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N-BENZYLISOQUINOLINE ALKALOIDS FROM *CERATOCAPNOS HETEROCARPA*

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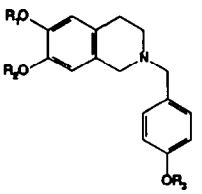
Key Word Index—*Ceratocapnos heterocarpa*; Fumariaceae; sendaverine; isosendaverine; capnosine; capnosinine; structure; synthesis.

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.

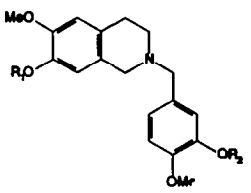
INTRODUCTION

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].

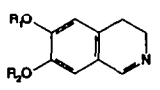
Ceratocapnos heterocarpa is a proven rich source for the less common isoquinoline alkaloids cularine [7] and 1,2-substituted berbines [8]. Further research on this species led to the isolation of three new *N*-benzylisoquinoline alkaloids, namely, isosendaverine (**4**), capnosine (**5**) and capnosinine (**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.



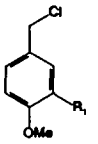
1 $R_1 = R_3 = Me$; $R_2 = H$
2 $R_1 = Me$; $R_2 = R_3 = H$
3 $R_1 + R_2 = CH_2$; $R_3 = Me$
4 $R_1 = H$; $R_2 = R_3 = Me$
9a $R_1 = Bn$; $R_2 = R_3 = Me$
9b $R_1 = R_3 = Me$; $R_2 = Bn$



5 $R_1 = R_2 = H$
6 $R_1 = Me$; $R_2 = H$
9c $R_1 = R_2 = Bn$
9d $R_1 = Me$; $R_2 = Bn$



7a $R_1 = Bn$; $R_2 = Me$
7b $R_1 = Me$; $R_2 = Bn$
7c $R_1 = R_2 = Me$



8a $R_1 = H$
8b $R_1 = OBn$

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]. ^{1}H 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°) was considerably different from that reported for sendaverine (140–141.5°); 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 $NaBH_4$ 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 ^{1}H 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 $C_{18}H_{21}NO_4$, 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 ^{1}H 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 ^{1}H 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 the methoxy group at C-4′, but not with 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 from the *N*-benzylisoquinoline fraction was capnosinine (**6**), which analysed for $C_{19}H_{23}NO_4$ and was spectroscopically similar to capnosine. The main differences were associated with an additional methyl group on the isoquinoline nucleus. Thus, the mass spectrum showed the base peak at *m/z* 192, consistent with a dimethoxyisoquinoline fragment. As expected, the aromatic part of the ^{1}H NMR spectrum showed two singlets (H-5 and H-8), and the ABX spectrum was almost identical with that found for capnosine; therefore, the nitrogen substituent was assumed to be identical in both alkaloids. As before, the structure of **6** was confirmed by total synthesis. Reaction between **7c** and **8b** followed by reduction gave **9d**, which was deprotected to yield capnosinine (**6**) undistinguishable from the natural product.

It is worth noting that the major biosynthetic pathway in *C. heterocarpa* is that initiated in crassifoline and leading to cularine and 1,2-substituted berbines. On the other hand, *N*-benzylisoquinolines might be related to the coclaurine–reticuline pathway [10], a scarcely significant route in the plant since only protopine, glaucine, dihydrosanguinarine 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 GF_{254} for TLC. ^{1}H and ^{13}C NMR signals were measured at 200 and 50 MHz, respectively. Proton chemical shifts are referred to the residual $CHCl_3$ (δ 7.24) signal, and carbon chemical shifts to the solvent ($^{13}CDCl_3 = 77$ ppm). ^{1}H and ^{13}C 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 CH_2Cl_2–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).

Isosendaverine (**4**). Pale yellowish crystals, mp 181–182° (MeOH). UV λ_{max}^{MeOH} nm (log ε): 204 (4.55), 226 (4.18), 284 (3.61). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.30 (2H, *d*, $J = 8.6$ Hz, H-2′ and H-6′), 6.86 (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-α), 3.51 (2H, *s*, H-1), 2.80–2.60 (4H, *m*, H-3 and H-4). ^{13}C NMR (50 MHz, $CDCl_3$): 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-α), 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 $C_{18}H_{21}NO_3$: 299.15213).

Capnosine (**5**). Yellowish powder, mp 105–106°. UV λ_{max}^{MeOH} nm (log ε): 208 (4.54), 228sh (4.14), 284 (3.85); + NaOH: 212 (4.64), 244sh (4.17), 286 (4.00). ^{1}H NMR (200 MHz, $CDCl_3$): 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-α), 3.54 (2H, *s*, H-1), 2.80–2.65 (4H, *m*, H-3 and H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 145.7, 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-α), 50.5 (C-3), 28.7 (C-4). EIMS *m/z* (rel. int.): 315 $[M]^+$ (14), 314 $[M-1]^+$ (17), 178 (100), 150 (44), 137 (79). HRMS *m/z* 314.1457 ($[M]^+$, calcd for $C_{18}H_{21}NO_4$: 315.1470). (Found: C, 67.95; H, 6.77; N, 4.09. $C_{18}H_{21}NO_4$ requires: C, 68.55; H, 6.71; N, 4.44%.)

