STRUCTURES OF DICTYSINE AND DEHYDRODICTYSINE

B. T. Salimov, B. Tashkhodzhaev, and M. S. Yunusov

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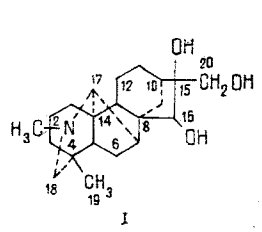
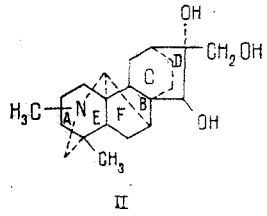
From the results of an x-ray structural analysis of free dictysine it has been established that dictysine is an alkaloid of the denudatine type with an α, β, γ-triol system at C_{15}, C_{16}, and C_{20}. The structures of the acetonides of dictysine and dehydrodictysine formed from dictysine and dehydrodictysine, respectively, in the process of separating the combined alkaloids, have been shown.

The alkaloid dictysine $C_{21}H_{33}NO_3$ has been isolated from the epigeal part of *Delphinium dictyocarpum* DC., collected in the upper reaches of the Koktal River (Dzhungarian Ala-Tau) [1]. On the basis of the spectra of dictysine and its trideutero analog, the ^{13}C NMR spectrum, and the chemical transformation structure (I) with a songorine skeleton and a triol system at C_{15}, C_{16}, and C_{20} has been proposed for the alkaloid [2]. However, an x-ray structural analysis of free dictysine has shown that the alkaloid is based not upon a songorine but on a denudatine skeleton [6], and dictysine has the structure (II).



I



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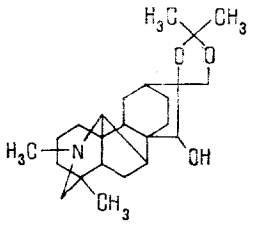
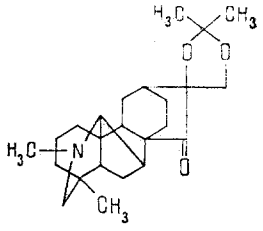
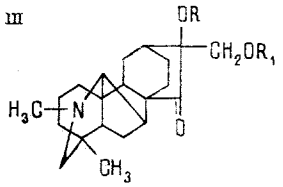


III



IV



V. R = R_1 = H

VI. R = H; R_1 = CH_3CO

VII. R = R_1 = CH_3CO

The geometry of the dictysine (II) molecule is shown in Fig. 1. The lengths of the bonds and the valence angles, and also the conformational parameters of dictysine agree well with those obtained for the related diterpene alkaloid denudatine [3] and its methiodide [4]. The conformations of the rings are as follows: A, E — chair; B, C, D — distorted boat (twist); the five-membered ring F has the envelope form. Linkages of the rings: A/B — trans; B/C — cis.

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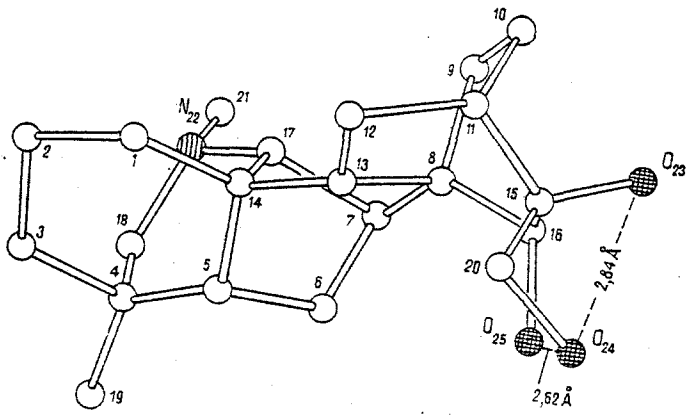


Fig. 1. Geometry of the dictysine molecule.

