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ALKALOIDS OF *Arundo donax*.

VI. THE STRUCTURE OF DONINE

V. U. Khuzhaev, S. F. Aripova,
and U. A. Abdullaev

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A new dimeric alkaloid donine with the composition $C_{19}H_{22}N_2O_4$ has been isolated from the epigeal part of Arundo donax. *On the basis of chemical transformations and UV, IR, mass, PMR, and ^{13}C NMR spectra the structure of diethyl N,N'-methylenebis(4-aminobenzoate) is proposed for this alkaloid.*

Continuing an investigation of the alkaloid composition of the plant *Arundo donax* L. gathered in the environs of the village of Kumkishlak in the Kashkadar'inskaya province of Uzbekistan [1, 2], by chromatographing the ether fraction on a column of alumina we have isolated donaxine and a new base with mp 126-128°C (methanol), which we have called donine (1).

Donine has the composition $C_{19}H_{22}N_2O_4$ (HRMS 342.1605). The UV spectrum of (1) contained two absorption maxima: at 246 and 201 nm (log ε 4.62 and 4.76). The IR spectrum of the base showed absorption bands of active hydrogen (3321 cm^{-1}), of methyl and methylene groups (2908-3045 cm^{-1}), of an ester carbonyl group (1703 cm^{-1}), and of a disubstituted benzene ring (1610, 860, 838, 770 cm^{-1}).

In the mass spectrum of (1), we observed the peak of the molecular ion (M^+ 342), with the maximum intensity, and the peaks of fragmentary ions with *m/z* 313, 296, 267, 250, 223, 221, and 132.

After donine had been treated with CD_3OD, there was a displacement of the peak of the molecular ion in the mass spectrum by 2 a.m.u. in the direction of high masses, which showed the presence of two active hydrogen atoms in the molecule.

In the PMR spectrum of (1) taken in deuteropyridine the signals of the protons of two symmetrical methyl groups appeared at 1.13 ppm in the form of a six-proton triplet. A two-proton singlet characteristic of a methylene group linked to nitrogen ($NH-CH_2-$) was seen at 3.89 ppm. A four-proton quartet from two methylene groups linked to methyl groups was observed at 4.20 ppm. The signals of aromatic protons appeared at 7.00 and 7.22 ppm in the form of a doublet of doublets (J = 8 Hz), (8H, dd, J = 8 Hz, H-Ar), which unambiguously showed *para*-substitution in the aromatic ring.

To confirm the structure of donine found in this way, we compared its PMR spectrum with that of ethyl aminobenzoate. Comparative analysis showed almost complete analogy in the nature of the signals of the two compounds.

In the ^{13}C NMR spectrum of (1) 10 carbon atom signals were observed (Table 1). From their chemical shifts, the signals in the spectrum could be grouped in the following way: four signals of tertiary carbons and two signals of quaternary carbons appeared in the aromatic part of the spectrum, and three signals of the secondary carbons of methylene groups and two signals of primary carbons of methyl groups in the aliphatic region. The carbon atoms of the carbonyl groups appeared at 154 ppm. The shift of the signal of the carbon atoms of the carbonyl groups is due to the inductive efect of the nitrogen atom.

The alkaline hydrolysis of (1) formed substance (2) (Scheme 1). The mass spectrum of (2) gave the peak of the molecular ion with *m/z* 286, differing from that of the initial base by 56 *m/z*, which showed the possible splitting out of two ethanol molecules on saponification.