Capnosinine (**6**). Crystals, mp 172–173° (EtOH). UV λ_{max}^{MeOH} nm (log ε): 210 (4.52), 230sh (4.19), 282 (3.80); + NaOH: 214 (4.54), 232sh (4.14), 284 (3.85). ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.95 (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′), 6.58 (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-α), 3.52 (2H, *s*, H-1), 2.80–2.60 (4H, *m*, H-3 and H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 147.7, 147.4, 145.8, 145.6

(C-6, C-7, C-3', C-4'), 131.7 (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 × OMe), 55.8 (C-α), 50.5 (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. $C_{19}H_{23}NO_4$ requires: C, 69.28; H, 7.04; N, 4.25%).

General procedure for synthesizing **9a–d**. A mixt. 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 evapn of solvent, the isoquinolinium salt was dissolved in MeOH (8 ml) and $NaBH_4$ (3.2 mmol) added at room temp. for 5 hr. Usual work-up provided the corresponding *O*-benzyl derivatives of the *N*-benzylisoquinolines (**9a–d**).

O-*Benzylsendaverine* (**9b**). From **7b** [13] and **8a**. Mp 65–67° (hexane–Me_2CO) (87%). ^{1}H NMR (200 MHz, $CDCl_3$): δ7.45–7.20 (5H, *m*, C_6H_5), 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*, OCH_2Ar), 3.79 (6H, *s*, 2 × OMe), 3.60 (2H, *s*, H-α), 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. $C_{25}H_{27}NO_3 \cdot H_2O$ requires: C, 73.69; H, 7.17; N, 3.44%.)

O-*Benzylsendaverine* (**9b**). From **7b** [13] and **8a**. Mp 87–88° (hexane–Me_2CO) (87%) [14]. ^{1}H NMR (200 MHz, $CDCl_3$): δ7.65–7.20 (5H, *m*, C_6H_5), 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*, OCH_2Ar), 3.82 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 3.59 (2H, *s*, H-α), 3.47 (2H, *s*, H-1), 2.80 (2H, *t*, *J* = 5.1 Hz, H-3), 2.70 (2H, *t*, *J* = 5.1 Hz, H-4). EIMS *m/z* (rel. int.): 389 $[M]^+$ (21), 388 $[M-1]^+$ (27), 268 (14), 121 (100), 91 (67).

7,3'-*Di*-O-*benzylcapnosine* (**9c**). From **7b** and **8b**. Amorphous powder (89%). ^{1}H NMR (200 MHz, $CDCl_3$): δ7.46–7.26 (10H, *m*, 2 × C_6H_5), 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*, OCH_2Ar), 5.08 (2H, *s*, OCH_2Ar), 3.87 (3H, *s*, OMe), 3.83 (3H, *s*, OMe), 3.57 (2H, *s*, H-α), 3.45 (2H, *s*, H-1), 2.80–2.50 (4H, *m*, H-3 and H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ148.9, 148.3, 148.0, 146.4 (C-6, C-7, C-3', C-4'), 137.2 (C-1''), 130.8 (C-1'), 128.4 (C-3'', C-5''), 127.7 (C-4''), 127.4 (C-2'', C-6''), 127.2, 126.7 (C-4a, C-8a), 121.8 (C-6'), 115.0, 112.4, 112.0, 111.5 (C-5, C-8, C-2', C-5'), 71.2, 70.9 (2 × OCH_2Ar), 61.9 (C-1), 56.0 (2 × OMe), 55.2 (C–α), 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, 74.30; H, 6.46; N, 2.76. $C_{32}H_{33}NO_4 \cdot H_2O$ requires: C, 74.83; H, 6.87; N, 2.17%.)

3'-O-*Benzylcapnosinine* (**9d**). From **7c** [15] and **8b**. Crystals, mp 137–138° (EtOH) (90%). ^{1}H NMR (200 MHz, $CDCl_3$): δ7.46–7.26 (5H, *m*, C_6H_5), 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*, OCH_2Ar), 3.87 (3H, *s*, OMe), 3.83 (3H, *s*, OMe), 3.80 (3H, *s*, OMe), 3.56 (2H, *s*, H-α), 3.47 (2H, *s*, H-1), 2.73 (2H, *t*, *J* = 5.4 Hz, H-3), 2.57 (2H, *t*, *J* = 5.4 Hz, H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ148.8, 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 (OCH_2Ar), 62.1 (C-1), 56.0, 55.9 (3 × OMe), 55.5 (C-α), 50.3 (C-3), 28.4 (C-4). EIMS *m/z* (rel. int.): 419 $[M]^+$ (3), 418 $[M-1]^+$ (5), 328 (39), 227 (30), 192 (100), 91 (54). (Found: C, 73.97; H, 6.96; N, 3.18. $C_{26}H_{29}NO_4$ 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 H_2O and extracted with Et_2O. The aq. layers were basified, extracted with CH_2Cl_2 and evapd to obtain pure samples of **1** (74%), **4** (81%), **5** (56%) and **6** (85%), identical with the natural alkaloids (TLC, mp, NMR and MS).

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