NEW ALKALOIDS FROM *BERBERIS ORTHOBOTRYS*[1]

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ABSTRACT.—*Berberis orthobotrys* has yielded the new aporphine-benzylisoquinolines 1-*O*-methylpakistanine (**2A**), kalashine (**3B**) which is the first such dimer substituted at C-11, and chitraline (**4A**). Known alkaloids present are pakistanamine (**1**), pakistanine (**3A**), berbamine, oxyacanthine and aromoline. A careful study of the dienone-phenol rearrangement of pakistanamine (**1**) has shown that aryl migration to the less hindered side of the dienone system is the heavily favored, but not the exclusive, process.

It had previously been shown that *Berberis baluchistanica* Ahrendt (Berberidaceae), native to northwestern Pakistan, produces the hitherto unknown alkaloids pakistanamine (**1**), which was and still is the only known proaporphine-benzylisoquinoline dimer, as well as pakistanine (**2A**), which was the first aporphine-benzylisoquinoline dimer known to be derived biogenetically from the condensation of two coclaurine type units (1). We became interested, therefore, in a systematic study of the Berberidaceae of Pakistan to determine if analogs of the two alkaloids could be found.

Our first effort in this direction centered upon *Berberis orthobotrys* Bienert ex Aitch. Six kilograms of roots were collected in Chitral, in the North-West Frontier Province, alongside the border with Afghanistan.[1a,b]

Extraction and work-up of this material, as described in the Experimental section, followed by repeated tlc on silica gel plates, indicated the presence of several alkaloid spots, some of which turned green after about 6–10 hours, pointing to the presence of aporphinoids with a phenolic function incorporated at C-1. The solvent systems chloroform-diethylamine (90:10) and chloroform-diethylamine (95:5) were found to be superior for the preparative tlc separation of what proved to be a series of aporphine-benzylisoquinoline alkaloids. A distinct advantage of preparative tlc over column chromatography is that the sensitive dimeric alkaloids were not exposed to light or adsorbant for long hours; therefore, at no stage was column chromatography used in the separation process.

The first alkaloid thus obtained was the known dimer pakistanamine (**1**) (1). Its isolation was an indication that some of the remaining alkaloids would probably prove to be analogs of the related aporphine-benzylisoquinoline dimer pakistanine (**3A**) (3).

The second alkaloid obtained was identified as 1-*O*-methylpakistanine (**2A**), which had previously been known not as a natural product, but as a semi-synthetic compound from the dienone-phenol rearrangement of pakistanamine (**1**) in 3N sulfuric acid at 70° C (1). This alkaloid was followed on the tlc plates by the known bisbenzylisoquinolines berbamine, oxyacanthine and aromoline,[3] all of which are also derived biogenetically from the condensation of coclaurine type monomers.

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[1]Parts of the information presented here have appeared in communication form, see (a) S. F. Hussain and M. Shamma, *Tetrahedron Lett.*, **21**, 3315 (1980); (b) S. F. Hussain, Lajber Khan and M. Shamma, *Heterocycles*, **15**, 191 (1981); and (c) S. F. Hussain, M. T. Siddiqui and M. Shamma, **21**, 4573 (1980). The present paper deals primarily with the alkaloid isolation procedure. The reader is referred to refs. 1a-c for the details of the structural elucidation.

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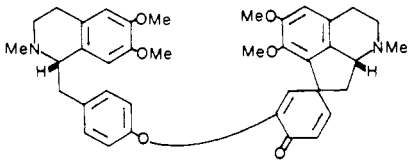
[3]For a listing of the bisbenzylisoquinoline alkaloids, see K. P. Guha, B. Mukherjee and R. Mukherjee, *J. Nat. Prod.*, **42**, 1 (1979).

The next two alkaloids proved to be the known pakistanine (**3A**) and the new base kalashine (**3B**); the fact that they both possessed phenolic groups at C-1 was indicated by the green tinge their spots developed with time on the tlc plates. In line with the presence of two phenolic groups in kalashine (**3B**), 1,10-di-*O*-acetylkalashine was prepared by treatment of the alkaloid with acetic anhydride in pyridine. Kalashine is of more than passing interest, since it is the first aporphine-benzylisoquinoline alkaloid to be substituted at C-11.[1a] Significantly, however, pakistanine (**3A**), in which C-11 is unsubstituted, was found to be about fifty times more abundant in the plant.



