Bisjatrorrhizine, a New Dimeric Protoberberine Alkaloid from *Jatrorrhiza palmata* [Lam.] Miers

By **M. L. Carvalhas,** Department of Pharmacology, Biological Centre, Calouste Gulbenkian Foundation, Oeiras, Portugal

A new quaternary protoberberine alkaloid, bisjatrorrhizine, has been isolated from *Jatrorrhiza palmata*. A structural assignment based on the analysis of the n.m.r. spectra of the original alkaloid and its methylation products, and on the mass spectral fragmentation pattern of the hydrogenation products, was confirmed by a partial synthesis of the alkaloid by catalytic oxidative coupling of jatrorrhizine chloride.

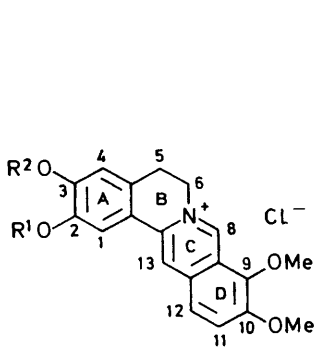
In a previous study of the alkaloids of the root of *Jatrorrhiza palmata*,[1] we have noted the existence of another alkaloid besides palmatine (1), columbamine (2), and jatrorrhizine (3).[2] We now report the isolation, identification, and synthesis of this compound.

The alkaloid can be detected in the methanolic extract of the root by t.l.c. and corresponds to 1·5% of the alkaloidal content. It is present in the mother liquors of jatrorrhizine and columbamine chlorides and can be separated by partition chromatography [3] and adsorption chromatography.[4] Also, in the iodide form, it can be separated from the other alkaloids, as a precipitate from their solution in methanol–acetone (1 : 1). Bisjatrorrhizine chloride $C_{40}H_{38}Cl_2N_2O_8,2H_2O$ was obtained from methanol as yellow-orange crystals, darkening above 270°.

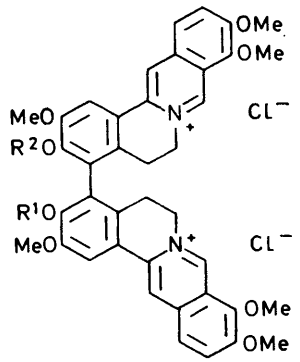
The u.v. and visible spectra are characteristic of a quaternary protoberberine alkaloid, and phenolic groups are indicated by the change to a deep red colour, as well as a bathochromic shift in the u.v., upon addition of base.[2,5] The material is highly sensitive to solvent

[1] M. L. Carvalhas and A. S. Graça, *J. Soc. Cien. Med. Lisboa*, 1968, **132**, 211.
[2] M. P. Cava, T. A. Reed, and J. L. Beal, *Lloydia*, 1965, **28**, 73.
[3] M. Shamma and B. S. Dudock, *J. Pharm. Sci.*, 1968, **57**, 262.
[4] B. J. Hunt and W. Ribgy, *Chem. and Ind.*, 1967, 1868.
[5] A. I. Scott, 'Interpretation of the Ultraviolet Spectra of Natural Compounds, Pergamon, Oxford, 1964, p. 95.

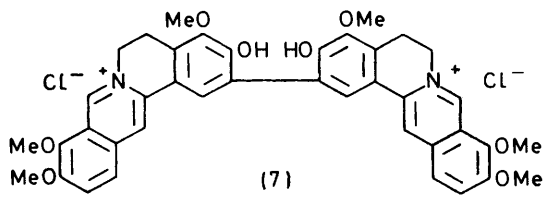
effects, much more than other G berberines studied.[6-8] The i.r. spectrum supported the presence of hydroxy-groups ($\nu_{max.}$ 3600—3400 cm^{-1}). The n.m.r. spectrum showed signals for the aromatic protons of rings C (δ 9·68 and 8·60) and D (δ 8·11 p.p.m.). Only one signal from the aromatic protons of ring A was observed. Two multiplets (δ 3·15 and 4·95) corresponded to the aliphatic protons of ring B. When bisjatrorrhizine was heated with deuteriochloric acid the n.m.r. spectrum showed no exchange in the aromatic region. Under the same conditions the C-1 proton in columbamine chloride (2) is exchanged. As exchange occurs only *ortho* and/or *para* to a phenolic hydroxy-group,[9] and as comparison of the n.m.r. spectrum of bisjatrorrhizine with those of other protoberberine alkaloids [10,11] indicates the absence of a C-4 proton, the alkaloid must be dimeric and have a hydroxy-group at C-3. Similarly the higher field signal of the $ArCH_2$·C system (δ 3·15) [10,11] is in agreement with a substituent at C-4.