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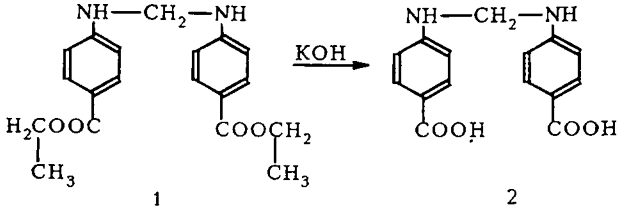
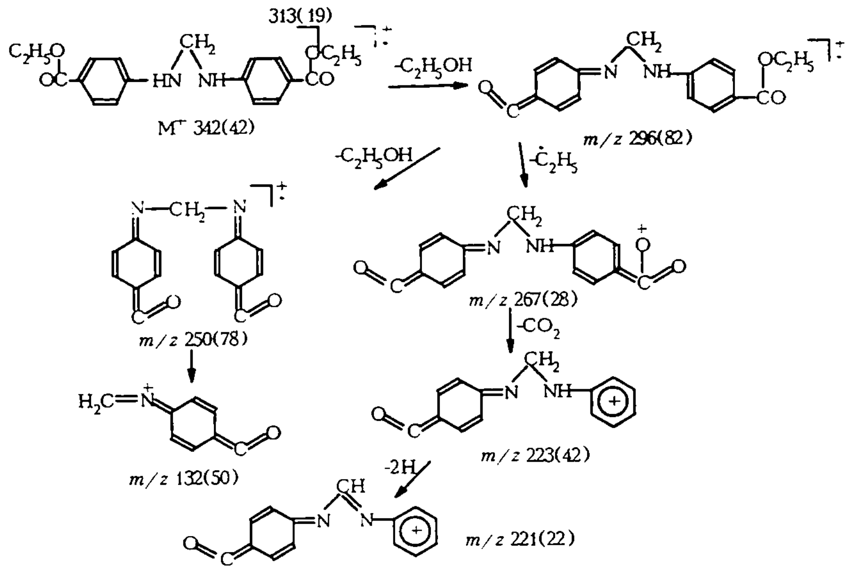


Scheme 1. Chemical transformation of donine (**1**).



Scheme 2. Mass-spectrometric fragmentation of donine (**1**).

Assignments of the signals of the protons and carbon atoms of the aromatic nuclei and also of $-CO$, $-OC_2H_5$, and CH_2 groups were made with the aid of the PMR and ^{13}C NMR spectra. A provisional composition of donine, $C_{10}H_{13}NO_2$, was established. However, the mass spectrometric results showed an almost doubled value of the molecular mass, and a measurement of the elementary composition of the molecular ion indicated doubled numbers of nitrogen and oxygen atoms (Table 2). Because of this, we subjected almost all the ions to elementary analysis. Thus, the first act of fragmentation of the M^+ ion was the ejection of C_2H_5 and C_2H_5OH fragments with the formation of ions having *m/z* 313 and 296, respectively. To our surprise, the ejection of the above-mentioned fragments was repeated from the ion with *m/z* 296, leading to ions with *m/z* 267 and 250. Such a route of the elimination of ethanol from a M^+ ion has been discussed in detail in [3] for the case of pyrrolidinecarboxylic acids.

In the spectrum of the deuterated analog of donine a shift of the peak of the M^+ ion in the direction of higher masses by 2 a.m.u. was observed; the shift of the peak of the $(M - C_2H_5OH)^+$ ion amounted to 1 a.m.u., while the peak of the $(M - C_2H_5OH)^+$ ion underwent no displacement [sic].* These facts indicate the participation of both active hydrogen ions in the successive elimination of C_2H_5OH molecules.

We may note that in both cases the elimination of the ethoxy grouping took place with the migration of hydrogen to the ethoxy group, and the intensity of the $(M - 45)^+$ and $(M - 46 - 45)^+$ peaks is included in the intensities of the isotopic ion peaks with *m/z* 296 and 250, respectively. It is reported in [3] that in the case of the mass spectra of ethyl 2-methylpyrrole-3-carboxylate and of 3-methylpyrrole-2-carboxylate, where a favorable situation is created for the migration of a hydrogen atom to the ethoxy group, an intense peak of the $(M - C_2H_5OH)^+$ is observed. In the case of the isomeric ethyl 2-methylpyrrole-4-carboxylate the peak of the $(M - C_2H_5O)^+$ ion is intense.