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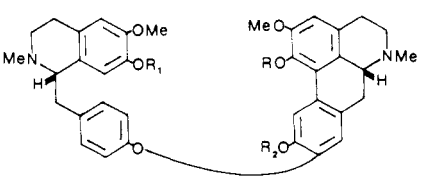
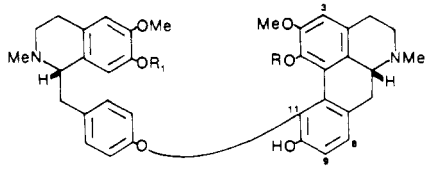




2A, R = R_1 = Me, R_2 = H
3A, R = R_2 = H, R_1 = Me
4A, R = R_1 = R_2 = H
5A, R = R_1 = R_2 = Me

2B, R = R_1 = Me
3B, R = H, R_1 = Me
4B, R = R_1 = H

Pakistanine (**3A**) and kalashine (**3B**) are structural isomers. At that stage of the work it was logical to infer that aporphine-benzylisoquinoline dimers of the "A" and "B" series could be derived in nature from dienone-phenol rearrangement of some proaporphine-benzylisoquinoline precursor. Pakistanine (**3A**) would then result from aryl migration to the less hindered side of the dienone system, a favored process; kalashine (**3B**) would be formed from the more difficult aryl migration to the more hindered side.[4]

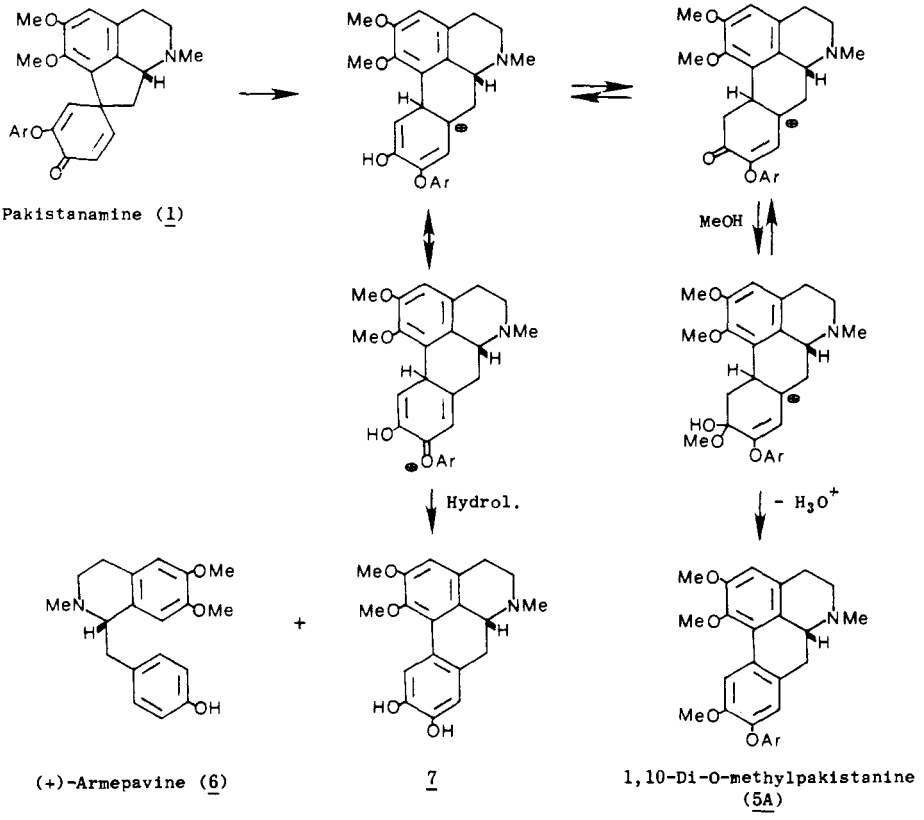
To test the above hypothesis, it was decided to reinvestigate the acid catalyzed dienone-phenol rearrangement of pakistanamine (**1**). A solution of **1** was heated, therefore, at 70° for ten hours. Work-up furnished the expected 1-*O*-methylpakistanine (**2A**) in about 95% yield. Significantly, however, two minor products, each isolated in less than 1% yield, were the hitherto unknown 1-*O*-methylkalashine (**2B**) and the previously known simple benzylisoquinoline (+)-armepavine (**6**).[1a] The first of these two minor products must indeed be formed from **1** by aryl migration to the more hindered side of the dienone; thus the analogy between the natural and the *in vitro* processes was proved. The genesis of (+)-armepavine (**6**) is somewhat more complex and is explained in the scheme. The small quantity of the diphenolic aporphine **7** formed as a by-product would tend

[4]For an attempt at rationalizing the biogenesis of the aporphine-benzylisoquinoline dimers derived from two coclaurine-type units, see ref. 1c above.

to further oxidize in air, since the molecule is a catechol, so this product was not isolated.

