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ALKALOIDS OF *Arundo donax*
VII. A SPECTROSCOPIC AND X-RAY STRUCTURAL INVESTIGATION OF ARUNDININE — A NEW DIMERIC ALKALOID FROM THE EPIGEAL PART OF *Arundo donax*

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From Arundo donax *L. we have isolated the new dimeric alkaloid arundinine, with the composition $C_{23}H_{28}N_4O$, the structure of which has been established on the basis of its physicochemical characteristics and ^1H and ^{13}C NMR spectroscopy, including 1D and 2D experiments in both homo- and heteronuclear regimes. The proposed structure — 3a-(3'-dimethylaminoethylindol-5'-yloxy)-1-methylpyrrolidino[1a,3a — b]indoline — has been confirmed by x-ray structural analysis (XSA).*

The isolation from the epigeal part and roots of *Arundo donax* L. (fam. Gramineae) gathered in the environs of the village of Kumkishlak, Kashkadar'inskaya oblast, and in the botanical gardens of Tashkent and Samarkand of 10 alkaloids of the pyrrolidine and indolylalkylamine series has been reported previously. The structures of six of them have been established on the basis of chemical transformations, spectral characteristics, and XSA [1].

The plant *A. donax* is widely used in folk medicine as a sudorific and diuretic agent and also for the treatment of feminine diseases. The valuable biological properties of the main alkaloid of *A. donax* — donaxine — have served as a stimulus to a search for new raw material sources of this plant in Uzbekistan. With this aim, we have studied for the first time the epigeal part and roots of *A. donax* in two vegetation periods from a new growth site — the Fergana valley of Uzbekistan. As a result of these investigations we have isolated eight alkaloids of known structure (donaxine, donaxarine, donaxaridine, donaxanine, donine, deoxyvasicinone, phenyl-β-naphthylamine, and arundine) [4] and a white crystalline base with mp 148—150°C, composition $C_{23}H_{28}N_4O$, readily soluble in acetone, chloroform, and methanol and moderately soluble in benzene and ethyl acetate, which we have called arundinine (**1**).

The UV spectrum of (**1**) showed absorption maxima at 206, 226, and 288 nm (log ε 4.49, 4.02, 3.48). The IR spectrum contained absorption bands of active hydrogen (3397 cm^{-1}), of —CH_3 and —CH_2 groups (3040—2826 cm^{-1}), and of the asymmetric stretching vibrations of a C—O—C bond, and also those of an aromatic ring (1609, 820, and 748 cm^{-1}). The mass spectrum included the peak of the molecular ion with *m/z* 376, while the peak of maximum intensity had *m/z* 173. It is important to note that the presence of peaks of ions with *m/z* 130 and 173 in the mass spectrum of (**1**) is typical for indolyl alkaloids. A comparative study of the general mass spectra of arundinine and donaxine [5], isolated from the same plant, and also the results of a determination of the elementary composition of (**1**) gave grounds for assuming the presence in the (**1**) molecule of the elements of the structure of donaxine.

The spectral characteristics of (**1**) and its molecular mass show that the structure of the alkaloid is of dimeric nature, one half of the dimer having the structure of donaxine substituted in the aromatic ring. The peak of an ion with *m/z* 204 having the elementary composition $C_{12}H_{16}N_2O$ shows that the link of the donaxine monomer to the other half of the structure of (**1**) is made through an oxygen function. The presence in the mass spectrum of the peak of an ion with *m/z* 190 having the elementary composition $C_{11}H_{14}N_2O$ shows the structure of the second half of the molecule as consisting of three rings and

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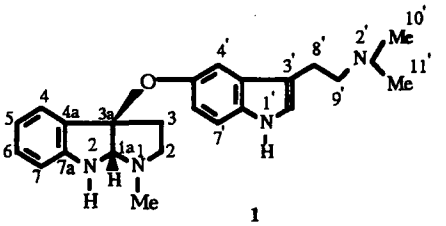
resembling the nucleus of the alkaloid alline from plants of the genus *Allium* [6], which contains a physostigmine skeleton [7].

