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STRUCTURE OF 22-DEOXOCUCURBITACINS ISOLATED FROM *Bryonia alba* AND *Ecbalium elaterium*

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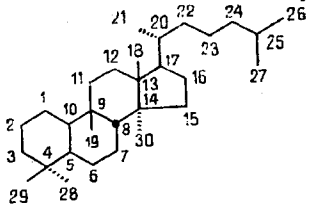
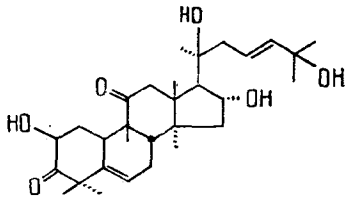
22-Deoxocucurbitacin D (II), dihydrocucurbitacin B, cucurbitacin L, and the previously unknown rhamnoglucosyl-22-deoxo-16,23-epoxycucurbitosides A (III) and B (IV) have been isolated from the roots of *Bryonia alba* and identified. Glucoside (III) has also been identified in the fruit of *Ecbalium elaterium* L.

It has been shown previously that the main components of an extract of the roots of *Bryonia alba* L., which are widely used in folk medicine for the treatment of various diseases are tetracyclic triterpenes — dihydrocucurbitacin D (Dh-D), 2- and 25-glucosyldihydrocucurbitacins D, and 2,25-diglucosyldihydrocucurbitacin D [1].

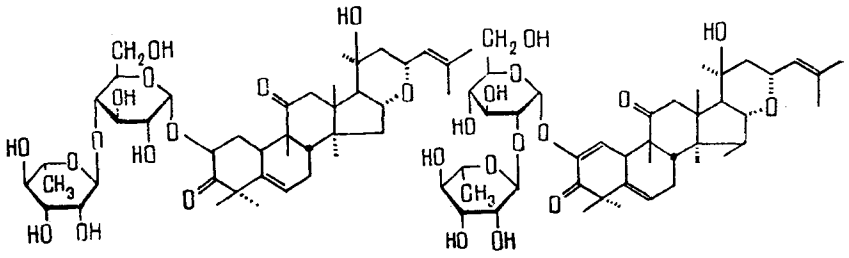
The cucurbitacins [2] exhibit a high cytotoxicity in cultures of tumor cells of the KB and Hela lines [3-6], show antitumoral [5, 6], antimicrobial [7], anthelminthic [8], purgative [9], antihepatic [10], and stimulating and tonic [11] actions, increase the permeability of capillaries and lower the blood pressure [11], inhibit the growth of plants [13], and are insect attractants [14]. It has been shown that the cucurbitacins inhibit the synthesis of DNA by selectively suppressing the inclusion of thymidine in the lymphocytes [15], and they inhibit the anaerobic glycolysis and respiration of tumor cells [16] and also the binding of [^{3}H]cortisol with the glucocorticosteroid receptors in HeLa cells [17]. The action of the cucurbitacins is also connected with their influence of the level of arachidonic acid in the tissues [18] and their inhibiting action on the liberation arachidonic acid and the biosynthesis of leukotrienes in human leukocytes [19].



I cucurbitane



II 22-Deoxocucurbitacin D



III—22-Deoxocucurbitoside A, IV—Deoxocucurbitoside B

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TABLE 1. PMR Spectra of the Cucurbitacin (II) and its Acetate and of Cucurbitacins (IIIb), (IVb), (IIIc), (IVc), and Dh-B