The dienone-phenol rearrangement of **1** took a somewhat different course when the reaction was run in methanol containing a small amount of 3N hydrochloric acid. Two products were obtained in almost equal amounts, namely, 1-*O*-methylpakistanine (**2A**) and the hitherto unknown 1,10-di-*O*-methylpakistanine (**5A**),[1a] formed through methanolysis of a reaction intermediate, as indicated in the scheme.

Scheme



The last and most polar alkaloid obtained from *B. orthobotrys* proved to be the new triphenolic base chitraline (**4A**), which again eventually developed a greenish coloration on tlc and whose acetylation yielded 1,10,7'-tri-*O*-acetylchitraline.[1b]

The above isolation results should be compared to those for *B. calliobotrys* Bienert ex Aitch., also collected in the North-West Frontier Province of Pakistan.[5] *B. calliobotrys* was found to produce pakistanamine (**1**), 1-*O*-methylpakistanine

[5]A small portion of the crude ethanolic extracts from *B. calliobotrys* was applied directly on a tlc plate. Development of the plate indicated the presence of **1**, **2A**, **3A**, **3B**, **4A**, thus clearly indicating that dienone-phenol rearrangement is a natural process, since no mineral acid had been added to us to the mixture up to that stage.

(**2A**), pakistanine (**3A**), kalashine (**3B**), chitraline (**4A**), and interestingly enough the new base khyberine (**4B**).[1c] Inspection of structures **2A**, **3A**, **3B**, **4A**, and **4B** makes it evident that in aporphine-benzylisoquinoline dimers derived biogenetically from two coclaurine type units, C-10 inevitably bears a phenol, while C-1 and C-7' may also be bonded to such a function when not connected to methoxyl groups.[4, 6]

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Tlc was on Merck silica gel F-254 plates. Spots were visualized under ultraviolet light or by spraying with chloroplatinate reagent. Due to the small quantities of alkaloids isolated, it was not possible to obtain crystalline samples for melting point determinations.

EXTRACTION PROCEDURE.—The ethanolic extract from 5 kg of dried roots (6 kg of wet roots) of *B. orthobotrys* was extracted twice with 10% hydrochloric acid (3 liters) and the aqueous layer was filtered from insoluble matter (Extract A). When the filtrate was cooled in a refrigerator for 48 h., a small amount (~1 g) of berberine separated out. Following filtration and evaporation, the extract was exhaustively extracted with chloroform (Extract B). The aqueous layer was then made alkaline (pH ~8) with ammonium hydroxide; the resulting precipitate, 36 g (Extract C) was filtered off. The filtrate was extracted with chloroform to provide 12 g of Extract D.

Tlc of fractions A, B, C, and D, in chloroform-diethylamine (90:10) indicated Extract C to be by far the richest in alkaloid content, showing numerous spots on spraying with the iodoplatinate reagent.

A portion of Extract C (9 g) was fractionated on 2 mm thick Merck F-254 tlc plates with chloroform-diethylamine (95:5). Six fractions (C-1 for the fastest moving to C-6 for the slowest) were separated. Each of these fractions was further purified on 0.5 mm and 0.25 mm plates with chloroform-diethylamine (90:10) and chloroform-diethylamine (95:5) as the developing solvent systems.

PAKISTANAMINE (**1**).—Fraction C-1 was comprised almost entirely of **1** (463 mg), which was further purified by preparative tlc with benzene-chloroform-diethylamine (5:4:1), R_f 0.43, and identified by comparison with an authentic sample (**1**).[2] A solution of pakistanamine while being evaporated must never be heated beyond 40° to prevent decomposition.

1-*O*-METHYLPAKISTANINE (**2A**).—Fraction C-2 contained a small amount of pakistanamine (**1**). However, the major part of the R_f 0.47 in chloroform-diethylamine (90:10) was identified as 1-*O*-methylpakistanine (**2A**), $C_{38}H_{42}N_2O_6$ (1.2 g) by comparison with an authentic sample; λ max (EtOH) 207, 230 sh, 270 sh, 280, 290 sh and 300 sh nm (log ε 4.78, 4.53, 4.30, 4.37, 4.25, and 4.08) (1).

