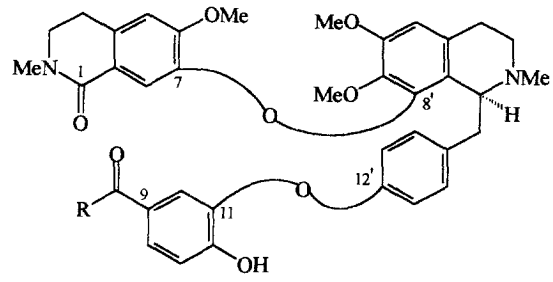
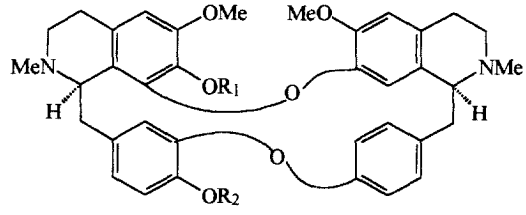
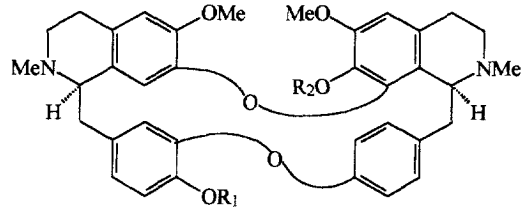


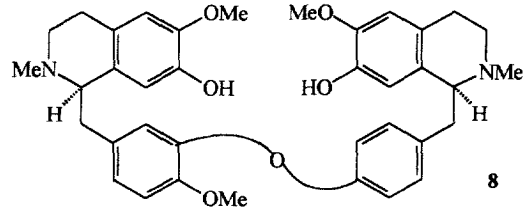
1, R = OMe
13, R = H



2, R_1 = Me, R_2 = H
3, R_1 = R_2 = Me
7, R_1 = R_2 = H



4, R_1 = H, R_2 = Me
5, R_1 = R_2 = Me
6, R_1 = R_2 = H



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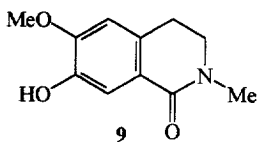
the presence of one AMX and one A_2B_2 system for the aromatic protons of the lower part, evidenced the 11 to 12′ biaryl ether bond. NOE between H-10 and the equivalents H-11′ and H-13′ confirmed the previous evidence. From this data the structure of (−)-tejedine (**1**) was established. In addition to **1**, baluchistanamine (**13**), the lactam-aldehyde, was also isolated. Both *seco*-alkaloids must have oxyacanthine (**4**) as their biosynthetic precursor. However, $KMnO_4$ or photochemical oxidation of **4**, followed by esterification with methanol, did not give **1**, but only a small amount of **13**. Tejedine, together with gilgitine and talcamine, are the known examples of *seco*-benzylisoquinoline alkaloids bearing the ester-lactam functionality [10]. Evidence that **1** was not formed during the extraction of the plant was obtained from the fact that the product obtained by extraction with ethanol and isolation, provided a CI mass spectrum that exhibited the expected $[M + H]^+$ at *m/z* 669, but no traces of the *m/z* 683 peak.

Quaternary alkaloids were precipitated with Mayer's reagent and recovered as chlorides. The protoberberinium salts jatrorrhizine (**14**) and palmatine (**15**) were isolated, in addition to berberine (**12**). The widespread quaternary aporphine, (+)-



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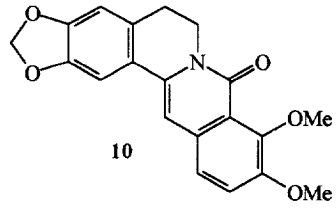

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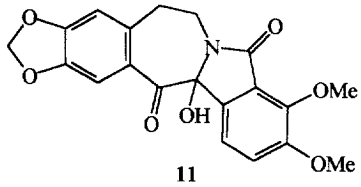

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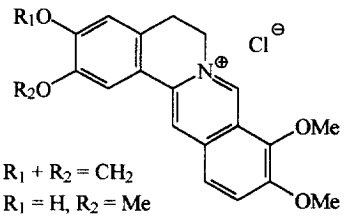
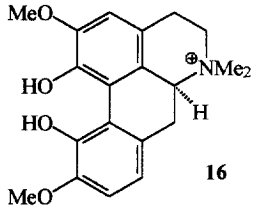


12, $R_1 + R_2 = CH_2$
14, $R_1 = H$, $R_2 = Me$
15, $R_1 = R_2 = Me$



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magnoflorine (**16**), was the main component of this fraction [11].