Proton	II	Acetate of (II)	Dh-B	III b	IVb	IIIc	IVc
1			—	—	5,92 (m)	—	5,92 (y 8)
2	4,43 (dd. 13,6)	5,46 (dd. 13,6)	4,48 (dd 13,6)	4 65 (dd 13,6)	—	—	—
6	5,78 (m)	5,75 (m)	5,85 (m)	5,78 (m)	5,78 (m)	5,75 (m)	5,70 (m)
12-α	3,15 (d. 15)	3,10 (d 15)	3,32 (d 15)	3,12 (d 15)	3,14 (d 15)	—	—
12-β	2,45 (d 15)	2,66 (d 15)	2,80 (d 15)	2,55 (d 15)	2,57 (d 15)	—	—
16	4,62 (t 7)	5,51 (t 7)	4,35 (t 7)	4 45 (t 7)	4,45 (t 7)	4,68 (t 7,5)	4,71 (t 7,2)
17	2,25 (d 7)	2,37 (t 7)	2,60 (d 7)	2,32 (d 7)	2,32)d 7)	—	—
23	5,73 (m)	4,56 (m)		4,55 (st 10, 10,4)	4,55 (st 10, 10,4)	4,55 (st 10, 10,4)	4,53 (st 10, 10,4)
24	5,73 (m)	5,66 (m)		5,15 (d 8)	5,15 (d 8)	5,15 (d 9)	5,15 (d 8)
26				1,71 (3H, s)	1,71 (3H, s)		
27				1,69 (3H, s)	1,69 (3H, s)		
2-OAc		2,11 (3H, s)					
16-OAc		2,03 (3H, s)					
25-OAc			2,00 (3H, s)				
Glc-1				4,85 (d 7)	4,95 (d 7)	4,56 (d 7,5)	4,55 (d 8)
Ph-1				5,36 (d 2,5)	5,42 (d 2,5)	5,60 (d 2,4)	5,30 (d 2,4)
Ph-6				1,25 (d 6)	1,25 (d 6)	1,25 (d 6)	1,25 (d 6)

Note. The spectra were obtained in $CDCl_3$ with TMS as internal standard.

In the present paper we give information on the isolation from the roots of *Bryonia alba* and the fruit of *Ecbalium elaterium* L. of minor cucurbitacins and their identification and the determination of the structure of previously unknown rhamnoglucosyl-22-deoxo-16,23-epoxycucurbitacins — 22-deoxocucurbitosides A (III) and B (IV).

The chromatography on silica gel of a chloroform—methanol extract of the roots of *Bryonia alba* led to the isolation of three substances of medium polarity which were identified on the basis of NMR (Tables 1 and 2), UV, and IR spectroscopy and mass spectrometry (Experimental part) as 2β,16α,20,25-tetrahydroxycucurbita-5,23(E)-diene-3,11-dione (22-oxocucurbitacin D, II), 25-acetoxy-2β,16α,20-trihydroxycucurbit-5-ene-3,11,22-trione (23, 24-dihydrocucurbitacin B), and 2,16α,20,25-**tetrahydroxycucurbita-1,5**-diene-3,11,22-trione (cucurbitacin L).

Cucurbitacins Dh-B and L are distributed comparatively widely [2] (their presence in the roots of *Bryonia alba* was also known [20, 21]), but so far as concerns 22-deoxycucurbitacin D it has been isolated only from the fungus *Lagenaria siceraria* [22].

22-Deoxocucurbitacin D was detected by the vanillin reagent on TLC in the form of a bright blue spot which is obviously characteristic for all the 22-deoxocucurbitacins. The same coloration is possessed by another, more polar, fraction of the extract of *Bryonia alba* roots giving a positive reaction for sugars with α-naphthol. TLC on silica gel impregnated with silver nitrate showed that the fraction consisted of two components, which have been called 22-deoxocucurbitosides A (III) and B (IV).

The acid hydrolysis of 22-deoxocucurbitosides A and B formed D-glucopyranose and L-rhamnopyranose in a molar ratio of 1:1 together with previously unknown aglycones, the ketolic anhydro-22-deoxocucurbitacin (IIIa) and the diosphenolic anhydro-22-deoxocucurbitacin (IVa). Aglycone (IVa) gave the coloration characteristic for diosphenolic cucurbitacins when it was detected on TLC with the aid of ferric chloride, and the UV spectrum showed absorption with a maximum at 270 nm (bathochromic shift to 314 nm in an alkaline medium); in the mass spectrum the peak of maximum intensity was that of an ion with m/z 164 formed as the result of a retro-Diels-Alder rearrangement in ring B. [23]. For the aglycone (IIIa), correspondingly, the peak of an X ion with m/z 166 was observed but there was no absorption band in the UV region or coloration on treatment with ferric chloride. The mass spectra of both compounds lacked the peaks of ions with m/z 403 for (IIIa) and m/z 401 for (IVa)) formed as the result of the cleavage of the C20—C22 bond and the loss of the side chain, which is very characteristic for the cucurbitacins [23]. The mass numbers of the molecular ions of the aglycones (IIIa) and (IVa) showed the presence of only five oxygen-containing functions.

