ing slutions; the cytisine and lupinine were purified by recrystallization from acetone and petroleum ether, and anabasine by vacuum distillation.

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ALKALOID SPECIOSEINE FROM Colchicum speciosum

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The fraction of weak bases from Colchicum speciosum Stev. (family Liliaceae) has yielded a new compound – specioseine. The structure of 10-demethylspeciosine has been established for this base by chemical and spectral methods.

The main alkaloids of the bulbs of showy autumn crocus are colchisine and colchamine, while the amount of speciosine is far smaller [1]. Among the minor alkaloids, 2-demethylcolchicine (alkaloid E), 3-methylcolchisine (C), N-formyl-N-deacetylcolchicine (B), 2-demethylcolchamine (S) [2, 3], colchameine [1], γ-lumicolchicine (J), N-methylcolchamine, 3-demethylcolchamine [4], and speciosamine [5] have been isolated. In addition, the presence of an unknown alkaloid with mp 215-217°C [1] and of unidentified minor alkaloids with a tropolone ring and without it have been reported [2, 4].

By the chromatographic separation of the alkali-soluble part of the weak-base fraction of the autumn crocus we have succeeded in isolating, together with speciosine, a new compound for which, on the basis of the results of a study of spectral characteristics and chemical transformations, the structure of 10-demethylspeciosine has been established. By analogy with other alkaloids of autumn crocuses with an unmethylated tropolone hydroxy group (colchiceine, colchameine, etc.) we have called it speicoseine.

Specioseine, with the composition $C_{27}H_{29}O_6N$, mp 169-171°C and $[\alpha]_D$ – 78° (c 1.01; $CHCl_3$) has in its UV spectrum absorption maxima at 247 and 348 nm (log ε 4.51 and 4.39), which are characteristic for the tropolone alkaloids of autumn crocuses [6, 7]. Its IR spectrum contains absorption bands of a tropolone carbonyl group (1612 cm^{-1}), of tropolone and aromatic rings (1590 and 1495 cm^{-1}, respectively), of methylene and methoxy groups (1450, 2840, 2950, and 3000 cm^{-1}) and of hydroxy groups (3380-3530 cm^{-1}), which are characteristic of the cholchicine alkaloids [8].

With ferric chloride, aqueous and alcoholic solutions of specioseine gave an olive green coloration which did not change on acidification. This showed the presence of a free tropolone hydroxy group in it [9].

The PMR spectrum of specioseine showed the signals of all the aromatic and aliphatic protons and also those of the N-methyl and methoxy groups that are characteristic of speciosine [10], with the exception of one methoxy group.

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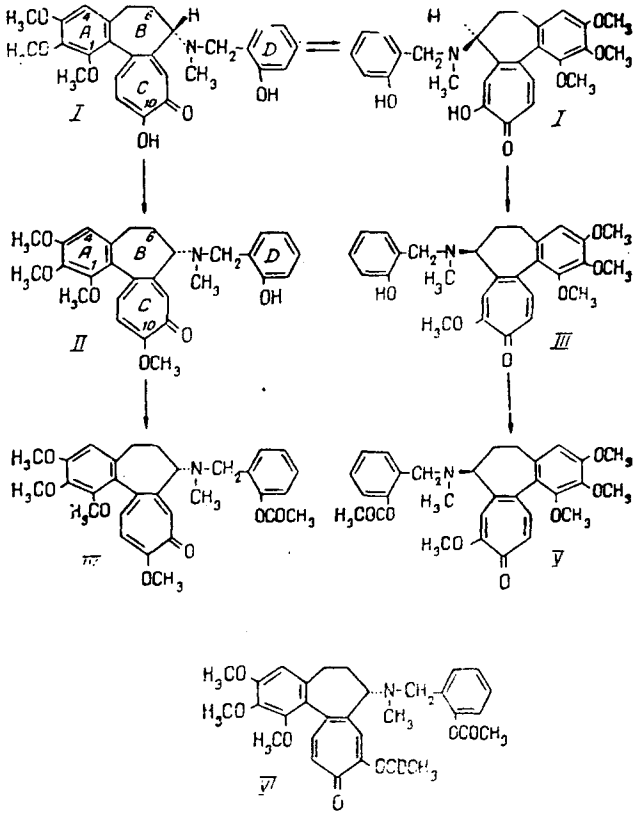
In the mass spectrum of specioseine there were intense peaks of ions with m/z 207, 107, and 106, which are characteristic for the N-benzyltropolone alkaloid speciosine and showed the structural closeness of these compounds. In actual fact, a comparison of the mass spectra of specioseine and speicosine showed that in the former the peaks of a number of ions (m/z 463 (M^+), 448 $(M - 15)^+$, 357 $(M - 10)^+$, 342 (etc.) were shifted in the direction of smaller masses by m/z 14 in comparison with the latter [11]. This also showed that specioseine was a demethylated derivative of speciosine.

