QUATERNARY PROTOBERBERINE ALKALOIDS FROM
CERATOCAPNOS HETEROCARPA

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Abstract—Three new quaternary protoberberine alkaloids, cis-caseamine N-oxide, cis-caseadine N-oxide and caseadinium chloride were isolated from Ceratocapnos heterocarpa. Their structures and stereochemistry were determined by spectral methods and chemical correlations.

INTRODUCTION

Protoberberine alkaloids occur naturally in two main types according to the oxygenation pattern on ring A. The more common 2,3-oxygenated alkaloids are biosynthetically derived from the benzylisoquinoline reticuline [1] and usually isolated in two oxidation stages, either as tetrahydro derivatives (berbines) or as protoberberinium salts [2]. The latter type, which bear substituents at positions 1 and 2, are known to occur in the reduced form and be related to cularine [3] and quettamine [4] alkaloids; also, they presumably derive from a 7,8-substituted benzylisoquinoline such as crassifoline [5, 6]. Their presence in Papaveraceae is seemingly limited to the genera Sarcocapnos, Ceratocapnos, Corydalis and Dicentra. Ceratocapnos heterocarpa Durieu is special in this respect because of its ability to produce not only the usual Papaveraceae alkaloids and cularines [7], but also the 1,2 substituted berbines, caseamine, clarkeadinine, caseadine [8] and malacitanine [9].

The present paper reports the stereostructure and molecular conformation of the new alkaloids cis-caseamine N-oxide (1) and cis-caseadine N-oxide (2), which were isolated from C. heterocarpa, together with caseadinium chloride (3), the first reported example of a berberinium salt with this type of substitution.

RESULTS AND DISCUSSION

The more polar fractions of the silica gel CC of the crude alkaloids yielded 1-3. From analytical data, the molecular formula C_{19}H_{21}NO_{5} was assigned to 1, consistent with the FAB mass spectrum [M+H]^{+} at m/z 344 and the [M]^{+} observed at m/z 343 in the EI mass spectrum. The peak [M-16]^{+} at m/z 327.1468 (C_{19}H_{21}NO_{5} requires: 327.1470) suggested the presence of an N-oxide function, whereas the base peak at m/z 178 and another prominent peak at m/z 150 were indicative of a berbine structure with a methoxyl group and a hydroxyl group on both rings A and D [10]. The \textsuperscript{1}H NMR spectrum recorded under neutral conditions showed H-14, H-8a and H-8β at very low-field due to the quaternary nitrogen atom and four aromatic protons (two ortho-coupled and two as a broad singlet). The presence of a 1-hydroxy-2-methoxy substituent on ring A was evident from the 2D COSY spectrum, which correlated the doublet at δ 6.65 with the benzyl hydrogens at C-5, and the doublet at δ 6.65 with a methoxyl group. The 10,11-substitution pattern on ring D was apparent from the \textsuperscript{1}H NMR spectrum recorded in an acid medium; in the aromatic region, the broad singlet was resolved and two para-oriented protons were observed. One of them (δ 6.66) was correlated with H-13eq and H-13w while the other (δ 6.69), as expected, was coupled to a methoxyl group and the benzyl hydrogens at C-8. On these grounds, structure 1 was anticipated to be (-)-caseamine N-oxide.

Compound 2 was obtained as amorphous powder. Comparison of \textsuperscript{1}H and \textsuperscript{13}C NMR data between 1 and 2 showed them to be structurally similar; however, the latter had three methoxyl groups. Analytical data and the FAB mass spectrum [M+1]^{+} at m/z 358 suggested the molecular formula to be C_{20}H_{23}NO_{5}. The EI mass spectrum showed the base peak at m/z 164, thus indicating the presence of two methoxyl groups at ring D. Therefore, the structure of 2 was concluded to be (-)-caseadine N-oxide.

The present paper reports the stereostructure and molecular conformation of the new alkaloids cis-caseamine N-oxide (1) and cis-caseadine N-oxide (2), which were isolated from C. heterocarpa, together with caseadinium chloride (3), the first reported example of a berberinium salt with this type of substitution.
constant between H-14 and both H-13. The large $J_{14-13}^\beta$ value found suggests a trans-diaxial relationship, so the cis-N-oxides of caseamine and caseadine must occur preferentially in conformation la [12, 13].

Compounds 1 and 2 were obtained by oxidation of (-)-caseamine and (-)-caseadine (also present in the plant) with MCPBA; a single stereoisomer which coincided with the naturally occurring alkaloids was isolated in both cases. Therefore, the absolute configuration for both N-oxides was concluded to be 7R, 14S.

