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KALASHINE, A NOVEL TYPE APORPHINE-BENZYLISOQUINOLINE ALKALOID

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Berberis orthobotrys produces the new dimer kalashine (1), together with the previously reported pakistanamine (2) and pakistanine (3). Kalashine is the first aporphine-benzylisoquinoline known to be substituted at C-11. Acid catalyzed rearrangement of pakistanamine (2) in 3N HCl leads to 1-O-methylpakistanine (6) together with small amounts of 1-O-methylkalashine (7) and (+)-armepavine (8). Rearrangement of 2 using methanol containing a little 3N HCl gives about equal amounts of 6 and 1,10-di-O-methylpakistanine (9).

One of the hallmarks of the twenty-three aporphine-benzylisoquinoline alkaloids presently known is that the hindered aporphine C-11 position is always unsubstituted. We wish to report here the isolation and structural elucidation of kalashine (1), the first aporphine-benzylisoquinoline alkaloid substituted at that site.[2]

Six kilograms of the roots of Berberis orthobotrys Bienert ex Aitch. (Berberidaceae) were collected near the Kalash valley of the Chitral region of Pakistan, adjoining the Afghan border.[3] The resulting five kilograms of dried roots were powdered and extracted with cold ethanol. Acid extraction followed by basification provided 36 g of basic extract. A 9 g portion of this extract was repeatedly fractionated on Merck silica gel F-254 tlc plates using $CHCl_3$:$HN(Et)_2$ (90:10) as the developing solvent.[4]

In addition to pakistanamine (2) which is the main alkaloid in the plant, and to the accompanying pakistanine (3),[5] a small amount (3.5 mg) of the colorless diphenolic base kalashine (1) was obtained, $C_{37}H_{40}N_2O_6$. The mass spectral fragmentation pattern of kalashine (1) is characteristic of an aporphine-benzylisoquinoline dimer with peak m/e 607 $(M - 1)^+$, and base peak m/e 206 corresponding to fragment a or to the A and B rings of the benzylisoquinoline moiety. The other intense fragments, m/e 403 $(M - a + H)^+$, 311 $(M - b - H)^+$, 296 $(M - c)^+$ and 107 $(c - a)^+$ suggested that the alkaloid was bonded at the lower portion of the benzylisoquinoline residue through a diphenyl ether linkage to the aporphine system.

The most notable feature of the nmr spectrum of kalashine (Table) is the absence of a downfield H-11 proton signal, thus denoting substitution at the aporphine C-11 position. Moreover, this spectrum which shows the presence of three methoxyl and two N-methyl groups, as well as of nine aromatic protons, could be readily interpreted by assuming that the benzyloxy portion of the benzylisoquinoline moiety was bonded to C-11 rather than to the more usual C-9 site.

To obtain further support for this conclusion, kalashine (1) was acetylated using acetic anhydride in pyridine at room temperature. The resulting 1,10-di-O-acetylkalashine (4), $C_{41}H_{44}N_2O_8$, m/e 690 $(M - 2H)^+$, 487, 443, 402 and 206 (base), exhibited an nmr spectrum whose H-9 doublet appeared at δ7.06 ($J_{8,9}$ = 8.0 Hz), downfield from the corresponding chemical shift of δ6.93 for kalashine (1), due to acetylation of the C-10 phenolic function (Table).[6]

The uv spectrum of kalashine, λ_{max}^{MeOH} 220, 272, 290sh and 304 (log ε 4.54, 4.04, 3.74 and 3.70) is also consonant with substitution at C-11 since it lacks the strong absorbance near 280 nm characteristic of 1,2,9,10-substituted aporphines and present in the spectrum of the accompanying alkaloid pakistanine (3).[7]

It was logical to infer at this stage that acid catalyzed dienone-phenol rearrangement of th unknown proaporphine-benzylisoquinoline dimer 5 in the plant could lead on the one hand to pakistanine (3), and on the other hand but to a lesser extent to kalashine (1).

To verify this hypothesis, the acid catalyzed rearrangement of pakistanamine (2) was reinvestigated. It had previously been shown that when 2 is warmed in 3N H_2SO_4 at 70° C for 28 hours, the product isolated is 1-O-methylpakistanine (6).[5] We have now found that warming pakistanamine (2) in 3N HCl at 70° C for 10 hours leads as expected to 1-O-methylpakistanine (6) in 95% yield. However, two minor products, each in about 1% yield were also obtained. The first proved to be the desired 1-O-methylkalashine (7), $C_{38}H_{42}N_2O_8$, m/e 620 $(M- 2H)^+$, 417, 310, 206 (base) and 190; λ_{max}^{MeOH} 222, 272 and 302 nm (log ε 4.51, 4.00 and 3.68); which indeed must have been formed from pakistanamine (2) by the less favored aryl migration to the more hindered side of the dienone system. The second minor product was simply the monomeric benzylisoquinoline (+)-armepavine (8) showing $\Delta\varepsilon_{nm}^{MeOH}$ $+3.88_{288}$, -3.29_{244}, -9.31_{226} and $+18.82_{212}$,[8] thus supplying independent support f the assignment of absolute configuration to the benzylisoquinoline portion of alkaloids 1-3.