On the basis of the facts given above, the structure of 10-demethylspeciosine (I, scheme) is proposed for specioseine.



When specioseine was methylated with diazomethane, two isomeric methyl ethers were formed, as in the case of cholchiceine [9]. One of them was identified as speciosine (II) by chromatographic methods and on the basis of its physical constants, while the second was isospeciosine (III). The direction of this reaction was shifted towards the formation of isospeciosine, the yield of which amounted to 80%, while that of speciosine was only ~20%. This can probably be explained by the influence of the hydroxybenzyl radical attached to the nitrogen atom on the distribution of the electron density in the tropolone ring.

The second hydroxy group of specioseine, located in ring D of the benzyl part of the molecule could not be methylated with diazomethane. Its presence was detected by acetylation: under the action of acetic anhydride, compounds (II) and (III) formed the monoacetyl derivatives (IV) and (V). The acetylation of specioseine itself led to the diacetyl derivative (VI). Analysis of the PMR spectrum of the latter showed that only one of the possible isomers had been formed, since the signals of the aromatic protons appeared as narrow singlets, and on chromatograms the substance gave a single spot. Judging from the relatively high value of the angle of rotation and the pronounced downfield shift of the signal of the H-B proton in the PMR spectrum, the compound obtained was probably diacetylisospecioseine (VI), i.e., a compound of the isocolchicine series. An acetyl group in the tropolone ring does not exert an appreciable influence of the angle of rotation of the substance, as can be seen from a comparison of the figures for acetylspeciosine, acetylisospeciosine, and diacetylspeciosine, and also those for colchicine and acetylcolchicine [12]. In this case, the value of the angle of rotation obviously depends mainly on the structure of ring C of the compound.

The transformations of the compound described above agree with the structure of 10-demethylspeciosine proposed for it. This structure was definitively confirmed by the formation of the substance from speciosine by hydrolysis in acid. It must be mentioned that under the conditions of isolation of the fractions of weak bases from the total alkaloids of the autumn crocus (see the Experimental section), no hydrolysis of speciosine takes place, as was checked in an appropriate mixture of colchicine, colchamine, and speciosine. This indicated the native nature of specioseine.

Two close isomers of specioseine – speciocolchine and specioritchine, having the structures of 2-demethylspeciosine and 3-demethylspeciosine, have recently been isolated form another species of autumn crocus – Colchicum ritchii (R. Br.) [13].

We determined the structures of the compounds obtained mainly on the basis of their PMR spectra. It is known [11] that in the PMR spectra of tropolone compounds of the normal series the signal of the H-8 proton, which appears in the form of a one-proton singlet, has the greatest downfield shift. In compounds of the iso series, however, the signal of the H-12 proton, which is a one-proton doublet, undergoes the greatest downfield shift. Furthermore, the spin-spin coupling constants of the H-11 and H-12 protons in the compounds of the iso series have larger values than those in the compounds of the normal series (J = 12-13 and 10-11 Hz, respectively). But the main difference consists in the fact that it is the H-11 proton in the compounds of the normal series and the H-8 proton in the compounds of the iso series that undergoes a powerful influence of the substituent at C-10. At the same time, the substituents at the nitrogen atom also exert a strong influence on the chemical shifts of the protons of the tropolone ring. We used this information to confirm the structures of the compounds obtained and their characteristics.

One of the monomethyl ethers of specioseine, having the signal of the H-8 proton at 7.53 ppm and that of the H-12 proton at 7.18 ppm ($J_{11,12}$ = 10 Hz) was assigned to the compounds of the normal series (speciosine), and the other, with the signal of the H-8 proton at 7.07 ppm and that of the H-12 at 7.27 ppm ($J_{11,12}$ = 12 Hz) to the compounds of the iso series (isospeciosine).

In the acetyl derivatives of the compounds, the analogous assignment of the signals of these protons was difficult. In their spectra, the signal of the H-proton was strongly shifted downfield (by 0.4-0.8 ppm). The signals of the H-11 and H-12 protons are usually masked by the signals of the protons of the benzyl moiety of the base.