The more polar compound 3 was isolated as a yellow, optically inactive powder. The UV absorptions at $\lambda_{max}$ 222, 310, 370 nm shifted to 254, 290, 310 and 398 nm on addition of NaOH, indicating the structure of a phenolic protoberberinium salt. The [M] at $m/z$ 338 was consistent with the molecular formula $C_{20}H_{20}NO_4$. The $^1H$ NMR spectrum showed three methoxyl groups, two aliphatic triplets and the expected six aromatic protons, with H-8 and H-13 at a very low-field ($\delta$ 9.16 and 9.08, respectively). A 1,2,10,11-substituted protoberberinium salt structure for 3 was thus suggested. The locations of the substituents on 3 were determined by borohydride reduction which yielded racemic caseadine, quantitatively. Attempts at obtaining 3 by direct oxidation of caseadine were unsuccessful. The oxidation of 1,2-substituted berbines proved to be more difficult than the oxidation of the more common 2,3-substituted derivatives; this has been related to their conformation in solution [14].

**EXPERIMENTAL**

**General.** Mps: uncorr. EIMS: direct inlet, 70 eV. FABMS: 2-hydroxyethyl disulphide as matrix. Silica gel 60 (70–230 mesh) was used for CC and silica GF254 for TLC. Optical rotations were measured at 20–22°. $^1H$ and $^{13}C$ NMR signals were measured at 200 and 50.3 MHz, respectively. Proton chemical shifts are referred to the residual CHCl$_3$ ($\delta$ 7.24) or Me$_2$CO signal ($\delta$ 2.04) and carbon chemical shifts to the solvent ($^{13}$CDCl$_3$ = 77 ppm). $^1H$ and $^{13}C$ NMR signals were assigned from 2D COSY and DEPT expts.

**Isolation.** The source of the plant material and part of the isolation procedure are reported elsewhere [8]. The new compounds 1 (240 mg), 2 (78 mg) and 3 (5 mg) were obtained by CC over silica gel using EtOAc-MeOH (2:1) as eluent, and subsequent purification by prep. TLC and recrystallization.

cis-(-)-Caseamine N-oxide (1). Pale yellowish crystals, mp 244–246° (MeOH), [\$]$_D$ = -216° (MeOH; c 0.05). UV $\lambda_{max}$ nm (log e) in MeOH: 208 (4.83), 230 (4.03), 286 (3.74); + NaOH: 208 (4.89), 238 (3.95), 252 (3.95), 288 (3.68). IR $\nu_{max}$ cm$^{-1}$ (KBr): 3240. $^1H$ NMR (200 MHz, CDCl$_3$ + MeOH-d$_4$): $\delta$ 6.65 (1H, d, $J$ = 8.3 Hz, H-3), 6.55 (1H, d, $J$ = 8.3 Hz, H-4), 6.45 (2H, s, H-9 and H-12), 4.73 (1H, d, $J$ = 15.4 Hz, H-8eq), 4.27 (1H, d, $J$ = 15.4 Hz, H-8az), 3.72 (3H, s, OMe), 3.70 (3H, s, OMe), 3.50 (1H, dd, $J$ = 18.5 and 6.5 Hz, H-13aq), 3.50–3.30 (2H, m, H-5 and H-6), 3.10–3.00 (1H, m, H-6'), 2.80–2.60 (1H, m, H-5'), 2.60 (1H, dd, $J$ = 18.5 and 11.3 Hz, H-13eq), 3.50–3.30 (2H, m, H-5 and H-6), 3.10–3.00 (1H, m, H-5), 2.80–2.60 (1H, m, H-5'), 2.60 (1H, dd, $J$ = 18.5 and 11.3 Hz, H-13eq). $^1H$ NMR (200 MHz, CDCl$_3$ + TFA): $\delta$ 6.84 (1H, d, $J$ = 8.4 Hz, H-3), 6.74 (1H, d, $J$ = 8.4 Hz, H-4), 6.69 (1H, s, H-9), 6.66 (1H, s, H-12), 5.21 (1H, dd, $J$ = 11.2 and 6.5 Hz, H-14), 5.13 (2H, s, H-8aq and H-8eq), 3.99 (1H, dd, $J$ = 12.8 and 7.9 Hz, H-6), 3.89 (3H, s, OMe), 3.88 (3H, s, OMe), 3.76 (1H, dd, $J$ = 18.3 and 6.5 Hz, H-13eq), 3.75 (1H, m, H-6'), 3.60 (1H, m, H-5), 3.01 (1H, dd, $J$ = 17.1 and 5.4 Hz, H-5'), 2.88 (1H, dd, $J$ = 18.3 and 11.2 Hz, H-13eq). $^{13}C$ NMR (50.3 MHz, CDCl$_3$ + TFA): $\delta$ 146.7, 145.1, 141.7 (C-1, C-2, C-10, C-11), 120.9, 120.5, 118.8, 117.2 (C-4a, C-8a, C-12a, C-14a), 119.8, 113.5, 110.8, 108.7 (C-3, C-4, C-9, C-12), 69.8 (C-8), 65.2
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(C-14), 56.1 (2 × OMe), 54.0 (C-6), 34.1 (C-13), 23.4 (C-5). EIMS m/z (rel. int.): 343 [M]+ (2), 328 (12), 327 [M − 16]+ (Found: 327.1468; C19H21NO4 requires: 327.1470) (53), 312 (7), 194 (16), 178 (100), 176 (33), 150 (57). FABMS m/z: 344 [M + H]+. (Found: C, 64.29; H, 6.60; N, 3.83. C19H21NO5.2/3 H2O requires: C, 64.22; H, 6.29; N, 3.94%).