Root-barks of *B. vulgaris* subsp. *australis* provided smaller amounts of alkaloids (8.5% based on dry plant) than those from subsp. *vulgaris* (16.2%) [3]. Alkaloid distribution was quite similar: *ca* 47% of quaternary protoberberines, 25% of *bis*-benzylisoquinolines and 12% of magnoflorine. The main differences lay in minor alkaloids and quaternary protoberberines; the latter consisted of 87% berberine and only 10% jatrorrhizine.

EXPERIMENTAL

General

Mps are uncorr. EIMS and CIMS: direct inlet, 70 eV. Silica gel 60 (5–40 μm) was used for vacuum column chromatography (VCC) and silica GF_{254} for TLC. ^1H and ^{13}C NMR were measured at 200 and 50 MHz, respectively. Proton chemical shifts are referred to residual $CHCl_3$ (δ 7.24) and carbon chemical shifts to the solvent ($^{13}CDCl_3$, δ 77.0). ^1H and ^{13}C NMR signals were assigned from 2D COSY, NOE and DEPT expts.

Plant material

Berberis vulgaris subsp. *australis* root bark was collected at the ripe fruit stage in Sierra Tejeda (Granada, southern Spain). The plant was identified by Prof. Baltasar Cabezudo and a voucher specimen is deposited in the herbarium of the Department of Plant Biology (MGC 24563).

Extraction and isolation

Air-dried, powdered root bark (400 g) was repeatedly extracted with MeOH at room temp. The combined MeOH extracts (8 l) were evapd. at red. pres. to a syrup. The residue was treated with 5% aq. HCl (1.5 l) and filtered, and the clear soln allowed to stand at 10° overnight. The yellow pp. thus obtained was filtered to afford Fr. A (15 g). Its acid soln was extracted with CH_2Cl_2 (3 × 200 ml). Organic extracts were dried and concd. to give Fr. B (1.3 g). Its acid soln was made alkaline (pH 8–9) with conc. NH_3 and extracted with $CHCl_3$ (8 × 200 ml). Evapn of solvent afforded Fr. C (8.6 g). The aq. soln was adjusted to pH 3–4 with conc. HCl and Mayer's reagent was added until precipitation ceased. The resulting yellow pp. was filtered, washed with cold H_2O and suspended in MeOH–H_2O. To this suspension, Amberlite IRA-400 (Cl^- form) was added until the pp. was completely redissolved. The resin was filtered off and the clear solution concd. to give Fr. D (10 g).

Fraction A. Fr. A was recrystalized from dil. HCl and found to consist of almost pure berberine chloride (**12**), mp 208–210° (lit. 209–210°) [3].

Fraction B. Fr. B was a complex mixt. that was resolved by VCC (silica gel, 47 g) using CH_2Cl_2–MeOH mixts of increasing polarity, the individual components being purified by prep. TLC (benzene–