EXPERIMENTAL

The composition of the mixture of alkaloids and the individuality of the compounds were studied by chromatography in a fixed layer of type LS 5/40 silica gel with 13% of gypsum in the following solvent systems: 1) chloroform–methanol–acetone–benzene–25% aqueous ammonia (10:8:6:3:3), and 2) chloroform–methanol–acetone–benzene–acetic acid (15:3:3:1).

UV spectra were taken on a SF-4A spectrometer in methanol; IR spectra on a UR-10 double-beam spectrometer in KBr tablets; NMR spectra on a XL-100 instrument in $CDCl_3$; and mass spectra on a Varian MAT-311 spectrometer.

Isolation of the Alkaloid Fraction. A solution of 3.0 kg of the total cruded (undried) alkaloids in 30 liters of chloroform, obtained by the method of [14] from 43 liters of concentrated dichloroethane extract of autumn crocus bulbs [15] was extracted with 10% sulfuric acid (5 × 5 liters). The sulfuric acid extract was made alkaline with 40% caustic soda solution to pH 4 and was extracted with chloroform (6 × 5 liters). A weak-base fraction was obtained. The chloroform extract of the weak bases was concentrated to 5 liters by the distillation of the chloroform and was extracted with 3% caustic soda solution (2 × 3 liters) and was washed with water (2 × 2 liters). The combined alkaline and aqueous extracts were acidified with 25% sulfuric acid to pH 1 and were again made alkaline with 25% ammonia to pH 8, after which they were extracted with chloroform.

All the processes in the isolation of the alkaloids were carried out in a continuous cycle with cooling.

The chloroform extracts of the weak bases, both those extracted by the solution of alkali and those remaining in the chloroform, were concentrated by the distillation of the solvent and were dried over sodium sulfate, and then the rest of the solvent was evaporated off. This gave, respectively, 46.3 and 97.4 g of mixtures of alkaloids. It was established by chromatographic methods that both fractions of weak bases contained mainly speciosine.

Specioseine (I). The alkali-soluble part of the weak-base fraction consisted of six compounds, with R_f 0.17, 0.43, 0.57, 0.67, 0.79 (the main one), and 0.86 (system 1). To separate it, 20 g of the mixture of substances was chromatographed on a column containing 560 g of type L 100/160 silica gel with elution successively by ether, ether–chloroform (1:1), chloroform, and chloroform–methanol (8:2). By crystallization from acetone, the chloroform and chloroform and chloroform–methanol eluates yielded 12 g of speciosine (R_f 0.79). From its mother solutions, by repeated separation, it was possible to isolate 0.96 g of the new compound specioseine (R_f 0.43), which was crystallized from acetone.

PMR spectrum (ppm): 7.70 (1H, s, H-8), 7.50 (1H, d, H-12, $J_{11,12}$ = 12 Hz), 6.70 (1H, d, H-11; $J_{11,12}$ = 12 Hz), 7.30-6.65 (5H, arom.), 6.50 (1H, s, H-4), 3.87 (6H, s, $2OCH_3$), 3.57 (3H, s, OCH_3), 2.15 (3H, s, N–Ch_3), 3.08 (1H, m, H-7).

Methylation of (I) to (II) and (III). With stirring, a saturated solution of diazomethane in ether was added to 0.6 g of (I) (R_f 0.36) in 5 ml of chloroform, and the solution was left for 12 h. The solvent was distilled off, and the mixture, consisting of two substances, with R_f 0.75 and 0.82 (system 2), was separated by chromatography on 10 g of alumina with elution by ether, ether–chloroform (1:1), and chloroform.

Isospeciosine (III). The ethereal eluates yielded 0.41 g of (III) with R_f 0.82, mp 184-186°C (from ether) and $[\alpha]_D$ −37° (c 0.62 in $CHCl_3$).

UV spectrum: 246 and 346 nm.

IR spectrum: 3400, 3200, 2950, 2840, 1605 cm^{-1}.

PMR spectrum: 7.27 (1H, d, H-12, $J_{11,12}$ = 12 Hz), 7.07 (1H, s, H-8), 7.20-6.63 (4H, arom.), 6.69 (1H, d, H-11, $J_{11,12}$ = 12 Hz), 6.47 (1H, s, H-4), 3.95 (3H, s, OCH_3), 3.83 (6H, s, $2OCH_3$), 3.60 (3H, s, OCH_3), 2.11 (3H, s, N–CH_3), 3.10, (1H, m, H-7).

Speciosine (II) was obtained from the following eluates, formed by ether–chloroform and by chloroform. Its yield was 0.1 g, mp 210-211°C (from acetone), $[\alpha]_D$ −26° (1.0; $CHCl_3$), R_f 0.76.

