Pakistanine and Pakistanamine, Two New Dimeric Isoquinoline Alkaloids[1]

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Abstract: *Berberis baluchistanica* Ahrendt (Berberidaceae) has yielded two novel dimeric alkaloids, pakistanine (**1**) and pakistanamine (**9**). Pakistanine is a new type of dimeric aporphine benzylisoquinoline while pakistanamine is the first known proaporphine benzylisoquinoline. Dienone–phenol rearrangement of pakistanamine followed by O-methylation yielded 1,10-di-*O*-methylpakistanine (**2**), which was also obtained by diazomethane O-methylation of pakistanine. The biogenetic sequence in the pakistanine–pakistanamine series must be benzylisoquinoline → bisbenzylisoquinoline → proaporphine benzylisoquinoline dimer → aporphine benzylisoquinoline dimer.

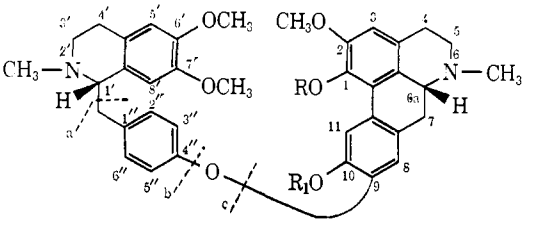
Members of the family Berberidaceae have been known to produce isoquinoline alkaloids of the benzylisoquinoline, bisbenzylisoquinoline, aporphine, protoberberine, protopine, phthalideisoquinoline, and taspine types,[3] and it was, therefore, of interest to study the alkaloids of *Berberis baluchistanica* Ahrendt, a plant native to Pakistan and which had heretofore not been investigated for its alkaloidal constituents. The roots of the plant were found to yield two new alkaloids, (+)-pakistanine and (+)-pakistanamine.

Structural Elucidation of Pakistanine. The ir spectrum of pakistanine (**1**), $C_{37}H_{40}N_2O_6$, showed *N*-methyl absorbance at 2800 cm^{-1} (3.57 μ), *O*-methyl absorbance at 2860 cm^{-1} (3.55 μ), as well as an intense OH stretching band at 3450 cm^{-1} (2.83 μ). The uv spectrum exhibited λ_{max}^{EtOH} 206 nm, 218, 270 sh, 277, and 307 (log ϵ 4.69, 4.61, 4.13, 4.21, and 4.07); and a marked bathochromic shift upon addition of base indicated the presence of at least one phenolic group.

The mass spectral fragmentation pattern of pakistanine (**1**) was characteristic of an aporphine benzylisoquinoline dimer[4] with a molecular ion peak *m/e* 608, and a base peak *m/e* 206, which corresponded to fragment a, *i.e.*, to the A and B rings of the benzylisoquinoline moiety. The other intense fragments, *m/e* 402 (M − a), 312 (M − b), 296 (M − c), and 107 (c − a), suggested that the molecule was bonded at the lower portion of the benzylisoquinoline residue through a diphenyl ether linkage to the aporphine system (Scheme I).

The nmr spectrum contained two *N*-methyl singlets at δ 2.51 and 2.56, three methoxyl singlets at δ 3.61, 3.83, and 3.88, four one proton aromatic singlets at δ 6.06, 6.54, 6.56, and 6.71, a four proton A_2B_2 quartet centered at δ 7.00 (J = 8.5 Hz; ics = 10 Hz), and a one proton aromatic singlet at δ 8.11. The upfield aromatic proton at δ 6.06 was in accord with the highly shielded C-8′ proton of the benzylisoquinoline moiety,[5] and the occurrence of a low field proton at δ 8.11 indicated

Scheme I. Mass Spectral Fragmentation of Pakistanine (**1**)



1, R = R_1 = H
2, R = R_1 = CH_3
3, R = CH_3; R_1 = H

that the C-11 position of the aporphine system was unsubstituted.[4] The four protons which gave the distinct A_2B_2 quartet could only be the two ortho-pair protons (C-2′′, 3′′, 5′′, and 6′′) of the benzyl system.[6]

Diazomethane O-methylation of pakistanine (**1**) afforded (+)-1,10-di-*O*-methylpakistanine (**2**), $C_{39}H_{44}N_2O_6$. Comparison of the mass spectrum of **1** with that of **2**, *m/e* 636 (M^+), 430 (M − a), 340 (M − b), 324 (M − c), and 206 (a, base), indicated that pakistanine (**1**) consisted of an armepavine-like unit bonded through a diphenyl ether linkage to a diphenolic aporphine.

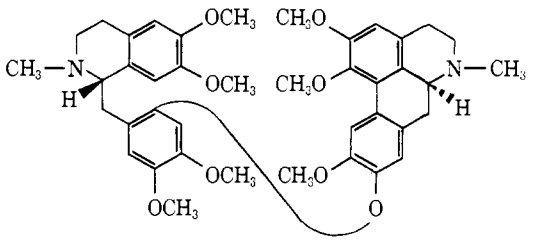
The nmr spectrum of 1,10-di-*O*-methylpakistanine contained two additional methoxyl resonances not present in the starting alkaloid, one at δ 3.72 and the other at 3.90, the former chemical shift being characteristic of a C-1 methoxyl in an aporphine system.[7]

Since the phloroglucinol test for a catechol system[8] was negative for pakistanine, and since aporphines are inevitably substituted at C-1 and C-2, a methoxyl group could be assigned to the C-2 position. Considering the data obtained so far, the structural features of pakistanine still to be determined were (a) the location of the remaining phenolic function in the aporphine moiety, (b) the terminus of the diphenyl ether linkage at the aporphine, and (c) the absolute configurations at the C-1′ and C-6a centers.

