α- AND β-SCOPODONNINES FROM Datura inoxia SEEDS

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A mixture of two alkaloids, d, ℓ-α- and β-scopodonnines, known earlier as synthetic products, has been isolated for the first time from the seed of Datura inoxia. The spatial structure of β-scopodonnine dimethiodide has been established by the x-ray structural method.

Continuing the separation of the total alkaloids of the seeds of Datura inoxia cultivated in the Darmina Sovkhov [communal farm] of Lekrasprom SSSR [All-Union Combine for the Production, Harvesting, and Processing of Medicinal Plants, Ministry of the Medical Industry of the USSR], Chimkent Province, by chromatography on columns of silica gel we have isolated a crystalline mixture of two bases, which were difficult to separate. By the use of rechromatography and preparative chromatography we obtained bases with mp 190-191°C and 178-179°C. Their IR spectra, with absorption bands at 700-710 and 775-770 cm^{-1} (mono- and 1,2-disubstituted benzene rings) and 1730 cm^{-1} (ester carbonyl group), were close. Their UV spectra each showed maxima at 254, 259, and 263 nm (log ε 2.63, 2.67, 2.66), which are characteristic for substituted benzene rings. The molecular masses of the bases, confirmed by the two peaks of molecular ions with m/z 570, corresponded to those of the dimer of apohyoscine [1] and showed the "dimeric" nature of the alkaloids. Their spectra contained peaks of ions characteristic for alkaloids with substituted tropane rings (154, 138, 94, and 81 a.m.u.). The nature of the fragmentation resembled that of apohyoscine. The strongest peaks in the mass spectra of the two bases were the same, and differences consisted only in the intensities of some of the peaks (Table 1).

The strongest peak in the spectrum of each of the two bases was that of an ion with m/z 138, corresponding to the residue of a 3,6,7-trisubstituted tropane ring. The PMR spectra of the alkaloids each contained two one-proton triplets (C3α'H and C3α"H) and two three-proton singlets due to the protons of two N-methyl groups. The signals of nine aromatic protons in the PMR spectrum of each of the bases appeared in the form of broadened singlets (9 H) at 7.26 and 7.28 ppm (Table 2).

The spectral characteristics of the two alkaloids show that they were α- and β-scopodonnines (I),* which have been synthesized previously by German scientists [2] although we are the first to have isolated them from a plant.

The isolation of the "dimeric" alkaloids α- and β-scopodonnines from a plant rich in ℓ-hyoscine and of α- and β-belladonnines from a plant containing considerable amounts of hyoscyamine has been discussed in the literature [3]. The idea has been expressed that the possibility of the isolation of alkaloids of this type from plants is more likely at later

TABLE 1. Mass Spectra of α- and β-Scopodonnines (the relative intensities of the peaks of the ions are given)

Alkaloid	m/z (%)									
	570 (M)	389	154	138	108	97	94	81	57	55
α-Scopodonnine	33	7	20	100	20	14	40	10	48	36
β-Scopodonnine	82	27	30	100	79	19	66	12	6	5

*The α- and β-forms of isatropic acid (or of the scopodonnines) are determined by the mutual syn and anti orientations, respectively, of the hydrogen atom at C4 relative to the carboxy substituent at C1.

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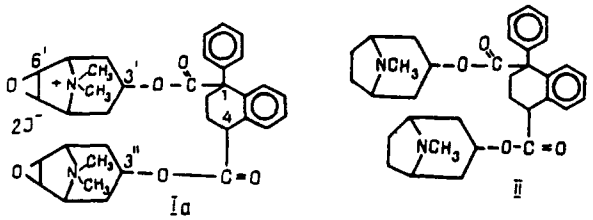
TABLE 2. NMR Spectra of α- and β-Scopodonnines

