N-BENZYLISOQUINOLINE ALKALOIDS FROM CERATOCAPNOS HETEROCARPA

RAFAEL SUAU, M. VICTORIA SILVA, INMACULADA RUIZ and MARIA VALPUESTA
Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain
(Received 2 August 1993)

Key Word Index—Ceratocapnos heterocarpa; Fumariaceae; sendaverine; isosendaverine; capnosine; capnosinine; structure; synthesis.

Abstract—Three new N-benzylisoquinoline alkaloids, namely isosendaverine, capnosine and capnosinine, were isolated from Ceratocapnos heterocarpa in addition to the known sendaverine. Their structure was established by spectroscopic methods and verified by total synthesis.

INTRODUCTION
Sendaverine (1), corgoine (2) and viguine (3), and some of their N-oxides and N-methyl derivatives are the sole N-benzylisoquinoline alkaloids known to date [1-5]. They have been isolated from various species of the Fumariaceae and all of them exhibit the common structural feature, monooxygenation at the para-position of the benzyl moiety. They are presumably biosynthesized from a monophenolic 1-benzyl isoquinoline, migration of the benzyl group from carbon to nitrogen taking place via a para-benzoquinone methide intermediate [6].

Ceratocapnos heterocarpa is a proven rich source for the less common isooquinoline alkaloids cularine [7] and 1,2-substituted berbines [8]. Further research on this species led to the isolation of three new N-benzylisoquinoline alkaloids, namely, isosendaverine (4), capnosine (5) and capnosinine (6), together with sendaverine (1). The alkaloids were identified by spectroscopic analysis and their structures confirmed by total synthesis. Interestingly, capnosine and capnosinine are the first compounds to exhibit a 3',4'-oxygenation pattern on the benzene ring.

RESULTS AND DISCUSSION
Isosendaverine (4), recrystallized from methanol, exhibited no optical activity and gave the molecular formula C_{18}H_{21}NO_3 by high resolution mass spectrometry. The benzylisoquinoline structure was suggested by the [M]^+ (m/z 299) fragmentation, in the form of two major ions at m/z 178, characteristic of a dioxygenated isoquinoline, and the base peak at m/z 121, in good agreement with a benzyl fragment bearing a methoxy group. The position of the benzyl substituent at C-1 was excluded from the intensity of [M]^+ (12%) and [M-1]^+ (22%) [9]. ^1H NMR revealed the two uncoupled methylene groups expected for the N-benzylisoquinoline. Furthermore, two aromatic singlets confirmed the 6,7-substitution pattern for the isoquinoline nucleus, while the para-methoxy benzyl substituent was inferred from the AB-system for the remaining four aromatic hydrogens. Location of the methoxy group at position 7 of the isoquinoline nucleus was excluded since the mp of 4 (181-182°C) was considerably different from that reported for sendaverine (140-141.5°C); thus, the isomeric 1,2,3,4-tetrahydro-6-methoxy-2-(4-methoxybenzyl)-7-isoquinolinol structure
was considered for compound 4. In order to confirm the tentative structure, total synthesis of isosendaverine (4) and sendaverine (1) was undertaken. The straightforward approach based on the alkylation-reduction sequence of the corresponding 3,4-dihydroisoquinoline was used for this purpose. The isoquinolinium salt obtained in the reaction between 7a and 8a was treated with NaBH₄ to give 9a. Removal of the protecting group yielded isosendaverine (4), identical with the naturally occurring metabolite. Condensation of 7b and 8a and reduction afforded 9b, the sendaverine precursor.

The next-eluted fraction was found to be a mixture of isosendaverine (4) and sendaverine (1), as deduced from GC-MS analysis and ¹H NMR comparison with synthetic samples of both alkaloids, the chemical shifts of the aromatic protons H-5 and H-8 (6.54 and 6.52 for sendaverine and 6.63 and 6.43 for isosendaverine) being the main difference between the two.

The next isolated N-benzylisoquinoline, capnosine (5), analysed for C₁₅H₂₁N₃O₄, and the two moieties of the molecule observed in the mass spectrum suggested an isoquinoline portion (m/z 178) analogous to 4, and a benzyl fragment including an additional oxygen atom (m/z 137). The ¹H NMR chemical shift of the aromatic protons at H-5 and H-8 suggested a 6-methoxy-7-hydroxy-substituted isoquinoline, as in sendaverine (1). The substituents on the aromatic ring of the benzylic portion of capnosine (5) were located at positions 3' and 4', as inferred from the ¹H NMR spectrum, with one meta- and two ortho-coupled aromatic protons. The 2D COSY experiment correlated the doublet at 6.77 (J = 8.2 Hz, H-5'), with not the doublet at 6.94 (J = 1.9 Hz, H-2'); thus, the phenol group must be located at C-3'. The proposed structure was confirmed by total synthesis. The isoquinolinium salt obtained by reaction between 7b and 8b was reduced to the tertiary amine 9c, which was deprotected by acid hydrolysis to afford synthetic capnosine (5). This compared positively with the natural alkaloid.

