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ISOLATION AND STRUCTURAL ELUCIDATION OF THE FIRST KNOWN C-HOMOPROTOBERBERINE

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Abstract

A novel C-homoprotoberberine alkaloid, Hediamine (**2**), has been isolated from the roots of *Berberis actinacantha* (Berberidaceae). Conclusive proof for the structure of hediamine was obtained by its comparison with the isomeric lactam (**4**), by chemical transformation, and by means of spectroscopic techniques (UV, IR, MS, NMR).

Introduction

The study of the alkaloids of the Berberidaceae is facilitated by the fact that relatively large amounts of these plants are widely available and are a rich repository of isoquinoline alkaloids. Therefore, it is possible to study the minor alkaloids present, i.e. those available only in very small amounts, and this can often afford an insight into the biosynthetic pathways for the principal alkaloids.

Berbines may be formed *in vivo* from a variety of tetrahydrobenzylisoquinolines [1], while they themselves may act as precursors to the protopines, the phthalideisoquinolines, the benzophenanthridines, and even to some of the aporphines [2]. A typical berine alkaloid is berberine (**11**) which is present in all plants belonging to the genus *Berberis* (Berberidaceae).

We have previously reported the isolation of two pseudobenzylisoquinoline alkaloids from the roots of *B. actinacantha* [3]. In this paper, we describe the isolation and characterization of a novel alkaloid, hediamine (**2**), $C_{21}H_{19}NO_5$, which is probably derived from berberine (**11**) in a series of steps. The importance of this lactam (**2**) lies in the fact that it is the first known C-homoprotoberberine.

Results and Discussion

The hediamine crystallized from chloroform as pale yellow needles. Its UV spectrum possessed absorption maxima at 329 nm, indicating the presence of a highly conjugated system. The spectrum remained unaffected by the addition of either acid or base. The IR spectrum of hediamine revealed a tertiary amide carbonyl functionality (1659 cm^{-1}). Its high resolution mass spectrometry disclosed a molecular formula of $C_{12}H_{19}NO_5$ for (**2**), showing the molecular ion at 365.1275 (calcd. 365.1258) as the base peak. This also indicates 13 double bond equivalents in the molecule.

The ^{1}H-NMR spectrum of hediamine recorded at 360 MHz in $CDCl_3$ showed overlapping absorption around δ 3.91 probably due to the methylene protons next to amidic carbonyl, the methylene protons adjacent to the amidic nitrogen and the methoxy singlets. A singlet at δ 5.99 was due to a methylenedioxy proton. Three aromatic-vinylic singlets at δ 6.64, 6.97 and 7.15, in addition to two doublets at δ 6.91 and 7.07 (J= 8.5 Hz) were also present. This pattern is characteristic of two aromatic nuclei substituted at 1,2,4,5 and 1,2,3,4, respectively and a double bond between the two aromatic rings. This is in good agreement with UV absorption at 329 nm which also suggests the presence of a stilbene system.

Consideration of all these data along with biogenetic consideration leads to the conclusion that structure (**2**) is the most likely structure of hediamine. The assignment of chemical shifts was confirmed by NMR NOEDS study in $CDCl_3$ at 360 MHz.

Keywords: Alkaloids; Berberidaceae; *Berberis actinacantha*; C-homoprotoberberine; Hediamine

It was reasoned that the key to the complete structure elucidation of hediamine (2) lay in its comparison with the isomeric lactam (4). For this purpose, the sodium borohydride reduction of puntarenine (1) carried out previously was repeated on a relatively large scale (200 mg). The main product was the expected racemic alcohol (3) already described in the literature [4], and two minor products were (8) and (2).

Dehydration of the main alcohol (3) in refluxing trifluoroacetic acid generated three products which were separated by TLC. The first was the desired pale yellow lactam (4) (29%), $C_{21}H_{19}NO_5$, ν_{max} ($CHCl_3$) 1603, 1630 and 1660 cm^{-1}, which proved to be very significantly different from hediamine (2). The second was a deep yellow isoquinolone (5) (33%), also analyzing for $C_{21}H_{19}NO_5$, ν_{max}($CHCl_3$) 1637 cm^{-1}. The third was the red ketolactam (6), obtained in a minor amount (3%), and analyzing for $C_{21}H_{17}NO_6$; ν_{max} ($CHCl_3$) 1650, 1665, and 1685 cm^{-1}.