Me_2CO–NH_3, 10:10:0.3). The alkaloids characterized were as follows. *Thalifoline* (**9**): (55 mg); Needles, mp 195–197° (lit. 210–211°) [12]. *8-Oxyberberine* (**10**): (190 mg); Yellow needles, mp 191–193° (EtOAc) (lit. 198–200°) [11]. *(±)-Chilenine* (**11**): (135 mg); Powder, mp 135–137° (lit. 114.5–116°) [9]. *Baluchistanamine* (**13**): (12 mg); Powder, mp 115–118° (lit. 122–124°) [11]. IR (KBr) ν_{max}: 3430 (OH), 1680 (CO aldehyde), 1635 (CO amide) cm^{-1}. CIMS (CH_4) *m/z*: 639 $[M + H]^+$. *(−)-Tejedine* (**1**): (99 mg); Amorphous powder, mp 132–134°. $[\alpha]_D$ −40.6° (MeOH, *c* 0.064). UV (MeOH), λ_{max} (log ϵ): 206 (4.83), 224 (4.79), 260 (4.36), 292 (3.99) nm; +NaOH: 208, 228 (sh), 274 (sh), 302 nm. IR (KBr) ν_{max}: 1710 (CO ester), 1640 (CO lactam) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.72 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-14), 7.51 (1H, *d*, *J* = 2.0 Hz, H-10), 7.23 (1H, *s*, H-8), 7.07 (2H, *bd*, *J* = 8.6 Hz, H-10′ and H-14′), 7.00 (1H, *d*, *J* = 8.5 Hz, H-13), 6.80 (2H, *bd*, *J* = 8.6 Hz, H-11′ and H-13′), 6.66 (1H, *s*, H-5), 6.49 (1H, *s*, H-5′), 3.87 (3H, *s*, OMe-6), 3.81 (3H, *s*, OMe-6′), 3.79 (3H, *s*, CO_2Me), 3.57 (3H, *s*, OMe-7′), 3.03 (3H, *s*, CONMe), 2.30 (3H, *s*, NMe). ^{13}C NMR ($CDCl_3$): δ 166.5 (*C*OOMe), 164.5 (*C*ONMe), 154.5 (*s*), 152.5 (*s*), 152.0 (*s*), 151.6 (*s*), 146.4 (*s*), 145.7 (*s*), 143.9 (*s*), 139.6 (*s*), 136.2 (*s*), 132.8 (*s*), 129.6 (*s*), 123.2 (*s*), 122.1 (*s*), 121.8 (*s*), 130.6 (*d*), 130.58 (*d*), 126.5 (*d*), 120.2 (*d*), 117.5 (*d*), 117.5 (*d*), 116.0 (*d*), 114.1 (*d*), 110.1 (*d*), 109.0 (*d*), 60.1 (C-1), 60.7 (OMe-7′), 56.0 (OMe-6′), 55.8 (OMe-6), 51.8 (COO*Me*), 48.1 (C-3), 44.0 (C-3′), 41.9 (CON*Me*), 40.2 (C-α), 35.0 (NMe), 27.5 (C-4′), 22.6 (C-4). HREIMS *m/z*: found $[M]^+$ 411.1917; $C_{23}H_{27}N_2O_5$ requires 411.1919. CIMS (CH_4) *m/z* (rel. int.): 669 $[M + H]^+$ (22), 411 (100). (Found: C, 66.82; H, 6.07; N, 4.11. $C_{38}H_{40}N_2O_9 \cdot H_2O$ requires: C, 66.44; H, 6.17; N, 4.08%).

Fraction C. Tertiary bases (7.4 g) were separated by VCC (silica gel, 165 g) and eluted with $CHCl_3$–MeOH–NH_3 (95:5:0.5). Three main frs were collected that were further purified by VCC and prep. TLC using benzene–Me_2CO–NH_3 10:10:0.3 as eluent. The following *bis*benzylisoquinolines were characterized, in order of elution. *(+)-Obaberine* (**5**): (150 mg); Amorphous white powder, mp 136–138°, $[\alpha]_D$ +238 (*c* 0.06, MeOH); [lit. 134–139°, $[\alpha]_D$ +312 ($CHCl_3$)] [4, 13]. EIMS *m/z* (rel. int.): 622 $[M]^+$ (13), 515 (7), 396 (22), 395 (81), 381 (43), 198 (100), 175 (57). *(+)-Isotetrandrine* (**3**): (120 mg); Amorphous powder, mp 171–174° $[\alpha]_D$ +110 (*c* 0.03, MeOH); [lit. 182–184°, $[\alpha]_D$ +158 ($CHCl_3$)] [14]. EIMS *m/z* (rel. int.): 622 $[M]^+$ (15), 485 (2), 430 (12), 395 (100), 381 (51), 198 (97), 175 (58). *(+)-Oxyacanthine* (**4**): (760 mg); Prisms, mp 205–208°. $[\alpha]_D$ +209 (*c* 0.065, MeOH); [lit. 212–214°, $[\alpha]_D$ +285.6 ($CHCl_3$)] [11]. EIMS *m/z* (rel. int.): 608 $[M]^+$ (16), 501 (3), 396 (25), 395 (49), 381 (40), 198 (100), 175 (51). *(+)-Berbamine* (**2**): (912 mg); Prisms, mp 145–147°. $[\alpha]_D$ +78 (*c* 0.051, MeOH); [lit. 168–173°, $[\alpha]_D$ +97 ($CHCl_3$)] [15]. EIMS *m/z* (rel. int.): 608 $[M]^+$ (16), 501 (3), 396 (25), 395 (49), 381 (40), 198 (100), 175 (51). *(+)-Aromoline* (**6**): (210 mg); Needles, mp 166–169° (CH_2Cl_2). $[\alpha]_D$ +337 (*c* 0.051, MeOH); [lit. 178–180°, $[\alpha]_D$ +318 (Et_2O)] [16]. EIMS *m/z* (rel. int.): 594 $[M]^+$ (9), 501 (3), 382 (43), 381 (90), 367 (78), 191 (100). *(+)-Obamegine* (**7**): (23 mg); Needles, mp 197–198°. $[\alpha]_D$ +148 (*c* 0.049, MeOH); [lit. 171–173°, $[\alpha]_D$ +143 ($CHCl_3$)] [17]. EIMS *m/z* (rel. int.): 594 $[M]^+$ (17), 402 (13), 382 (30), 381 (100), 367 (30), 191 (85), 174 (17). *(+)-Thaligrisine* (**8**): (10 mg); Amorphous, mp 120–122°. $[\alpha]_D$ +58 (*c* 0.043, MeOH); [lit. $[\alpha]_D$ +57 (MeOH)] [18]. EIMS *m/z* (rel. int.): 610 $[M]^+$ (1), 418 (1), 192 (100), 175 (4).

