SINDAMINE, PUNJABINE AND GILGITINE: THREE NEW SECOBISBENZYLISOQUINOLINE ALKALOIDS

John E. Leet, S. Fazal Hussain,[1] Robert D. Minard and Maurice Shamma*

Department of Chemistry, The Pennsylvania State University,

University Park, Pennsylvania 16802, U.S.A.

Abstract: Three new secobisbenzylisoquinolines found in Berberis lycium Royle (Berberidaceae) are (+)-sindamine (3), (-)-punjabine (4) and (-)-gilgitine (5). Alkaloids 4 and 5 are the first seco dimeric alkaloids derived from in vivo oxidation of a bisbenzylisoquinoline precursor incorporating three diaryl ether bridges.

Secobisbenzylisoquinolines may be considered to be in vivo catabolic products of bisbenzylisoquinolines. So far, only two of these have been described in the literature, namely (-)-baluchistanamine (1),[2] and (-)-revolutinone (2).[3] We describe here three additional secobisbenzylisoquinolines, (+)-sindamine (3), (-)-punjabine (4) and (-)-gilgitine (5). Punjabine and gilgitine are of particular interest since they are the first seco alkaloids resulting from oxidative cleavage of a bisbenzylisoquinoline precursor possessing three diaryl ether bridges.

Berberis lycium Royle (Berberidaceae), collected in the Murree Hills of northern Pakistan, has as its two major tertiary alkaloids the known bisbenzylisoquinolines (+)-berbamine (6) and (+)-oxyacanthine (7). Careful fractionation of the minor alkaloidal constituents provided the monophenolic base (+)-sindamine (3), $C_{37}H_{38}O_8N_2$, ν max ($CHCl_3$) 1605 and 1645 cm^{-1} (lactam carbonyl) and 1695 cm^{-1} (conjugated aldehyde carbonyl). Whereas a substantial bathochromic shift and hyperchromic effect were observed in the UV spectrum of (-)-baluchistanamine (1) when measured in basic solution, the UV spectrum of sindamine (3) underwent a relatively small bathochromic shift upon addition of base (Table), suggesting that the phenolic group does not lie in a para relationship to the aldehyde function.

The 360 MHz ($CDCl_3$) NMR spectrum of sindamine has been outlined around expression 3 and clearly shows the presence of two N-methyl singlets, one of which is appreciably downfield (δ 3.04) since it represents a methyl group bonded to a non-basic, lactam nitrogen. There is also an aromatic A_2B_2 system representing four protons, which is centered at δ 7.03 and 7.81, J_o = 8.8 Hz. The large difference in chemical shifts for these protons is diagnostic of a p-aryloxybenzaldehyde system. Finally, a one proton downfield singlet at δ 9.91 represents the aldehyde proton.

The mass spectrum of sindamine (3) displays a small molecular ion peak m/z 638, while the m/z 411 base peak is due to portion a of the molecule. Portion b is represented by the m/z 227 peak (Table).

The CD spectrum (Table) with a descending tail beyond 220 nm is indicative of a benzylisoquinoline possessing the R configuration. In accord with this stereochemical assignment, the acetate ester of sindamine shows a positive specific rotation, $[\alpha]_D^{25}$ +38° (c 0.04, MeOH).[4]

Since (-)-baluchistanamine (1) is the seco dimer corresponding to the bisbenzylisoquinoline (+)-oxyacanthine (7), it was reasoned that sindamine could be derived from (+)-berbamine (6). Indeed, acetylation of berbamine with acetic anhydride in pyridine, followed by potassium permanganate in acetone oxidation gave a 25% overall yield of the aldehydo lactam acetate 3a, identical with the acetate ester of sindamine (Table).

The second seco dimer we report from the same plant is the monophenolic (-)-punjabine (4), $C_{35}H_{32}O_7N_2$, which biogenetically can be considered an oxidation product of a bisbenzylisoquinoline of the micranthine type (8). The IR spectrum of 4 shows ν max ($CHCl_3$) 1620 and 1645 cm^{-1} (lactam carbonyl) and 1690 cm^{-1} (conjugated aldehyde carbonyl). The UV spectrum undergoes a large bathochromic shift accompanied by a hyperchromic effect in basic solution (Table), reflecting a para relationship between the phenolic function and the aldehyde group.

The 200 MHz ($CDCl_3$) NMR spectrum of punjabine is described around expression 4. Salient features are the single methoxyl singlet at δ 3.87, as well as the relatively close A_2B_2 system of four protons centered at δ 6.94 and 7.18, $J_o \approx 8.5$ Hz, representing the aromatic protons of ring C to which no aldehyde group is attached.