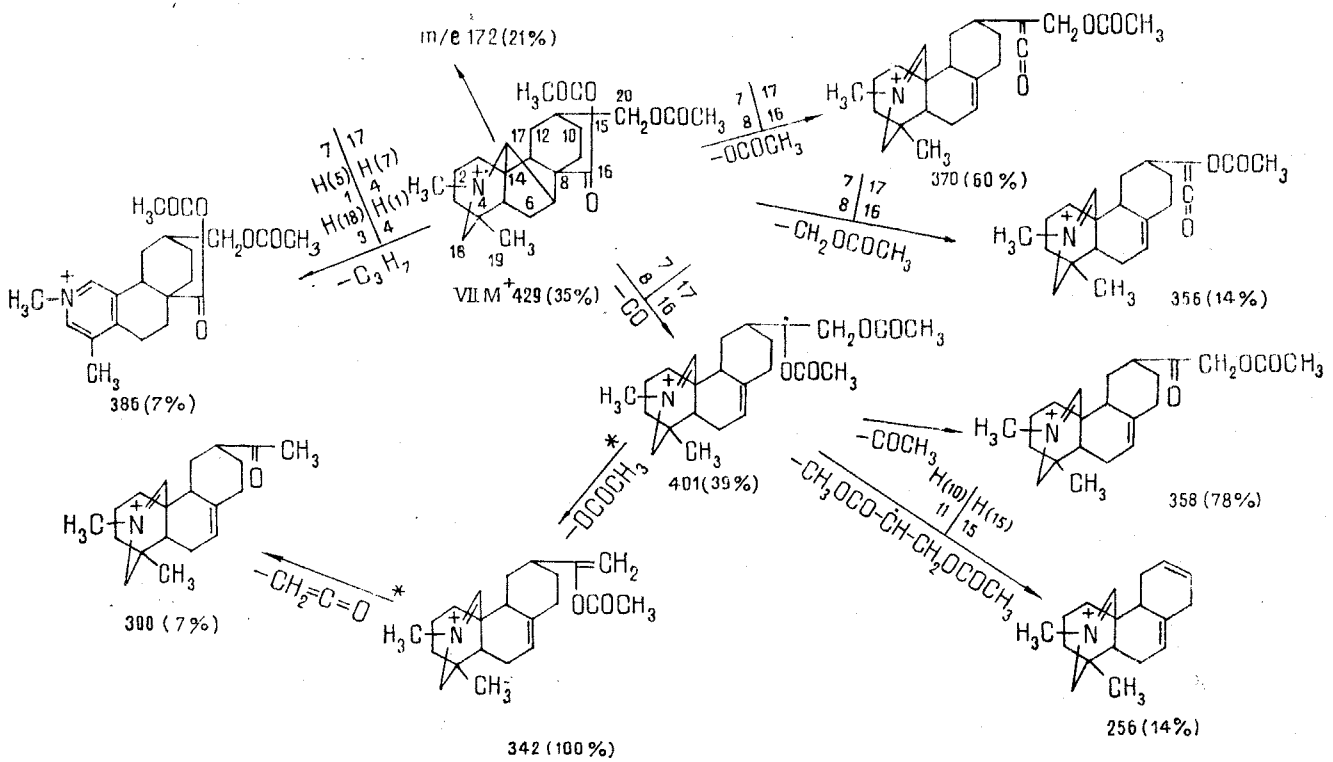
All three active hydrogens participate in the formation of inter- and intramolecular hydrogen bonds. The O(24)...O(25) distance (2.62 Å) shows the presence of a strong intramolecular H-bond.

On continuing the separation of the combined alkaloids of the epigeal part of *D. dictyocarpum*, in addition to those obtained previously, we isolated two more bases: $C_{24}H_{37}NO_3$ (III), mp 151-153°C (acetone), $[\alpha]_D^{20}$ −102 (c 3.3; $CHCl_3$), and $C_{24}H_{35}NO_3$ with mp 143-145°C (acetone), $[\alpha]_D^{20}$ −58° (c 0.4; $CHCl_3$).

Base (III) dissolves readily in chloroform and less readily in ethanol and acetone. Its IR spectrum has absorption bands at 3550 cm^{-1} (OH group) and 1090 cm^{-1} (C—O ether bonds). The NMR spectrum shows three-proton singlets at (ppm) 0.63, 1.28, and 1.38 (3 C—CH_3) and 2.19 (N—CH_3), one-proton singlets at 3.24 and 3.92, and two one-proton doublets at 3.67 and 4.37 ppm (J = 10 Hz). The mass spectrum of (III), which contains the peaks of ions with m/z 387 (M^+, 100%), 372, 358, 344, 312, 300, 256, and 172, is similar to that of dictysine. What has been said above, and also the difference by 40 a.m.u. in the molecular weights of (III) and (II) give grounds for assuming that (III) is dictysine acetonide. In actual fact, when (III) was treated with 20% H_2SO_4, dictysine was obtained. Compound (III) was apparently formed from (II) in the process of separating the combined alkaloids (see the Experimental part [1]), as is confirmed by the formation of (III) from dictysine and acetone in the presence of perchloric acid.