The aporphine benzylisoquinoline alkaloids belong-

(1) For a preliminary communication, see M. Shamma, J. L. Moniot, S. Y. Yao, G. A. Miana, and M. Ikram, *J. Amer. Chem. Soc.*, **94**, 1381 (1972).
(2) (a) The Pennsylvania State University; (b) Peshawar University.
(3) M. Shamma, "The Isoquinoline Alkaloids," Academic Press, New York, N. Y., 1972, p 521.
(4) Reference 3, p 232.
(5) M. Tomita, T. Shingu, K. Fujitani, and H. Furukawa, *Chem. Pharm. Bull.*, **13**, 921 (1965).
(6) V. Deulofeu, J. Comin, and M. J. Vernengo in "The Alkaloids," Vol. X, R. H. F. Manske, Ed., Academic Press, New York, N. Y., 1968, p 418.
(7) W. H. Baarchers, R. R. Arndt, K. Pachlers, J. A. Weisbach, and B. Douglas, *J. Chem. Soc.*, 4778 (1967).
(8) H. A. Schroeder, *J. Chromatogr.*, **30**, 537 (1967).

ing to the thalicarpine (**4**) series exhibit uv maxima near 282 nm.[4] This pattern is essentially that predicted for a 1,2,9,10-tetrasubstituted aporphine.[9] However, the uv spectrum of pakistanine (**1**) exhibited absorption maxima at 218, 270 sh, 277, and 307 nm, closely resembling the pattern of a 1,2,10-trisubstituted aporphine.[9] The lack of a meta splitting for the aromatic protons in pakistanine (**1**) precluded the possibility of a 1,2,8,10-tetrasubstituted aporphine moiety. The apparent delinquency in the uv spectrum of pakistanine can be rationalized on the ground that the *oxygen of the diphenyl ether will resonate with the less hindered adjoining aromatic chromophore*, with hindrance being caused primarily by substituents ortho to this oxygen. This thesis was supported by the close similarity of the uv spectrum of pakistanine (**1**) to that of 9-phenylboldine (**5**), λ_{max}^{EtOH} 218 nm, 270 sh, 275, and 303 (log ϵ 4.30, 4.23, 4.25, and 3.82), which also incorporates a 1,2,9,10-tetrasubstituted aporphine system. In compounds **1** and **5**, however, the aporphine ring D is the more hindered side of the diphenyl ether, so that the pattern observed is that of a 1,2,10-substituted aporphine.[9]



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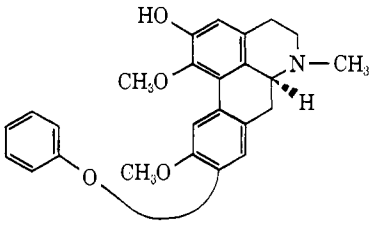

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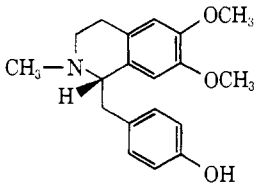
Upon addition of base, the uv spectrum of pakistanine (**1**) showed a bathochromic shift to λ_{max}^{EtOH} 230 nm, 315 sh, and 335. The absence of a hyperchromic effect between 315 and 330 nm indicated that the aporphine C-9 site was not occupied by a phenolic function, so that the remaining phenol could be at C-10, with C-9 being the terminus of the diphenyl ether linkage.[10]

The ORD curve for pakistanine consisted of one positive and one negative Cotton effect between 240 and 600 nm, $[\alpha]_{315}$ +2736° (pk) and $[\alpha]_{252}$ −11208° (tr), which was essentially the reverse of that for (+)-thalicarpine (**4**), $[\alpha]_{310}$ −441° (tr), $[\alpha]_{286}$ +831° (pk), $[\alpha]_{280}$ −1186° (tr), and $[\alpha]_{250}$ +7966°. This suggested that the stereochemistry for at least one portion of the pakistanine molecule was the reverse of that for thalicarpine (**4**).

In order to establish firmly the stereochemistry at the two asymmetric centers of pakistanine, the limited supply of the alkaloid was O-methylated, and the resulting 1,10-di-*O*-methylpakistanine (**2**) was subjected to sodium in liquid ammonia cleavage. The phenolic product was indistinguishable in terms of melting point, tlc R_f values, uv and mass spectra, and ORD curve from an authentic sample of L-(+)-armepavine (**6**). The nonphenolic product was characterized as D-(−)-2,10-dimethoxyaporphine (**7**), being the enantiomer of L-(+)-2,10-dimethoxyaporphine derived from the lithium in liquid ammonia cleavage of L-(+)-glaucine (**8**). The absolute configuration for (−)-pakistanine is thus firmly established to be as indicated in expression **1**.



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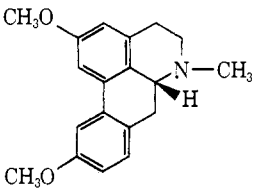

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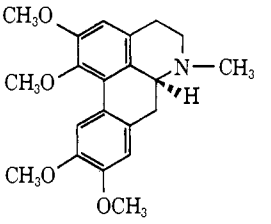

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It is clear that the oxygen of the diphenyl ether linkage of pakistanine (**1**) originates biogenetically from the armepavine side of the molecule. The absolute configuration for the aporphine moiety of pakistanine is, therefore, in agreement with the observation that ring D monooxygenated aporphines occurring outside the Papaveraceae family are usually levorotatory and possess the D-(−) configuration.[11] In fact, pakistanine (**1**) is not only an aporphine benzylisoquinoline alkaloid with a new oxygenation pattern and a different stereochemistry from those previously described in the literature; but its isolation from *B. baluchistanica* represents the first report of the presence of an aporphine benzylisoquinoline dimer outside the Ranunculaceae and Hernandiaceae families.

