The steroid glycosides were extracted with hot methanol. The extract was cooled and filtered. The total volume was made up to 25 ml. The completeness of the conversion of the oligofurostanol glycosides into oligospirostanol glycosides was checked by the TLC method.

CONCLUSIONS

A method has been developed for the quantitative determination of oligospirostanosides in a cell culture of Dioscorea deltoidea with the aid of HPLC on a LiChrosorb RP-18 column. Elution was performed with mixtures of acetonitrile and water (50-75%), and detection was carried out at 207 nm.

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STRUCTURE OF THE PRODUCTS OF OXIDATION OF VINDOLINE BY THE SARETT REAGENT

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The oxidation of vindoline with the Sarett reagent has given five lactams the structures of which have been established on the basis of the results of instrumental methods of analysis (the x-ray structural method, and the PMR, mass, and IR spectroscopy). Two of the vindoline derivatives – 4β-acetoxy-7,8-dihydroxy-16-methoxy-3α-methoxycarbonyl-3β,6β-epoxy-7,8,9,19-tetradehydrovincaminoreine 7,9-betaine and 4β-acetoxy-16-methoxy-3α-methoxycarbonyl-1-methyl-7,8-dioxo-3β,6β-epoxyaspidospermidine – have been obtained for the first time and are new compounds.

We have shown previously that vindoline (I) – an alkaloid of Catharanthus roseas (Madagascar periwinkle) is oxidized by chromic acid with the formation of various reaction products, depending on the pH of the medium [1-3]. The present work was devoted to determining the structures of the reaction products obtained on the oxidation of (I) with the Sarett reagent, which leads to the formation of a mixture of several substances. Four compounds (II, III, IV, and V) have been isolated by column chromatography on silica gel.

On the basis of IR, PMR, and mass spectra, (II) was shown to be identical with a lactam of the composition $C_{25}H_{30}N_2O_7$ that was obtained as an intermediate product in the synthesis of vindoline [4]. To confirm the structure of (II) and to establish its stereochemistry, we have made an x-ray structural study of a bromine derivative (IIa) of the lactam. Compound (II) was brominated in ether. Rhombic crystals of (IIa) were obtained from a mixture

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0009-3130/88/2404-0467 $12.50

TABLE 1. Coordinates of the Atoms ($\times 10^3$, Br $\times 10^4$)

Atom	Molecule A			Molecule B		
	X	Y	Z	X	Y	Z
Br	2236(1)	1642(4)	867(4)	3370(1)	1856(4)	885(4)
O1	—5(1)	77(2)	—113(2)	272(1)	—508(2)	—39(2)
O2	197(1)	—51(2)	203(2)	402(1)	91(2)	—48(2)
O3	12(1)	—59(2)	132(2)	372(1)	—524(2)	—49(2)
O4	—28(1)	—116(2)	—20(2)	331(1)	—583(2)	—171(2)
O5	9(1)	183(2)	97(2)	335(1)	—491(2)	127(2)
O6	50(1)	161(2)	227(2)	381(1)	—357(2)	184(2)
O7	11(1)	135(2)	—391(2)	165(1)	—421(2)	—55(2)
N1	76(1)	—106(3)	—6(3)	339(1)	—282(3)	—142(3)
N9	56(1)	152(3)	—257(3)	215(1)	—297(3)	13(3)
C2	56(1)	—43(4)	—87(3)	300(1)	—334(4)	—113(3)
C3	20(1)	34(4)	—30(3)	309(1)	—442(4)	—40(3)
C4	37(1)	148(4)	20(3)	317(1)	—398(4)	72(3)
C5	38(1)	216(4)	—75(3)	272(1)	—377(4)	108(3)
C6	—4(1)	198(4)	—117(3)	254(1)	—502(4)	67(3)
C7	—8(1)	226(4)	—236(3)	211(1)	—505(4)	73(3)
C8	18(1)	165(4)	—310(3)	196(1)	—413(4)	24(3)
C10	86(1)	65(4)	—302(3)	205(1)	—207(4)	—85(3)
C11	107(1)	13(4)	—221(3)	248(1)	—168(4)	—124(3)
C12	86(1)	55(4)	—125(3)	277(1)	—226(4)	—55(3)
C13	116(1)	45(4)	—29(3)	309(1)	—148(4)	—32(3)
C14	152(1)	113(4)	—16(3)	310(1)	—43(4)	24(3)
C15	179(1)	83(4)	64(3)	340(1)	36(4)	23(3)
C16	171(1)	—28(4)	129(3)	373(1)	9(4)	—44(3)
C17	134(1)	—93(4)	112(3)	376(1)	—94(4)	—104(3)
C18	109(1)	—59(4)	27(3)	342(1)	—170(4)	—93(3)
C19	68(1)	173(4)	—147(3)	253(1)	—275(4)	45(3)
C20	44(1)	359(4)	—50(3)	269(1)	—374(4)	230(3)
C21	82(1)	379(4)	18(3)	287(1)	—245(4)	272(3)
C22	183(1)	—161(4)	270(3)	437(1)	66(4)	—102(3)
C23	70(1)	—241(4)	—5(3)	357(1)	—309(4)	—241(3)
C24	—3(1)	—54(4)	39(3)	337(1)	—523(4)	—92(3)
C25	—56(1)	—189(4)	40(3)	404(1)	—594(4)	—98(3)
C26	16(1)	178(4)	201(3)	366(1)	—467(4)	181(3)
C27	—16(1)	185(4)	273(3)	389(1)	—564(4)	235(3)