The last component of the N-benzylisoquinoline fraction was capnosine (6), which analysed for C₁₅H₂₃N₂O₄ and was spectroscopically similar to capnosine. The main differences were associated with an additional methyl group on the benzylic portion of capnosine (5) and was confirmed by total synthesis. Reaction between 7c and 8b followed by reduction gave 9d, which was deprotected to yield capnosine (6) indistinguishable from the natural product.

It is worth noting that the major biosynthetic pathway in C. heterocarpa is that initiated in cocrinine and leading to cularine and 1,2-substituted berbines. On the other hand, N-benzylisoquinolines might be related to the coacoline–reticuline pathway [10], a scarcely significant route in the plant since only protopine, glaucine, dihydroisouquinarine and oxosanguinarine have been isolated in minor amounts [11].

EXPERIMENTAL.

General. Mps: uncorr. EIMS: direct inlet, 70 eV. Silica gel 60 (70–230 mesh) was used for CC and silica GF₃₄₄ for TLC. ¹H and ¹³C NMR signals were measured at 200 and 50 MHz, respectively. Proton chemical shifts are referred to the residual CHCl₃ (δ 7.24) signal, and carbon chemical shifts to the solvent (δCDCl₃ = 77 ppm). ¹H and ¹³C NMR signals were assigned from 2D COSY and DEPT expts.

Isolation. For a description of plant material and extraction conditions, see ref. [7]. The crude alkaloid extract was subjected to CC over silica gel and the fraction eluted with C₂H₅OH–EtOAc (1:5) (4.8 g) was subsequently purified by CC and TLC to obtain the new compounds isosendaverine (16 mg), capnosine (7 mg) and capnosinine (10 mg).

Isoendaverine (4). Pale yellowish crystals, mp 181–182° (MeOH). UV λmax nm (log e): 204 (4.55), 226 (4.18), 284 (3.61). ¹H NMR (200 MHz, CDCl₃): δ 7.30 (2H, d, J = 8.6 Hz, H-2' and H-6'), 6.68 (2H, d, J = 8.6 Hz, H-3' and H-5'), 6.63 (1H, s, H-5), 6.43 (1H, s, H-8), 3.79 (6H, s, 2 × OMe), 3.61 (2H, s, H-8), 3.51 (2H, s, H-1), 2.80–2.60 (4H, m, H-3 and H-4). ¹³C NMR (50 MHz, CDCl₃): δ 159.0 (C-4'), 145.0 (C-6), 144.2 (C-7), 130.4 (C-2', C-6'), 130.1 (C-1'), 127.2 (C-4a), 125.8 (C-8a), 114.3 (C-5'), 113.8 (C-3', C-5'), 109.8 (C-8), 61.8 (C-1'), 56.1, 55.3 (2 × OMe), 55.4 (C-2a), 50.6 (C-3), 28.2 (C-4). EIMS m/z (rel. int.): 299 [M⁺] (12), 298 [M−1]+ (22), 178 (16), 150 (37), 121 (100). HRMS m/z 299.1519 [(M⁺)], calcd for C₁₅H₁₅NO₄: 299.1521.

Capnosine (5). Yellowish powder, mp 105–106°. UV λmax nm (log e): 208 (4.54), 228sh (4.14), 284 (3.83). + NaOH: 212 (4.64), 244sh (4.17), 286 (4.00). ¹H NMR (200 MHz, CDCl₃): δ 6.94 (1H, d, J = 1.9 Hz, H-2'), 6.85 (1H, dd, J = 1.9 and 8.2 Hz, H-6'), 6.77 (1H, d, J = 8.2 Hz, H-5'), 6.53 (1H, s, H-5), 6.50 (1H, s, H-8), 3.85 (3H, s, OMe on C-4'), 3.80 (3H, s, OMe on C-6'), 3.55 (2H, s, H-1), 2.80–2.65 (4H, m, H-3 and H-4). ¹³C NMR (50 MHz, CDCl₃): δ 154.7, 145.5, 145.2, 143.7 (C-6, C-7, C-3', C-4'), 131.5 (C-1'), 127.5 (C-4a), 125.6 (C-8a), 120.6 (C-6'), 115.5, 112.3, 110.7, 110.4 (C-5, C-8, C-2', C-5'), 62.1 (C-1), 55.9 (2 × OMe), 55.4 (C-2a), 50.5 (C-3), 28.7 (C-4). EIMS m/z (rel. int.): 315 [M⁺] (14), 314 [M−1]+ (17), 178 (100), 150 (44), 131 (79). HRMS m/z 314.1457 [(M⁺)], calcd for C₁₅H₁₅NO₄: 314.1470. (Found: C, 67.95; H, 6.77; N, 4.09. C₁₅H₁₅NO₄ requires: C, 68.55; H, 6.71; N, 4.44%)