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*The second "$(M - C_2H_5OH)^+$" probably should be "$(M - C_2H_5O)^+$" — Translator.

TABLE 1. Chemical Shifts of the ^{13}C Atoms in the NMR Spectrum of Donine (in C_5H_5N, ppm, 0 — TMS)

CH		CH_2		CH_3		Quat. carbons	
atom carbon	ppm	atom carbon	ppm	atom carbon	ppm	atom carbon	ppm
C-3	129.7[a]					C-1	138.5
C-4	119.3[a]	C-8	60.64	C-9	14.70	C-2	149.8
C-5	123.4[a]	C-10	40.80			C-7	154.7
C-6	135.5[a]						

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*Possible alternative assignment of the signals.

TABLE 2. Results of a Mass-Spectrometric Study of Donine

m/z of the ions	Mass, m/z		Composition
	found	calculated	
M^+ 342	342.1605	342.1580	$C_{19}H_{22}N_2O_4$
313	313.1189	313.1188	$C_{17}H_{17}N_2O_4$
296	296.1162	296.1161	$C_{17}H_{16}N_2O_3$
267	267.0774	267.0770	$C_{15}H_{11}N_2O_3$
250	250.0748	250.0742	$C_{15}H_{10}N_2O_2$
223	223.0905	223.0917	$C_{14}H_{11}N_2O$
221	221.0726	221.0705	$C_{14}H_9N_2O$
132	132.0445	130.0449	C_8H_6NO

The comparative results on the formation of the $(M - C_2H_5OH)^+$ ions showed that donine could be a derivative of either *ortho*- or *para*-aminobenzoic acid. However the signals of the protons of the substituted benzene nucleus in the PMR spectra unambiguously showed the *para*- arrangement of the substituents. In this case, so far as concerns the ejection of a C_2H_5OH molecule from M^+, a part is possibly played by the state of the aromatic nucleus, which spontaneously passes into the *para*-quinoid form, as shown in Scheme 2.

On the basis of the mass, PMR, and ^{13}C NMR spectra it may be stated that donine is a dimeric compound formed from two molecules of ethyl *para*-aminobenzoate linked through a nitrogen atom by a methylene group and having the structure of diethyl N,N'-methylenebis(4-aminobenzoate).

EXPERIMENTAL

General Observations. The chromatographic separation of the total alkaloids was conducted on column of alumina (Brockmann activity grade II). The following systems were used for TLC: 1) petroleum ether—benzene (1:1); 2) benzene—chloroform (1:1); 3) benzene—methanol (9:1). IR spectra were taken on a Perkin-Elmer model 2000 spectrometer in tablets with KBr, mass spectra on a MKh-1310 spectrometer (EI source), PMR spectra on a Tesla BS 567 A/100 instrument, and ^{13}C NMR spectra on a Bruker AM/400 MHz instrument (Py-d_5, 90°) in the regime of complete decoupling from protons and under J-modulation conditions.

Isolation of Donine (1). The material (6 g) from the acetone mother liquor after the separation of donaxine was chromatographed on a column of alumina. The alkaloids were eluted with chloroform, and 15 fractions of 150-200 ml were collected. Fractions 1-3 (0.900 g) were rechromatographed on a column of alumina (system 1). Fractions 3-8 (20-30 ml each) yielded 0.080 g of donine, mp 126-128°C (methanol), readily soluble in chloroform, methanol, ethanol, and benzene, and sparingly soluble in petroleum ether and hexane; R_f 0.2 (TLC, Al_2O_3, system 2), R_f 0.82 (Al_2O_3, system 3), giving a blue coloration with the Dragendorff reagent.

Saponification of Donine. A solution of 0.03 g of donine in 5% methanolic KOH was heated on the water bath for 60 min. The solvent was distilled off, the residue was diluted with water, and the alkaloids were extracted with chloroform. After the chloroform had been distilled off, 0.01 g of base (**2**) was obtained (yield 33%).

The D-analog of donine was obtained by briefly steeping a sample in CD_3OD.

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