Upon extended treatment with trifluoroacetic acid, lactam (4) isomerized into (5), while in base the reverse transformation occurred. In addition, on a silica gel TLC plate which was developed in the common solvent $CHCl_3$-MeOH (95:5 v/v), the pale yellow lactam (4) tended to oxidize to red ketolactam (6), thus explaining the formation of the minor red product. A parallel oxidation occurred but less readily, with the isoquinolone (5). Finally, sodium borohydride reduction of the deep yellow isoquinolone (5) led to the saturated lactam (7) (53%), $C_{21}H_{21}NO_5$, ν_{max} ($CHCl_3$) 1650 cm^{-1}, together with the pale yellow lactam (4) (25%), which is in good agreement with rearrangement of isoquinolone (5) to lactam (4) in basic media.

Returning now to sodium borohydride reduction of puntarenine (1), besides alcohol (3) as the main product (75%), a minor product was alcohol (8) (7.5%), isomeric with (3). The NMR spectrum of (8) is noteworthy since the H-1 and H-13 absorptions appear further downfield relative to the alcohol (3), namely at δ 6.91 and 7.06, respectively. This reflects the fact that the C-15 alcohol in (8) is quasi-equatorial and is close to H-1 and H-13. Additionally, $J_{14,15}$ in (8) is 4.59, corresponding to a dihedral angle approximating 145°.

The third product of the reduction of puntarenine, obtained in only 9% yield, unexpectedly proved to be hediamine (2). In retrospect, it can be stated that sodium borohydride reduction of puntarenine (1) proceeds mainly by hydride addition at the C-15 carbonyl center, but a side reaction consists of hydride attack at C-14 resulting in the formation of anion (9). This anion can then undergo intramolecular addition at C-15, and subsequent loss of water would lead to hediamine (2). In contrast, sodium borohydride reduction of dehydropuntarenine (10) generated mostly alcohol (3) and a small yield of the isometric (8), but no hediamine (5).

There is a possibility that the sodium borohydride reduction of puntarenine (1) gives a clue to the biogenesis of hediamine. Again, an intermediate such as (9) could be involved in the *in vivo* conversion of puntarenine (1) into hediamine (2). Such a hypothesis however, still remains to be proven.

Experimental Section

Analytical and preparative TLC were carried out on silica gel GF254 Merck precoated plates. Column chromatography was on Merck G-60 silica gel. Mass spectra were determined at 70 eV on an AEI MS-902 double-focusing spectrometer. All ^{1}H-NMR spectra were obtained in $CDCl_3$ solution either at 200 or at 360 MHz on a Bruker spectrometer. All ultraviolet spectra were taken in methanol solutions unless stated otherwise, and infrared spectra were obtained in chloroform solution. The specimen of *B. actinacantha* used in this investigation was collected in spring from Locurr Hill, immediately east of Santiago, Chile. A voucher specimen was deposited in the herbarium of the Natural History Museum in Santiago.

Dried, powdered roots were extracted successively with light petroleum ether and with ethanol by percolation. Evaporation of the solvent of the ethanolic extract was carried out with the minimum application of heat. The extracts were dissolved in 3N HCl, and then filtered. The acid solution was basified with ammonium hydroxide and the crude alkaloids extracted with chloroform. The chloroform was evaporated and the residue was brought on silica gel (70-230 mesh) column. Elution was performed with chloroform containing increasing percentages of methanol and the final purification by extensive thin layer chromatography on pre-prepared Merck silica gel plates.

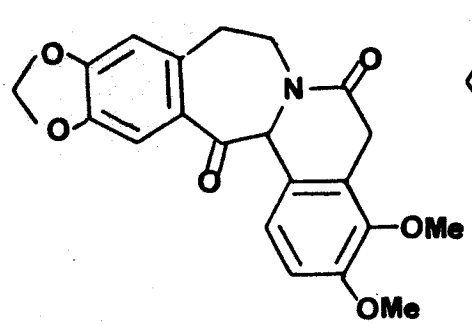
Reduction of Puntarenine (1) with Sodium Borohydride

Puntarenine (200 mg) was suspended in methanol (40 ml) and a large excess of sodium borohydride was added. The mixture was allowed to stir at room temperature for 3 hours. The methanol was evaporated under reduced pressure to afford a pale yellow residue (192 mg), which was a mixture of three compounds, showed by TLC, using 6% MeOH/$CHCl_3$ as solvent system. They were separated by preparative TLC, and identified by means of spectroscopic techniques, to be trans-dihydropuntarenine (3) (148 mg), cis-dihydropuntarenine (8) (14 mg), and hediamine (2) (18 mg).