(1) $R^1 = R^2 = Me$
(2) $R^1 = H, R^2 = Me$
(3) $R^1 = Me, R^2 = H$



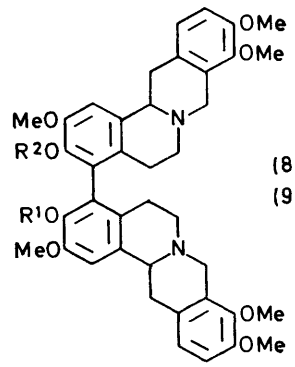
(4) $R^1 = R^2 = H$
(5) $R^1 = H, R^2 = Me$
(6) $R^1 = R^2 = Me$



(7)

In order to confirm this suggestion, methylation of the phenolic groups with dimethyl sulphate [12,13] was attempted. After isolation *via* column chromatography we obtained a fully methylated compound (6) and only one monophenolic compound (5). The u.v. and visible spectra of these compounds confirmed their protoberberine character. In the n.m.r. spectra, the aromatic region and (particularly) the number of methoxy-groups provided strong evidence of a dimeric structure.

It was not possible to obtain the mass spectrum of the original quaternary alkaloid owing to its involatility and thermal instability. However, hydrogenation [14] gave two isomeric products (8) with identical u.v., i.r., and n.m.r. spectra. The u.v. spectrum was as expected for a tetrahydroprotoberberine alkaloid and the i.r. showed a strong hydroxy-absorption (3600—3400 cm^{-1}) and Bohlmann bands (2800—2700 cm^{-1}). The n.m.r. spectrum ($CDCl_3$) (aromatic region) showed two protons *ortho* to each other. Another AB system near δ 4·0 p.p.m. was obscured by the methoxy-signal and is characteristic of tetrahydroprotoberberines with 9,10-substitution.[15]



(8) $R^1 = R^2 = H$
(9) $R^1 = Me, R^2 = H$

The mass spectra of the two hydrogenation products exhibited the same molecular ions and the following major fragments:

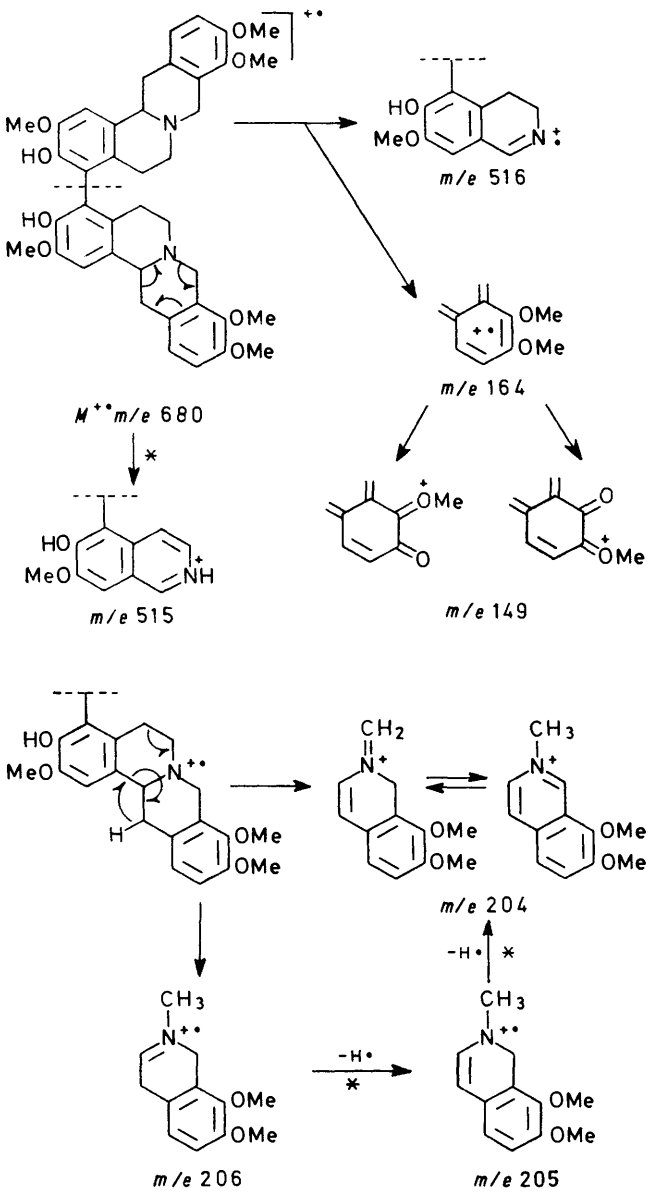
Fragment	*m/e*	680	516	515	390 *	206
Relative intensity (%)	(R_F 0·34)	86	23	68		100
	(R_F 0·24)	100	39	98		37
Fragment	*m/e*	205	204	164	149	
Relative intensity (%)	(R_F 0·34)	56	73	75	63·5	
	(R_F 0·24)	3	14	67·5	59	