TABLE 2. Valence Angles, ω, deg

Angle	ω	Angle	ω	Angle	ω
C13C18C17	124(2)	C12C2C3	108(2)	C19C5C20	109(2)
C13C18N1	105(2)	N1C2C3	107(2)	C4C5C20	111(2)
C17C18N1	127(2)	C2N1C18	112(2)	C4C5C6	106(2)
C18C17C16	119(2)	C2N1C23	119(2)	C5C4O5	114(2)
C17C16C15	118(2)	C18N1C23	122(2)	C5C4O3	97(2)
C17C16O2	122(2)	C12C11C10	105(2)	C3C4O5	107(2)
O2C16C15	116(2)	C11C10N9	106(2)	C6O1C3	110(2)
C16C15C14	126(2)	C10N9C19	109(2)	C4C3O1	104(2)
C16C15Br	118(2)	C10N9C8	119(2)	O1C3C24	110(2)
BrC15C14	124(2)	C19N9C8	127(2)	C10C3C2	111(2)
C15C14C13	121(2)	N9C19C12	101(2)	C2C3O1	107(2)
C14C13C18	118(2)	C12C19C5	118(2)	C4C3C24	119(2)
C12C13C14	127(2)	N9C19C5	116(2)	C2C3C24	107(2)
C12C13C18	113(2)	N9C8C7	120(2)	C3C24O3	125(2)
C16O2C22	117(2)	O7C8C7	127(2)	O3C24O4	124(2)
C13C12C2	99(2)	N9C8C7	113(2)	C3C24O4	110(2)
C13C12C11	111(2)	C8C7C6	113(2)	C24O4C25	116(2)
C13C12C19	117(2)	C7C6C5	114(2)	C5C20C21	111(2)
C2C12C11	110(2)	C7C6O1	111(2)	C4O5C26	121(2)
C2C12C19	110(2)	C5C6O1	99(2)	O5C26C27	123(2)
C19C12C11	108(2)	C6C5C19	113(2)	O5C26O6	117(2)
C12C2N1	104(2)	C19C5C4	111(2)		

of ether and ethanol with $a = 33.99(2)$, b = 11.274(6), c = 12.185(7) Å, z = 8, space group $P2_12_12_1$. The structure was interpreted by the heavy-atom method and was refined by the method of least squares in the full-matrix anisotropic(Br)—isotropic approximation to R = 0.124 for 2300 reflections with $F^2 \geq 3\sigma$. The coordinates of the atoms are given in Table 1. The structure is composed of two crystallographically independent molecules (A and B) linked with one another by van der Waals interactions and having similar geometric parameters and stereochemically similar (individual values of the valence angles are given in Table 2). The structure of (IIa) with bond lengths averaged over the two crystallographically independent mole-