Dehydration of Dihydropuntarenine (3)

Dihydropuntarenine (3) (17.75 mg) was dissolved in trifluoroacetic acid (6 ml) and refluxed for 1 hour. The acid

Table I. Spectral characteristics of the alkaloids



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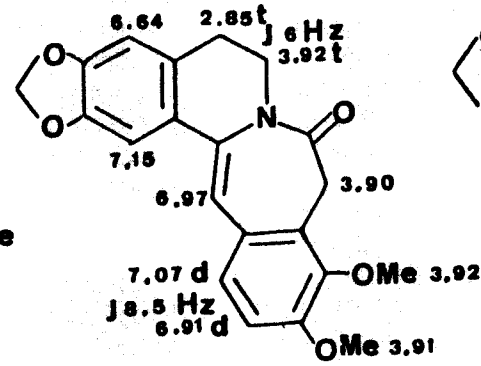

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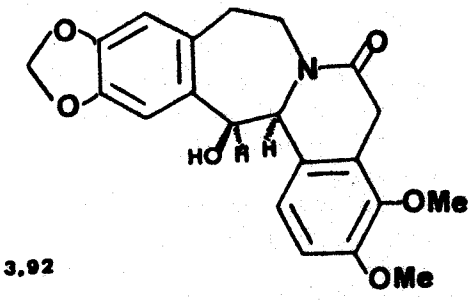

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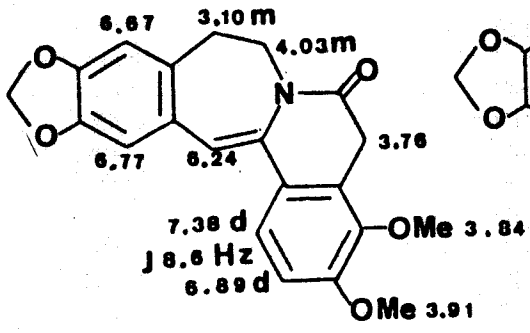

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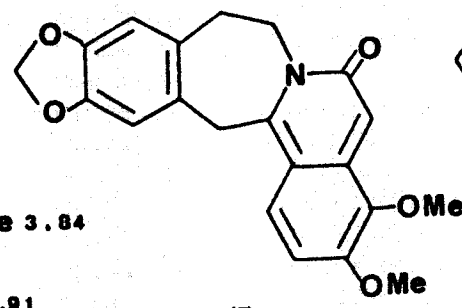

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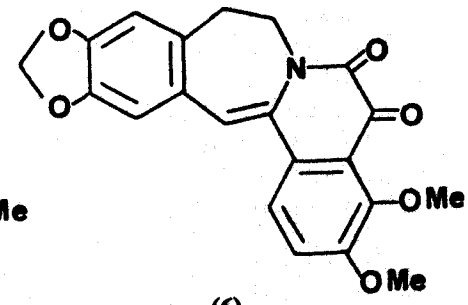

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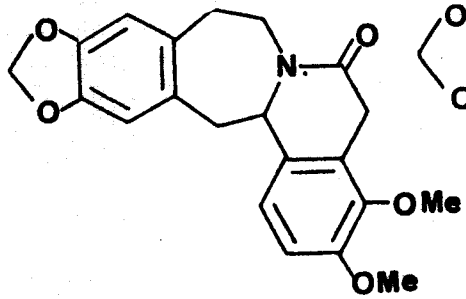

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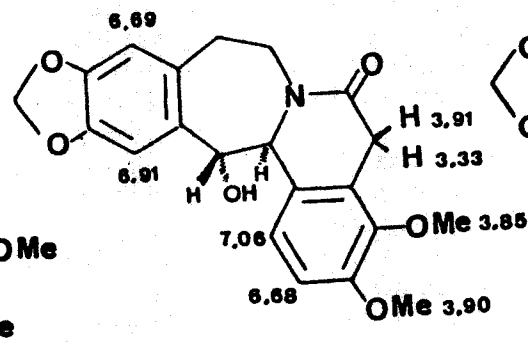

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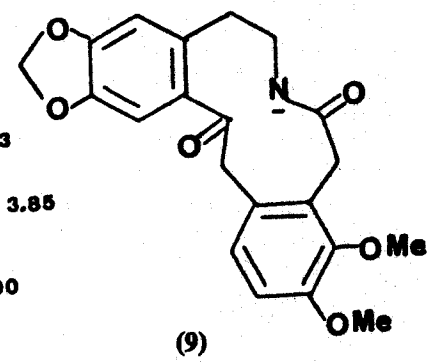