22-Deoxocucurbitoside B gave no positive reaction with ferric chloride, which indicated the replacement of the diosphenolic hydroxyl by a glucoside residue and this was confirmed by the presence in the UV spectrum of an absorption band with a maximum at 258 nm.

The acetylation of 22-deoxycucurbitosides A and B formed the hexaacetates (IIIb) and (IVb) (the NMR spectra showed the presence of six acetyl groups, Table 1), from which it follows that these glycosides each contained a disaccharide residue and that the molecules of (III) and (IV) lacked free hydroxy groups at C16.

The first of these conclusions was confirmed by the mass spectrometry of the acetates (IIIb) and (IVb) and of the methyl ethers (IIIc) and (IVc). The spectra contained strong peaks of the ions of the disaccharide residue (m/z 561 for (IIIb) and (IVb) and m/z 393 for (IIIc) and (IVc)) and of a terminal rhamnose residue (m/z 273 for (IIIb) and (IVb) and m/z 189 for (IIIc) and (IVc)), and the peaks of the ions of a terminal glucose residue were absent, which is in harmony with the conclusion drawn from the **resistance of glycosides** (III) and (IV) to the action of glucosidase.

The absence of free hydroxy groups at C16 and C2 was confirmed by the nonappearance of the corresponding signals of the C16 and C2 protons at 5.15-5.20 and 5.48 ppm in the spectra of the acetates (IIIb) and (IVb) [24]. At the same time, the PMR spectra contained the signals of a methine proton at C23, of an olefinic proton at C24, and of a dimethyl grouping at a double bond at C25,C26, showing the structure of the anhydrocucurbitacins — formed, as is assumed, as the result of the dehydration-cyclization of a precursor with a Δ^{23}-16,25-dihydroxy grouping [22].

A comparison of the ^{13}C NMR spectra of 22-deoxocucurbitosides A and B (Table 2) with literature information for the cucurbitacins [1, 24, 25], the cucurbitacin glycosides [1, 26, 27], and some other glycosides [28-31] showed:

TABLE 2. ^{13}C NMR Chemical Shifts of the Cucurbitacins Dh-B, (II), (III), and (IV)*

Carbon	Dh-B	II	III	IV	Carbon	Dh-B	II	III	IV
1	36,8 t	36 0 t	35,6 t	123 7d	23	30,2 t	121,0 d	74,4 d	74,4 d
2	71,6 d	72,7d	77,8 d	147,2 s	24	35,3 t	143 4 d	126,7 d	126,4 d
3	212,5 s	212 8 s	211,8 s	198 2 s	25	81,4 s	70,8 s	136,8 s	135,7 s
4	48,6 s	48,0 s	49 7 s	47,0 s	26	26,0 q	29,4 q	29,8 q	30 0 q
5	140,4 s	140,4 s	141,9 s	137,6 s	27	26,0 q	29 7 q	29 3 q	29,0 q
6	120,4 d	120,4 d	121,0 d	122,1d	28	20,0 q	20,0 q	20,7 q	20,7 q
7	24 1 t	23,9 t	24,7 t	24,9 t	29	29,4 q	29,9 q	29,3 q	27,9 q
8	42 7d	42,7 d	43,7 d	42,7 d	30	20 3 q	20,0 q	20,2 q	20,2 q
9	48,6 s	48,4 s	49,8 s	49,0 s	$COCH_3$	170,5 s			
10	34,2 d	33,7 d	34,9 d	36 2 d	$\overline{C}OCH_3$	22,1 q			
11	213 0 s	213,0 s	215,6 s	216,4 s	1′			100,9 d	99,5 d
12	49,2 t	49,8 t	49,1 s	47,1 t	2′			72,2 d	79,3d
13	50,8 s	50,3 s	50 5 s	50,5 s	3′			71 5 d	71,5 d
14	50,8 s	48,6 s	52 4 s	50,8 s	4′			79,0 d	70,8d
15	46,2 t	46,0 t	46,7 t	46,9 t	5′			69,7 d	71,9d
16	70,7d	71,6 d	76,5 d	76,5 d	6′			62,8t	61,9 t
17	60,7 d	60,7 d	56,2 d	56,2 d	1″			101,9 d	101,9 d
18	21,2 q	21,2 q	21,3 q	21,9 q	2″			72,6 d	72,6 d
19	19,0 q	18,8 q	18,4 q	18,4 q	3″			72,2d	72,2 d
20	79,0 s	75,5 s	77,5 s	77,5 s	4″			74,0d	74 0d
21	25,5 q	25,5 q	25,8 q	26,1 q	5″			69,4d	69,4 d
22	214,0 s	44,4 t	41,9 t	41 6t	6″			17,8 q	17,8 q