The mass spectrum with molecular ion m/z 592 prominently displays benzylic cleavage with formation of base peak m/z 365 (portion a) and peak m/z 227 (portion b) (Table). Information on the absolute configuration of the alkaloid is conveyed by the CD spectrum, which includes a positive Cotton effect, culminating in a maximum at 222 nm, denoting the S configuration (Table).[5] This conclusion is further supported by the negative specific rotation of punjabine, $[\alpha]_D^{25}$ -40° (c 0.48, MeOH).[4]

The aldehyde group of a secobisbenzylisoquinoline may undergo in vivo reduction to the alcohol stage, or else may be oxidized to a carboxylic acid which may in turn undergo esterification. The latter sequence indeed applies in the case of our third alkaloid, (-)-gilgitine (5), $C_{36}H_{34}O_8N_2$, whose 200 MHz ($CDCl_3$) NMR spectrum (see expression 5) includes an ester methoxyl singlet at δ 3.81. The remaining features of the spectrum bear a distinct similarity to that of punjabine.

The UV spectrum of gilgitine (Table) exhibits a large bathochromic shift in base, denoting a para relationship between the phenolic function and the ester group. The IR spectrum has

ν max ($CHCl_3$) 1620 and 1645 cm^{-1} (lactam carbonyl) and 1710 cm^{-1} (conjugated ester carbonyl). The mass spectrum shows molecular ion peak m/z 622, benzylic cleavage results in base peak m/z 365 (portion a) as seen with punjabine, and m/z 257 (portion b). The specific rotation of gilgitine could not be measured accurately due to paucity of material, however the general shape of the CD curve (Table) is identical to that of (-)-punjabine (4) (Table). Both alkaloids must, therefore, possess the same absolute configuration as well as the same sign of rotation.[4]

All of the lactamic secobisbenzylisoquinoline alkaloids described thus far possess one common feature, namely that in vivo oxidation has occurred at the less sterically hindered benzylic site, i.e. at that half of the molecule in which C-8 is unsubstituted. It is interesting to note in this respect that in vitro oxidation of a bisbenzylisoquinoline using potassium permanganate in acetone also proceeds preferentially at the less hindered side of the dimer, the product being a lactamic secobisbenzylisoquinoline.[6]

TABLE: Spectral Characteristics of New Alkaloids

(+)-Sindamine (3): λ max (MeOH) 208, 259, 270, 283 nm (log ε 4.62, 4.09, 4.07, 4.04); λ max ($MeOH,OH^-$ 212, 272, 294 nm (log ε 4.86, 4.05, 4.08); ms m/z 638 (M^+) (1.2), 411 (100), 365 (24), 227 (9), 206 (13), 204 (31); CD $\Delta\varepsilon$(nm) (MeOH) 0(300), -1.7(255), 0(245), +5(232), 0(220).

(+)-Sindamine Acetate Ester (3a): $C_{39}H_{40}O_9N_2$, λ max (MeOH) 221 sh, 259, 270 nm (log ε 4.48, 4.11, 4.10); ms m/z 680 (M^+) (0.1), 411 (100), 365 (17), 269 (0.4), 227 (2.4), 206 (4), 204 (9); CD $\Delta\varepsilon$(nm) (MeOH) 0(300), -1(258), 0(245), +3(233), 0(220).

(-)-Punjabine (4): λ max (MeOH) 231, 274, 325 nm (log ε 4.81, 4.32, 3.98); λ max ($MeOH,OH^-$) 228, 253 sh, 295, 339 nm (log ε 4.78, 4.57, 4.11, 4.50); ms m/z 592 (M^+) (0.3), 365 (100), 227 (12); CD $\Delta\varepsilon$(nm) (MeOH) 0(300), -3.6(280), -12.6(247), 0(232), +10.4(222).

(-)-Gilgitine (5): λ max (MeOH) 224, 250 sh, 285, 325 (log ε 4.32, 3.96, 3.57, 3.22); λ max ($MeOH,OH^-$) 208, 252 sh, 297 nm (log ε 4.66, 3.99, 3.82); ms m/z 622 (M^+) (0.1), 621 (0.3), 365 (100), 257 (2); CD $\Delta\varepsilon$(nm) (MeOH) 0(285), -4(249), 0(232), +2(220).