It must also be mentioned that arundinine is the first dimeric alkaloid of the *Arundo donax* series in which the bond between the monomeres is effected through an ether bridge. On the basis of UV, IR, and mass spectroscopy and ^{1}H and ^{13}C NMR results, including 1D and 2D experiments both in homo- and heteronuclear regimes, structure (1), which follows from a logical analysis of the whole combination of available information, is proposed for arundinine:



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The PMR spectrum of arundinine in $CDCl_3$ is represented by two groups of signals: those of aromatic protons and those of aliphatic protons. Two one-proton singlets from two NH groups are observed at 7.94 and 4.24 ppm and also the signals of a methine proton at 4.93 ppm (s). The signals of the amine protons consist of lines broadened to 12.8 and 7.40 Hz, while the signal of the methine proton is a line with a width of 2.9 Hz. Double resonances relating the amine protons exhibit small SSCCs of N2-H with the H-1a, H-4, and H-7 protons (≈0,5 Hz) and of N1′—H with the H-2′, H-4′, and H-7′ protons (2.3 Hz, ≈0.5 Hz, and very small, respectively). Small SSCCs from the H-2′ proton on the signals of the H-6′ proton and on the methylene pair of H-8′ protons of the side-chain of the alkaloid were also detected, by differential methods. When the H-1a methine proton was irradiated, however, no hidden SSCCs were detected, but two weak Overhauser effects appeared: 3.9% on the H-4′ proton and 2.4% on the H-6′ proton of the second half of the dimeric alkaloid; i.e., the spatial structure of the alkaloid in solution permits a fairly close arrangement of the NH amine proton and the benzene ring of the second half of the dimer.

The aliphatic part is represented by two N-methyl singlets (three-proton from the cyclic nitrogen and six-proton from the nitrogen of the side-chain) and six complex multiplets of eight methylene protons. The H-8′ and H-9′ methylene protons form an *AA′BB′* spin system with degeneracy in relation to the chemical shifts but with retention of the differences in the SSCCs; $J_{AB} \neq J_{AB'}$. This is connected with a retardation of the rotation of the side chain around the C8′—C9′ bond because of the heavy termination of the side-chain — $N(CH_3)_2$. In view of this, for the methylene protons it is possible to give only the values of the chemical shifts and the sum of the SSCCs. The signals of the H-2 and H-3 methylene protons, however, in spite of their partial overlapping, permit the complete reproduction of their chemical shifts. In order to refine the parameters, this four-spin system was calculated fully with the aid of the LAOCON-5 spin-simulation program. A comparison of the experimental and calculated spectra is given in Fig. 1.

The aromatic part of the spectrum is represented by eight well-resolved signals. A homocorrelation two-dimensional experiment (COSY-45) and the long-range constants of the amine protons permit a complete assignment of all the signals with the indication of all the SSCCs of the two individual aromatic systems and of one olefinic proton — H-2′. The latter appears in the form of a doublet with the SSCC 2.28 Hz. A detailed description of these spin systems is given in Table 1.

Table 1 also gives the chemical shifts of the NMR signals on the ^{13}C carbon isotope. The assignment of the ^{13}C carbon signals was made on the basis of two-dimensional heterocorrelation spectra with respect to short-range (HMQC) and long-range (HMBC) constants.

The following conclusions can be drawn on the stereochemistry of the arundinine molecule. The presence of small Overhauser effects of H-1a on the H-4′ and H-6′ protons may correspond to some "mean effective distance" of the H-1a proton from these aromatic protons, H-4′ and H-6′, of approximately 3—5 Å. Consequently, in solution in $CDCl_3$ the arundinine molecule has a characteristic configuration in which the H-1a proton and the C3a—O ether bond are *cis*-oriented on the C1a—C3a bond. The first half of the alkaloid turns around the C3a—O bond in such a way that the H-1a proton is oriented in the direction of the C5′ carbon. Rotation of the first half of the alkaloid around the C3a-O bond is impossible because of the voluminous substituents on the C3a carbon on the side of the C4a and C3 carbons. The second half of alkaloid (1) is planar and is oriented perpendicularly to the C3a—O bond. It cannot rotate around the C5′—O bond, either, because of its large volume and the linear substituent at the C3′ carbon. Only two possible orientations remain for it, the choice between which cannot be made otherwise than by means of XSA.

TABLE 1. Chemical Shifts and SSCCs of Arundinine in $CDCl_3$ (TMS - 0)