Table I summarizes the nmr resonances of pakistanine and its derivatives.

Structural Elucidation of Pakistanamine (9), the First Known Proaporphine Benzylisoquinoline Dimer. The new alkaloid pakistanamine (**9**) was obtained from *B. baluchistanica* and isolated as the picrate salt. Ion exchange provided the crystalline hydrochloride salt, $[\alpha]_D^{25}$ +20° (MeOH). Upon conversion to the free base, $C_{38}H_{42}N_2O_6$, the amorphous pakistanamine readily darkened to a deep purple color.

The ir spectrum of pakistanamine contained absorbance at 1640 cm^{-1} (6.10 μ) and 1670 cm^{-1} (6.00 μ) suggestive of the presence of a dienone moiety.[12] The uv spectrum of the free base exhibited λ_{max}^{EtOH} 206 nm, 225 sh, 280, and 310 sh (log ϵ 4.86, 4.63, 4.12, and 3.61), with a shift to λ_{max}^{EtOH} 230 sh nm 282, 310 sh, and 340 sh upon addition of base.

The nmr spectrum of pakistanamine (**9**) contained signals attributable to the cyclohexadienone ring protons at δ 6.16 (1 H, d, $J_{8,12}$ = 2.5 Hz) for the C-8 H, at

(9) M. Shamma in "The Alkaloids," Vol. IX, R. H. F. Manske, Ed., Academic Press, New York, N. Y., 1967, p 1.

(10) M. Shamma, S. Y. Yao, B. R. Pai, and R. Charubala, *J. Org. Chem.*, **36**, 3253 (1971).

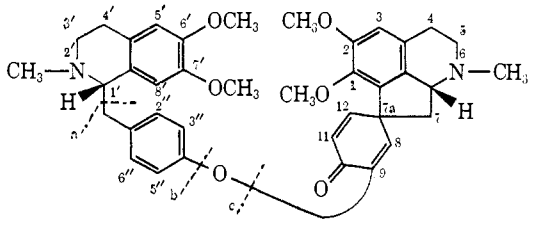
(11) Reference 3, p 194.

(12) Reference 3, p 175.

Table I. Nmr Resonances of Pakistanine, Pakistanamine, and Related Bases[a]

Compd	Methylamino N-2′	Methylamino N-6	Methoxyl C-1	Methoxyl C-2[b]	Methoxyl C-6′[b]	Methoxyl C-7′	Aromatic Proton C-8′	Aromatic Proton C-3[c]	Aromatic Proton C-5′[c]	Aromatic Proton C-8	Aromatic Proton C-2″3″5″6″	C-11
Pakistanine (1)	2.51	2.56		3.83	3.88	3.61	6.06	6.54	6.56	6.71	7.00 q (8.5) [10]	8.11
1-*O*-Methylpakistanine (3)	2.51	2.56	3.73	3.84	3.88	3.63	6.10	6.57	6.61	6.71	7.02 q (10) [11]	8.10
1,10-Di-*O*-methylpakistanine[d] (2)	2.51	2.57	3.72	3.84	3.88	3.63	6.10	6.57	6.63	6.80	7.00 q (9.5) [11]	8.20
1-*O*-Methyl-10-*O*-acetylpakistanine	2.50	2.53	3.71	3.85	3.89	3.67	6.19	6.58	6.64	6.82	7.04 q (8.5) [11]	8.21
1,10-Di-*O*-acetylpakistanine	2.55	2.76		3.85	3.85	3.59	5.99	6.51	6.56	6.80	7.06 q (9) [10]	7.73
1-*O*-Methyl-10-deoxypakistanine[e] (12)	2.53	2.55	3.69	3.84	3.89	3.65	6.04	6.58	6.62	6.87 d (2.5)	7.04 q (10) [12]	8.30 d
Pakistanamine[f] (9)	2.52	2.35	3.64	3.80	3.82	3.57	6.07	6.55	6.62	6.16 d (2.5)	7.02	6.32 d (10)
11,12-Dihydropakistanamine (10)	2.51	2.37	3.73	3.81	3.83	3.56	6.06	6.55	6.59	6.12	6.68 q (8.5) [10]	

[a] Spectra were determined on a Varian A-60A in $CDCl_3$ solution; values given in δ units relative to tetramethylsilane as internal standard; multiplicity of signals is designated as follows: d, doublet; dd, doublet of doublets; q, quartet. Numbers in parentheses denote coupling constants in hertz; numbers in brackets indicate internal chemical shifts in hertz. [b] The values are interchangeable for these methoxyl signals. [c] The values are interchangeable for these aromatic protons. [d] C-10 OMe at δ 3.90. [e] C-10 at δ 6.84 (dd) (2.5 and 10 Hz). [f] C-12 at δ 7.06 (dd) (2.5 and 10 Hz).



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δ 6.32 (1 H, d, $J_{11,12}$ = 10 Hz) for the C-11 H, and at δ 7.06 (1 H, dd, $J_{8,12}$ and $J_{11,12}$ = 2.5 and 10 Hz, respectively) for the C-12 H.[12] The relative positions for each of the assigned protons were confirmed by decoupling experiments. When the C-8 proton was irradiated, the absorption caused by the C-12 proton collapsed to a doublet ($J_{11,12}$ = 10 Hz) while the C-11 proton resonance remained as a doublet ($J_{11,12}$ = 10 Hz). Irradiation of the C-11 proton resulted in collapse of the C-12 proton signal to a doublet ($J_{8,12}$ = 2.5 Hz). Similarly, when the C-12 proton was irradiated, the absorptions due to the C-8 and C-10 protons collapsed to singlets.