cis-(−)-Caseadine N-oxide (2). Amorphous powder, mp 156 °C. [α]D −199° (MeOH; c 0.06). UV λmax nm (log e) in MeOH: 206 (4.00), 230sh (3.22), 284 (2.79); +NaOH: 206 (4.10), 230sh (3.22), 286 (2.78). 1HNMR (200 MHz, acetone-d6): δ 6.70 (1H, s, H-9), 6.67 (1H, s, H-12), 6.47 (1H, d, J = 8.5 Hz, H-3), 6.40 (1H, d, J = 8.5 Hz, H-4), 5.20 (1H, dd, J = 11.7 and 6.1 Hz, H-14), 4.83 (1H, d, J = 15.5 Hz, H-8ax), 4.52 (1H, d, J = 15.5 Hz, H-8eq), 3.76 (3H, s, OMe on C-10), 3.72 (3H, s, OMe on C-11), 3.54 (3H, s, OMe on C-2), 3.55 (1H, dd, J = 17.3 and 6.1 Hz, H-13eq), 3.80–3.20 (2H, m), 2.80–2.60 (2H, m), 2.65 (1H, dd, J = 17.3 and 11.7 Hz, H-13ax). 13CNMR (50.3MHz, CDCl3): δ 149.3, 148.5, 146.7, 144.1 (C-1, C-2, C-10, C-11), 122.9, 121.7, 121.2 (C-4a, C-8a, C-12a, C-14a), 117.3, 110.8, 110.2, 108.9 (C-3, C-4, C-9, C-12), 72.2 (C-8), 66.0 (C-14), 56.2, 56.0, 55.6 (3 × OMe), 55.3 (C-6), 35.7 (C-13), 24.4 (C-5). EIMS m/z (rel. int.): 341 [M − 16]+ (Found: 341.1625; C20H23NO4 requires: 341.1627) (22), 164 (100), 121 (42). FABMS m/z: 358 [M+H]+. (Found: C, 64.10; H, 6.76; N, 3.32. C20H23NO6.1/2 H2O requires: C, 64.00; H, 6.67; N, 3.73%).

Caseadinium chloride (3). Yellowish solid, mp 192 °C. UV λmax nm (log e) in MeOH: 204, 224sh, 228, 310sh, 370; +NaOH: 204, 254, 290, 310sh, 398. 1HNMR (200 MHz, CDCl3 + TFA): δ9.16 (1H, s, H-8), 9.08 (1H, s, H-13), 7.45 (1H, s), 7.30 (1H, s), 7.00 (1H, d, J = 8.2 Hz), 6.88 (1H, d, J = 8.2 Hz), 4.74 (2H, t, J = 5.4 Hz, H-6,6'), 4.14 (3H, s, OMe), 4.07 (3H, s, OMe), 4.00 (3H, s, OMe), 3.18 (2H, t, J = 5.4 Hz, H-5,5'). EIMS m/z (rel. int.): 338 [M]+ (Found: 338.1345; C20H20NO4 requires: 338.1392) (19), 323 (13).

Oxidation of (−)-caseadine. A soln of caseadine (41 mg) in CHCl3 (4 ml) was stirred with MCPBA (40 mg) at room temp. for 5 hr. The reaction mixt. was diluted with CHCl3, washed with 5%aq. K2CO3, dried and the solvent evapd to obtain an N-oxide (23 mg, 54%) which was identical with the natural cis-(−)-caseadine N-oxide.

Reduction of caseadinium chloride (3). Compound 3 (1 mg) was treated with NaBH4 in a MeOH soln at room temp. for 1 hr. Acidification of the reaction medium and extraction (CHCl3) yielded an amorphous powder (1 mg) which was identified as (−)-caseadine by comparison (TLC, NMR and MS) with an authentic sample.

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