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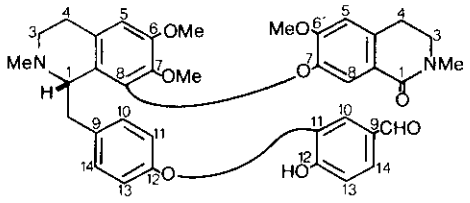

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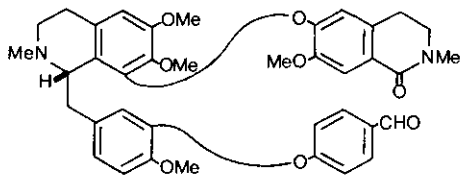

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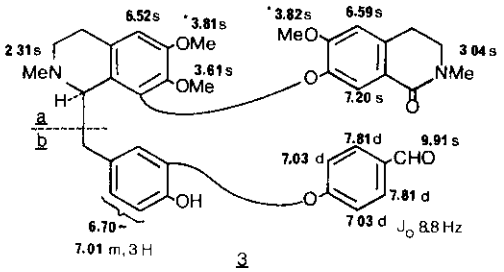

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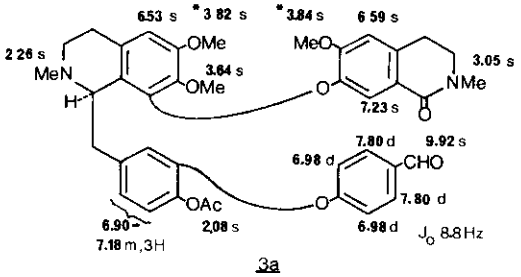

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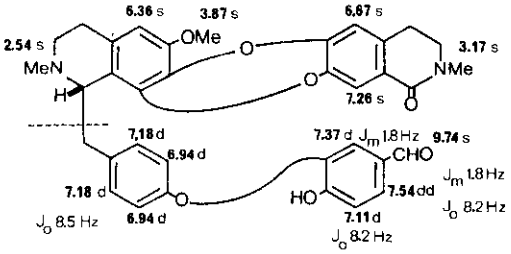

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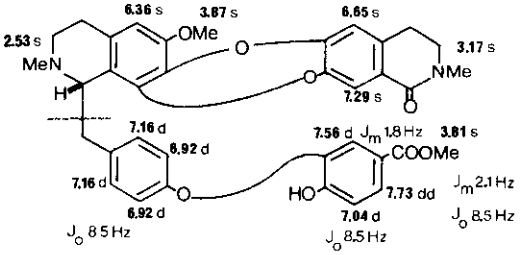

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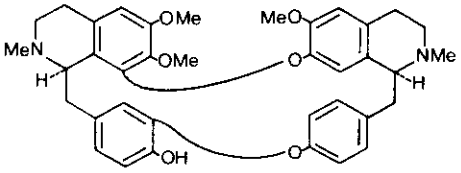

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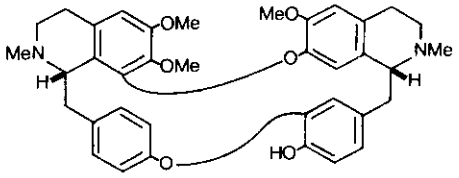

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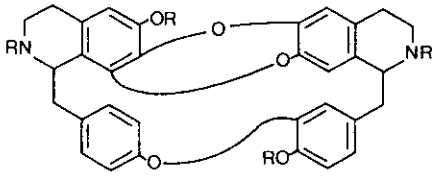

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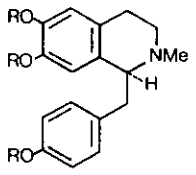

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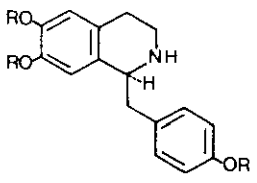

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Experimental[7]

Extraction and Purification: The dried ethanolic extract from 10 kg dried plant material (roots) was dissolved in 5% HCl and filtered to remove insoluble material. The filtrate was chilled to allow for precipitation of berberine, and then filtered again. The filtrate was extracted with chloroform. The organic phase was dried over sodium sulfate, filtered, and the solvent evaporated, leaving 55 g of residue A which was mainly non alkaloidal in nature. The acidic aqueous phase was basified to pH=8 (ammonium hydroxide), and extracted with chloroform. The organic layer was again dried and the solvent evaporated to furnish 50 g of tertiary alkaloidal extract B.

Extract B was chromatographed on a column of silica gel, and one half to one liter fractions were collected by elution first with chloroform, followed by increasing proportions of methanol in chloroform. All of the succeeding thin layer chromatography (tlc) was on Merck Silica Gel G F-254 glass plates. All compounds obtained are amorphous unless indicated otherwise.