The nmr spectrum of the alkaloid also contained two *N*-methyl singlets at δ 2.35 and 2.52, four methoxyl singlets at δ 3.57, 3.64, 3.80, and 3.82, three aromatic proton singlets at δ 6.07, 6.55, and 6.62, and a sharp four-proton singlet at δ 7.02.

The ten major fragments in the mass spectrum of pakistanamine (**9**) in the order of decreasing relative intensities were *m/e* 206 (a, base), 190 ($C_{11}H_{12}NO_2$), 608 (M − 14), 107 (C_7H_7O), 91 (C_7H_7), 416 (M − a), 310 (M − c), 267 (M − c − C_2H_5N), 622 (M^+), and 326 (M − b). Expression **9** illustrates the overall fragmentation pattern of pakistanamine, so that the mass spectrum strongly suggested that an armepavine-like benzylisoquinoline unit constitutes one-half of the molecule. Noteworthy was the loss of CO in the mass spectrum, a feature typical of proaporphine systems.[13]

Reduction of pakistanamine hydrochloride with palladium on carbon afforded the crystalline 11,12-dihydropakistanamine (**10**), which showed an intense absorbance at 1690 cm^{-1} (5.92 μ) in the ir spectrum, indicative of the presence of a conjugated carbonyl function (Scheme II).

Since proaporphines undergo the dienone–phenol rearrangement with mineral acids to yield aporphines,[14] pakistanamine was warmed to 70° in 3 *N* sulfuric acid. The resulting dextrorotatory aporphine benzylisoquinoline dimer **3** exhibited an ir spectrum which contained hydroxyl absorbance at 3555 cm^{-1} (2.82 μ). The uv spectrum, λ_{max}^{EtOH} 207 nm, 225 sh, 270 sh, 277, and 304 (log ϵ 4.88, 4.75, 4.28, 4.37, and 4.16), was shifted in the presence of base to λ_{max}^{EtOH} 212 nm, 230 sh, 258, 280, 305 sh, and 335. The striking similarity in the ir and uv spectra of **3** and pakistanine (**1**) suggested that these aporphine benzylisoquinoline dimers might possess the same basic skeleton and substitution pattern. The mass spectrum of **3** contained fragments attributable to the aporphine moiety having 14 mass units more than the corresponding fragments from **1**. The only significant difference in the nmr spectra of **3** and pakistanine (**1**) was an additional high field C-1 methoxyl resonance at δ 3.73 present in the former, so that **3** was proposed to be 1-*O*-methylpakistanine.[7] Indeed treatment of **3** with diazomethane yielded 1,10-di-*O*-methylpakistanine (**2**), identical in terms of mixture melting point and uv, ir, and nmr spectra as well as specific rotation and ORD curves with an authentic sample of 1,10-di-*O*-methylpakistanine derived from the diazomethane O-methylation of pakistanine (**1**).

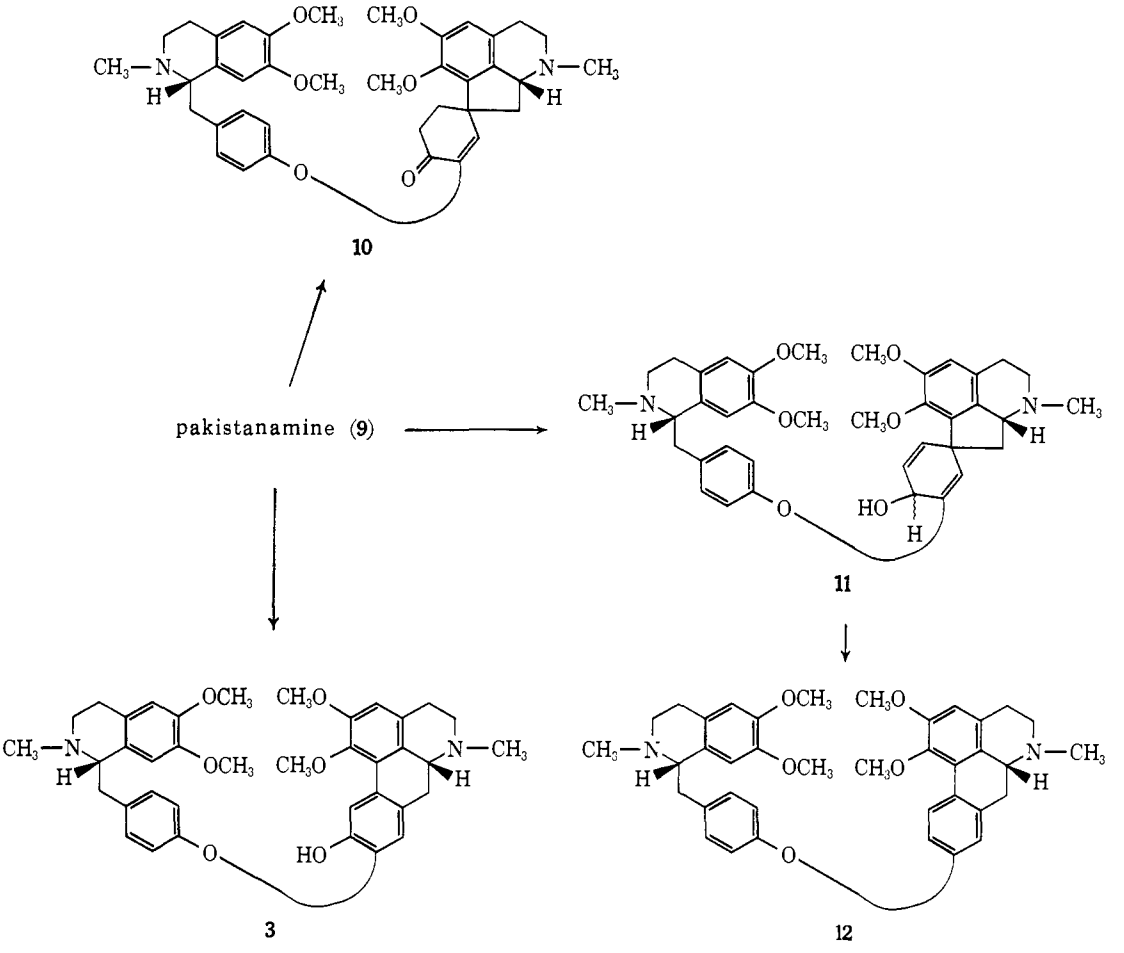
Additionally, brief sodium borohydride reduction of pakistanamine (**9**) afforded a mixture of pakistanaminols **11** which underwent the dienol–benzene rearrangement with acid to yield 1-*O*-methyl-10-deoxypakistanine (**12**), $C_{38}H_{42}N_2O_5$ (Scheme II).