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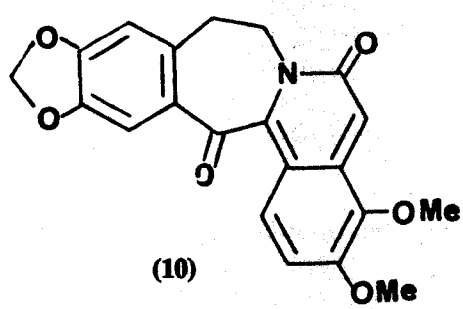

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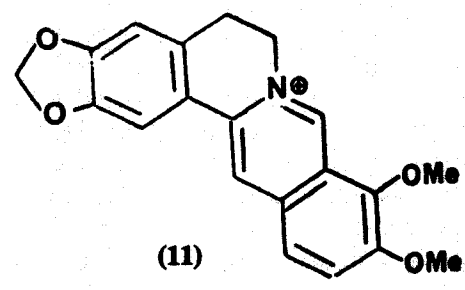

(11)

TFA) was evaporated under reduced pressure. The residue (17.1 mg) comprised three compounds which were separated by preparative TLC using 6% methanol chloroform as solvent system, and identified to be compounds (**4**) 4.7 mg), (**5**) (5.7 mg), and (**6**) (0.6 mg).

The Rearrangement of Compound (4) to Compound (5)

A solution of compound (**4**) (1.5 mg) in trifluoroacetic acid (6 ml) was refluxed for 3 hours. TFA was evaporated and the residue was checked on TLC. It was mostly compound (**5**), 93%.

The Rearrangement of Compound (5) to Compound (4)

To a solution of compound (**5**) (2 mg) in methanol (6 ml), a drop of concentrated sodium hydroxide was added. The yellow color of compound (**5**) disappeared after a few seconds. The mixture was allowed to stir for 30 min. at room temperature. The solvent was evaporated and the residue was dissolved in chloroform (20 ml), washed with water, and then dried over anhydrous sodium sulphate. Evaporation of the solvent gave mostly compound (**4**) (1.5 mg).

Reduction of Isoquinolone (5) with Sodium Borohydride

Compound (**5**) (7.5 mg) was dissolved in methanol (20 ml) and a large excess of sodium borohydride was added. The reaction mixture was allowed to stir at room temperature for 3 hours. The methanol was evaporated and the residue was purified by preparative TLC. Two compounds were identified, one of them was lactam (**4**) (1.9 mg) and the other was 15-deoxypuntarenine (**7**) (4 mg).

Reduction of Dehydropuntarenine (10) with Sodium Borohydride

Dehydropuntarenine (**10**) was prepared from platinum black dehydrogenation of puntarenine (**1**) in refluxing mesitylene [5].

Sodium borohydride (20 mg) was added to a solution of dihydropuntarenine (**10**) (3 mg) in methanol (16 ml), and the mixture stirred for 3 hours. Then the solvent was removed and the residue dissolved in saturated solution of sodium sulphate (20 ml), and extracted with chloroform (4 x 20 ml). The combined chloroform layer was dried over anhydrous sodium sulphate, filtered, and the solvent was evaporated. The residue was purified by preparative TLC. Two compounds were identified, which were in fact cis- and trans-dihydropuntarenine (8, 3). By repeating the experiment with a larger amount of starting material (12 mg) the ratio of (1:9) was observed.

Hediamine (**2**); EIMS: m/z 366 (M^+ + 1,24), 365 (M^+, 100), 350 (27), 336 (25), 206 (23), 182 (8); ^{1}H-NMR ($CDCl_3$, 360 MHz): δ (ppm)= 2.85 (2H, t, J= 6 Hz, H-5), 3.91 (3H, s, CH_3O at C-11), 3.92 (3H, s, CH_3O at C-10), 5.99 (2H, s, OCH_2O at C-2,3), 6.64 (1H, s, H-4), 6.91 (1H, d, J= 8.5 Hz, H-12), 6.97 (1H, s, H-14), 7.07 (1H, d, J= 8.5 Hz, H-13), 7.15 (1H, s, H-1); IR: $\nu_{max}^{CHCl_3}$ cm^{-1}= 1595, 1659, UV: λ_{max} (MeOH)= 329 nm (log ε= 3.85). High-resolution MS, M^+, calcd. 365.1258, found 365.1275.

