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(−)-CASEAMINE FROM *CERATOCAPNOS HETEROCARPA*: STRUCTURE AND TOTAL SYNTHESIS

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Key Word Index—*Ceratocapnos heterocarpa*; Fumariaceae; protoberberine alkaloid; caseamine; clarkeanidine; synthesis.

Abstract—(−)-Caseamine was the major alkaloid isolated from *Ceratocapnos heterocarpa*. Its structure was elucidated by spectroscopic methods and by total synthesis.

INTRODUCTION

The alkaloid (−)-caseamine was isolated in 1938 from *Corydalis caseana* [1] and its structure was proposed 30 years later, incorrectly as **1** [2, 3] thus leaving it as an alkaloid of unknown structure [4]. (−)-Caseamine, together with (−)-caseadine (**2**) [2, 3], (−)-caseanadine (**3**) [5, 6] and (−)-clarkeanidine (**4**) [6], constitute a small group of protoberberine alkaloids that bear the unusual 1,2-oxygenated substitution pattern and they are related to the cularine type of alkaloids [7] through the benzylisoquinoline crassifoline (**5**) as the common precursor [8].

In this report we describe the isolation and the unambiguous structure of (−)-caseamine (**6**), elucidated by spectral studies and by total synthesis of its racemic form, as well as synthetic confirmation of the structure of clarkeanidine (**4**).

RESULTS AND DISCUSSION

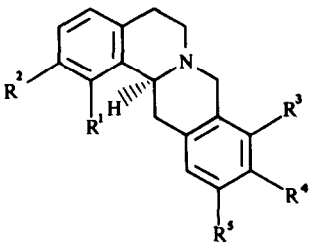
Ceratocapnos heterocarpa D. (Fumariaceae) is a climbing herb very localized in a few parts of the south of Spain. Its alkaloid extract was partially soluble in hot ethanol. The insoluble fraction was crystallized from chloroform–methanol, to afford a protoberberine alkaloid that was tentatively recognized as (−)-caseamine. According to previously reported observations [3] the mass spectrum of **1** located one methoxy and one hydroxy group in the A-ring (*m/z* 178) and a similar substitution in the D-ring (*m/z* 150). The ^{1}H NMR spectrum of **1** suggested the 1,2,10,11-substitution pattern, since the appearance of H-14 (δ3.99) at lower field than δ3.80, implies substitution at C-1 [9], while the high field of the protons at C-8 (δ3.98, *dd* and 3.85, *dd* for H_{eq}-8 and H_{ax}-8) indicated the absence of a substituent at C-9 [10]. The alternative 9,10-substitution was also excluded by the ^{13}C NMR data, since C-8 appeared at very low field (δ57) [9]. However, the relative position of the hydroxy and methoxy substituents could not be deduced from these data.

The 2D-COSY spectrum showed coupling between the doublet at δ6.72 (H-3) and one methoxy group (at C-2) indicating its *ortho*-relationship. The other doublet at higher field (6.62) showed benzylic coupling with the protons at C-5, consequently the hydroxy function on the A-ring should be located at C-1. On the other hand the aromatic proton at 6.53 (*s*, H-9) was coupled with the other methoxy group and with the benzylic protons at C-8. Therefore, the D-ring has the methoxy group at C-10 and the hydroxy group at C-11. NOE confirmed this result, establishing that (−)-caseamine (**6**) is a 1,11-dihydroxy-2,10-dimethoxy tetrahydroprotoberberine.

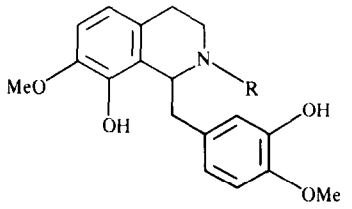
Further proof for the hydroxy group at C-1 was obtained from the ^{1}H NMR spectrum of (−)-caseamine diacetate (**7**). A greater paramagnetic shift ($\Delta\delta$ = +0.36 ppm) was observed for the signal of the aromatic proton at C-4 (*para* one acetyl group) than the other aromatic proton signals. As expected the methylation of **6** (diazomethane) was much faster for the hydroxyl at C-11 than for the C-1 hydroxy group thus giving a mixture of (−)-caseadine (**2**) (identified with an authentic sample) and (−)-*O*-methyl caseadine (**8**) whose spectral and physical data were in full agreement with those previously reported for the synthetic product [2, 3].

In order to confirm the structure of **6**, its total synthesis was undertaken following the biosynthetic route. The benzylisoquinoline **9** was prepared by the Reissert approach according to the procedure previously described [11]. Reduction and removal of the protecting groups of **9**, were carried out in a single reaction to afford (±)-norcrassifoline (**10**) in high yield. Compound **10** was characterized by its physical and spectral data. The EIMS did not exhibit a molecular ion, but the CIMS showed the expected M + 1 at *m/z* 316.