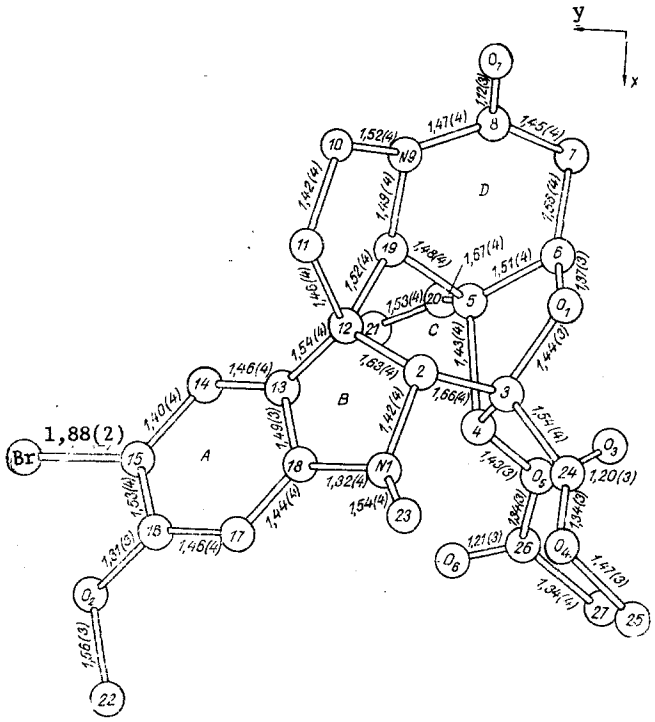


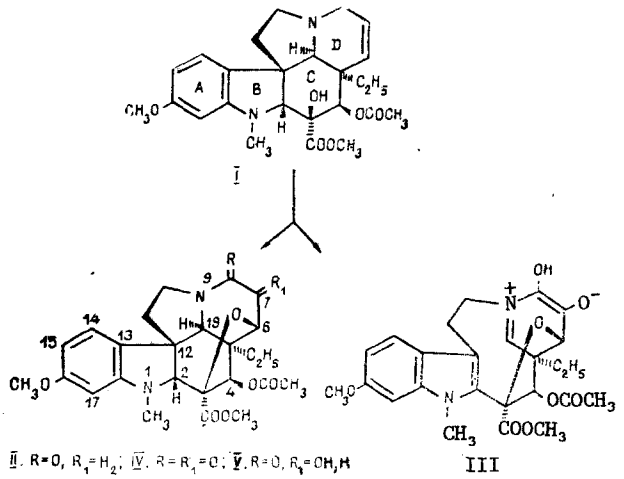
Fig. 1. Structure of the bromine derivative of the lactam (IIa).

cules is shown in Fig. 1. The absolute configuration was determined by Hamilton's test: For the inverted structure r = 0.126, i.e., with a probability greater than 99.5% the absolute configuration of the seven asymmetric centers is C2—R, C3—R, C4—S, C5—S, C6—S, C12—R, C19—R.

The structures of compounds (III), (IV), and (V) were established by comparison with the results of the instrumental methods of analyzing lactam (II). According to their PMR spectra, each of the substances mentioned retained the ethyl, acetoxy, N-methyl, methoxy, and methoxycarbonyl groups. The structure of the aromatic ring was unchanged [the doublet signals of H-14 (J = 8 Hz) and H-17 (J = 3 Hz) and the quartet of H-15 (J = 8 Hz, J = 3 Hz) were present]. The PMR spectrum of (III) was characterized by the presence, in addition to the signals mentioned, of four one-proton singlet signals which were assigned to the protons at C-4 (5.93 ppm), C-6 (3.93 ppm), and C-19 (8.20 ppm), and the signal of the proton of a hydroxy group (3.77 ppm). The origin of the signals of the mobile proton was established by deuterium exchange. In the mass spectrum, the peak of the molecular ion with m/z 484 was recorded, together with the peaks of ions confirming the structure as 4β-acetoxy-7,8-dihydroxy-16-methoxy-3α-methoxycarbonyl-3β,6β-epoxy-7,8,9,19-tetradehydrovincaminoreine 7,9-betaine.