*The spectra of Dh-B and (II) were obtained in $CDCl_3$ and those of compounds (III) and (IV) in CD_3OD with TMS as internal standard.

– the absence of a carbonyl group at C22 (the triplet of a methylene group was observed at 41.9 ppm and there was a upfield shift of the singlet from the neighboring C20 carbon atom);

– the presence of an additional trisubstituted double bond at C24–C25 outside the tetracyclic skeleton, together with the C5–C6 trisubstituted double bond of ring B and the C1–C2 trisubstituted conjugated double bond in ring A of the 22-deoxycucurbitoside B molecule; the values of the chemical shifts for C1, C2, and C5 also indicate substitution of the diosphenol hydroxyl by a carbohydrate residue;

– the absence of free hydroxy groups at C2 and C16 (downfield shift of the C2 and C16 doublets by 5–7 ppm);

– the α-configuration of the anomeric carbon atoms of the glucopyranosyl (δC1′ – 99.5 and 100.9 ppm) and the rhamnopyranosyl (δC″ – 101.9 ppm) residues (the possibility of the glycosylation of the only tertiary hydroxy at C20 must be excluded because of the presence of a singlet at 77.5 ppm in each of the spectra); and

– the nature of the linkages of the monosaccharide units – (1 → 4) and (1 → 2) in the molecules of 22-deoxocucurbitosides A and B, respectively.

The last conclusion follows from the values of the glycosylation shifts [28, 29] for C4′ ($\Delta\delta_A$ + 9.0) and for C2′ (Δδ + 7.1) of the glucosyl residues, while for the rhamnosyl residues the chemical shifts of the carbon atoms correspond to an unsubstituted rhamnopyranoside [30, 31].

The structure of the rhamnoglucosyl residue was also confirmed by the results of the GLC-mass spectrometry of the alditol acetates [32] derived from the partially methylated sugars obtained as the result of the acid hydrolysis of the exhaustively methylated glycosides (IIIc) and (IVc).

Glycoside (III) – 2-[O-α-L-rhamnopyranosyl-(1 → 4)-α-D-glucopyranosyloxy]-3,11-dioxo-16α, 23α-epoxycucurbita-5,24-diene-2β,20-diol – was isolated from the fruit of *Ecbalium elaterium* and identified similarly.

It must be mentioned that there are only two reports in the literature on the detection of 22-deoxocucurbitacins in plants: in addition to the 22-deoxocucurbitacin D mentioned above (*Lagenaria siceraria* [22]), anhydro-22-deoxo-2-epiisocucurbitacin D has been isolated from the juice of the fruit of *Ecbalium elaterium* [33].

EXPERIMENTAL

The optical rotations of the samples were measured on a Polamat A polarimeter (GDR), and UV and IR spectra were recorded on Specord UV-Vis and UR-20 spectrophotometers (GDR), respectively, PMR and ^{13}C NMR spectra on a Varian XL-200 instrument, and mass spectra on a MKh1320 instrument.

KSK silica gel (100-120 μ) for column chromatography was treated with 10% HNO_3 (100°C, 10 h), with water, with 2% ammonia solution, with water, and with methanol and was activated at 180°C for 7 h.

