Tetrahedron Letters Vol. 21, pp 4573 - 4576 0040-4039/80/1122-4573$02.00/0

KHYBERINE AND THE BIOGENESIS OF DIMERIC APORPHINE-BENZYLISOQUINOLINE ALKALOIDS

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The new aporphine-benzylisoquinoline khyberine (4B) is present in Berberis calliobotrys Aitch. ex Bienert in about one part per million. An effort is made to present a complete scheme for the biogenesis of aporphine-benzylisoquinoline dimers which arise from the condensation of two coclaurine units.

Following the original isolation of the proaporphine-benzylisoquinoline pakistanamine (1) and the aporphine-benzylisoquinoline pakistanine (3A) from Berberis baluchistanica Ahrendt (Berberidaceae),[2] we have carefully screened several additional Berberis species, all collected in northern or northwestern Pakistan, for their alkaloidal contents. B. orthobotrys Bienert ex Aitch. recently yielded three new alkaloids, 1-O-methylpakistanine (2A), chitraline (4A),[3] and very importantly kalashine (3B),[4] together with the known pakistanine (3A).[3,4] Kalashine is of particular interest since it demonstrates that a dienone-phenol rearrangement to the more hindered side of the dienone system is possible in nature. Noteworthy is the fact that kalashine (3B) is present in B. orthobotrys to the extent of only five parts per million, which is about one hundred times less than its more abundant companion pakistanine (3A), thus indicating that dienone-phenol rearrangement involving aryl migration to the more hindered side of the dienone system is not as favored as the opposite aryl migration to the less hindered side.

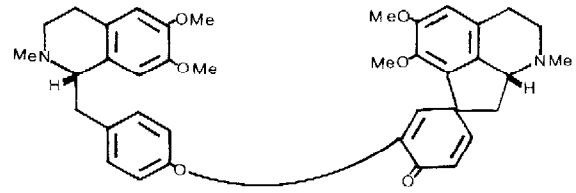
The study of B. zabeliana Schneider was less rewarding, yielding only the dimer chitraline (4A). Our efforts turned, therefore, towards a detailed study of the alkaloids of B. calliobotrys Bienert ex Aitch. Seven kilograms of the wet roots were collected near Chitral, in the Northwest Frontier Province of Pakistan, north of the Khyber Pass, to afford six kilograms of dried material. Ethanol extraction then yielded 325 g of crude extracts. One half of this amount was subjected to acid work-up followed by preparative tlc on Merck silica gel plates of one third of this fraction (10 g). Several alkaloids were obtained which were characterized as the known pakistanamine (1), 1-O-methylpakistanine (2A), pakistanine (3A), chitraline (4A), and kalashine (3B). Near the origin of the thin layer chromatogram, however, it was possible to detect a faint greenish spot, denoting a C-1 hydroxylated aporphinoid appreciably more polar than any of its congeners.[5] It was also observed that 1-O-methylpakistanine (2A), pakistanine (3A), and chitraline (4A), in which C-11 is unsubstituted, present a purplish-blue fluorescence under long-wave length uv light; whereas C-11 substituted aporphinoids such as kalashine (3B) as well as 1-O-methylkalashine (2B), obtained as a minor product by heating pakistanamine (1) in 3N HCl,[4] exhibit a greenish-blue fluorescence under the same conditions. The new phenolic and polar aporphinoid displayed a greenish-blue fluorescence, denoting that it belonged to the "B" series of dimeric aporphine-benzylisoquinolines. Perusal of the structural scheme reproduced below made it evident that the new alkaloid had to possess structure 4B. Before this fact could be ascertained, however, it was

necessary to obtain the alkaloid in pure form, free of accompanying bisbenzylisoquinolines and quaternary protoberberinium salts. This was achieved through a succession of three separate and distinct tlc operations using first $CHCl_3$-$HN(C_2H_5)_2$ (90:10), then $CHCl_3$-MeOH (85:15), followed again by $CHCl_3$-$HN(C_2H_5)_2$ (90:10). In this fashion, one milligram of colorless, microcrystalline, khyberine, mp 145-147° ($CHCl_3$-MeOH), was obtained, corresponding to about one part per million of the living plant. All of the structural work was then carried out using this one milligram of material. The mass spectrum of khyberine is in full agreement with structure 4B and shows m/e 593 $(M - 1)^+$, denoting the molecular formula $C_{36}H_{38}O_6N_2$ and loss of a proton, 403 $(M - a + H)^+$, 402 (M - a), 296 (M - c), 192 (base, a), and 107 (c - a). The uv spectrum, λ_{max}^{MeOH} 220sh, 264sh, 272, 292sh and 304 nm (log ε 4.53, 3.97, 4.02, 3.80 and 3.70), is very close to that for kalashine (2B) and 1-O-methylkalashine (2B),[4] pointing to 1,2,10,11-substitution around the aporphine nucleus. The $CDCl_3$ nmr spectrum at 200 MHz (FT) yielded conclusive evidence in favor of structure 4B and has been summarized in the Table, together with the data available for the other aporphinoids in question. The absence of a downfield proton peak near δ8.0 denotes substitution at C-11, and the lack of a three-proton singlet near 3.40 indicates the presence of a phenol rather than a methoxyl at C-7'. Finally, the cd spectrum of khyberine (4B) in methanol shows $\Delta\varepsilon_{nm}$: $+3.24_{308}$, $+3.06_{292}$, $+3.42_{275}$, -54.0_{234}, and $+36.0_{214}$, a pattern strongly reminiscent of that for kalashine (3B) and 1-O-methylkalashine (2B), thus indicating the identical absolute configuration.[4]