The molecular weights are as expected for hydrogenated compounds derived from structure (4) or (7), and comparison with the mass spectra of tetrahydroprotoberberine alkaloids [15] indicates 9,10-dimethoxy-substitution since the ion *m/e* 149, characteristic of this substitution pattern, is very intense. The presence of a biphenyl linkage is consistent with the high relative intensities of the molecular ions and also with production of fragment ions of *m/e* 515 by a retro-Diels–Alder reaction. The differences in relative intensities of the ions *m/e* 206, 205, and 204 are probably due to differences in the stereochemistry of these alkaloids at C-13a. The suggested fragmentation pattern is shown in the Scheme.

Similarly, the mass spectrum of the hydrogenated

[6] Z. Gasparec and K. Weber, *Croat. Chem. Acta*, 1967, **39**, 175.
[7] E. Sebe, S. Abe, N. Murase, and Y. Shibata, *J. Chinese Chem. Soc.*, 1968, 135.
[8] M. Shamma, M. J. Hillman, and D. C. Jones, *Chem. Comm.*, 1969, **69**, 779.
[9] C. Y. Chen, D. B. MacLean, and R. H. F. Manske, *Tetrahedron Letters*, 1968, 349.
[10] K. Jewers and A. Manchanda, personal communication.
[11] V. Preiningher and L. Hruban, *Coll. Czech. Chem. Comm.*, 1970, **35**, 124.
[12] M. P. Cava and A. T. Reed, *J. Org., Chem.*, 1967, **32**, 1640.
[13] R. W. Doskotch, M. Y. Malik, and J. L. Beal, *J. Org. Chem.*, 1967, **32**, 3253.
[14] R. H. F. Manske, ' The Alkaloids,' vol. 9, Academic Press, New York, 1967, p. 54.
[15] C. Y. Chen and D. B. MacLean, *Canad. J. Chem.*, 1968, **46**, 2501.

compound from the partially methylated derivative agrees with the proposed structure (9), showing ions at *m/e* 694 ($M^{+\cdot}$ 100%), 529 (63), 164 (20), and 149 (37).



SCHEME

Thus, on the basis of the n.m.r. and mass spectra this new protoberberine alkaloid has either the dimeric structure (4) or (7), the latter being unlikely on biogenetic grounds. Structure (4) was confirmed by synthesis, involving *ortho* oxidative coupling [16] of the phenolic group of jatrorrhizine (3).

EXPERIMENTAL

M.p.s were taken with a Leitz 350 apparatus. I.r. spectra were obtained with Perkin-Elmer 257 and 225 spectrometers and u.v. spectra with a Bausch–Lomb 605 recording spectrophotometer. N.m.r. spectra were determined with a Varian HA 100 instrument and a Jeolco 60 MHz spectrometer; 12N-deuteriochloric acid was used for investigating proton exchange. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Mass spectra were obtained with an A.E.I. MS9 instrument by direct insertion. T.l.c. was carried out on silica gel G (Merck) plates developed with ethanol–water–ammonia (30 : 15 : 5; sandwich-chamber system [17]) (system A) or chloroform–methanol (96 : 4 v/v) (system B). Micro-analysis were performed by M. Cameron, Chemistry Department, University of Glasgow.

Preliminary Separation of the Alkaloids.—Dried, pulverised Colombo roots (25 kg), from Mozambique, were extracted with methanol by multiple contact (24 h). The dark brown extract was treated as described in ref. 1 to obtain a crude mixture of alkaloid iodides (221·3 g). From these (40 g) were obtained the chlorides (33·5 g) [by ion-exchange (Amberlite IRA-410)], which were chromatographed over acid alumina (Woelm, grade I) with chloroform and chloroform–methanol as eluants,[1] to give palmatine (1), columbamine (2), and jatrorrhizine (3). Bisjatrorrhizine (4) was present in the mother liquors of compounds (2) and (3).