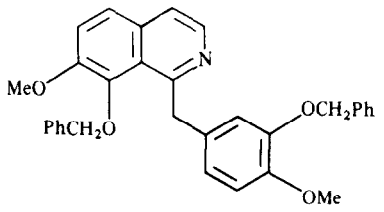
Competitive *ortho*- and *para*-cyclization was observed when **10** was heated with 37% formaldehyde, to afford (±)-caseamine (**6**) and (±)-clarkeanidine (**4**), which showed an identical mp and spectral data to those reported [6].



1 R^1, R^2 = OH,OMe; R^3 = H; R^4, R^5 = OMe,OH
2 R = OH; R^3 = H; R^2 = R^4 = R^5 = OMe
3 R^1 = OH; R^5 = H; R^2 = R^3 = R^4 = OMe
4 R^1 = R^3 = OH; R^2 = R^4 = OMe; R^5 = H
6 R^1 = R^5 = OH; R^3 = H; R^2 = R^4 = OMe
7 R^1 = R^5 = OAc; R^3 = H; R^2 = R^4 = OMe
8 R^1 = R^2 = R^4 = R^5 = OMe; R^3 = H



5 R = Me
10 R = H



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EXPERIMENTAL

General procedures. All mps: uncorr. Optical rotations were recorded at 18–20° using a 1 dm cell. ^{1}H and ^{13}C NMR spectra were obtained at 200 and 50.3 MHz, respectively. EIMS spectra, direct inlet, 70 eV. TLC were performed on silica gel 60 F 254 plates. CC was carried on silica gel 60 (70–230 mesh).

Plant material. The plant material used in this work was collected in April and May 1984 in Moron de la Frontera (Sevilla, Spain), and was identified by Prof. Baltasar Cabezudo, Department of Vegetal Biology, University of Malaga, Spain. A voucher specimen is on deposit in its Herbarium.

Isolation of (−)-caseamine. The air-dried and powdered whole plant (1.5 kg) was extracted with hot MeOH. Acid-base fractionation of the extract, gave an alkaloid fraction (5.06 g). Extraction on this fraction with EtOH left a white solid residue (1.3 g) which was crystallized from $CHCl_3$–MeOH. Mp 246–247°, (lit. [2] mp 257°); $[\alpha]_D$ −365° (*c* 0.04; $CHCl_3$); UV λ_{max}nm (log ε) in EtOH: 215 (4.21), 224 sh (4.12), 284 (3.77); +NaOH: 218 (4.37), 287 sh (3.73), 296 (3.75); IR ν_{max}cm^{-1}: 3475, 1610, 1580, 1530, 1500, 1275, 1220, 800, 775. ^{1}H NMR ($CDCl_3$): δ 6.72 (1H, *d*, *J* = 8.3 Hz, H-3), 6.64 (1H, *s*, H-12), 6.62 (1H, *d*, *J* = 8.3 Hz, H-4), 6.53 (1H, *s*, H-9), 5.80, 5.43 (each 1H, *br s*, 2 × OH), 3.99 (1H, *dd*, *J* = 11.5 and 3.6 Hz, H-14), 3.98 (1H, *d*, *J* = 15 Hz, H_{eq}-8), 3.85 (1H, *d*, *J* = 15 Hz, H_{ax}-8), 3.86, 3.84 (each 3H, *s*, 2 × OMe), 3.61 (1H, *dd*, *J* = 16.3 and 3.6 Hz, H_{eq}-13), 3.20-2.50 (5H, *m*); ^{13}C NMR (DMSO-d_6): δ 146.0, 145.3, 144.8 (C-2, C-10, C-11), 143.0 (C-1), 128.0, 127.2, 125.8, 124.8 (C-4a, C-8a, C-12a, C-14a), 118.8 (C-4), 115.3 (C-12), 110.3 (C-3, C-9), 57.0 (C-8), 56.2, 55.9 (2 × OMe), 55.7 (C-14), 47.9 (C-6), 31.5 (C-13), 29.3 (C-5). EIMS *m/z* (rel. int.): 327 $[M]^+$ (25), 178 (100), 150 (17), 135 (6).

Acetylation of (−)-caseamine. A soln of caseamine (100 mg) in pyridine (3 ml) was treated with Ac_2O (1 ml) at room temp. for 1 week. After the usual treatment, the crude product was purified on prep. TLC (AcOEt), to give a diacetate (**7**, 44 mg, 35%), as an oil. ^{1}H NMR ($CDCl_3$): δ 6.98 (1H, *d*, *J* = 8.4 Hz, H-4), 6.82 (1H, *d*, *J* = 8.4 Hz, H-3), 6.74 (1H, *s*, H-12), 6.64 (1H, *s*, H-9), 4.13 (1H, *d*, *J* = 16 Hz, H_{eq}-8), 3.95 (1H, *dd*, *J* = 11 and 4 Hz, H-14), 3.89 (1H, *d*, *J* = 16 Hz, H_{ax}-8), 3.79, 3.78 (each 3H, *s*, 2 × OMe), 3.10 (1H, *dd*, *J* = 16 and 4 Hz, H_{eq}-13), 3.10–2.90 (3H, *m*), 2.76 (1H, *dd*, *J* = 16 and 11 Hz, H_{ax}-13), 2.70 (1H, *m*), 2.28 (6H, *s*, 2 × OAc).

