Cucumarioside G_4. The total glycosides (9.8 g) were chromatographed repeatedly on silica gel in the chloroform–methanol–water (65:24:4) system, and then the fraction containing the (I) was separated by the HPLC method on a Zorbax-ODS column, 4.8 × 250 mm, at a rate of elution of 1 ml/min with the eluent water–acetone (73:27). This gave 98 mg of (I) with mp 211-213°C, $[\alpha]_{578}^{20}$-11° (c 0.1; pyridine).

The desulfation of (I) by solvolysis in a mixture of pyridine and dioxane (1:1) was carried out by the procedure described in [4]. The derivative obtained was finally purified by chromatography on silica gel in the chloroform–methanol (8:1) system. This gave (II) with mp 265-267°C, $[\alpha]_{578}^{20}$-12° (c 0.1; pyridine).

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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XLIV. STRUCTURE OF CYCLOCARPOSIDES A AND C

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UDC 547.918:547.626

The structures of two new cycloartane glycosides – cyclocarposides A and C, isolated from the herb Astragalus coluteocarpus Boiss. – have been established on the basis of spectral characteristics and chemical transformations. Cyclocarposides A and C are: 20R,24S-epoxycycloartane-3β,6α,17β,25-tetraol 3-O-(2-O-acetyl-β-D-xylopyranoside)6-O-(2-O-acetyl-α-L-rhamnopyranoside) and 20R,24S-epoxycyloartane-3β,6α,16β,25-tetraol 3-O-(2-O-acetyl-β-D-xylopyranoside) 6-O-α-L-rhamnopyranoside, respectively.

Continuing investigations of cycloartane methylsteroids and their glycosides from plants of the genus Astragalus (Leguminosae), we have determined the structures of two new glycosides isolated from Astragalus coluteocarpus Boiss. [1] which we have called cyclocarposide A (III, substance 3) and C (IV), substance 5).

The presence in the PMR signals of the new glycosides (III) and (IV) of signals characteristic for the methylene protons of a tetrasubstituted cyclopropane and also of seven methyl groups showed that the glycosides under consideration were cycloartane derivatives [2, 3]. The IR spectra of cyclocarposides A and C each contained an absorption band at 3055 cm^{-1} assigned to the methylene of a three-membered ring [4].

The correctness of the assignment of the glycosides under discussion to the cycloartane series was also confirmed by the fact that on acid hydrolysis both glycosides formed cyclosieversigenin (I) [3]. It was shown by the GLC method [5] that cyclocarposides A and C each

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Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 694-698, November-December, 1992. Original article submitted April 20, 1992.

TABLE 1. Chemical Shifts (δ, ppm), Multiplicities, and SSCCs (J, Hz) of the Protons of Cyclocarposides A (III) and C (IV) (C_5D_5N – TMS)

Position of the protons	Compound	
	III	IV
H-3	3,23 dd(12; 5)	3,17 dd (11; 5)
H-6	3,45 td (9;4)	3,36 td (9; 4)
H-16	4,90 q (7,6;7,6; 7,6)	4,82 q (7,5; 7,5; 7,5)
H-17	2,41 d (7,6)	2,33 d (7,5)
2H-19	0,16; 0,32 d (4)	0,10; 0,26 d (4)
H-22	2,95 q (10; 10; 10)	2,84 q (9,7; 9,7; 9,7)
H-24	3,81 dd(10; 6)	3,73 dd, (10; 6)
CH_3- groups	0,87 s	0,81 s
	1,01 s	0,89 s
	1,21 s	1,14 s
	1,22 s	1,16 s
	1,29 s	1,16 s
	1,34 s	1,22 s
	1,47 s	1,40 s
CH_3COO	1,95 s	1,92 s
	2,09 s	—
	β-D-Xylp residue	
1	4,67 d (8)	4,58 d (8)
2	5,35 t (8)	5,21 t (8)
3,4	4,05–4,30	3,90—4,20
5a	3,62 t (10)	3,56 t (10)
5e	4,50 dd, (10;5)	4,28 dd (10; 3)
	α-L-Rhap residue	
1	5,09 br.s	5,18 br.s
2	5,61*	4,39*
3,4,5	4,05—4,30	3,90—4,20
6	1,51 d (6)	1,42 d (6)

*Doublet with broadened components (^3J = 4 Hz).

contained D-xylose and L-rhamnose residues in a ratio of 1:1. This conclusion was in full agreement with the ^1H and ^{13}C NMR spectra of glycosides (III) and (IV) (Tables 1 and 2).