Punjabine (4): Fractions 40-41, eluted with 3% MeOH in $CHCl_3$, were combined to give 40 mg of material. This was subjected to tlc using $CHCl_3$-MeOH (95:5) and then $CHCl_3$:DEA (85:15) to afford 8 mg punjabine.

Baluchistanamine (1) and Sindamine (3): Column fractions 61-65 (4% MeOH in $CHCl_3$) gave 188 mg. TLC was first with $CHCl_3$-MeOH (90:10) and then with $CHCl_3$-DEA (100:10), and with $CHCl_3$-MeOH (85:15). Baluchistanamine (22 mg) and sindamine (2 mg) were thus obtained.

Berbamine (6): Fractions 79-85 (5% MeOH in $CHCl_3$) were combined to give 2.5 g. A small portion of this material (150 mg) was subjected to tlc using $CHCl_3$-MeOH (85:15) and then C_6H_6-$CHCl_3$-DEA (5:4:1) to provide 47 mg berbamine, mp 155-155.5° C (EtOH).

Gilgitine (5) and Oxyacanthine (7): Fractions 103-117 (7% MeOH in $CHCl_3$) were amalgamated to supply 2.7 g. This material was loaded on a small Silica Gel F-254 column using C_6H_6-CH_3COCH_3-NH_4OH (20:20:0.5). Fractions measuring 30 mL were collected. Fractions 16-21 were combined and further purified by tlc using $CHCl_3$-DEA (90:10) to afford 3 mg gilgitine. Fractions 51-60 were combined and subjected to tlc first using $CHCl_3$-DEA (90:10), and then $CHCl_3$-MeOH-NH_4OH (90:10:1) to give 7 mg of oxyacanthine.

Acetylation of Berbamine (6): Pyridine (0.5 mL), acetic anhydride (1.0 mL) and berbamine (26 mg) were mixed and the solution allowed to stand overnight. Work-up afforded a nearly quantitative yield of berbamine acetate ester.

Potassium Permanganate Oxidation: A 27 mg sample of berbamine acetate ester was dissolved in 30 mL acetone, and a solution of potassium permanganate (30 mg) in acetone (20 mL) was added dropwise over 0.5 h with stirring. After 4 h, the mixture was filtered, the solvent evaporated, and the residue purified by tlc using $CHCl_3$-MeOH (80:20). Collection of the highest R_f band afforded

7 mg secoberbamine acetate aldehydo lactam, identical with sindamine acetate ester (3a).

TLC R_f Values of New Alkaloids: Using the system CH_3CN-C_6H_6-$CH_3COOCH_2CH_3$-CH_3OH-NH_4OH (40:30:20:5:5 v/v): alkaloid 3 0.55, derivative 3a 0.71, alkaloid 4 0.16, alkaloid 5 0.34.

References and Footnotes

1. Permanent address: PCSIR Laboratories, Peshawar, Pakistan.
2. M. Shamma, J.E. Foy and G.A. Miana, J. Am. Chem. Soc., 96, 7809 (1974).
3. J. Wu, J.L. Beal and R.W. Doskotch, J. Nat. Prod., 43, 270 (1980).
4. The paramount factor controlling the sign of rotation of most alkaloids incorporating a tetrahydrobenzylisoquinoline skeleton is the location of the lower pendant aromatic ring. For example, species 9 which belongs to the S configuration, and which has its lower ring preferentially anti to the nitrogen atom, will be dextrorotatory. However, nor compound 10 where the lower ring lies syn to the nitrogen will be levorotatory, even though it possesses the identical absolute configuration. A similar situation also applies to the aporphines and the tetrahydroprotoberberines since an aporphine of the S configuration will be dextrorotatory, while the corresponding tetrahydroprotoberberine will be levorotatory. This generalization can even be extended to the secobisbenzylisoquinolines. In alkaloids 1-5, the lower aromatic ring will lie syn to the basic nitrogen due to substitution at C-8. It follows that species 1, 2, 4, and 5, which partake of the S configuration are levorotatory, while 3 (or its acetate ester) is dextrorotatory since it possesses the R configuration. Further applications of this rule to other new secobisbenzylisoquinoline alkaloids will be presented in subsequent papers.
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6. M. Shamma and J.E. Foy, Tetrahedron Lett., 2249 (1975).
7. In diagrams 3 and 3a above, chemical shifts with identical superscripts are interchangeable.

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