Interestingly enough, when pakistanamine (2) was heated at 70° C in methanol containing 5% 3N HCl, two products in almost equal amounts were obtained. These proved to be 1-O-methylpakistanine (6), and the new derivative 1,10-di-O-methylpakistanine (9) which must have been formed through methanolysis of the protonated dienone-phenol reaction intermediate.

Noteworthy is the observation that kalashine (1), pakistanine (3), 1-O-methylpakistanine (6) 1-O-methylkalashine (7), and 1,10-di-O-methylpakistanine (9), show closely related patterns in their CD curves, pointing to the identical absolute configuration.[9]

We believe that kalashine (1) as well as pakistanine (3) are true alkaloids rather than artefacts produced during our alkaloid isolation process since we could not find in the plant any trace of their putative phenolic dienone precursor 5, even though substantial amounts of pakistanamine (2) were isolated.[10] More significantly, we have carried out an extraction and analysis of the alkaloids of E. calliobotrys Bienert ex Aitch.,[6] which also grows in Chitral, without the use of acid at any stage. Among the alkaloids present were pakistanamine (2) and pakistanine (3), thus indicating that the dienone-phenol rearrangement is a natural process.[11]

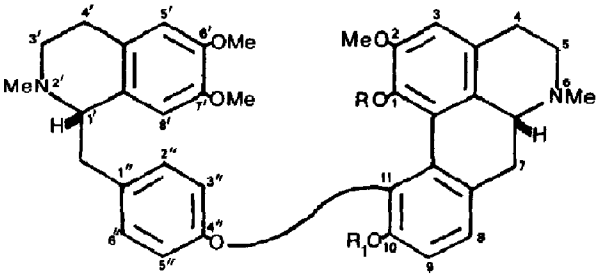
The characterization of kalashine (1) has an important bearing on present theories of aporphine biogenesis. Since the aporphine moiety in 1 is 1,2,10,11-substituted, it is obvious that monomeric 1,2,10,11-tetrasubstituted aporphines could also be formed in nature by a similar dien phenol rearrangement involving aryl migration to the more hindered side of the dienone system.

TABLE

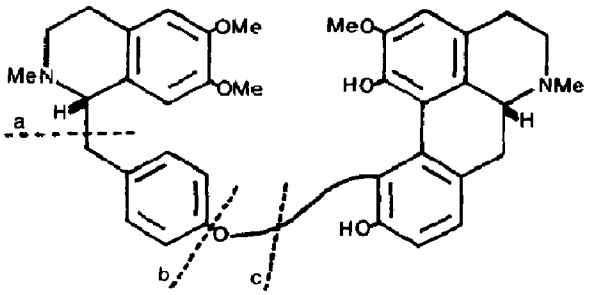
NMR Resonances at 200 MHz (FT) in $CDCl_3$ with TMS as Internal Standard

	Methylimino		Methoxyl				Aromatic Protons						
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"
Kalashine (1)	δ2.47	2.56	-	3.82	3.83	3.40	5.85	6.53	6.56	7.14d	6.93d	6.88d	6.67d
1-O-Methyl-kalashine (7)	2.45	2.55	3.67	3.77	3.82	3.42	5.85	6.52	6.52	7.08d	6.97d	6.81d	6.57d
1,10-Di-O-acetyl-kalashine (4)	2.48	2.57	-	3.77	3.82	3.44	5.91	6.68	6.52	7.19d	7.06d	6.85d	6.43d
Pakistanine (3)	2.51	2.55	-	3.85	3.92	3.64	6.13	6.57	6.57	6.72	-	7.10d	6.98d
1-O-Methyl-pakistanine (6)	2.50	2.55	3.72	3.84	3.89	3.64	6.11	6.57	6.61	6.70	-	7.10d	6.99d
1,10-Di-O-methyl-pakistanine (9)	2.51	2.55	3.70	3.84	3.90	3.62	6.06	6.56	6.63	6.76	-	7.07d	6.94d