The IR spectra of cyclocarposides A and C 3ach contained, in addition to the absorption band mentioned above, absorption bands characteristic of an ester grouping. The ^1H and ^{13}C NMR spectra of the new glycosides (III) and (IV), containing two 3-proton singlets at 1.95 and 2.09 ppm and one 3-proton singlet at 1.92 ppm, respectively, and also signals at 21.29, 20.99, 170.75, 170.14, and at 21.17 and 169.95 ppm, showed that cyclocarposides A and C were di- and monoacetates.

The alkaline hydrolysis of each of the glycosides gave one and the same glycoside, which was identified as cyclocarposide (II).

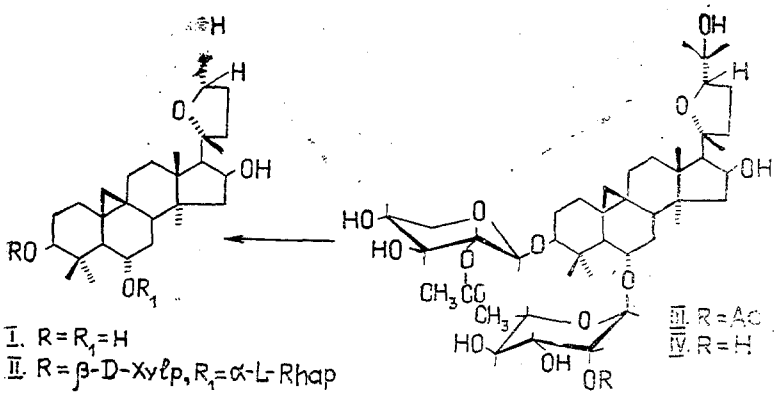
The positions of the acetyl groups were elucidated by a study of the ^1H and ^{13}C NMR spectra and also double homonuclear resonance spectra.

In the ^1H NMR spectrum of cyclocarposide A the signals of protons geminal to acetoxy functions were observed at 5.35 and 5.61 ppm in the form of a triplet with an SSCC of 8 Hz and a doublet with broadened components (^3J = 4 Hz). The presaturation of the anomeric proton of the D-xylose residue (4.67 ppm) converted the triplet at 5.35 ppm into a doublet. The preirradiation of the anomeric proton of the L-rhamnose residue (5.09 ppm) led to the conversion of the doublet with broadened components at 5.61 ppm into a sharp doublet. Consequently, the signals under consideration related to protons located at C-2 of the D-xylose and L-rhamnose residues, respectively, and the acetyl groups were located in the same positions.

TABLE 2. Chemical Shifts of the Carbon Atoms of Compounds (I-IV) (δ, ppm; O – TMS)