Proton	^{13}C, ppm	^{1}H, ppm	SSCC, Hz
1a	84.59	4.933	
3a	96.96	-	
4a	130.41	-	
4	124.89	7.301	7.42(H-5), 1.32(H-6), 0.52(H-7)
5	119.28	6.770	7.42(H-4), 7.54(H-6), 0.98(H-7)
6	129.63	7.111	7.80(H-7), 0.98(H-5), 0.52(H-4), 0.52(N2-H)
7	110.30	6.620	
7a	151.01	-	8.35(H-2a), 6.50(H-3e), 5.10(H-3a)
2_{eq}	51.87	2.893	8.35(H-2e), 8.00(H-3e), 5.42(H-3a)
2_{ax}		2.687	12.05(H-3a), 8.00(H-3a), 6.50(H-2a)
3_{eq}	40.87	2.767	12.05(H-3e), 5.42(H-2a), 5.10(H-2e)
3_{ax}		2.331	2.28(N1'-H)
2'	122.20	6.900	
3'	114.16	-	
3'a	127.52	-	2.28(H-6')
4'	111.16	6.828	
5'	149.55	-	8.71(H-7'), 2.28(H-4')
6'	116.78	6.696	8.71(H-6')
7'a	109.03	7.079	
7'	132.29	-	Σ SSCC=16.09
8'	60.00	2.732	Σ SSCC=16.57
9'	23.52	2.444	
NCH_3	37.47	2.474	
$N(CH_3)_2$	45.34	2.297	
N2-H	-	4.236	
N1'-H	-	7.939	

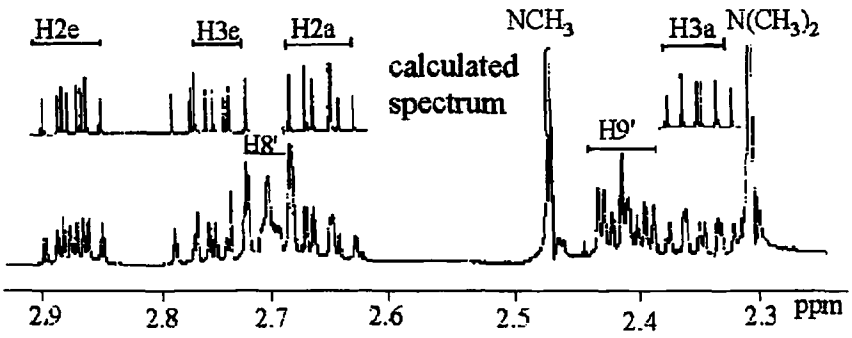

Fig. 1. Aliphatic part of the PMR spectrum of arundinine in $CDCl_3$.

With the aim of determining the spatial structure of arundinine unambiguously, we made an x-ray structural analysis of a single crystal of it. Figure 2 shows the structure of the arundinine molecule according to the XSA results. As can be seen from this figure, the molecule contains fragments of the alkaloids alline (the N1, C1a—C8, N2, and O atoms) [6] and donaxine (the N1', C2'—C9', C-10', C-11', and N2' atoms) [5]; i.e., the dimeric alkaloid is formed from known monomers as the result of the dehydration of the hydroxy group of alline and an aromatic proton of donaxine. It can be seen from an analysis of the torsion angles and Fig. 2 that the donaxine half of the molecule has a planar form, while in the alline half the five-membered ring with the N-methyl group assumes a half-chair form (the N1 and C2 atoms deviate in opposite directions by +0.036 and −0.032 Å, respectively). In this part, the indoline nucleus is *cis*-linked with the five-membered heterocycle, which agrees with what is found in alline itself [6]. However, the N-methyl group in (1) is β-oriented, by which it differs from what is observed

TABLE 2. Coordinates ($\times 10^3$) of the Nonhydrogen Atoms in the (1) Molecule

Atom	x	y	z	Atom	x	y	z
O	336(7)	-349(5)	495(2)	N2	554(7)	-77(5)	356(2)
N1'	1069(7)	-765(5)	370(2)	N1	364(7)	45(5)	492(2)
N2'	618(7)	-636(5)	38(2)	C1a	476(7)	-98(5)	450(2)
C2'	1005(7)	-810(5)	287(2)	C3a	269(7)	-202(5)	448(2)
C3'	817(7)	-730(5)	274(2)	C4a	266(8)	-211(5)	352(2)
C4'	547(7)	-526(5)	372(2)	C4	86(7)	-275(5)	305(3)
C5'	548(7)	-447(5)	460(2)	C5	96(8)	-266(6)	217(3
C6'	715(8)	-469(5)	516(2)	C6	251(8)	-207(5)	163(2)
C7'	906(7)	-572(5)	490(2)	C7	425(8)	-134(5)	198(2)
C3'a	750(7)	-629(5)	353(2)	C7a	411(7)	-140(5)	293(2)
C7'a	914(7)	-643(5)	405(2)	C2	202(8)	-2(5)	565(2)
C8'	679(7)	-751(5)	197(2)	C3	91(7)	-116(5)	515(2)
C9'	699(8)	-615(5)	125(2)	C8	533(8)	135(5)	534(2))
C10'	365(8)	-633(6)	40(3)				
C11'	676(9)	-522(6)	-28(3)				
			Chloroform molecules				
Cl1	751(8)	-97(5)	960(2)	Cl5	352(7)	707(5)	776(2)
Cl2	1097(7)	61(5)	907(2)	Cl6	-54(7)	634(5)	744(2)
Cl3	749(7)	90(5)	800(2)	C1	807(8)	66(6)	911(2)
Cl4	315(8)	395(5)	745(2)	C2	211(8)	590(6)	718(2)