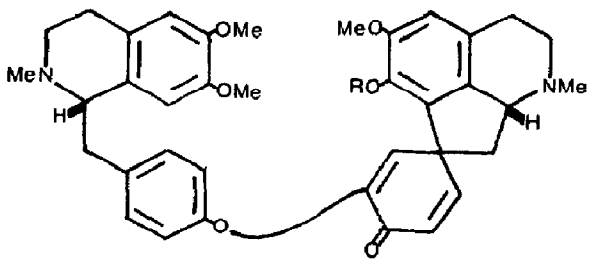
Chemical shift assignments for H-3 and H-5' are interchangeable. For compound 4, C-1 acetoxyl singlet δ2.36, C-10 acetoxyl singlet δ2.02. Compounds 3, 6, and 9, H-11 singlets δ8.13, 8.11 and 8.20, respectively. Compound 9 C-10 methoxyl singlet δ3.88. $J_{8,9}$ is 8.0 Hz for compounds 1, 4 and 7; and $J_{2'',3''}$ and $J_{5'',6''}$ is 8.7 Hz for each compound in the Table.



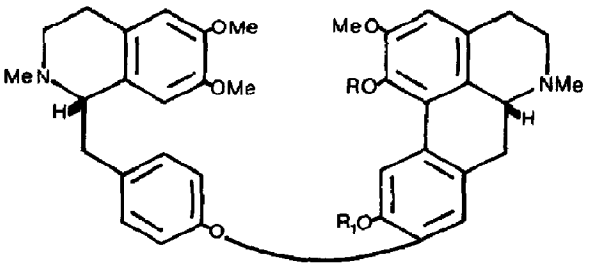
1, R = R_1 = H
4, R = R_1 = Ac
7, R = Me, R_1 = H



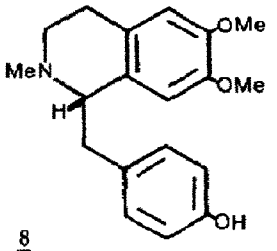
Mass spectral breakdown for kalashine (1).



2, R = Me
5, R = H



3, R = R_1 = H
6, R = Me, R_1 = H
9, R = R_1 = Me



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References and Footnotes

1. Permanent address: PCSIR Laboratories, Peshawar, NWFP, Pakistan.
2. For a complete listing of aporphine-benzylisoquinoline alkaloids, see H. Guinaudeau, M. Lebœuf and A. Cavé, J. Natural Products, 42, 133 (1979).
3. For an excellent description of the mountains and parts of the flora and fauna of Chitral as well as of the neighboring district of Gilgit, see George B. Schaller, Stones of Silence, Journeys in the Himalayas, The Viking Press, New York (1980).
4. TLC R_f values in this system are as follows: Compound 1 0.18, compound 3 0.24, compound 4 0.55, compound 6 0.47, and compound 9 0.58. Kalashine and its diacetate derivative were obtained in such small amounts that they could not be crystallized.
5. M. Shamma, J.L. Moniot, S.Y. Yao, G.A. Miana and M. Ikram, J. Am. Chem. Soc., 95, 5742 (1973
6. S.F. Hussain, M.T. Siddiqui, G. Manikumar and M. Shamma, Tetrahedron Lett., 723 (1980).
7. For a summary of the uv and nmr spectra of aporphine-benzylisoquinoline dimers, see M. Shamma, The Isoquinoline Alkaloids, Academic Press, New York (1972), pp. 238-239; M. Shamma and J.L. Moniot, Isoquinoline Alkaloids Research, 1972-1977, Plenum Press, New York (1979), p. 165; and Ref. 2 above.
8. W.-N. Wu, J.L. Beal and R.W. Doskotch, Lloydia, 40, 508 (1977).
9. The cd $\Delta\varepsilon_{nm}^{MeOH}$ values are as follows: Kalashine (1) $+9.54_{280}$, -50.59_{235} and $+41.04_{214}$; 1-O-methylkalashine (7) $+6.57_{285}$, -5.47_{260}, -60.81_{236} and $+37.25_{212}$; pakistanine (3) $+17.62_{3}$ $+32.04_{274}$, -52.86_{244} and $+80.10_{212}$; 1-O-methylpakistanine (6) $+12.82_{280}$, -61.18_{241} and $+24.67_{220}$; and 1,10-di-O-methylpakistanine (9) $+14.49_{277}$, -76.07_{240} and $+66.65_{212}$. The absolute configuration of pakistanine (3) and pakistanamine (2) has been established previously, see Ref. 5 above.
10. Pakistanine (3) could, alternatively, originate biogenetically from O-demethylation of 1-O-methylpakistanine (6), in which case it would clearly be a natural product and not an artefact of isolation.
11. All elemental analyses are by mass spectroscopy.

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