C atom	Compound			
	I	II	III	IV
1	32,72	32,20	32,04	32,06
2	31,30	30,06	29,99	29,93
3	78,21	87,83	88,25	88,05
4	42,28	42,26	41,95	41,95
5	53,86	52,03	51,95	51,98
6	68,27	79,16	79,75	79,25
7	38,69	34,55	34,62	34,70
8	47,21	46,16	46,34	46,77
9	20,84	20,65	20,66	20,73
10	29,80	28,70	28,76	28,69
11	26,29[a]	26,38	26,41	26,40
12	33,31	33,27	33,28	33,29
13	44,89	45,00	45,03	45,03
14	46,09	46,16[a]	46,16	46,19
15	46,69	46,67	46,74	46,30
16	73,35	73,33	73,36	73,36
17	58,26	58,23	58,32	58,30
18	21,51	21,47	21,56	21,57
19	31,00	30,22	30,34	30,38
20	87,17	87,15	87,19	87,18
21	28,46	28,42	28,05	28,02
22	34,81	34,88	34,91	34,90
23	26,29[a]	25,95	25,90	25,92
24	81,57	81,67	81,74	81,72
25	71,19	71,22	71,30	71,30
26	27,04*	27,05*	27,09*	27,07*
27	28,09*	28,11*	28,15*	28,13*
28	20,17	20,16	20,21	20,21
29	29,28	28,51	28,53	28,52
30	16,16	17,05	16,95	16,96
	β-D-Xylp residue			
1		107,43	105,01 (−2,42)	104,95 (−2,48)
2		75,38	76,24 (+0,86)	76,21 (+0,83)
3		78,44	75,61 (−2,82)	75,55 (−2,89)
4		71,14	71,24	71,23
5		66,96	67,12	67,10
	α-L-Rhap residue			
1		103,88	100,65 (−3,23)	104,00
2		72,87	74,34 (+1,47)	72,98
3		72,56	70,60 (−1,96)	72,60
4		73,71	73,91	73,81
5		70,06	70,09	70,12
6		18,16	18,07	18,17
CH_3			21,29	21,17
COO			20,99 170,75 170,14	169,95

*The signals marked with the same letters are superposed with one another, and those marked with asterisks have been assigned uncertainly.



This conclusion was confirmed by a comparative analysis of the ^{13}C NMR spectra of cyclocarposide A (III) and cyclocarposide (II). As can be seen from Table 2, considerable changes were observed in the values of the chemical shifts of the C-1–C-3 atoms of the monosaccharide residues of cyclocarboside A as compared with those of cyclocarposide. The magnitudes and signs of these changes unambiguously determined the position of the acetyl groups at C-2 in both monosaccharide residues.

Thus, cyclocarposide A had the structure of 20R,24S-epoxycycloartane-3β,6α,17β,25-tetraol 3-O-(2-O-acetyl-β-D-xylopyranoside) 6-O-(2-O-acetyl-α-L-rhamnopyranoside).

In the PMR spectrum of cyclocarposide C (IV), the signal of the proton geminal to the acetoxy function was observed at 5.21 ppm in the form of a triplet with an SSCC of 8 Hz. In the double homonuclear resonance spectrun taken with preirradiation of the anomeric proton of the β-D-xylopyranoside residue (4.58 ppm), the signal at 5.21 ppm was converted into a doublet. This means that the signal under consideration related to a proton at C-2 of the β-D-xylopyranose residue, and the acetyl group was present in the same position.

As was to be expected, in the ^{13}C NMR spectrum of cyclocarposide C the signal of the C-2 atom of the β-D-xylopyranoside residue had undergone a downfield shift and the signals of the C-1 and C-3 atoms upfield shifts in comparison with their positions in the spectrum of cyclocarposide. This fact served as an additional confirmation of the conclusion concerning the position of the acetyl group in the new glycoside (IV).

Thus, we are justified in concluding that cyclocarposide C is 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-(2-O-acetyl-β-D-xylopyranoside) 6-O-α-rhamnopyranoside.

EXPERIMENTAL

For general remarks, see [6]. The following solvent systems were used: 1) chloroform–methanol, (15:1); and 2) chloroform–methanol–water (70:23:4).

For the GLC, TLC, and CC conditions, see [5].

^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 and Tesla BS-567A instruments. ^{13}C NMR spectra NMR spectra were also obtained under the conditions of J-modulation.

For the isolation and separation of the triterpenoids of Astragalus coluteocarpus, see [1].

Cyclocarposide A (III) – substance 3. $C_{45}H_{72}O_{15}$, mp 224-226°C (from methanol) $[\alpha]_D^{23}$ -31.7 ± 2° (c, 0.57; methanol). ν_{max}^{KBr}, cm^{-1}: 3600-3235 (OH), 3055 (CH_2 of a cyclopropane ring), 1730, 1260 (ester groups). GLC [5] showed that glycoside (III) contained D-xylose and L-rhamnose residues in a ratio of 1.00:1.13. The ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2.