Capnosinine (6). Crystals, mp 172–173° (EtOH). UV λmax nm (log e): 210 (4.52), 230sh (4.19), 282 (3.80). + NaOH: 214 (4.54), 232sh (4.14), 284 (3.85). ¹H NMR (200 MHz, CDCl₃): δ 6.95 (1H, dd, J = 1.9 Hz, H-2'), 6.85 (1H, dd, J = 1.9 and 8.2 Hz, H-6'), 6.77 (1H, d, J = 8.2 Hz, H-5'), 6.58 (1H, s, H-5), 6.46 (1H, s, H-8), 3.88 (3H, s, OMe), 3.85 (3H, s, OMe), 3.80 (3H, s, OMe), 3.58 (2H, s, H-2'), 3.52 (2H, s, H-1), 2.80–2.60 (4H, m, H-3 and H-4).

¹³C NMR (50 MHz, CDCl₃): δ 147.7, 147.4, 145.8, 145.6.
Benzylisoquinoline alkaloids from Ceratocapnos heterocarpa

(2e, 6, C-7, C-3', C-4'), 13.17 (C-1'), 126.9, 126.4 (C-4a, C-8a), 120.6 (C-6'), 115.5, 111.8, 110.6, 109.9 (C-5, C-8, C-2', C-5), 62.2 (C-1'), 56.0 (3 x OMe), 55.8 (C-3), 50.3 (C-3'), 28.4 (C-4). EIMS m/z (rel. int.): 329 [M]⁺ (8), 328 [M – 1]⁺ (11), 192 (100), 164 (58), 137 (59). (Found: C, 69.00; H, 7.32; N, 4.06. C₁₂H₁₄NO₂ requires: C, 69.28; H, 7.04; N, 4.25%).

General procedure for synthesizing 9a–d. A mixture of the 3,4-dihydroisoquinoline (7a–c) (1 mmol) and the benzyl chloride (8a–b) (1.1 mmol) in MeCN (10 ml) was heated under reflux for 2 hr. After evaporation of solvent, the isoquinoline salt was dissolved in MeOH (8 ml) and NaBH₄ (3.2 mmol) was added at room temp. for 5 hr. Usual work-up provided the corresponding O-benzyl derivatives of the N-benzylisoquinolines (9a–d).

O-Benzylsendarverine (9b). From 7b [13] and 8a. Mp 65–67°C (hexane–Me₂CO) (87%). H NMR (200 MHz, CDCl₃): 6.75–7.20 (5H, m, C₆H₅), 7.28 (2H, d, J = 8.6 Hz, H-2' and H-6'), 6.85 (2H, d, J = 8.6 Hz, H-3' and H-5'), 6.61 (1H, s, H-5), 6.49 (1H, s, H-8), 5.08 (2H, s, OCH₂Ar), 3.79 (3H, s, OMe), 3.60 (2H, s, H-5'), 3.51 (2H, s, H-1'), 2.80–2.60 (4H, m, H-3 and H-4). EIMS m/z (rel. int.): 389 [M]⁺ (18), 388 [M – 1]⁺ (23), 268 (10), 121 (100), 91 (91). (Found: C, 73.45; H, 6.91; N, 3.48. C₁₂H₁₄NO₂ requires: C, 73.69; H, 7.17; N, 3.44%).

O-Benzylsendarverine (9b). From 7b [13] and 8a. Mp 87–88°C (hexane–Me₂CO) (87%) [14]. H NMR (200 MHz, CDCl₃): 6.75–7.20 (5H, m, C₆H₅), 7.28 (2H, d, J = 8.6 Hz, H-2' and H-6'), 6.85 (2H, d, J = 8.6 Hz, H-3' and H-5'), 6.60 (1H, s, H-5), 6.50 (1H, s, H-8), 5.05 (2H, s, OCH₂Ar), 3.82 (3H, s, OMe), 3.79 (3H, s, OMe), 3.59 (2H, s, H-5'), 3.47 (2H, s, H-1'), 2.80–2.60 (4H, m, H-3 and H-4). EIMS m/z (rel. int.): 389 [M]⁺ (21), 388 [M – 1]⁺ (27), 268 (14), 121 (100), 91 (67).