For preparative TLC and analytical micro-TLC, silica gel of the same brand was used and the following solvent systems: 1) chloroform–acetone (2:1); 2) chloroform–acetone (1:1); 3) chloroform–acetone (6:1); 4) chloroform–methanol–water (30:15:1); 5) chloroform–acetone–methanol–water–acetic acid (5:2:1:0.5); 6) chloroform–acetone (5:1); 7) hexane–acetone (2:1); 8) chloroform–acetone (20:0.8). The substances were revealed on the chromatograms: a) with 0.5% vanillin in 15% H_3PO_4 followed by heating at 100°C (5-10 min); 6) with 5% $FeCl_3 \cdot 6H_2O$ in 50% MeOH; c) with iodine vapor; d) with 0.5% α-naphthol in methanol–water (1:1 followed by spraying the plates with 5% H_2SO_4 and heating them at 120°C.

In the PC of the sugars, the butanol–pyridine–benzene–water (5:1:3:3, upper phase) system was used, and the substances were detected with a solution of aniline phthalate (aniline–phthalic acid–butanol–diethyl ether–water (9.2:16:490:490: 20)).

The GLC (Chrom-41) of the TMS derivatives of the sugars and their methyl glycosides was performed on a column (1200 × 3 mm) containing 3% SE-30/Gas Chrom Q (125-150 μ) at 170°C, with a flame-ionization detector. The carrier gas was helium (50 ml/min).

Compounds (II), (III), and (IV) were acetylated by treating samples with a 1:1 mixture of acetic anhydride and pyridine (20°C, 7 h) after which the reaction mixtures were diluted with water, acidified with 2 N HCl, and extracted with ethyl acetate.

The conversion of the α-ketolic cucurbitacins into the diosphenolic compounds was performed by alkaline treatment as described by Enslin et al. [22]. The methylated alditol acetates were analyzed by GLC using a column containing 10% PEGS/Gas Chrom Q (170°C) and by GLC-mass spectrometry using a column with 3% SE-30/Gas Chrom Q (LKB-9000).

Isolation of 22-Deoxycucurbitacin D (II), 23,24-Dihydrocucurbitacin B, and Cucurbitacin L. A chloroform–methanol (1:1) extract of the roots of *B. alba* was evaporated to dryness and the residue (63.4 g) was subjected to separation in the two-phase system of chloroform–methanol–water (200:100:75). The lower, chloroformic phase was evaporated and the residue (22 g) was deposited on a column containing 100 g of silica gel. The column was eluted with benzene, benzene–chloroform (4:1), chloroform, chloroform–methanol (24:1, 19:1, and 14:1), and methanol (100 ml). The separation was monitored by TLC (system 1, detection by reagents a and b). Benzene–chloroform (4:1) eluted from the column a mixture of substances containing 23,24-dihydrocucurbitacin B (yellow coloration with reagent a on TLC), chloroform eluted a mixture of compounds containing cucurbitacin L (yellow coloration with reagent a and green coloration with reagent b), and chloroform–methanol (24:1) eluted a mixture of substances containing 22-deoxocucurbitacin D (bright blue coloration with reagent a).

The fraction containing 22-deoxocucurbitacin D (4400 mg) was subjected to rechromatography on a column of silica gel (25 g). The substances were eluted from the column with chloroform (200 ml) and with chloroform–methanol (100:1), (600 ml), (200:3) (203 ml), (100:2) (200 ml), and (100:6) (200 ml).

The last 200 ml of the (100:1) solvent system eluted 245 g of a mixture of substances from which, by preparative TLC on silica system (system 2, c) 69 mg of pure 22-deoxocucurbitacin D (II) was obtained with R_f 0.5 (system, a) $\nu_{max}^{CHCl_3}$, cm^{-1}: 1695, 1710, 3480; $\lambda_{max}^{CH_3OH}$ – no absorption. Mass spectrum, m/z (%): 502 (M^+, 7), 484 ($[M-H_2O]^+$, 12), 469 ($[M-H_2O-CH_3]^+$, 466 ($[M-2H_2O]^+$, 5), 451 ($[M-2H_2O-CH_3]^+$, 9), 403 (cleavage of the C20–C22 bond, 60), 385($[403-H_2O]^+$, 30), 367 ($[403-2H_2O]^+$, 14), 343(8), 237(8), 219(20), 189(18), 187(23), 166(17), 135(30), 109(40), 82 (cleavage of the C20–C22–OH bond, 100). When the cucurbitacin (II) was treated with 4 N sodium methanolate in methanol at 25°C for 1 h, the diosphenolic derivative was formed with $\lambda_{max}^{CH_3OH}$, nm: 300 (ε 1000).