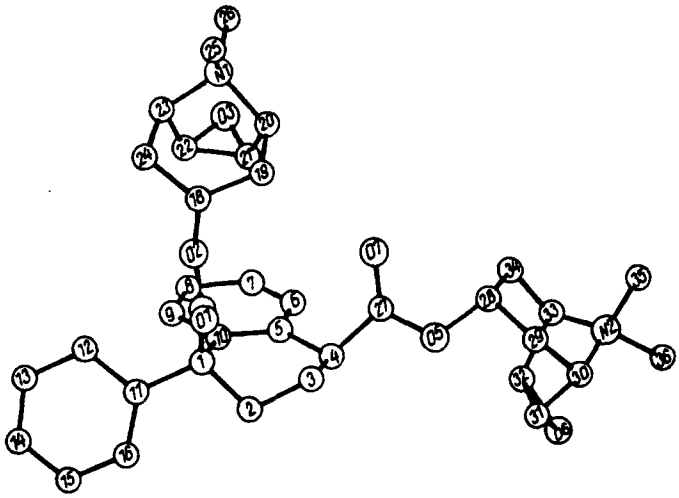
Alkaloid	Chemical shifts, δ scale, ppm						
	NCH₃	NCH₃	3'-Hα 3"-Hα	H-1', 1" H-5', 5"	H-2', 2"; H-6', 6" H-4', 4"; H-7', 7"	C—H	Ar
α-Scopodonnine	2,41 s	2,45 s	4,97 t 5,09 t	3,00 q	1,20 – 2,25 m	3,82	7,28 (7H), s
β-Scopodonnine	2,44 s	2,49 s	5,07 t 5,14 t	3,08 q	1,35 – 1,30 m	3,84 t	7,26 (7H), s

stages of the vegetation process than in the earlier periods of vegetation, and this has been observed in our case.



Ia II

It was reported in 1858 that when atropine was subjected to prolonged heating a new dimeric base called belladonnine (II) was formed which later proved to be a mixture of d,-ℓ-α- and β-belladonnines [4]. In 1973 both isomers were isolated for the first time from the plant Physochlaina alaica [5]. Later, in a similar way, two products were obtained from an alkaloid of the scopolamine series, ℓ-hyoscine, one of which was named scopodonnine [3]. The second product was not characterized. We have repeated this reaction and have isolated two products which are apparently isomers. In terms of mixed melting points and spectral characteristics, these proved to be identical with the natural bases that are the subject of this paper.



Structure of the cation in β-scopodonnine dimethiodide.

According to the literature, the alkaline hydrolysis of scopodonnine with mp 190-191°C [2] yielded β-isatropic acid, from which it follows that the initial alkaloid had to be assigned to the β-series. However, it is known from the literature that the alkaline hydrolysis of α- and β-belladonnines always leads to the β-isatropic acid. Apparently, under the conditions of hydrolysis there is a mutual transformation of α- and β-isatropic acids. Consequently, the production of one or other acid in the hydrolysis of a base may lead to an erroneous conclusion relative to the stereochemistry of the initial alkaloid.

On the basis of what has been said above and because it is impossible from the UV, IR, mass, and NMR spectra of the two alkaloids and their hydrolysis products to state unambiguously which of the isomers of (I) is the α- and which the β-form and also in order to refine certain stereochemical features of the structure of bimolecular "dimeric" alkaloids of this

TABLE 3. Bond Lengths (α, Å) and Valence Angles (ω, deg) in the Structure of (Ia)