UV spectrum: 247 and 340 nm.

IF spectrum: 3190, 2930, 2830, 1605, 1540, 1470 cm^{-1}.

PMR spectrum: 7.53 (1H, s, H-8), 7.18 (1H, d, $J_{11,12}$ = 10 Hz, H-12), 7.00-6.63 (4H, arom.), 6.68 (1H, d, $J_{11,12}$ = 10 Hz, H-11), 6.46 (1H, s, H-4), 3.91 (3H, s, OCH_3), 3.87 (6H, s, $2OCH_3$), 3.50 (3H, s, OCH_3), 2.16 (3H, s, N–CH_3), 3.08 (1H, m, H-7).

Mass spectrum: (m/z): 477 (M^+), 462, 371, 356, 342, 312, 208, 207, 107, 106.

Acetylspeciosine (IV). A mixture of 0.1 g of (II) (R_f 0.76), 1 ml of acetic anhydride, and 0.1 g of freshly calcined sodium acetate was left at 45-50°C for 12 h. The excess of acetic anhydride was eliminated by the addition of methanol and evaporation. The residue was dissolved in water, and the solution was made alkaline with ammonia and was extracted with chloroform. The chloroform extract was dried over sodium sulfate and purified by passage through a layer of alumina, and the solvent was distilled off. This gave (IV) with R_f 0.82 (system 2) and $[\alpha]_D$ −42° (c 0.94; $CHCl_3$).

IR spectrum: 1750 cm^{-1} ($OCOCH_3$).

PMR spectrum: 7.94 (1H, s, H-8), 7.42-6.34 (6H, arom.), 6.32 (1H, s, H-4), 3.70 (9H, s, $3OCH_3$), 3.40 (3H, s, OCH_3), 2.02; 1.80 (3H×2, ss, NCH_3, $OCOCH_3$).

Acetylspeciosine (V) was obtained from 0.15 g of (III) (R_f 0.82) under the conditions for the acetylation of (II). This gave (V) with R_f 0.92 and $[\alpha]_D$ −100° (c 1.01; $CHCl_3$).

IR spectrum: 1755 cm^{-1} ($OCOCH_3$).

PMR spectrum: 7.86 (1H, s, H-8), 7.60-6.90 (6H, arom.), 6.52 (1H, s, H-4), 3.90 (9H, s, $3OCH_3$), 3.65 (3H, s, OCH_3), 2.18 (3H, s, N–CH_3), 2.07 (3H, s, $OCOCH_3$), 3.20 (1H, m, H-7).

Diacetylspecioseine (VI). The acetylation of 0.1 g of (I) (R_f 0.36) was performed under the conditions described above. The product was (VI) with R_f 0.90 and $[\alpha]_D$ −106° (c 0.94; $CHCl_3$).

IR spectrum: 1755 cm^{-1} ($2OCOCH_3$).

PMR spectrum: 8.28 (1H, s, H-8), 7.62-6.80 (6H, arom.), 6.50 (1H, s, H-4), 3.88; 3.86; 3.58 (each 3H, ss, $3OCH_3$), 2.20 (6H, s, $N{-}CH_3$, $OCOCH_3$), 2.00 (3H, s, $OCOCH_3$), 3.12 (1H, m, H-7).

Hydrolysis of Speciosine (II) to Specioseine (I). A mixture of 3 g of speciosine and 20 ml of 3% hydrochloric acid was heated at 100°C for 2 h. The rsulting solution was made alkaline with ammonia and extracted wtih chloroform. A compound with mp 169-171°C (from acetone) was isolated and was identified from its R_f value and PMR spectrum as specioseine (I).

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8-ACETYLEXCELSINE – A NEW ALKALOID FORM

Aconitum kirinense

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The alkaloid 8-acetylexcelsine has been isolated from the epigeal part of Aconitum kirinense Nakai. Its structure has been established with the aid of chemical transformations and spectral characteristics.

We have investigated the alkaloids of the epigeal part of the plant Aconitum kirinense gathered at the end of the vegetation period in the environs of the village of Chernyatino, Oktyabr'skii region, Maritime Territory. An alkaloid has been obtained from this plant previoiusly and an empirical formula has been derived for it [1]. After the extraction of the air-dry plant with aqueous ethanol, 1.9% of alkaloids was obtained. An amorphous base (I) with the composition $C_{24}H_{35}O_7$ (449.24094, HRMS) was isolated from the total alkaloids. The IR spectrum of (I) had absorption bands of hydroxy and ester groups while the PMR spectrum revealed the signals of N-ethyl, acetoxy, and two methoxy groups.

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