The uv spectrum of **12** was typical of a 1,2,9-trisubstituted aporphine system with maxima at 228, 281, and 299 sh nm.[9] An ABX system in the nmr spectrum (Table I) confirmed the monosubstituted pattern in ring D of the aporphine moiety. The extremely low field C-11 proton appeared as a doublet ($J_{10,11}$ = 10 Hz) centered at δ 8.30, the C-8 proton as a doublet at δ 6.87 ($J_{8,10}$ = 2.5 Hz), while the C-10 proton was a well-defined doublet of doublets centered at δ 6.84 ($J_{8,10}$ and $J_{10,11}$ = 2.5 and 10 Hz, respectively). These data are consistent with a dimeric structure in which the aporphine C-9 position is joined by means of a diphenyl ether bond to an armepavine unit. Consequently, the terminus of the diphenyl ether bridge at C-9 of pakistanamine (**9**) was firmly established.

Although the absolute configuration of pakistanamine at C-6a and C-1′ was settled through chemical correlation with 1,10-di-*O*-methylpakistanine, it should

(13) M. Tomita, A. Kato, T. Ibuka, and H. Furukawa, *Tetrahedron Lett.*, 32, 2825 (1965).
(14) K. Bernauer, *Helv. Chim. Acta*, 47, 2122 (1964).

Scheme II



be pointed out that the stereochemistry of pakistanamine at C-7a is still unknown.

Biogenesis of Pakistanine and Pakistanamine. The fact that both pakistanine (**1**) and pakistanamine (**9**) were isolated from the same plant source strongly indicates that in the pakistanine–pakistanamine series dimerization to a bisbenzylisoquinoline must precede formation of the aporphine system. Intermolecular phenolic oxidative coupling of two trioxygenated tetrahydrobenzylisoquinoline units such as **13** provides the bisbenzylisoquinoline **14**. Intramolecular phenolic coupling would then lead to the proaporphine benzylisoquinoline dimer **15** which could rearrange to the aporphine benzylisoquinoline dimer **16**. The observation that dimer **14** corresponds to the alkaloid berbamunine found in *Berberis amurensis* Rupr. var. *japonica*,[15] a close relative of *B. baluchistanica*, lends further credence to this postulated biogenetic pathway.[16]

Experimental Section

Melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrometer. Ultraviolet spectra were measured on a Coleman Hitachi 124 instrument. The nmr data were obtained using a Varian A-60A spectrometer. $CDCl_3$ was the nmr solvent, and TMS the internal standard. Mass spectra were on an AEI MS-902 instrument. All tlc was on Merck silica gel 254 plates.

Isolation of Pakistanine (1) and Pakistanamine (9). The dried ground root of *Berberis baluchistanica* (3.75 kg) was extracted with ethanol, and the dried extract was dissolved in 5% hydrochloric acid, extracted with ether, basified with ammonium hydroxide, and reextracted with ethyl acetate. The ethyl acetate was evaporated, and column chromatography of the residue on neutral alumina using benzene–ethyl acetate–ethanol mixtures afforded seven fractions.

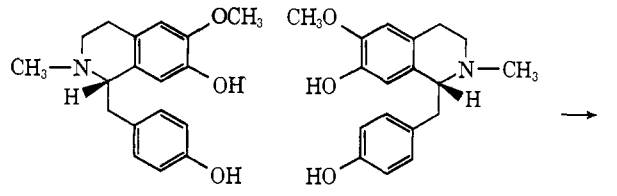
a. Pakistanine (1). Concentration of the fifth and sixth fractions afforded a white crystalline mass which upon recrystallization from ethanol gave 0.5 g of **1** as white needles: mp 154–156°; $[\alpha]^{25}D$ +106° (*c* 0.57, MeOH); ORD (*c* 0.053, MeOH) $[\alpha]_{360}$ +226°, $[\alpha]_{315}$ +2736° (pk), $[\alpha]_{252}$ −11208° (tr), and $[\alpha]_{240}$ −6698°; high-resolution mass spectrum: *m/e* 608.2803 (calcd for $M^+ = C_{37}H_{40}N_2O_6$, *m/e* 608.2885).

b. Pakistanamine (9). Fractions 1–4 from the above column were combined, evaporated, dissolved in methanol, and treated with a saturated solution of picric acid. The total precipitate was collected and recrystallized from ethanol to afford 0.5 g of pakistanamine picrate as yellow crystals, mp 158–162° dec. Ion exchange using Amberlite CG-400 (Mallinckrodt) resin and methanol–acetone (1:9) as the eluent afforded 0.280 g of a crude hydrochloride salt which was converted to the free base. Purification by preparative layer chromatography on sodium hydroxide impregnated silica gel plates using chloroform–ethyl acetate–methanol (3:3:4) as solvent afforded the amorphous free base which darkened on standing. This major alkaloid was dissolved in ether and treated with dry hydrogen chloride to afford, after recrystallization from methanol, 0.130 g of pakistanamine hydrochloride as colorless crystals: mp 215°; HCl salt $[\alpha]^{25}D$ +20° (*c* 0.34, MeOH); HCl salt ORD (*c* 0.060, MeOH) $[\alpha]_{360}$ +82°, $[\alpha]_{285}$ +2200° (pk), $[\alpha]_{240}$ −2333°; high-resolution mass spectrum for pakistanamine free base *m/e* 622.3012 (calcd for $M^+ = C_{38}H_{42}N_2O_6$, *m/e* 622.3042).