Bond	r	Bond	r	Angle	ω	Angle	ω	Angle	ω
C1—C2	1.56	C20—C21	1.47	C10C1C2	109	C11C12C13	111	N1C23C24	110
C1—C10	1.53	C21—O3	1.43	C11C1C2	111	C12C13C14	121	C22C23C24	127
C1—C11	1.55	C21—C22	1.43	C11C1C10	114	C13C14C15	120	C18C24C23	113
C1—C17	1.56	C22—O3	1.43	C17C1C2	106	C14C15C16	120	O4C27O5	123
C2—C3	1.49	C22—C23	1.51	C17C1C10	110	C11C16C15	120	O4C27C4	126
C3—C4	1.57	C23—N1	1.56	C17C1C11	107	O1C17O2	124	O5C27C4	111
C4—C5	1.52	C23—C24	1.53	C1C2C3	111	O1C17C1	123	C27O5C28	117
C4—C27	1.51	C25—N1	1.50	C2C3C4	110	O2C17C1	112	O5C28C29	104
C5—C6	1.41	C26—N1	1.47	C3C4C5	115	C17O2C18	118	O5C28C34	112
C5—C10	1.38	C27—O4	1.21	C3C1C27	109	O2C18C19	109	C29C28C34	117
C6—C7	1.38	C27—O5	1.36	C5C4C27	112	O2C18C24	105	C28C29C30	112
C7—C8	1.43	C28—O5	1.43	C4C5C6	119	C19C18C24	116	N2C30C29	111
C8—C9	1.38	C28—C29	1.56	C4C5C10	122	C18C19C20	114	N2C30C31	104
C9—C10	1.43	C28—C34	1.58	C6C5C10	119	N1C20C19	108	C29C30C31	104
C11—C12	1.40	C29—C30	1.55	C5C6C7	125	N1C20C21	105	O6C31C30	117
C12—C13	1.38	C30—N2	1.50	C6C7C8	118	C19C20C21	107	O6C31C32	105
C13—C14	1.37	C30—C31	1.51	C7C8C9	120	O3C21C20	117	C30C31C32	106
C14—C15	1.36	C31—O6	1.51	C8C9C10	120	O3C21C22	60	C31O6C32	63
C15—C16	1.40	C31—C32	1.52	C5C10C1	121	C20C21C22	109	O6C32C31	62
C17—O1	1.21	C32—O6	1.40	C1C10C9	119	C21O3C22	60	O6C32C33	120
C17—O2	1.32	C32—C33	1.53	C5C10C9	120	O3C22C21	61	C31C32C33	107
C18—O2	1.48	C33—N2	1.55	C1C11C12	123	O3C22C23	117	N2C33C32	101
C18—C19	1.49	C33—C34	1.48	C1C11C16	119	C21C21C23	117	N2C33C33	109
C18—C24	1.55	C25—N2	1.50	C12C11C16	118	N1C23C22	104	C32C33C34	113
C19—C20	1.55	C36—N2	1.56					C28C34C33	111
C20—N1	1.54								

TABLE 4. Coordinates (×10⁴) of the Nonhydrogen Atoms of Structure (Ia)

Atom	x	y	z	Atom	x	y	z
C1	4702(14)	6168(8)	8673(9)	C24	3121(16)	3136(9)	8457(10)
C2	4329(17)	7479(9)	9162(11)	C25	0313(20)	2022(9)	7586(11)
C3	2589(18)	7442(9)	8835(11)	C26	—3405(21)	1651(13)	5369(15)
C4	1932(15)	7024(8)	7695(11)	C27	0324(16)	6861(9)	7327(9)
C5	239 (15)	6579(9)	7111(9)	C28	—1092(16)	7392(9)	7163(11)
C6	2937(18)	6226(11)	6469(13)	C29	—2408(21)	8287(12)	782 (13)
C7	2616(21)	5609(12)	5475(11)	C30	—2814(19)	9383(9)	7332(11)
C8	3088(19)	5308(9)	5047(11)	C31	—1463(24)	9413(11)	7213(16)
C9	4357(17)	5609(9)	6061(10)	C32	—1636(2)	8771(12)	6185(10)
C10	3985(14)	6226(8)	7555(5)	C33	—3945(2)	8942(11)	5760(13)
C11	6400(15)	6477(9)	9293(9)	C34	—2810(17)	7133(9)	6734(11)
C12	7445(16)	7338(9)	9510(11)	C35	—5334(35)	8924(2)	6221(15)
C13	8943(2)	7372(18)	9828(14)	C36	—4731(2)	9386(14)	5815(17)
C14	9441(2)	6916(17)	9607(13)	N1	0593(12)	2398(7)	6824(9)
C15	8169(23)	6358(16)	9289(14)	N2	—3963(14)	8628(8)	6297(9)
C16	6041(16)	6504(11)	8968(11)	O1	4067(13)	5874(7)	9827(8)
C17	4000(14)	5713(9)	8736(9)	O2	3490(10)	4859(6)	8281(6)
C18	2591(16)	4107(9)	8462(9)	O3	0968(11)	3982(6)	6553(6)
C19	0967(18)	4112(9)	8400(11)	O4	—0267(12)	6911(7)	7098(9)
C20	0265(16)	3435(8)	6905(9)	O5	—0419(12)	7626(6)	7483(7)
C21	1999(14)	3785(9)	6386(9)	O6	—1683(16)	9777(8)	6363(10)
C22	2213(15)	3251(9)	6111(11)	I1	3507(1)	7164(1)	8144(1)
C23	2171(14)	2539(9)	6975(10)	I2	2773(1)	—0037(1)	7467(1)