The formation of an acetonide grouping with the involvement of the hydroxy groups at C_{15} and C_{20} is confirmed by the following transformations. The oxidation of (III) with chromium trioxide gave a compound which, according to its IR and mass spectrum, was the ketone (IV) (ν_{max}^{KBr} 1735 cm^{-1}). The latter, on reaction with 20% H_2SO_4, was converted into dehydrodictysine (V). The acetylation of (V) with acetic anhydride in pyridine led to the monoacetate (VI), the further acetylation of which with acetyl chloride gave the diacetate (VII). The mass-spectrometric fragmentation of (VII) (see Scheme) took place similarly to that of songorine (5) and dictysine [2] and their derivatives. The somewhat specific nature of the fragmentation of (VII) is due to the presence of the C_{16} carbonyl group.

The base with mp 143-145°C readily dissolved on chloroform and less readily in acetone. Its IR spectrum contains a strong absorption band of a carbonyl group at 1735 cm^{-1} and the absorption band of C—O ether bonds at 1090 cm^{-1}. The NMR spectrum contains three-proton singlets (ppm) at 0.64, 1.40, and 1.48 (3 C—CH_3) and 2.18 (N—CH_3), a one-proton singlet at 3.14 and two one-proton doublets at 3.75 and 3.91 with J = 10 Hz. The mass spectrum of the base contains the peaks of ions with m/z 385 (M^+), 370, 357, 299 (100%), 256, and 172. The IR, NMR, and mass spectra of this compound and of dehydrodictysine acetonide (IV) were practically identical. A direct comparison also showed their identity. In view of the ease of formation of dictysine acetonide (III) from (II) under the conditions of separating the combined alkaloids, it may be assumed that dehydrodictysine acetonide is apparently a secondary product of the dehydrodictysine (V) present in the plant.



Scheme of the mass-spectrometric fragmentation of dehydrodictysine diacetate (VII) (relative intensities are given in parentheses; *denotes metastable transitions).

EXPERIMENTAL

The homogeneity of the substances was checked by chromatography in a thin layer of type KSK silica gel in the benzene–methanol (4:1) and chloroform–methanol (20:1) systems and on alumina of "for chromatography" grade in the chloroform–methanol (50:1) system. IR spectra were recorded on a UR-20 instrument in tablets with KBr; NMR spectra in $CDCl_3$ solution on a JMN-4H-100/100 Mz instrument with HMDS as internal standard (the values are given in the δ scale); and mass spectra on a MKh-1310 instrument fitted with a system for the direct introduction of the sample into the ion source. Specific rotations were measured on a JASCO J-20 spectropolarimeter.

Isolation of Dictysine and Dehydrodictysine Acetonides. Fractions 1-2 (7.8 g) from separation according to the basicity of the mother liquor after the elimination of the eldeline [1], were chromatographed on a column of silica gel (1:70). The alkaloids were eluted with chloroform–methanol (100:1) (fractions 1-14), (50:1) (fractions 15-25), and (25:1) (fractions 26-30), 300-ml fractions being collected. The treatment of fractions 18-30 with acetone yielded 2.1 g of dictysine acetonide. Fractions 4-5 (1.5 g) were rechromatographed on a column of alumina (1:100). The alkaloids were eluted with ether (fractions 1-12) and with ether–methanol (100:1) (fractions 13-24) and (50:1) (fractions 25-32), 30-ml fractions being collected. The acetone treatment of fraction 6 yielded 50 mg of dehydrodictysine acetonide, and the treatment of fractions 26-29 with ether led to 0.24 g of eldeline.

Fraction 3 (3.6 g) from the separation according to the basicity of the mother liquor after the elimination of the eldeline [1], was chromatographed in a similar manner to fractions 1-2, and yielded 0.84 g of dictysine acetonide. Fractions 4-5 (10.6) from the separation according to the basicity of the mother solution after the elimination of the eldeline [1] was chromatographed on a column of alumina (1:70). The alkaloids were eluted with ether (fractions 1-8) and with ether–methanol (100:1) (fractions 9-25), 150-ml fractions being collected. The acetone treatment of fractions 1-3 yielded 0.54 g of dictysine acetonide, and the ether treatment of fractions 11-17 yielded 6.4 g of eldeline.

Hydrolysis of (III). A solution of 50 mg of dictysine acetonide in 5 ml of 20% H_2SO_4 was left at room temperature for 24 h, after which 25 ml of water was added and it was washed with an equal volume of ether. With cooling, the acid solution was made alkaline with sodium carbonate and was shaken with chloroform. The combined chloroform extracts, after the solvent has been distilled off, gave 40 mg of a product readily crystallizing from chloroform and methanol. By treatment with methanol, 13 mg of a substance with mp 181-183°C, identical with dictysine in all respects, was isolated.