Fraction D. Quaternary alkaloids (8.2 g) were separated by VCC (silica gel, 56 g) with CH_2Cl_2–MeOH (4:1) as the initial solvent and with gradual increases in the MeOH concn. The frs eluted were purified by recrystallization to obtain the following alkaloids. *Jatrorrhizine chloride* (**14**): (1.9 g); Dark orange crystals, mp 203–205° (MeOH) (lit. 210°) [11]. *Palmatine chloride* (**15**): (530 mg); Orange crystals, mp 201–202°(MeOH) (lit. 205°) [11]. *Berberine chloride* (**12**): (1.5 g). *(+)-Magnoflorine chloride* (**16**): (4.1 g); White prisms, mp 202–206°(dec.) [14].

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REFERENCES

1. Webb, D. A. and Akeroyd, J. R., in *Flora Europeae*, Vol. I, ed. D. A. Webb. University Press, Cambridge, 1993, p. 295.
2. Ivanovska, N. and Philipov, S., *Int. J. Immunopharmaco.*, 1996, **18,** 553.
3. Slavik, J. and Slavikova, L., *Collect. Czech. Chem. Commun.*, 1995, **60,** 1034.
4. Guha, K. P., Mukherjee, B. and Mukherjee, R., *J. Nat. Prod.*, 1979, **42,** 1.
5. Schiff, P. L., Jr., *J. Nat. Prod.*, 1983, **46,** 1.
6. Schiff, P. L., Jr., *J. Nat. Prod.*, 1987, **50,** 529.
7. Schiff, P. L., Jr., *J. Nat. Prod.*, 1991, **54,** 645.
8. Perkin, W. H. Jr., *J. Chem. Soc.* 1918, 722.
9. Dorn, C. R., Koszyk, F. J. and Lenz, G. R., *J. Org. Chem.*, 1984, **49,** 2642.
10. Rozwadowska, M. D., in *The Alkaloids*, Vol. 33, ed. A. Brossi. Chap. 4. Academic Press, New York, 1988.
11. Southon, I. W., Buckingham, J., *Dictionary of Alkaloids*. Chapman and Hall, London, 1989.
12. Doskotch, R. W., Schiff, P. L. Jr. and Beal, J. L., *Tetrahedron*, 1969, **25,** 469.
13. Siwon, J., Verpoorte, R., van Beek, T., Meerburg, H. and Svendsen, A. B., *Phytochemistry*, 1981, **20,** 323.

14. Singh, R. S., Kumar, P. and Bakuni, D. S., *J. Nat. Prod.*, 1981, **44,** 664.
15. Akasu, M., Itokawu, H. and Fujita, M., *Phytochemistry*, 1976, **15,** 471.
16. Wu, W., Beal, J. L. and Doskotch, R. W., *J. Nat. Prod.*, 1976, **39,** 378.
17. Owusu, P. D., Slatkin, D. J., Knapp, J. E. and Schiff Jr., P. L., *J. Nat. Prod.*, 1980, **44,** 61.
18. Guinaudeau, H., Freyer, A. J., Shamma, M., Hüsnü, K. and Baser, A., *Tetrahedron*, 1984, **40,** 1975.