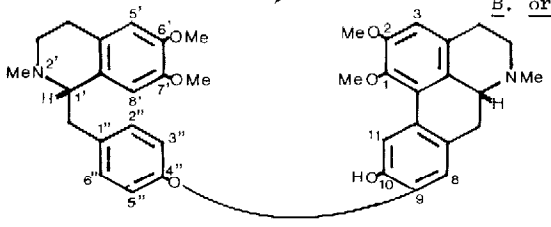
Portions of the remaining half of the crude ethanol extracts were applied directly on a silica gel tlc plate. Development with $CHCl_3$-$HN(C_2H_5)_2$ (90:10) showed the presence of pakistanamine (1), 1-O-methylpakistanine (2A), pakistanine (3A), chitraline (4A), and kalashine (3B). The spot corresponding to khyberine (4B) was blurred due to the minute amounts of this alkaloid which were present, and which were superimposed on accompanying bisbenzylisoquinolines and quaternary protoberberinium salts. The detection of alkaloids 2A-4A and 3B under these conditions demonstrates that the dienone-phenol rearrangement is a natural process, and is not due to acid work-up during the isolation process.

A biogenetic dilemma presents itself at this juncture. Given that pakistanamine (1) is the only proaporphine-benzylisoquinoline known, and that all of the aporphine-benzylisoquinolines in question bear a phenolic function at C-10, it is tempting to speculate that pakistanamine (1) first rearranges to 2A along with the less favored 2B. Sequential O-demethylation then takes over to furnish 3A and 4A, as well as 3B and 4B. It must be borne in mind, however, that a precursor of pakistanamine (1) could very well be a bisbenzylisoquinoline alkaloid such as the known (+)-berbamunine (6), derived from the dimerization of two coclaurine units, which could undergo intramolecular oxidative coupling to the putative proaporphine-benzylisoquinoline 5. Compound 5 incorporates phenolic functions at C-1 and C-7', and its rapid dienone-phenol rearrangement would then lead to 4A and 4B. Through sequential O-methylation, these could in turn furnish 3A and then 2A on the one hand, and 3B and then 2B on the other. Intermediate 5 could also suffer direct O-methylation to furnish pakistanamine (1). It is, of course, always possible that the true biogenetic sequence will prove to be a complex interlacing of these two sequences.[6]

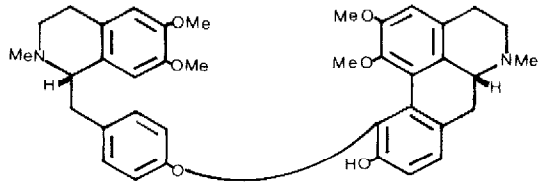
Acknowledgment: This research was supported by grant CA-11450 from the National Cancer Institute, National Institutes of Health, USPHS.



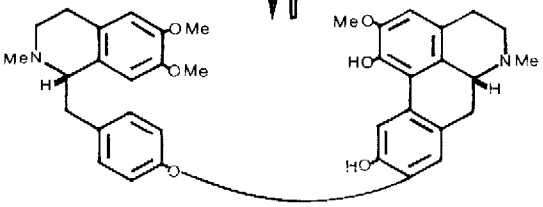
Pakistanamine (1)
B. baluchistanica
B. calliobotrys
B. orthobotrys



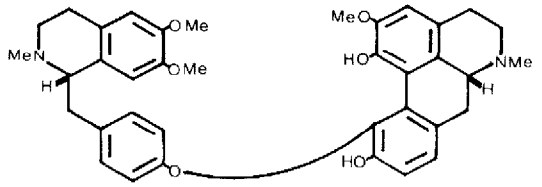
1-O-Methylpakistanine (2A)
B. calliobotrys
B. orthobotrys



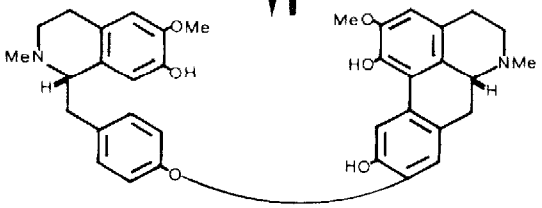
1-O-Methylkalashine (2B)
(prepared from 1)