Methylation of (−)-caseamine. Treatment of a methanolic soln of caseamine (50 mg) with an ethereal solution of CH_2N_2, gave two products. The faster running on TLC (AcOEt–hexane, 9:1) was identified as (−)-*O*-methyl caseadine (**8**) (11 mg, 20.3%). Mp 183–184° (EtOH), (lit. [2] mp 186°) and positive comparison (UV[2], ^{1}H NMR [2], MS [2, 3] with data reported for the synthetic product. ^{1}H NMR ($CDCl_3$): δ 6.85 (1H, *d*, *J* = 8.4 Hz, H-3), 6.77 (1H, *d*, *J* = 8.4 Hz, H-4), 6.56 (2H, *s*, H-9, H-12), 4.14

(1H, *d*, $J = 16$ Hz, H_{eq}-8), 4.10 (1H, *dd*, $J = 12$ and 4.1 Hz, H-14), 3.85 (1H, *d*, $J = 16$ Hz, H_{ax}-8), 3.84 (6H, *s*, 2 × OMe), 3.82 (6H, *s*, 2 × OMe), 3.35 (1H, *dd*, $J = 16.4$ and 4.1 Hz, H_{eq}-13), 3.15–2.60 (5H, *m*), 2.70 (1H, *dd*, $J = 16.4$ and 12 Hz, H_{ax}-13). The second product was identified as (−)-caseadine (**2**) by comparison with an authentic sample.

Preparation of (±)-*nor-crassifoline* (**10**). To a satd ethanolic soln of **9** (58.5 mg) were added a few drops of conc HCl and the mixture refluxed (12 hr). This was followed by the addition of a suspension of Zn-Hg amalgam (Zn 540 mg, $HgCl_2$ 23 mg, conc HCl 0.02 ml and H_2O 0.67 ml), and aq. 20% HCl (3 ml) and the mixture again refluxed for 3 hr. Basification of the reaction and extraction (CH_2Cl_2) afforded a brown foam (30 mg, 80%) that was crystallized from CH_2Cl_2 MeOH. Mp 169–170°. (Found: C, 68.81; H, 7.04; N, 4.24; $C_{18}H_{21}O_4N$ requires: C, 68.55; H, 6.71; N, 4.42%). UV λ_{max}nm (log ε) in MeOH: 227 (4.12), 282 (3.74); + NaOH: 223 (4.25), 288 (3.77), 296 sh (3.70). IR ν_{max}cm^{-1}: 3550, 3450, 3340, 1625, 1600, 1500, 1450, 800. ^{1}H NMR ($CDCl_3$): δ 6.81 (1H, *d*, $J = 1.7$ Hz, H-2′), 6.76 (1H, *d*, $J = 8.1$ Hz, H-5′), 6.70 (1H, *dd*, $J = 8.1$ and 1.7 Hz, H-6′), 6.70 (1H, *d*, $J = 8.3$ Hz, H-6), 6.59 (1H, *d*, $J = 8.3$ Hz, H-5), 4.37 (*dd*, $J = 10.5$ and 2.8 Hz, H-1), 3.82, 3.81 (each 3H, *s*, 2 × OMe), 3.30-2.80 (3H, *m*, H-3, H-3′, H-4′), 3.18 (1H, *dd*, $J = 13.8$ and 2.8 Hz, CH-Ph), 2.71 (1H, *dd*, $J = 13.8$ and 10.5 Hz, CH-Ph), 2.70 (1H, *m*, H-4). CIMS (NH_3), *m/z* (rel. int.): 216 $[M+H]^+$ (100), 178 (13). EIMS *m/z* (rel. int.): 178.0865 $[M]^+$ (100) (calc. for $C_{10}H_{12}NO_2$: 178.0865), 163 (14), 137 (7), 122 (2).

Mannich cyclization of (±)-*nor-crassifoline*. Procedure A: a water soln of the hydrochloride of **10** (25.6 mg) was heated on the steam-bath while 37% formaldehyde (0.1 ml) was added. After heating for 1 hr, the reaction was basified (NH_4OH) and extracted to afford two compounds, which were separated by prep. TLC. The upper band was identified as (±)-clarkeanidine (4 mg, 16.8%) by mp, UV, NMR, and MS comparison with the reported data [9]. The lower band, gave (±)-caseamine (14 mg, 58.5%), identical with the natural (−)-caseamine (mp, UV, NMR and MS).

Procedure B: to a soln of the Clark–Lubbs buffer, the hydrochloride of **10** (33.5 mg), 37% formaldehyde (1.7 ml) and MeOH (2.2 ml) were added. The mixture was refluxed for 1 hr, and worked up as above. (±)-Clarkeanidine (8 mg, 26%) and (±)-caseamine (11 mg, 35.3%), were isolated.

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