Isolation of Bisjatrorrhizine (4).—(*a*) A column of cellulose powder (Whatman CF11) (100 g) saturated with water–methyl ethyl ketone was equilibrated with the same system. Alkaloid chlorides (1 g) in the stationary phase (2 ml) were added to dry cellulose powder and chromatographed. Fractions 7—15 (each 150 ml) contained compounds (1), (2), and (3); fractions 25—37 were shown by t.l.c. (system A; R_F 0·32) to contain bisjatrorrhizine. Evaporation left material (52 mg) which was recrystallised from methanol (yield 15 mg).

(*b*) The mother liquors of compounds (2) and (3) yielded material (2·9 g) which was chromatographed on silica gel (Whatman SG 31) (150 g). Elution with ethanol–water–ammonia (30 : 19 : 1) afforded compounds (2) and (3); changing the solvent composition to 30 : 18 : 2 gave the alkaloid (4). Evaporation of the solvent left a dark red residue (40 mg), which was dissolved in methanol and dilute hydrochloric acid. Crystallisation from methanol gave yellow-orange crystals (25 mg).

(*c*) From the crude alkaloid iodides (170 g) in methanol–acetone (1 : 1), impure bisjatrorrhizine iodide (3 g) was slowly precipitated. This was converted by ion-exchange into the chloride, and repeated recrystallisation from methanol–ether yielded *crystals* (850 mg), darkening above 270°, $\lambda_{max.}$ (H_2O) 420 (log ε 5·08), 345 (5·76), 263 (5·73), 228 (5·72), and 275sh nm (5·72), $\lambda_{max.}$ (0·01N-$NaHCO_3$–H_2O) 378 (5·66), 353 (5·66), 255 (5·74), and 230sh nm (5·67), $\lambda_{max.}$ (0·01N-NaOH–H_2O) 490 (4·99), 385 (5·86), and 247 nm (5·80), $\nu_{max.}$ (KBr) 3600—3400, 1605, 1500, 1360, 1335, and 1280 cm^{-1}, δ ($CF_3{\cdot}CO_2H$; Me_4Si standard) 9·68 (2H, s), 8·60 (2H, s), 8·11 (4H, s), 7·80 (2H, s), 4·32 (6H, s, 2MeO), 4·19 (12H, s, 4MeO), 3·15 (4H, m), and 4·95 p.p.m (4H, m). (Found: C, 61·35; H, 5·2; N, 3·3. $C_{40}H_{38}Cl_2N_2O_8.2H_2O$ requires C, 61·45; H, 5·4; N, 3·6%).*

Hydrogenation of Bisjatrorrhizine (4).—Sodium borohydride (15 mg) was added to a solution of bisjatrorrhizine chloride (116 mg) in methanol. Immediately the solution

* This alkaloid presumably has optical activity, but no precautions were taken during recrystallisation, and rotation values would not therefore be meaningful.

[16] J. M. Bobbit, K. H. Weisgraber, A. S. Steinfeld, and S. G. Weiss, *J. Org. Chem.*, 1970, **35**, 2884.
[17] E. Stahl, 'Thin-Layer Chromatography,' Springer-Verlag Berlin, 1970, p. 70.

became colourless. After 15 min it was diluted with water and extracted with ether (3 × 50 ml). Removal of the solvent left a white residue which was dissolved in 3% acetic acid (15 ml), basified with ammonia, and extracted with ether. The extract was evaporated to leave a residue (106 mg) which gave two spots on t.l.c. (system B; R_F 0·34 and 0·24) and was chromatographed on silica gel (Merck, 0·08 mm) (20 g). Elution with chloroform–methanol (99 : 1 v/v) gave a tetrahydro-derivative (8) (R_F 0·34) (47 mg), crystals, m.p. 180—185° (decomp.) (from methanol–ether), $\lambda_{max.}$ (EtOH) 283 (log ε 4·95) and 227sh nm (5·66), $\nu_{max.}$ (KBr) 3520—3420, 2940, 2840, 2750 (Bohlmann band), 1495, 1280, and 1085 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 3540, 2940, 2750, AB, J 9 Hz), 6·84 (2H, s), 5·45 (2H, exchanged by D_2O), 3·84 (4H, AB, J 16 Hz), 3·95 (6H, s, 2MeO), 3·85 p.p.m. (12H, s, 3MeO $[\alpha]_D$ 0° ($CHCl_3$). Elution with chloroform–methanol (98 : 2 v/v) afforded the isomer of R_F 0·24 (29 mg) as an amorphous powder, with the same spectroscopic data.