Dehydrodictysine Acetonide (IV). A solution of 350 mg of dictysine acetonide in 25 ml of acetone was cooled with ice, and then a cooled solution of 350 mg of CrO_3 in 25 ml of acetone was added and the mixture was left at room temperature for 48 h. The residue after evaporation of the solvent was dissolved in 5% H_2SO_4 and the solution was washed with ether. Then, with cooling, the acid solution was made alkaline with sodium carbonate and was shaken with ether. The combined ethereal extracts were dried over Na_2SO_4. The residue after the ether had been distilled off (320 mg) was chromatographed on a column of silica gel (1:70). By elution with chloroform–methanol (100:1), 44 mg of dehydrodictysine acetonide with mp 141-143°C (acetone) was obtained.

Hydrolysis of (IV). A solution of 90 mg of dehydrodictysine acetonide in 5 ml of 20% H_2SO_4 was left at room temperature for 24 h. After the working up described above, 50 mg of chromatographically homogeneous dehydrodictysine (V) was obtained. Mass spectrum: m/z 345 (M^+), 328, 317, 316, 314, 300 (100%), 286, 274, 258, 256, 172.

Dehydrodictysine Monoacetate (VI). A mixture of 40 mg of dehydrodictysine, 2 ml of acetic anhydride, and 0.5 ml of pyridine was left at room temperature for 24 h. After the usual working up, 43 mg of a chromatographically homogeneous substance was obtained. Mass spectrum: m/z 387 (M^+), 372, 358, 359, 355, 342, 341, 327, 314, 313, 300, 299 (100%), 384, 382 283, 270, 256, 172.

Dehydrodictysine Diacetate (VII). A solution of 37 g of dehydrodictysine monoacetate in 2 ml of acetyl chloride was left at room temperature for 48 h. After the usual working up, 40 mg of chromatographically homogeneous substance was obtained. Mass spectrum: m/z 429 (M^+), 401, 370, 358, 356, 342 (100%), 300, 298, 282, 256, 172.

CONCLUSION

It has been established that dictysine is an alkaloid of the denudatine type with an α, β,γ-triol system at C_{15}, C_{16}, and C_{20}. The structures of dictysine and dehydrodictysine acetonides have been established.

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SYNTHESIS OF REGULAR POLYPEPTIDES INCLUDING POLYFUNCTIONAL AMINO ACIDS

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Polypeptides of regular structure with the sequences His-Glu, Ser-Glu, Try-Glu, His-Tyr, and Glu-His have been synthesized by the 2,4,5-trichlorophenyl ester method. Polymerization was carried out in dimethyl sulfoxide and dimethylformamide. The polymers were purified by dialysis against water. In the process of synthesizing the monomers, the α-amino groups of the acids were protected by ortho-nitrophenylsulfenyl (o-NPS-), benzyloxycarbonyl (Z-), and tert-butoxycarbonyl (t-BOC-) groups. The imidazole group of histidine was Z-protected, and the γ-hydroxy group of glutamic acid and the phenolic OH group of tyrosine were benzyl-protected (Bzl-).

Polypeptides of regular structure have shown themselves to be good catalytic models in the study of the rate of hydrolytic cleavage of the ester bond [1, 2]. The most convenient method for obtaining them is that of activated esters [3, 4]. The present investigation was devoted to the synthesis of peptide monomers — 2,4,5-trichlorophenyl (2,4,5-OTcp) esters containing serine, tryptophan, histidine, and glutamic acid residues, and their subsequent polycondensation.

In the process of synthesizing the peptide monomers, the α-amino groups of the amino acids were protected by o-nitrophenylsulfenyl (o-NPS-), benzyloxycarbonyl (Z-), and tert-butoxycarbonyl (t-BOC-) groupings, and the γ-carbonyl group of glutamic acid and the phenolic hydroxy group of tyrosine were benzyl-protected (Bzl-). The imidazole (im) group of histidine was protected by a Z group. The addition of 2,4,5-trichlorophenol to the N-protected amino acids and the synthesis of the dipeptides were carried out with the aid of the condensing agent dicyclohexylcarbodiimide (DCC). The protected peptides were purified by repeated recrystallization from organic solvents and, in some cases, by passage through a column filled with silica gel, which permitted the separation of the initial compounds that had not reacted during the process and other impurities and by-products.

The protective groups — Z, o-NPS, and t-BOC — of the activated amino acid and peptide esters were eliminated before polycondensation by the action of HBr in glacial acetic acid, 2.5 N HCl in ethyl acetate, and trifluoroacetic acid, respectively.

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