22-Deoxocucurbitacin D diacetate (IIa) – R_f 0.7 (system 1, a). Mass spectrum, *m/z* (%): 568 ([M—H_2O]$^+$, 1) 550 ([M—2H_2O]$^+$, 3), 487 (cleavage of the C20—C22 bond, 50), 427 ([487—AcOH]$^+$, 48), 411(20), 385(80), 367 ([487—2AcOH]$^+$, 46), 349(10), 338(18), 325(40), 309(15), 307(17), 283(10), 265(15), 219(32), 43(100) . The fraction containing the 23,24-dihydrocucurbitacin B (420 mg) was rechromatographed on a column of silica gel (5 g). The substances were eluted from the column with benzene—chloroform (100:10; 100:15; 100:25; and 100:50) (200 ml each). The last two solvent systems eluted 171 mg of 23,24-dihydrocucurbitacin B with R_f 0.6 (system 3, a); mp 150-161°C, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1740, 1721, 3460. Mass spectrum, *m/z* (%): 500 ([M—AcOH]$^+$, 6), 482 ([M—AcOH—H_2O]$^+$, 20), 467 ([M—AcOH—Me]$^+$, 5), 444 (10), 403 (cleavage of the C20—C22 bond, 100), 385 ([403—H_2O]$^+$, 60), 369 ([403—2H_2O]$^+$, 32), 343(10), 325(15), 309(3), 237(18), 219(20), 166(30), 142(42), 113(90), 87(80).

The fraction containing cucurbitoside L (500 mg) was dissolved in 20 ml of chloroform, the solution was extracted with 5% NaOH (5 × 5 ml), and the aqueous phase was neutralized with 2 N HCl and re-extracted with chloroform (3 × 100 ml). The chloroform extract was dried and evaporated to dryness, and the residue (88 mg) was subjected to preparative TLC (system 1, c). The substances were eluted from the silica gel with ethyl acetate. As a result, 61 mg of cucurbitacin L was obtained which gave a positive reaction with ferric chloride and had R_f 0.4 (system 1, a, b); mp 122-127°C; $\lambda_{max}^{CH_3OH}$, nm: 271 (ε 4500). Mass spectrum *m/z* (%): 498 ([M—H_2O]$^+$, 12); 480 ([M—2H_2O]$^+$, 20), 465 ([M—2H_2O—CH_3]$^+$, 25); 401 cleavage of the C20—C22 bond, 50), 385 ([401—H_2O]$^+$, 30), 368 ([401—H_2O—CH_3]$^+$, 16), 341(34), 219(36), 164(100), 142 (cleavage of the C20—C22—OH bond, 70).