Cyclocarposide C (IV) – substance 5. $C_{43}H_{70}O_{14}$, mp 257-259°C (from methanol), $[\alpha]_D^{23}$ –61 ± 2° (c 0.59; pyridine). ν_{max}^{KBr}, cm^{-1}: 3600-3230 (OH); 3055 (CH_2 of a cyclopropane ring); 1740, 1255 (ester group). It was found by the GLC method [5] that glycosides (IV) contained D-xylose and L-rhamnose residues in a ratio of 1.00:1.02. The ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2.

Cyclosieversigenin (I) from (III). Cyclocarposide (A) (40 mg) was hydrolyzed with 10 ml of a 0.5% methanolic solution of sulfuric acid at 60°C for 1 h. After working up and chromatography on a column in system 1, the genin fraction of the hydrolysate yielded 12 mg of cyclosieversigenin (I), mp 239-241°C (from methanol) $[\alpha]_D^{23}$ + 52 ± 2° (c 0.8; methanol).

Cyclocarposide (II) from (III). Glycoside (III) (30 mg) was hydrolyzed with 8 ml of a 0.1% methanolic solution of sodium hydroxide at room temperature for 0.5 h. After working up and chromatography on a column in system 2, the reaction products yielded 23 mg of cyclocarposide (II), mp 284-285°C (from methanol), $[\alpha]_D^{23}$-238 ± 2° (c 0.9; pyridine).

Cyclosieversigenin (I) from (IV). Cyclocarposide C (30 mg) was subjected to acid hydrolysis under the same conditions as for cyclocarposide A, and 10 mg of cycloseviersigenin was obtained.

Cyclocarposide (II) from (IV). The alkaline hydrolysis of cyclocarposide C (25 mg) with 10 ml of a 0.1% methanolic solution of sodium hydroxide at room temperature for 1 h followed by chromatography on a column in system 2 gave 15 mg of cyclocarposide (II).

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SYNTHESIS OF TRITIUM-LABELED PHYTOHORMONES

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UDC 547.857.581.192.7

A method has been developed for obtaining tritium-labeled analogues of β-indolylacetic acid and of 6-benzylaminopurine by the method of isotopic catalytic exchange. The localization of cytokinin- and auxin-binding proteins in cotton seedlings has been investigated.

One of the main methods of identifying receptor proteins in plant and animal tissues is that of isotopic indicators using labeled analogues of corresponding hormones possessing a high molar activity — not less than 10 Ci/mole [1]. In the present paper we describe the introduction of a tritium label into β-indolylacetic acid (IAA) and 6-benzylaminopurine (BAP) and the localization of the corresponding hormone-binding proteins in cotton seedlings.

The generally adopted method of introducing a tritium label into complex organic compounds is the method of catalytic $^1H \rightarrow {}^3H$ exchange. The intensity of the exchange reaction, the degree of labeling with tritium, and the final specific activity are determined by a number of factors, among which the most important is the efficiency of the catalyst [2].

Table 1 gives the dependence of the efficiency of isotopic exchange on the nature of the catalyst. It can be seen from Table 1 that the highest value of the molar ratio activity (A_{mol}) was achieved in the catalyst PdO/Al_2O_3. Isotopic exchange also took place in the presence of organic compounds, pyridine being more effective than triethylamine. The addition of triethylamine to the solid-phase catalyst PdO scarcely changed the value of A_{mol} for IAA and BAP. A subsequent investigation of the dependence of isotopic exchange on the amount of catalyst showed that an increase in the ratio of catalyst to compound raised the molar activity of IAA and, to a smaller degree, that of BAP (Table 2).

It must be mentioned that with an increase in the amount of catalyst the nonspecific chemosorption of BAP and IAA on its surface rose. This phenomenon, together with radiolysis, lowered the yield of the desired product, thereby limiting the use of large amounts of catalyst.

The nature of the solvent also had a definite influence on the resulting activity (Table 3).

As can be seen from Table 3, the molar activities of ^3H-BAP and ^3H-IAA increased substantially in dioxane, particularly when triethylamine was added to the reaction mixture. The high rate of exchange in an alkaline medium can be explained by the assumption that, together with protonated forms, nonprotonated forms of benzopyrrole and of benzylaminopurine

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