7β-De-O-benzylsendarverine (9e). From 7b and 8b. Amorphous powder (89%). H NMR (200 MHz, CDCl₃): 6.76–7.26 (10H, m, 2 x C₆H₅), 7.05 (1H, d, J = 1.9 Hz, H-2'), 6.89 (1H, dd, J = 1.9 and 8.2 Hz, H-6'), 6.82 (1H, d, J = 8.2 Hz, H-5'), 6.61 (1H, s, H-5), 6.49 (1H, s, H-8), 5.12 (2H, s, OCH₂Ar), 5.08 (2H, s, OCH₂Ar), 3.87 (3H, s, OMe), 3.83 (3H, s, OMe), 3.57 (2H, s, H-5), 3.45 (2H, s, H-1'), 2.80–2.50 (4H, m, H-3 and H-4). C₁₂NMR (50 MHz, CDCl₃): 148.9, 148.3, 148.0, 143.6 (C-6, C-7, C-3', C-4'), 137.1 (C-1'), 130.9 (C-1), 128.4 (C-3', C-5'), 127.7 (C-4'), 127.4 (C-2', C-6'), 127.2 (C-1'), 112.6 (C-4a, C-8a), 121.8 (C-6'), 115.0, 112.4, 111.5 (C-5, C-8, C-2', C-5'), 71.2, 70.9 (2 x OCH₂Ar), 61.9 (C-1'), 56.0 (2 x OMe), 55.2 (C-3), 50.1 (C-3'), 28.4 (C-4). EIMS m/z (rel. int.): 495 [M]⁺ (0.6), 494 [M – 1]⁺ (1.4), 404 (14), 268 (37), 227 (27), 91 (100). (Found: C, 73.40; H, 6.46; N, 2.76. C₁₂H₁₄NO₂ requires: C, 74.83; H, 6.87; N, 2.17%).

3β-O-Benzyleucapnosine (9d). From 7e [15] and 8b. Crystals, mp 137–138°C (EtOH) (90%). H NMR (200 MHz, CDCl₃): 6.76–7.26 (5H, m, C₆H₅), 6.99 (1H, d, J = 1.5 Hz, H-2'), 6.90 (1H, dd, J = 1.5 and 8.2 Hz, H-6'), 6.83 (1H, d, J = 8.2 Hz, H-5'), 6.58 (1H, s, H-5), 6.45 (1H, s, H-8), 5.14 (2H, s, OCH₂Ar), 3.87 (3H, s, OMe), 3.83 (3H, s, OMe), 3.80 (3H, s, OMe), 3.56 (2H, s, H-3a), 3.47 (2H, s, H-5'), 2.73 (2H, t, J = 5.4 Hz, H-3), 2.57 (2H, t, J = 5.4 Hz, H-4). C₁₂NMR (50 MHz, CDCl₃): 6.148.8, 148.0, 147.7, 147.1 (C-6, C-7, C-3', C-4'), 137.1 (C-1'), 130.9 (C-1), 128.4 (C-3', C-5'), 127.7 (C-4'), 127.4 (C-2', C-6'), 126.7, 126.2 (C-4a, C-8a), 121.8 (C-6'), 114.9, 111.5, 111.4, 109.5 (C-5, C-8, C-2', C-5'), 70.9 (OCH₂Ar), 62.1 (C-1'), 56.0, 55.9 (3 x OMe), 55.5 (C-3a), 50.3 (C-3), 25.4 (C-4). EIMS m/z (rel. int.): 419 [M]⁺ (3), 418 [M – 1]⁺ (5), 328 (39), 277 (30), 192 (100), 91 (54). (Found: C, 73.97; H, 6.96; N, 3.18. C₁₂H₁₄NO₂ requires: C, 74.43; H, 6.97; N, 3.34%).

General procedure for debenzylation of 9a–d. To solns of the O-benzyl derivatives 9a–d (0.9 mmol) in EtOH (20 ml) were added dropwise conc HCl (20 ml) and the mixts refluxed (3 hr). After cooling, the reaction media were quenched with H₂O and extracted with Et₂O. The aq. layers were basified, extracted with CH₂Cl₂ and evapd to obtain pure samples of I (74%), 4 (81%), 5 (56%) and 6 (85%), identical with the natural alkaloids (TLC, mp, NMR and MS).

Acknowledgement.—This research work was supported by the Spanish Comisión Interministerial de Ciencia y Tecnología (Project PB90-0816).