Isolation of 22-Deoxocucurbitosides A (III) and B (IV). A methanolic extract (94 g) of the roots of *B. alba* was subjected to separation in 375 ml of the chloroform—methanol—water (400:200:150) solvent system, the upper (aqueous phase) was evaporated, and the residue (61.4 g) was dissolved in 150 ml of water and extracted with butanol (3 × 100 ml). After the butanol had been distilled off, the substance (17 g) was dissolved in 16 ml of chloroform—methanol (15:1) and deposited on a column of silica gel (120 g). The column was eluted with chloroform—methanol (15:1) (500 ml) and (14:1) (3500 ml), and with acetone (500 ml). The (14:1) system eluted from the column 1100 mg of a mixture of substances containing cucurbitosides A and B, which were then purified by column chromatography on Sephadex LH-20 (Farmacia). The sorbent (60 g) was previously saturated with 60 ml of the lower phase of the chloroform—methanol—water (15:129:171) system. The substances were deposited on the column in 5 ml of the upper phase and were eluted with 380 ml of the same solvent system, as a result of which 289 ml of a mixture of 22-deoxocucurbitosides A and B was obtained (R_f 0.4 in system 4, a, d, and R_f 0.6 in system 5 a, d). Cucurbitosides A (III) and B (IV) were separated with the aid of preparative TLC on silica gel impregnated with silver nitrate (5%) in system 4, c (R_f 0.41 and 0.31, respectively). The substances were eluted from the silica gel with chloroform—methanol (1:1) and were subjected to gel filtration on columns of Sephadex LH-20 in methanol. This gave the amorphous 22-deoxocucurbitoside A (III), $[\alpha]_D^{20}$ + 51,9° (*c* 6,1 ; ethanol) and the amorphous 22-deoxocucurbitoside B (IV), $[\alpha]_D^{20}$ + 22,3° (*c* 2,88 ; ethanol), $\lambda_{max}^{ethanol}$, nm: 255.

Hexaacetates of 22-Deoxocucurbitosides A and B. R_f 0.6 (system 6, a). Mass spectrum, *m/z* (%): 771 ([M—RhO]$^+$, 2), 770 ([M—RhOH]$^+$, 3), 753 ([M—RhO—H_2O]$^+$, 2), 752 ([M—RhOH—H_2O]$^+$, 3), 693 ([M—RhOH—H_2O—AcO]$^+$, 4), 621 (3), 561 ([ROGlc]$^+$, 23), 519 (2), 482 ([M—RhOGlc—H]$^+$, 6), 480(5), 467 ([M—RhOGlc—OH]$^+$, 5), 319(15), 273 ([Rh]$^+$, 100), 231(12), 213(15), 171(30), 169(25), 153(75), 111(45), 43(100).

The exhaustive methylation of 22-deoxocucurbitosides A and B was performed by a modified Hakomiri method [34]. To 115 mg of dry sodium hydride was added 1 ml of anhydrous DMSO, and the mixture was stirred in an atmosphere of N_1 at 60°C for 30 min; then, at 20°C, 0.5 ml of a solution of the cucurbitosides (~20 mg) in DMSO was added. The mixture was stirred for 2 h, and then 1 ml of freshly distilled methyl iodide was added and the new mixture was kept at 20°C for 6 h and was diluted with 100 ml of water and extracted with ether. The ethereal extract was washed with water and evaporated, and the residue was subjected to chromatography on a column of silica gel (10 g). The substances were eluted from the column with hexane—acetone (99:1; 80:20; 70:30; 65:35; 60:40; ...; 20:80; 15:85; 10:90; and 5.95). As a result, the methyl ethers of 22-deoxocucurbitoside A (IIIc), R_f 0.5 (system 7, a) and of 22-deoxocucurbitoside B (IVc), with R_f 0.4 (system 7, a) were obtained. Mass spectrum of (IIIc), *m/z* (%): 556(3), 526(2), 494(1), 480 ([M—RhOGlcOH]$^+$, 10), 467(12), 393 ([RhOGlc]$^+$, 15), 361 ([RhOGlc—MeO]$^+$, 25), 319(10), 265(15), 233(10), 205(12), 201(25), 189 (Rh$^+$, 100), 173(25), 157(35), 109(35), 101(60), 88(100). Mass spectrum of (IVc), m/z (%): 646(1), 585(2), 512(1), 478 ([M—RhOGlcOH]$^+$, 25), 465(10), 393

([RhOGlc]+, 16), 361 ([RhOGlc—MeO]+, 22), 319(10), 264(13), 205(12), 201(20), 189 (Rh+, 100), 173(20), 157(30), 109(35), 101(50), 88(90).

The acid hydrolysis of the methyl ethers of the 22-deoxocucurbitosides and the acetylation of the polyols obtained by the reduction of the partially methylated sugars formed as the result of hydrolysis were performed by a known procedure [35]. 1,5-Diacetyl-2,3,4-trimethyl-6-deoxyhexitol, 1,2,5-triacetyl-3,4,6-trimethylhexitol, and 1,4,5-triacetyl-2,3,6-hexitol were identified by GLC and GLC-mass spectrometry [32].