type an x-ray structural investigation was necessary, and we performed this for a single crystal of the dimethiodide (Ia) of the base with mp 190-191°C (I).

The space group of the structure of the cation of (Ia) is shown in Fig. 1, from which it can be seen that both esterified tropane rings have the cis orientation relative to the mean plane of the 1,2,3,4-tetrahydronaphthalene system. The anti(trans)- arrangement of the hydrogen atom at C4 relative to the carboxy substituent at C1 follows from this. Thus, the base with mp 190-191°C investigated structurally is β-scopodonnine and consequently, the other base, with mp 178-179°C, is α-scopodonnine.

According to the space group P1 in which (Ia) crystallizes, the unit cell contains molecules of both optical antipodes of the alkali. Consequently, it would be unjustified

to assign definite values to the relative orientations of the C1–C17 and C4–C27 bonds in the chemical formulas of α- and β-scopodonnines (and also of α- and β-belladonnines) as is found in the literature [5].

The geometric parameters of the cation of (Ia) are given in Table 3. The bond lengths and valence angles were determined with an error not greater than 0.03 Å and 2°, respectively. No anomalous deviations from the standard values [6] are observed in the bond lengths.

In the cation of (Ia), the 1,2,3,4-tetrahydronaphthalene system has a clearly nonplanar structure with a substantial departure of the C2 atom by 0.64 Å from the plane of the other atoms (C1 and C3–C10) which is satisfied with an accuracy of ±0.06 Å. Thus, the C1–C5, C10 ring has the C2-sofa conformation. The phenyl substituent at C1 is planar to within ±0.02 Å. The nitrogen-containing six- and five-membered rings of the tropane series have, respectively, the chair and envelope conformations (with the departure of the nitrogen atoms from the planes of the other atoms of the ring). Both substituted tropane systems have their own noncrystalline planes of symmetry passing through the O2C18N1C25C26O3 (±0.02 Å) and O5C28N2C35C36O6 (±0.04 Å) atoms. The planar ester groups linking the tropane systems with the 1,2,3,4-tetrahydronaphthalene nucleus are somewhat twisted relative to the above-mentioned planes of symmetry (14 and 39°). The C1–C17 and C4–C27 bonds have the axial and equatorial orientations, respectively, relative to the cyclohexane ring of the tetrahydronaphthalene system. At the same time they have a mutual cis arrangement which, as mentioned above, has permitted the unambiguous assignment of the isomer of (I) investigated to β-scopodonnine.

EXPERIMENTAL

UV spectra were taken in ethanol on a Hitachi spectrophotometer, and IR spectra on a UR-20 instrument in the form of molded tablets with potassium bromide. Mass spectra were taken on a MKh 1310 instrument with an ionizing voltage of 60 V, and NMR spectra on a JNM-4H 100/100 MHz instrument in deuterochloroform. Chemical shifts are given on the δ scale relative to HMDS. Type KSK silica gel (125 and 250 μm) was used for column chromatography.