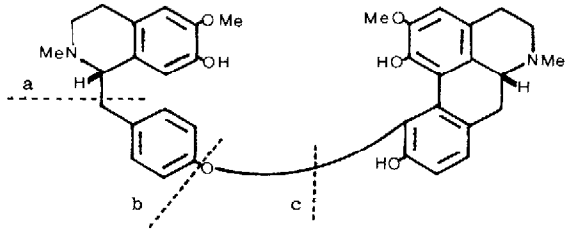
Pakistanine (3A)
B. baluchistanica
B. calliobotrys
B. orthobotrys



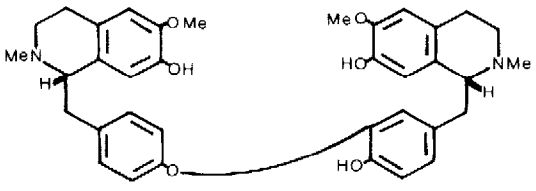
Kalashine (3B)
B. calliobotrys
B. orthobotrys



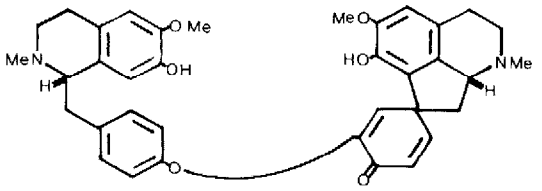
Chitraline (4A)
B. calliobotrys
B. orthobotrys
B. zabeliana



Khyberine (4B)
B. calliobotrys



(+)-Berbamunine (6)



5

TABLE. NMR Resonances at 200 MHz (FT) in $CDCl_3$

	Methylimino		Methoxyl				Aromatic Proton						
Compound	N-2'	N-6	C-1	C-2	C-6'	C-7'	H-8'	H-3	H-5'	H-8	H-9	H-2",6"	H-3",5"
1-O-Methyl-pakistanine (2A)	2.50	2.55	3.72	3.84	3.89	3.64	6.11	6.57	6.61	6.70	-	7.10d	6.99d
Pakistanine (3A)	2.51	2.55	-	3.85	3.92	3.64	6.11	6.57	6.57	6.72	-	7.10d	6.98d
Chitraline (4A)	2.50	2.51	-	3.86	3.92	-	6.37	6.54	6.57	6.75	-	7.01d	6.96d
1-O-Methyl-kalashine (2B)	2.45	2.55	3.67	3.77	3.82	3.42	5.85	6.52	6.52	7.08d	6.98d	6.81d	6.57d
Kalashine (3B)	2.47	2.56	-	3.82	3.83	3.40	5.85	6.53	6.56	7.14d	6.98d	6.88d	6.67d
Khyberine (4B)	2.41	2.55	-	3.83	3.83	-	6.16	6.51	6.56	7.14d	7.00d	6.89d	6.69d

All values are on the δ scale. Chemical shift assignments for H-3 and H-5' are interchangeable. Chemical shifts for the H-11 singlets are δ8.11, 8.13, and 8.12, for compounds 2A, 3A, and 4A, respectively. $J_{2",3"}$ and $J_{5",6"}$ are about 8.7 Hz for each of the compounds in the Table. $J_{8,9}$ is 8.0 Hz for compounds 2B, 3B, and 4B.

References and Footnotes

1. Permanent address: PCSIR Laboratories, Peshawar, Pakistan.
2. M. Shamma, J.L. Moniot, S.Y. Yao, G.A. Miana and M. Ikram, J. Am. Chem. Soc., 95, 5742 (1973).
3. S.F. Hussain, L. Khan and M. Shamma, Heterocycles, in press.
4. S.F. Hussain and M. Shamma, Tetrahedron Lett., in press.
5. The R_f values in $CHCl_3$-$HN(C_2H_5)_2$ (90:10) using Merck silica gel F-254 preprepared glass plates are as follows: 1-O-Methylpakistanine (2A) 0.47, 1-O-methylkalashine (2B) 0.36, pakistanine (3A) 0.27, kalashine (3B) 0.18, chitraline (4A) 0.10, and khyberine (4B) 0.07.
6. For a complete listing of aporphine-benzylisoquinoline alkaloids and their nmr and uv spectra, see G. Guinaudeau, M. Leboeuf and A. Cavé, J. Natural Products, 42, 133 (1979). See also M. Shamma, The Isoquinoline Alkaloids, Academic Press, New York (1972), pp. 205-206, and M. Shamma and J.L. Moniot, Isoquinoline Alkaloids Research 1972-1977, Plenum Press, New York (1979), p. 165.

(Received in USA 28 July 1980)