The acid and enzymatic hydrolysis of 22-deoxocucurbitosides A and B with a glucosidase preparation isolated from bryony roots were carried out by a method described previously [1]. After the solution had been neutralized with Dowex-1 (OH^-) and the aqueous phase had been extracted with ethyl acetate, the solvent was evaporated off and the residue was chromatographed on silica gel columns with elution by benzene—ethyl acetate (20:1; 10:1; 9:1; 8:1; 7:1; and 6.1). As a result, 22-deoxocucurbitoside A yielded the aglycone (IIIa), and 22-deoxocucurbitoside B the aglycone (IVa). (IIIa) had R_f 0.4 (system 8, a) and the mass spectrum *m/z* (%): 484 (M^+, 3), 467 ([M—HO]+, 3), 382(2), 368(2), 354(3), 312(5), 284(3), 279(10), 256(15), 213(8), 211(8), 166(25), 149(50), 111(25), 101(80), 88(100). (IVa-Rf 0.4 (system 8, a, b) and the mass spectrum *m/z* (%): 482 (M^+, 10), 464 ([M—H_2O]+, 8), 449 ([M—H_2O—Me]+, 9), 246(5), 239(6), 203(3), 204(12), 189(15), 186(16), 164(100).

Isolation of 22-Deoxocucurbitoside A from the Fruit of *Ecbalium elaterium* L. A methanolic extract of the fruit of *E. elaterium* was evaporated to dryness and the residue (1 g) was dissolved in 2 ml of water and deposited on a column of Woelm polyamide (10 g). The column was eluted with water (80 ml) and with methanol (100 ml); the last 20 ml of water contained 22-deoxocucurbitoside A. The aqueous solution was washed with chloroform (3 × 20 ml) and evaporated, the residue was dissolved in 20 ml of ethyl acetate, and the solution was washed with water. Evaporationof the ethyl acetate yielded 10 mg of pure 22-deoxocucurbitoside A.

SUMMARY

An extract of the roots of *Bryonia alba* L. contained 22-deoxoxcucurbitacins 2β,16α,20,25-tetrahydroxycucurbita-5,23(E)-diene-3,11-dione (II) and the previously unknown 20-hydroxy-2β-[O-α-L-rhamnopyranosyl-(1 → 4)-α-D-glucopyranosyloxy]-16α,23α-epoxycucurbita-5,24-diene-3,11-dione (III) and 20-hydroxy-2β-[O-α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranosyloxy]-16α,23α-epoxycucirbita-1,5,24-triene-3,11-dione (IV). Glycoside (III) has also been identified in an extract of the fruit of *Ecobalium elaterium* L.

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STRUCTURE OF ALLINE

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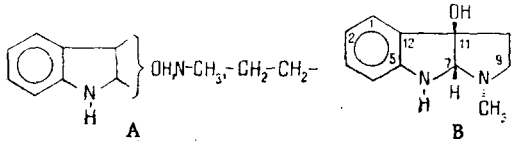
The spatial structure of the alkaloid alline has been determined by x-ray structural analysis. It has been established that alline has a physostigmine skeleton containing one tryptamine unit.

A base $C_{11}H_{14}N_2O$ has been isolated previously from the epigeal part of *Allium odorum* L. (family *Liliaceae*); it has proved to be new and we have called it alline (I).

UV spectrum of (I): λ_{max} 245, 303 nm (log ε 3.68, 3.14). The IR spectrum showed absorption bands at (cm^{-1}) 3350 (OH, NH); 1615, and 1495 (aromatic ring); and 710 and 760 (1,2-disubstituted benzene ring). The PMR spectrum of (I) had signals in the 7.12-6.45 ppm region (m, 4 H, Ar—H), singlets at 4.18 (1 H), 4.03 (2 H) (OH, NH, CH), and 2.21 ppm (s, 3 H, N—CH_3), and multiplets at 2.7 and 2.0 ppm (2 H each, N—CH_2—CH_2—). On the basis of its UV, IR, and PMR spectra and its composition, the partial structure (A) can be suggested for alline.



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