Compound (IV) also had a molecular weight of 484 amu. The PMR spectrum included the signals of protons at C-19 (3.57 ppm), C-2 (4.00 ppm), C-6 (4.21 ppm), and C-4 (5.53 ppm). The IR spectrum contained absorption bands at 1760, 1750, and 1720 cm^{-1}, corresponding to carbonyl groups.

Substance (V) was characterized by M^+ 486 amu, and in the PMR spectrum there were singlet signals of protons at C-2 (4.15 ppm), C-4 (5.75 ppm), and C-19 (3.57 ppm), the doublet of a proton at C-6 (4.37 ppm, J = 3.9 Hz), the doublet of a proton geminal to a secondary hydroxy group (4.92 ppm, J = 3.9 Hz), and the broadened singlet of the proton of a hydroxy group (4.41 ppm). It was established by the double proton-proton resonance method that the doublet nature of the H-6 and H-7 signals was due to their vicinal interaction. The presence of a hydroxy group in the C-7 position was confirmed by the formation of an acetyl derivative in the PMR spectrum of which, in addition to the appearance of singlet signals of the protons of two acetyl groups at 1.99 and 2.11 ppm, a paramagnetic shift of the H-7 signal (5.51 ppm, doublet, J = 3.9 Hz) was observed. There is a mention of compound (V) in [5], but no information was given on its physicochemical properties and spectral characteristic either in the paper cited or in subsequent publications.



II, R=O, $R_1=H_2$; IV, $R=R_1=O$; V, R=O, R_1=OH,H

III

EXPERIMENTAL

The course of the reaction and the purity of the products obtained were monitored chromatographically in a thin layer of Silufol UV-254 silica gel in the chloroform–methanol (9:1) system. The products were detected with cerium(IV) ammonium sulfate. The preparative separation of the substances was effected on a column of silica gel A 40/100 (Czechoslovakia).

X-ray analysis was carried out on a Syntex P1 diffractomer, λ Cu K_α, graphite monochromator, $\theta/2\theta$ scanning, $3 \geq 2\theta \geq 120°$. PMR spectra were measured on a Bruker WH-360 instrument (360 MHz) in $CDCl_3$, δ scale, ppm, with TMS as internal standard. Mass spectra were taken on a Varian MAT-311A spectrometer in the following regime: energy of the ionizing electrons 70 eV; cathode emission current 300 μA; accelerating voltage 3 kV; temperature of the ion source and temperature of introduction of the sample 180-200°C. IR spectra were taken on a Specord 75IR spectrophotometer of suspensions in paraffin oil and in $CHCl_3$. Melting points were determined on a Boëtius microheated stage. Elementary analysis was carried out on a Hewlett–Packard model 185B CHN-analyzer. The results of the analyses of all the compounds agreed with the calculated figures.

Oxidation of Vindoline by the Sarett Reagent. To 5 ml of pyridine and 1 ml of methylene chloride was added 0.3 g of chromium trioxide, and the mixture was stirred at room temperature for 15 min. Then 0.912 g of vindoline and 20 ml of methylene chloride was added to the Py·CrO_3 complex obtained. After 3.5 h, a 5% solution of sodium metabisulfite was added to the reaction mixture and the organic layer was separated off, washed with water, dried with anhydrous sodium sulfate, and evaporated in vacuum. The residue was chromatographed on a column. After elution with petroleum ether–chloroform (35:15), 0.28 g (29.0%) of substance (III), with the composition $C_{25}H_{28}N_2O_8$ was obtained. mp 255-257°C (ethanol), $\nu_{max}^{CHCl_3}$ 3400, 1790, 1750, 1660, 1630 cm^{-1}. PMR spectrum (δ, ppm): 0.82 t (3H, CH_2–CH_3), 1.74 m (2H, CH_2–CH_3), 2.09 s (3H, $OCOCH_3$), 2.67 s (3H, N–CH_3), 3.77 s (1H, OH), 3.78 s (3H, OCH_3), 3.91 s (3H, OCH_3), 3.93 s (1H, H-6), 5.93 s (1H, H-4), 6.22 d (1H, J = 3 Hz, H-17), 6.34 dd (1H, J = 8 Hz, J = 3 Hz, H-15), 6.86 d (1H, J = 8 Hz, H-14), 8.20 s (1H, H-19). Mass spectrum, m/z (%): 484 (M^+, 7), 244(44), 243(53), 232(10), 213(27), 201(11), 200(15), 188(90), 175(12), 174(81), 172(14), 159(14), 144(13), 131(12).