BERBAMINE AND OXYACANTHINE.—Fraction C-3 exhibited three distinct spots with R_f 0.47, 0.37 and 0.31; chloroform-diethylamine (90:10) was the solvent. The fastest moving compound with R_f 0.47 was immediately identified as **2A** (2 mg). The other two compounds, on further purification by tlc with chloroform-diethylamine (95:5) were found to correspond to berbamine (3 mg) and oxyacanthine (14 mg), respectively, by comparison with authentic samples.[3]

AROMOLINE, PAKISTANINE (**3A**) and KALASHINE (**3B**).—Fraction C-4 showed three major tlc spots with R_f 0.30, 0.24 and 0.18 with chloroform-diethylamine (90:10). The spots with R_f 0.24 and 0.18 turned green overnight. The three compounds were separated by tlc with chloroform-diethylamine (95:5). The fastest moving compound (27 mg) was identified as aromoline by comparison with an authentic sample.[3]

Following further fractionation by tlc with the system chloroform-diethylamine (90:10), the band with R_f 0.24 was found to consist of pakistanine (**3A**), $C_{37}H_{40}N_2O_6$, (167 mg); λ max (EtOH) 206, 218, 270 sh, 277 and 307 nm (log ε 4.69, 4.61, 4.13, 4.21 and 4.07), (1) while the slowest band R_f 0.18, was due to the dimer kalashine (**3B**), $C_{37}H_{40}N_2O_6$, (3.5 mg), λ max (MeOH) 220, 264 sh, 272, 290 sh, and 304 nm (log ε 4.54, 3.95, 4.04, 3.74 and 3.70).[1a]

1,10-DI-*O*-ACETYLKALASHINE.—A mixture of **3B** (1.5 mg), acetic anhydride (0.5 ml), and pyridine (0.25 ml) was kept overnight at room temperature. Work-up by tlc afforded the desired diacetyl derivative, $C_{41}H_{44}N_2O_8$.[1a]

CHITRALINE (**4A**).—Repeated purification of fraction C-5 in chloroform-diethylamine (90:10) and (95:5) afforded the colorless amorphous dimer **4A**, $C_{36}H_{38}N_2O_6$, (20 mg), λ max

[6]A study of the alkaloid content of *B. zabeliana*, also from northwestern Pakistan, yielded only chitraline (**4A**).

(MeOH) 220 sh, 268 sh, 278, 292 sh and 304 nm (log ε 4.51, 4.03, 4.10, 3.94 and 3.96). 1,10,7'-Tri-*O*-acetylchitraline, $C_{42}H_{44}N_2O_9$, was obtained under standard acetylating conditions as described above, followed by tlc in chloroform-diethylamine (95:5).[1b]

DIENONE-PHENOL REARRANGEMENT OF PAKISTANAMINE (**1**) TO 1-*O*-METHYLPAKISTANINE (**2A**) AND 1-*O*-METHYLKALASHINE (**3B**).—A solution of **1** (304 mg) in 3N hydrochloric acid (15 ml) was heated at 70° for 10 h. The solution was cooled and basified with ammonium hydroxide to pH ~8, and extracted with chloroform. Removal of the organic solvent and a tlc screen of the reaction products indicated one major and two minor components. The fast moving major component was separated by tlc in chloroform-diethylamine (90:10) and shown to be **2A** (249 mg) (1). The two superimposed slow moving bands were separated again by tlc, this time, with chloroform-diethylamine (95:5) as the solvent. The upper band (1.72 mg) was characterized as 1-*O*-methylkalashine (**2B**), $C_{38}H_{42}N_2O_6$, λ max (MeOH) 222, 272 and 302 nm (log ε 4.51, 4.00 and 3.68). The lower band (1.33 mg) proved to be (+)-armepavine (**6**) by comparison with an authentic sample.[1a]

DIENONE-PHENOL REARRANGEMENT OF **1** to **2A** and 1,10-DI-*O*-METHYLPAKISTANINE (**5A**).—A solution of **1** (68 mg) in methanol (10 ml containing 5% 3N hydrochloric acid) was refluxed gently for 12 h. Work-up was followed by tlc with benzene-chloroform-diethylamine (5:4:1); two major fractions in about equal amounts were obtained. The upper fraction was identified as 1,10-di-*O*-methylpakistanine (**5A**), λ max (EtOH) 215, 270 sh, 277 and 301 (log ε 4.60, 4.27, 429 and 4.09). The lower fraction consisted of **2A**.[1a]

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