1,10-Di-*O*-methylpakistanine (2). A solution of **1** (16.4 mg) in ether–methanol (1:1, 10 ml) was treated with excess ethereal diazomethane and the mixture was allowed to stand for 48 hr at 0° in the dark, and then evaporated. The light green residue was purified by preparative layer chromotography on silica gel plates using methanol–chloroform (1:9) to give after recrystallization from ether 13.4 mg of **2** as fine colorless needles: mp 139–141°; $[\alpha]^{25}D$

(15) M. Tomita and H. Kugo, *J. Pharm. Soc. Jap.*, 77, 1075, 1079 (1963).

(16) This biogenetic sequence probably does not apply to the biosynthesis of dimeric alkaloids of the thalicarpine class.



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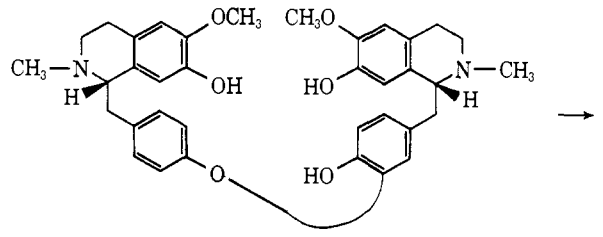

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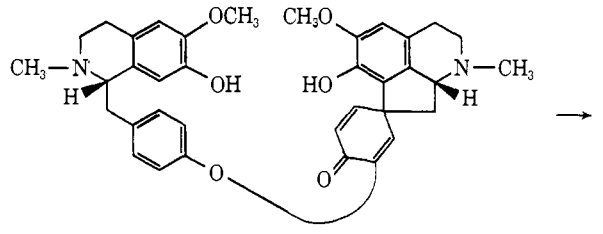

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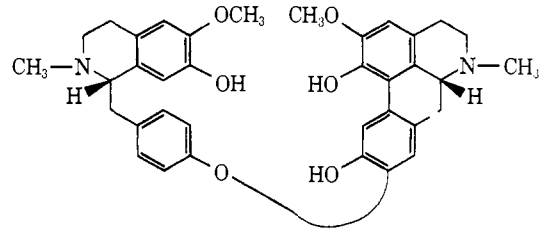

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+66° (*c* 0.40, MeOH); ORD (*c* 0.061, MeOH) $[\alpha]_{320}$ +246°, $[\alpha]_{300}$ +607° (pk), $[\alpha]_{250}$ −7049° (tr), $[\alpha]_{240}$ −6066°; $\nu_{max}^{CHCl_3}$ 2855 and 2800 cm^{-1}; λ_{max}^{EtOH} 215, 270 sh, 277, and 301 (log ε 4.60, 4.27, 4.29, and 4.09); mass spectrum *m/e* 636 (M^+), 430, 340, 324, 206 (base), 190, and 107; high-resolution mass spectrum *m/e* 636.3191 (calcd for $M^+ = C_{39}H_{44}N_2O_6$, *m/e* 636.3198).

1,10-Di-*O*-acetylpakistanine. A mixture of pakistanine (6 mg), pyridine (0.5 ml), and acetic anhydride (0.5 ml) was allowed to stand overnight. Work-up and purification by preparative layer chromatography using methanol–chloroform (1:9) afforded 5 mg of an amorphous base: ORD (*c* 0.031, MeOH) $[\alpha]_{310}$ +161°, $[\alpha]_{290}$ +452° (pk), $[\alpha]_{250}$ −4194°; $\nu_{max}^{CHCl_3}$ 1770 cm^{-1} (5.66) (acetate C=O), 2790 cm^{-1} (3.58) (NCH_3), and 2845 cm^{-1} (3.51) (OCH_3); λ_{max}^{EtOH} 215 nm, 275, and 305 (log ε 4.53, 4.23, and 3.89); mass spectrum *m/e* 692 (M^+), 486 (M − a), 444 (M − a − C_2H_2O), 402 (M − a − $2C_2H_2O$), 295, 206 (a, base); high-resolution mass spectrum *m/e* 691.3031 (calcd for $M^+ - 1 = C_{41}H_{43}N_2O_8$, *m/e* 691.3008).

Sodium–Liquid Ammonia Cleavage of 1,10-Di-*O*-methylpakistanine (2). A solution of **2** (31 mg) in dry THF (15 ml) was added dropwise to a stirred solution of sodium metal (0.1 g) in ammonia (150 ml) at −75° under a nitrogen atmosphere. A blue color persisted for 40 min, at which time the mixture was allowed to stand overnight. The residue was dissolved in 2% aqueous sodium hydroxide and extracted with ether. The ether extracts were dried and evaporated to dryness to yield 16 mg of a dark yellow oil (fraction A). The aqueous solution was treated with ammonium chloride and extracted with ether, and the ether extracts were dried and evaporated to yield 8.1 mg of a brown oil (fraction B).