Petroleum ether–chloroform (1:1) eluted the diketo derivative (IV) with the composition $C_{25}H_{28}N_2O_8$ (4β-acetoxy-16-methoxy-3α-methoxycarbonyl-1-methyl-7,8-dioxo-3β,6β-epoxyaspidospermidine). After crystallization from ether, 0.063 g (6.5%) was obtained with mp 241-243°C; ν_{max}: 1760, 1750, 1720 cm^{-1}. PMR spectrum (δ, ppm): 3.57 s (1H, H-19), 4.00 s (1H, H-2), 4.21 s (1H, H-6), 5.53 s (1H, H-4). Mass spectrum, m/z (%): 485 (M^+ +1, 10), 484 (M^+, 5), 459(93), 458(99), 456(100), 427(29), 399(67), 397(93), 297(96), 282(51), 267(41), 214(95), 200(92), 189(98), 174(93), 159(94), 144(92), 124(49).

Petroleum ether–chloroform (1:4) eluted 0.295 g (31.2%) of lactam (II) with the composition $C_{25}H_{30}N_2O_7$. mp 197-200°C (ether). ν_{max} 1740, 1640 cm^{-1}. PMR spectrum (δ, ppm): 3.14 m (1H, H-7), 3.61 s (1H, H-19), 4.07 s (1H, H-2), 4.34 m (1H, H-6), 4.40 m (1H, H-7), 5.57 s (1H, H-4). Mass spectrum, m/z (%): 470 (M^+, 100), 282(13), 189(65), 188(77), 175(29), 174(91), 161(35), 160(23), 159(49), 138(6), 121(14), 110(21), 108(15).

Chloroform eluted 0.15 g (12.8%) of the hydroxylactam (V), with the composition $C_{25}H_{30}N_2O_8$. mp 224-227°C (ether); $\nu_{max}^{CHCl_3}$ 3500, 1740, br. 1650 cm^{-1}. PMR spectrum (δ, ppm): 3.57 s (1H, H-19), 4.15 s (1H, H-2), 4.37 d (1H, J = 3.9 Hz, H-6), 4.41 br.s (1H, OH), 4.92 d (1H, J = 3.9 Hz, H-7), 5.57 s (1H, H-4). Mass spectrum, m/z (%): 487 (M^+ +1, 19), 486 (M^+, 10), 215(15), 214(11), 212(11), 204(10), 202(13), 200(15), 189(23), 188(100), 187(46), 175(11), 174(50), 168(11), 160(17), 158(22), 144(20), 130(15), 115(21).

The acetylation of the hydroxylactam (V) was performed in pyridine and acetic acid at room temperature for 10 h. The yield was quantitative. After crystallization from ethanol, an acetyl derivative with the composition $C_{27}H_{32}N_2O_9$ with mp 206-207°C was obtained. PMR spectrum (δ, ppm: 1.99 s (3H, $OCOCH_3$), 2.11 s (3H, $OCOCH_3$), 3.60 s (1H, H-19), 4.17 s (1H, H-2), 4.30 d (1H, J = 3.9 Hz, H-6), 5.51 d (1H, J = 3.9 Hz, H-7), 5.75 s (1H, H-4). M^+ 529.

CONCLUSIONS

The oxidation of vindoline by the Sarett reagent takes place in ring D with the formation of lactams. The following new compounds have been obtained for the first time: 4β-acetoxy-7,8-dihydroxy-16-methoxy-3α-methoxycarbonyl-3β,6β-epoxy-7,8,9,19-tetradehydrovincaminoreine 7,9-betaine and 4β-acetoxy-1-methyl-16-methoxy-3α-methoxycarbonyl-7,8-dioxo-3β,6β-epoxyaspidospermidine.

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