Isolation of α- and β-Scopodonnines. The total alkaloids, amounting to 0.28% of the weight of the raw material, were obtained by the usual chloroform extraction from 16 kg of the ground seeds of Datura inoxia (introduction) that had previously been defatted with petroleum ether (bp 30-40°C) [7]. The benzene-soluble material (14.7 g), after the separation of a mixture of hyoscine and hyoscyamine in the form of perchlorates, was chromatographed on a column of silica gel with elution successively by hexane, benzene, ethyl acetate, chloroform, chloroform–methanol (0.5:1), and methanol. A crystalline mixture of two bases in an amount of 0.2 g was isolated from individual fractions of the chloroform eluate. The fractional crystallization of the material from ethyl acetate and acetone yielded crystals of two bases with mp 190-191°C (β-scopodonnine) and mp 178-179°C (α-scopodonnine).

β-Scopodonnine Dimethiodide (Ia). A mixture of 0.1 g of β-scopodonnine, 5 ml of acetone, and 0.5 ml of methyl iodide was boiled in the water bath. After 10 min, crystals of β-scopodonnine dimethiodide deposited, with mp 220-222°C (decomp.).

X-Ray Structural Analysis of β-Scopodonnine Dimethiodide (Ia). The space group and the parameters of the unit cell were determined from precession photographs and were refined on a Syntex $P2_1$ diffractometer using CuK_α radiation: a = 9.808(2), b = 14.426(6); c = 15.495(5) Å; α = 107.39(5); β = 111.40(2); γ = 92.24(3)°; V = 1921(1) Å^3; d_{calc} = 1.479 g/cm^3; space group $P\bar{1}$, Z = 2.

A full set of experimental reflections (5343) with θ < 58° was obtained on the above-mentioned diffractometer (θ/2θ scanning). The calculations made use of 3841 reflections with $|F| > 4\sigma(|F|)$. The structure was determined by the direct method using the SHELXS-86 program [8] and was refined in the full-matrix isotropic–anisotropic approximation by the SHELX-76 program [9] (both programs in the PC MSDOS version). The H atoms, the initial positions of which were calculated, were refined isotropically. The final value of the discrepancy index R was 0.128 (R_w = 0.117). The coordinates of the nonhydrogen atoms are given in Table 4.

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DETERMINATION OF SOLASODINE IN CELL CULTURES OF Solanum laciniatum BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A method is proposed for the quantitative determination of solasodine by HPLC on a Millikhrom domestic microcolumn chromatograph. The amounts of solasodine in various cell lines of Solanum laciniatum have been determined. It has been shown that the genetic transformation leads to a substantial increase in the amount of solasodine in the biomass. Amounts of solasodine in multishoot cultures are comparable with its amount in normal mature plants.

The steroid alkaloid solasodine is the starting material for obtaining a whole series of drugs [1]. In Solanum laciniatum plants it is found both in the pure form and in the form of the glycoalkaloids solasonine and solamargine [2]. In view of the practical significance of solasodine throughout the world, the possibility is being widely studied of obtaining it by the methods of biotechnology [3]. Solasodine is synthesized in cultures of cell tissues of S. laciniatum in amounts far smaller than in normal plants [4]. One of the methods of raising the level of synthesis of alkaloids in a tissue culture is genetic transformation [5].

We have previously obtained a tissue culture and have performed the genetic transformation of S. lacinatum cells by various strains of Agrobacterium tumefaciens. The transgenic lines obtained as the result of genetic transformation differed from ordinary callus cells by their capacity for growing in a hormone-free medium [6]. A culture of differentiated cells – a multishoot culture [7] – has been obtained. In the present communication we give an analysis of the transgenic lines obtained for their level of steroid alkaloids.

In world practice, a number of methods are used for the analysis of alkaloids, the most effective of which is high-pressure liquid chromatography [2].

We have investigated the following cell lines: (I) – calluses growing in a hormone-containing medium; (II) – tumor cells obtained by transformation by a strain of agrobacterium from pTi A6, growing in a hormone-free medium; (III) – a multishoot culture obtained by transformation by a mutant strain of agrobacterium from the pTi A6 tms, growing in a hormone-free medium. To analyze the levels of alkaloids in the lines investigated we used a Millikhrom domestic microcolumn liquid chromatograph.

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