a. L-(+)-Armepavine (6). Fraction B was chromatographed on plates and the major uv fluorescent band was isolated and eluted with chloroform–methanol (4:1). Evaporation of the eluate afforded 3.7 mg of **6** as an oil, identical in terms of tlc R_f values, uv, mass, and solution ir spectra, and ORD curves with an authentic sample of L-(+)-armepavine. A portion of **6** was dissolved in ether, treated with dry hydrogen chloride gas, and evaporated, and the residue was recrystallized from ethanol to afford colorless crystals of hydrochloride, mp 154–5° [lit.[17] mp 156°].

b. (−)-2,10-Dimethoxyaporphine (7). Fraction A was chromatographed on tlc plates using chloroform–methanol (8:1), and the major band, corresponding in R_f to an authentic sample of 2,10-dimethoxyaporphine, was isolated to yield 2.1 mg of **7** as a light yellow oil. The ultraviolet spectrum, λ_{max}^{EtOH} 266 nm, 272, 298 sh, 310, and 318 (log ε 4.09, 4.11, 3.69, 3.75, and 3.75), and the mass spectrum, *m/e* 295 (M^+), 294, 280, 274, 251, and 97, as well as the solution ir spectrum, were identical with those of an authentic sample of (+)-2,10-dimethoxyaporphine. The ORD curve of **7** contained a negative Cotton effect centered at 245 nm while that for authentic (+)-2.10-dimethoxyaporphine showed a positive Cotton effect at this wavelength; high-resolution mass spectrum *m/e* 295.1591 (calcd for $M^+ = C_{19}H_{21}NO_2$, *m/e* 295.1573).

9-*O*-Phenylboldine (5). A mixture of (+)-boldine (0.5 g), bromobenzene (0.2 g), copper oxide (0.3 g), and pyridine (2 ml) was heated to reflux at 150° in an oil bath under a nitrogen atmosphere for 48 hr. The cooled reaction mixture was diluted with chloroform, filtered, and evaporated to dryness. The residue was chromatographed on tlc plates. The band with the highest R_f, after isolation and crystallization from ethanol, yielded 0.023 g of **5**: mp 155–157°; high-resolution mass spectrum *m/e* 403.1801 (calcd for $M^+ = C_{25}H_{25}NO_4$, *m/e* 403.1785).

11,12-Dihydropakistanamine (10). Pakistanamine hydrochloride (20 mg) was hydrogenated overnight using Pd/C and glacial acetic acid. Work-up gave 14 mg of white crystals: mp 85–86° (methanol); ORD (*c* 0.048, MeOH) $[\alpha]_{360}$ + 63°, $[\alpha]_{280}$ +250° (pk), $[\alpha]_{260}$ −563° (tr), $[\alpha]_{240}$ +1875°; λ_{max}^{EtOH} 206 nm, 225 sh, 279, and 310 sh (log ε 4.71, 4.41, 3.94, and 3.50); mass spectrum *m/e* 624 (M^+), 418, 328, 312, 269, 238, 206 (base), 192, 107; high-resolution mass spectrum *m/e* 624.3155 (calcd for $M^+ = C_{38}H_{44}N_2O_6$, *m/e* 624.3195).

Dienone–Phenol Rearrangement of Pakistanamine (9) to 1-*O*-Methylpakistanine (3). A solution of **9** in 3 *N* H_2SO_4 (5 ml) was warmed on an oil bath at 70° for 28 hr. The solution was basified with NH_4OH to pH 8 and extracted with chloroform. Drying, filtration, and evaporation afforded a white residue which was recrystallized from ether: mp 117°, $[\alpha]^{25}D$ +85° (*c* 0.40, MeOH); ORD (*c* 0.069, MeOH) $[\alpha]_{360}$ +130°, $[\alpha]_{310}$ +1507° (pk), $[\alpha]_{245}$ −11,087° (tr), $[\alpha]_{240}$ −10,884°; λ_{max}^{EtOH} 207 nm, 225 sh, 270 sh, 277, and 304 (log ε 4.88, 4.75, 4.28, 4.37, and 4.16); $\lambda_{max}^{EtOH-KOH}$ 212 nm, 230 sh, 258, 280, 305 sh, and 335; mass spectrum *m/e* 622 (M^+), 416, 326, 310, 267, 206 (base), and 190; high-resolution mass spectrum *m/e* 622.3005 (calcd for $M^+ = C_{38}H_{42}N_2O_6$, *m/e* 622.3042.)

Acetylation of 1-*O*-Methylpakistanine (3) to 1-*O*-Methyl-10-*O*-acetylpakistanine. The acetylation was carried out at room temperature, overnight, using acetic anhydride in pyridine. Recrystallization from methanol afforded fine while crystals: mp 180° dec; ORD (*c* 0.045, MeOH) $[\alpha]_{360}$ +44°, $[\alpha]_{286}$ +1622° (pk), $[\alpha]_{240}$ −10,356°; λ_{max}^{EtOH} 207 nm, 230 sh, 270 sh, 280, 290 sh, and 300 sh (log ε 4.78, 4.53, 4.30, 4.37, 4.25, and 4.08); mass spectrum *m/e* 664 (M^+), 458, 416, 309, 266, 206, 190, and 107; high-resolution mass spectrum *m/e* 664.3096 (calcd for $M^+ = C_{40}H_{44}N_2O_7$, *m/e* 664.3147).

Conversion of 1-*O*-Methylpakistanine (3) to 1,10-Di-*O*-methylpakistanine (2). An ether–methanol solution of **3** (16.6 mg) was treated with ethereal diazomethane for 48 hr. Purification of the product by recrystallization from methanol yielded 13 mg of **2**, mp 139–141°. Direct comparison (mixture melting point, tlc R_f values, uv, ir, nmr, and mass spectra, as well as ORD curves) showed this material to be identical with authentic **2** derived from **1**.