Methylation of Bisjatrorrhizine (4).—Dimethyl sulphate (570 μl) was added to bisjatrorrhizine chloride (227 mg) in water (227 ml) containing N-sodium hydroxide (6 ml) and the mixture was stirred at 40° for 48 h. Additional dimethyl sulphate and sodium hydroxide were added to ensure an excess of reagents. After precipitation with potassium iodide the alkaloids were converted into chlorides (216 mg). T.l.c. (system A) showed the presence of a mixture of the starting material and (40%) two products (5) (R_F 0·47) and (6) (R_F 0·20). This was chromatographed on acid alumina (23 g) with chloroform and chloroform–methanol as eluants. Elution with a 98 : 2 (v/v) system gave bispalmatine (6) as yellow needles (11 mg), darkening above 240° (decomp.) (from methanol–ether), $\lambda_{max.}$ (H_2O) 410 (log ε 4·96), 341 (5·70), 273 (5·75), and 225 nm (5·71), $\nu_{max.}$ 3420—3440, 1605, 1500, 1370, 1355, and 1270 cm^{-1}, δ (CF_3·CO_2H) 9·60 (2H, s), 8·65 (2H, s), 8·04 (4H, s), 782 (2H, s), 4·21 (6H, s, 2MeO), 4·10 (12H, s, 4MeO), 3·86 (6H, s, 2MeO), 4·80 (4H, m), and 2·98 p.p.m. (4H, m). Elution with chloroform–methanol (96 : 4 v/v) gave the monophenol (5), brown crystals, darkening above 200° (decomp.) (from methanol–ether), $\lambda_{max.}$ (H_2O) 414 (log ε 4·97), 343 (5·70), 274 (5·74), and 227 nm (5·69), $\lambda_{max.}$ (0·01N-$NaHCO_3$–H_2O) 375 (5·54), 340 (5·59), and 265 nm (5·68), $\lambda_{max.}$ (0·01N-NaOH–H_2O) 378 (5·58), 344 (5·58), and 265 nm (5·68), $\nu_{max.}$ 3400—3440, 1610, 1505, 1370, 1340, 1270, and 1115 cm^{-1}, δ (CF_3·CO_2H) 9·66 (1H, s), 9·585 (1H, s), 8·66 (1H, s), 8·60 (1H, s), 7·96 (2H, s), 7·94 (2H, s), 7·83 (1H, s), 7·75 (1H, s), 4·30 (6H, s, 2MeO), 4·17 (12H, s, 4MeO), 3·88 (3H, s, MeO), 4·90 (4H, m), and 3·05 p.p.m. (4H, m).

Hydrogenation of the Monophenol (5).—As described for compound (4), the partially methylated alkaloid (5) (10 mg) was hydrogenated prior to mass spectrometric analysis.

Synthesis of Bisjatrorrhizine (4).—Platinum oxide (155 mg) was hydrogenated at room temperature in ethanol. The platinum black obtained was added to 0·3M-sodium hydrogen carbonate (146 ml) and oxygenated for 15 min. Jatrorrhizine chloride (1 g) dissolved in water was added to the solution and oxygenated for 8 h. After filtration and evaporation, inorganic salt was precipitated with methanol and filtered off. After acidification with hydrochloric acid, the alkaloids were precipitated with methanolic potassium iodide. After centrifugation the precipitate was extracted with methanol and acetone to remove unchanged jatrorrhizine (3). The alkaloid chloride (435 mg) was obtained by ion-exchange; recrystallisation yielded a compound spectroscopically identical with the natural alkaloid.

The technical assistance of Mrs. M. A. Figueira is gratefully acknowledged. We thank Professor A. Ralha for his advice and criticism, Dr. R. J. P. Williams and Mr. A. V. Xavier (Inorganic Chemistry Laboratory, Oxford University) for the use of their 60 MHz n.m.r. spectrometer, Dr. R. T. Aplin (Dyson Perrins Laboratory, Oxford University) for the determination and interpretation of the mass spectra, and Dr. A. Lobo for obtaining the 100 MHz n.m.r. spectra at Imperial College (London).

[1/915 *Received*, 4*th June*, 1971]