Lactam (**4**); EIMS: m/z 366 (M^+ + 1,23), 365 (M^+, 100), 364 (M^+-1, 11), 350 (27), 336 (11), 322 (15), 306 (9), 205 (5), 168 (7); ^{1}H-NMR ($CDCl_3$, 360 MHz): δ (ppm)= 3.10 (2H, m, H-5), 3.76 (2H, s, H-9), 3.84 (3H, s, CH_3O at C-10), 3.91 (3H, s, CH_3O at C-11), 4.03 (2H, m, H-6), 5.96 (2H, s, OCH_2O at C-2,3), 6.24 (1H, s, H-15), 6.67 (1H, s, H-4), 6.77 (1H, s, H-1), 6.89 (1H, d, J= 8.6 Hz, H-12), 7.38 (1H, d, J= 8.6 Hz, H-13); IR: $\nu_{max}^{CHCl_3}$ cm^{-1}= 1603, 1630, 1660; UV: λ_{max} (MeOH)= 221, 254, 334 nm.

Isoquinolone (**5**); EIMS: m/z 366 (M^+ + 1,23), 3.65 (M^+, 100), 3.64 (M^+-1, 9), 351 (11), 350 (42), 336 (6), 322 (22); 306 (11), 168 (7), 162 (8), 32 (18), 28 (89); ^{1}H-NMR ($CDCl_3$, 200 MHz); δ 3.24 (2H, t, J= 6.5 Hz, H-5), 3.83 (3H, s, CH_3O at C-10), 3.97 (3H, s, CH_3O at C-11), 4.53 (2H, s, H-15), 5.10 (2H, t, J= 6.5 Hz, H-6), 5.91 (2H, s, OCH_2O at C-2,3), 6.54 (1H, s, H-14), 6.71 (1H, s, H-1), 6.85 (1H, d, J= 9.5 Hz, H-12) 6.91 (1H, s, H-9), 5.57 (1H, d, J= 9.5 Hz, H-13); IR: $\nu_{max}^{CHCl_3}$ cm^{-1}= 1637; UV: λ_{max} (MeOH)= 242, 267, 319, 334, 442 nm.

Ketolactam (**6**); EIMS: m/z 380 (M^+ +1, 24), 379 (M^+, 100), 352 (12), 351 (52), 350 (14), 336 (66), 322 (27), 308 (11), 292 (15); ^{1}H-NMR (CD Cl_3, 200 MHz): δ (ppm)= 3.15 (2H, m, H-5), 3.96 (6H, s, 2xCH_3O at C-10, 11), 4.25 (2H, m, H-6), 6.00 (2H, s, OCH_2O at C-2,3), 6.58 (1H, s, H-15), 6.82 (1H, s, H-1), 7.27 (1H, d, J= 8.9 Hz, H-13), 7.63 (1H, d, J= 8.9 Hz, H-12); IR: $\nu_{max}^{CHCl_3}$ cm^{-1}= 1485, 1650, 1665, 1685, UV: λ_{max} (MeOH)= 234, 312, 352 nm.

15-Deoxypuntarenine (**7**), EIMS: m/z 367(M^+, 3), 206 (24), 205 (91), 192 (35), 191 (7), 163 (16), 162 (100), 161 (16); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ (ppm)= 3.51 (1H, d, J_{gem}= 20.9 Hz, H-9), 3.94 (1H, d, J_{gem}=20.9 Hz, H-9'), 5.95 (2H, bs, OCH_2O at C-2,3), 6.65 (1H, s, H-4), 6.70 (1H, s, H-1), 6.87 (1H, d, J= 8.5 Hz, H-13) 6.93 (1H, d, J= 8.5 Hz, H-12), IR $\nu_{max}^{CHCl_3}$ cm^{-1}= 1650.

Cis-dihydropuntarenine (**8**), EIMS: m/z 384 (M^+ + 1, 20), 383 (M^+, 87), 354, 354 (15), 220 (20), 207 (70), 206 (86), 205 (84), 193 (13), 192 (100), 191 (41), 190 (27), 178 (35), 177 (72), 176 (51), 163 (17), 149 (25), 148 (32), 147 (20), 135 (14), 119 (15), 91 (26), 77 (14); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ (ppm)= 3.33 (1H, d, J_{gem}= 20.09 Hz, H-9), 3.85 (3H, s, CH_3O at C-10), 3.90 (3H, s, CH_3O at C-11), 3.91 (1H, d, J= 20.09 Hz, H-9) 4.51 (1H, d, J= 4.59 Hz, H-14), 4.59 (1H, d, J= 4.59, Hz, H-15), 6.69 (1H, s, H-4),

6.88 (1H, d, J= 8.4 Hz, H-12), 6.91 (1H, s, H-1), 7.06 (1H, d, J= 8.4 Hz, H-13), IR: $\nu_{max}^{CHCl_3}$ cm^{-1}= 1634, 3605.

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