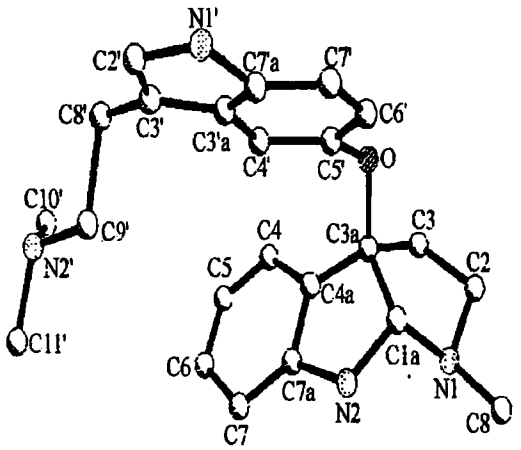

Fig. 2. Spatial structure of arundinine from the results of XSA.

in alline [6]. The mutual positions of the two monomers in space favor an approach of the H-1a atom (alline moiety) to the aromatic atom H-6′ (donaxine moiety) (the torsion angle C1a—C3a—O—C5′ is -56° and the H-1a···H-6′ distance is 2.99 Å). This situation is also shown by the Overhauser effects found on analyzing the NMR spectra, which demonstrates the conformational stability in solution of the two monomers relative to one another.

Two chloroform molecules located at Van-der-Waals distances from the base participate in the packing of the arundinine molecule. No intermolecular interactions involving active H atoms and unshared electrons are observed in the crystal.

EXPERIMENTAL

Isolation of Arundinine. The isopropanol extraction of 5 kg of the epigeal part of *A. donax* gathered in the period of vigorous growth gave a total of 23 g (0.46%) of bases. Chromatographic separation of the total alkaloids was carried out on a column of alumina (Brockmann activity grade II).

The mixture of bases (23 g) was deposited on a column containing 350 g of Al_2O_3. The alkaloids were eluted from the column successively with hexane, hexane—ether, chloroform, and chloroform—methanol. Fractions 16—40 of the chloroform eluates yielded 40 mg of arundinine, R_f 0.85 and 0.10 (TLC, Al_2O_3 and silica gel, respectively, chloroform—methanol (9:1) system).

General Observations. IR spectra were taken on a Perkin-Elmer Fourier IR spectrometer (model 2000) in KBr tablets; mass spectra on a MKh-1310 spectrometer using a system for the direct injection of the specimen into the ion source at a temperature of the ionization chamber of 150°C and of the evaporator tube of 70—80°C, with an ionizing energy of 70 eV.

PMR spectra were recorded on a Varian UNITY-400$^+$ spectrometer with a working frequency for protons of 400 MHz. Homocorrelation spectra for the partial suppression of diagonal peaks were recorded by the COSY-45 method. Indirect recording of the peaks of carbon signals in heterocorrelation spectra was carried out by the standard HMQC and HMBC methods. The values of the SSCCs were refined by the LAOCON-5 integration program.

X-Ray Structural Analysis. Colorless crystals of arundinine in the form of elongated prisms were grown from chloroform solution. The unit cell parameters and the space group were determined on a Syntex-$P2_1$ diffractometer using CuK_α radiation: a = 6.113(1), b = 8.720(2), c = 14.378(3) Å; α = 89.54(3), β = 83.58(3), γ = 82.83(3)°; V = 755.6 Å^3, d_{calc} = 1.352 g/cm^3, space group P1, Z = 4. After primary treatment, the set of experimental reflections obtained on the above-mentioned diffractometer numbered 1361, with the structural amplitudes $|F| \geq 2\sigma(F)$. The structure was interpreted by the direct method and was refined in the full-matrix anisotropic approximation for the nonhydrogen atoms. The positions of the hydrogen atoms were calculated and refined in the isotropic approximation. All the calculation were made by the SHELXTL-PC program.

Table 2 gives the coordinates of the nonhydrogen atoms from the last stage of MLS refinement (R = 0.134 and R_w = 0.130). The smallness of the amount of substances isolated made it difficult to obtain single crystals of the necessary quality for an x-ray structural experiment, and for this reason it was impossible to obtain a good set of statistical figures and, consequently, to decrease the value of the discrepancy factor.

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