Reduction of Pakistanamine to Diastereomeric Pakistanaminols (11), and Dienol–Benzene Rearrangement to 1-*O*-Methyl-10-deoxypakistanine (12). A mixture of pakistanamine hydrochloride (10.66 mg) and sodium borohydride (10 mg) in ethanol was stirred at 25° for 15 min. The product after work-up consisted of two diastereoisomeric pakistanaminols (**11**). This mixture was immediately treated with 3 *N* H_2SO_4 (5 ml) at 70° for 25 hr and was worked up according to the procedure described above for the dienone–phenol rearrangement. Crystallization of the product from methanol gave fine white crystals of **12**: mp 93°; $[\alpha]^{25}D$ +67° (*c* 0.33, MeOH); ORD (*c* 0.035, MeOH) $[\alpha]_{360}$ +143°,

(17) T. Kametani, "The Isoquinoline Alkaloids," Hirokawa Publishing Co., Tokyo, 1968, p 33.

$[\alpha]_{290}$ +800 (pk), $[\alpha]_{240}$ −2657°; λ_{max}^{EtOH} 228 nm, 281, and 299 sh (log ε 4.55, 4.36, and 4.06); mass spectrum *m/e* 606 (M^+), 400, 293, 236, 220, 206 (base), 192, and 91; high-resolution mass spectrum *m/e* 605.2992 (calcd for $M^+ - 1 = C_{38}H_{41}N_2O_5$, *m/e* 605.3014).

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Biosynthesis of Ergosterol in Yeast. Evidence for Multiple Pathways[1]

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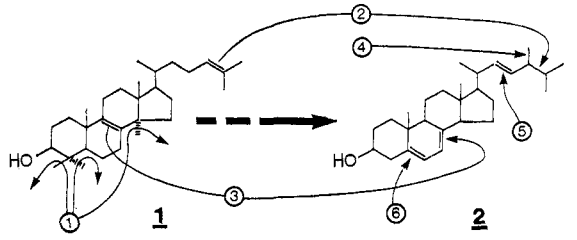
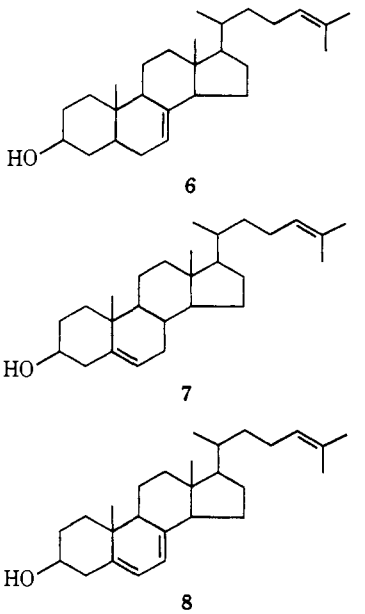
Abstract: The conversion of lanosterol to ergosterol in *Saccharomyces cerevisiae* has been investigated. Time-course analysis of the sterol content and feeding–trapping experiments with suspected intermediates led to the discovery of several alternative pathways in the latter stages of ergosterol biosynthesis. Maintenance of the yeast under anaerobic conditions depleted the sterol content of the organism. The sterols most rapidly consumed under these conditions were those possessing $\Delta^{5,7}$ unsaturation. During anaerobic maintenance squalene accumulated. A subsequent change to aerobic conditions was accompanied by accelerated sterol production. Time-course analysis of the changing sterol composition during aeration indicated that the initial structural modifications following the formation of lanosterol involved nuclear demethylation at C_4 and C_{14} as well as alkylation at C_{24}. In order to investigate subsequent modifications synthesis of suspected 4,14-desmethyl-24-alkyl sterol intermediates (unlabeled and ^{14}C or ^{3}H labeled) possessing varied unsaturation, *e.g.*, Δ^8, Δ^7, $\Delta^{5,7}$, and $\Delta^{24(28)}$, was carried out. Chromatographic separation and analysis of *S. cerevisiae* sterol mixtures led to the discovery of five previously unreported sterols (**14** and **16–19**) in this organism. Feeding and trapping experiments with suspected intermediates and previously reported yeast sterols revealed that several alternative pathways are operative in the latter stages of the lanosterol to ergosterol bioconversion. Based on relative incorporation efficiencies, the major route from fecosterol to ergosterol involves $\Delta^8 \rightarrow \Delta^7$ isomerization, introduction of unsaturation at C_{22}, then at C_5, and finally reduction of the 24-methylene. The first of these transformations was shown to be reversible while the last three were essentially nonreversible.

The enzymatic conversion of lanosterol (**1**) to ergosterol (**2**) in yeast (*Saccharomyces cerevisiae*) requires six general transformations:[2] (1) removal of the three methyl groups in lanosterol at C_4 and C_{14}; (2) alkylation at C_{24} with concomitant reduction at C_{25} and generation of a $\Delta^{24(28)}$-methylene; (3) isomerization of the Δ^8 double bond to Δ^7; (4) reduction of the $\Delta^{24(28)}$ double bond generating a C_{24}-methyl; (5) introduction of a Δ^{22} double bond; and (6) introduction of a Δ^5 double bond.



A priori, these transformations could occur in any order. A variety of studies, particularly those involving investigation of enzyme–substrate specifications, have provided an insight into the approximate order in which these transformations occur. By analogy with cholesterol biosynthesis, it has been assumed that nuclear demethylation is the initial step in the lanosterol to ergosterol conversion. Gaylor, *et al.*, have recently provided evidence that demethylation precedes $\Delta^8 \rightarrow \Delta^7$



(1) Preliminary communication: M. Fryberg, A. C. Oehlschlager, and A. M. Unrau, *Biochem. Biophys. Res. Commun.*, **48**, 593 (1972).
(2) L. J. Mulheirn and P. J. Ramm, *Chem. Soc. Rev.*, **27**, 259 (1972).