TRITERPENE GLYCOSIDES OF Astragalus AND OF THEIR GENINS.

X. CYCLOGALEGIGENIN FROM Astragalus galegiformis

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The flowers of the plant Astragalus galegiformis L. have yielded a new isoprenoid – cyclogalegigenin – the structure of which has been established on the basis of chemical transformations and spectral characteristics as 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol.

The plant Astragalus galegiformis L. has been studied previously for flavonoid glycosides [1]. We have now studied the isoprenoids of this plant.

From an ethanolic extract of the inflorescences of A. galegiformis four substances were isolated which were called in order of increasing polarity compounds A, B, C, and D. Isoprenoid A proved to be a genin, and we have called it cyclogalegigenin (I). The other substances were of glycosidic nature.

The Smith degradation of the total glycosides [2] led to cyclogalegigenin (I), and the structure of the substance is described in the present paper.

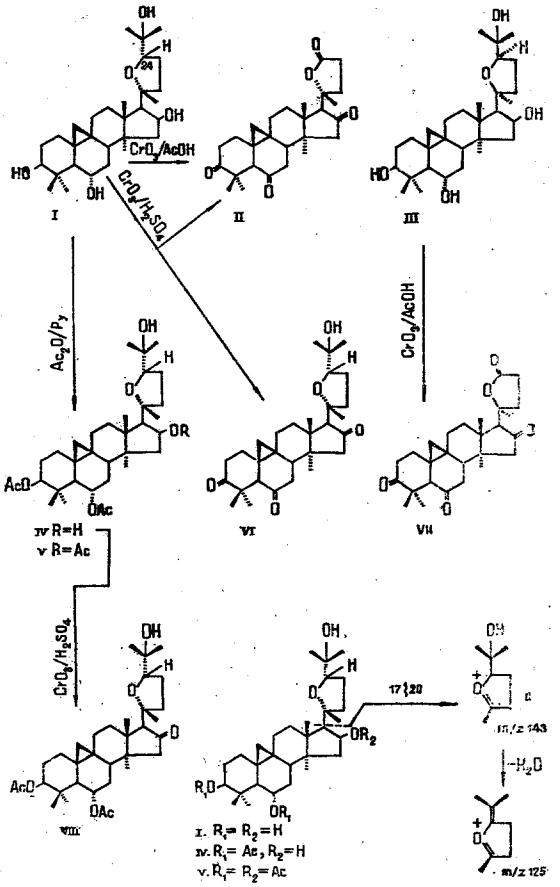

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TABLE 1. Chemical Shifts of the Protons of Cyclogalegigenin (I), Cyclosiversigenin (III), and Their Derivatives (δ, ppm, 0 – HMDS)

Compound	Positions of the protons						—OAc
	H-3	H-6	H-16	2H-19	H-24	CH_3 group	
I	3,55 q (^3J=4,8; 11,2 Hz) [3,24 q (^3J=4,8; 11,2 Hz)]	3,69 sx (^3J=3,6; 9,6; 9,6 Hz) [3,48 sx (^3J=3,8; 9,8; 9,8 Hz)]	4,70 sx (Σ^3J=21 Hz) [4,54 sx (Σ^3J=21 Hz)]	0,22; 0,50 d (^2J=4,2 Hz) [0,3 ; 0,45 d (^2J=4,6 Hz)]	3,83 t (Σ^3J=15 Hz) [3,77 t (Σ^3J=15 Hz)]	0,89; 1,17; 1,23; 1,24; 1,40; 1,57; 1,78 [0,84; 0,90; 1,06; 1,17; 1,19; 1,20; 1,34]	—
II	—	—	—		—	0,96; 1,11; 1,14; 1,26; 1,41	—
III	~3,57 m*	~3,57 m*	4,90 q (^3J at 7,0 Hz)	0,23; 0,51 d (^2J=4,0 Hz)	3,78 q	0,90; 1,18; 1,20; 1,25; 1,33; 1,45; 1,78	—
IV	[~4,5 m]*	[~4,6 m]*	[~4,5 m]*	[0,30; 0,57 d (^2J=4,5 Hz)]	[3,74 t (Σ^2J=15 Hz)]	[0,79; 0,85; 0,94; 1,06; 1,18; 1,29; 1,34]	[1,92; 1,98]
V	[~4,5 m]*	[~4,6 m]*	[5,37 m]	[0,30; 0,55 d (^2J=4,5 Hz)]	[3,60 t (Σ^3J=15 Hz)]	[0,80; 0,94 (CH_3×2); 1,08; 1,19; 1,23; 1,29]	[1,93; 1,98; 2,00]
VI	—	—	—		3,75 t (Σ^3J=15 Hz)	0,91; 1,13 (CH_3×3); 1,18; 1,24; 1,43	—
VII	—	—	—		—	0,93; 1,01; 1,16; 1,32; 1,45	—
VIII	~4,68 m*	~4,68 m*	—	0,43 d (^2J=4,5 Hz)	3,78 t (Σ^3J=15 Hz)	0,87; 0,91; 0,96; 1,16 (CH_3×3); 1,29	1,89; 1,94

Note. The spectra were taken in $CDCl_3$ or C_5D_5N. The indices given in square brackets were obtained using $CDCl_3$. The signals denoted by asterisks are superposed upon one another in the horizontal rows. The signals of the methyl groups have singlet natures. Abbreviations: d – doublet; t – triplet; q – quartet; sx – sextet; m – multiplet.

The PMR spectrum (C_5D_5N) of the isoprenoid (I) distinctly showed the signals of seven methyl groups in the strong field. In addition to these, protons coupled in the manner of a AB system resonated at 0.22 and 0.50 ppm in the form of two one-proton doublets (Table 1). In agreement with this, the IR spectrum of the compound under consideration had absorption at 3040 cm^{-1}, which is characteristic for a cyclopropane ring [3].

The facts given and the molecular formula of genin (I), $C_{30}H_{50}O_5$, permitted us to assign cyclogalegigenin to the methylsteroids of the cycloartane series [4]. Compounds of this class have been found previously in other species of Astragalus [5, 6].

The acetylation of cyclogalegigenin (I) with acetic anhydride in pyridine led to the formation of the diacetate (IV) and the triacetate (V).

The mass spectra of cyclogalegigenin (I) and of its acetates (IV) and (V) each have at the 100% peak that of an ion with m/z 143 (ion a) and the ion of a fragment with m/z formed by the subsequent ejection of a molecule of water.

These facts show that the side chain of cyclogalegigenin (I) is close in structure to the side chain of cyclosiversigenin (III) [5] and cycloasgenin A [6]. A confirmation of this is the presence in the PMR spectra of the genin (I) and of its acetates (IV) and (V) of a one-proton triplet at 3.83, 3.74, and 3.60 ppm, respectively, assigned to H-24 [5-11].

Analysis of the PMR spectra of the genin (I) and of the diacetate (IV) and the triacetate (V) showed that the cyclogalegigenin molecule contains three secondary hydroxy groups. In the PMR spectrum ($CDCl_3$) of the isoprenoid (I) the protons located geminally to these groups resonate at 3.24, 3.48, and 4.54 ppm. This assignment is substantiated by downfield shifts of these signals in the spectra of the diacetate (IV) (~4.5 and ~ 4.6 ppm) and the triacetate (V) (~4.5; ~4.6 and 5.37 ppm).

Consequently, in cyclogalegigenin (I) the oxygen functions are distributed in the following way: Thereare three secondary hydroxy groups in the pentacyclic part of the molecule and one tertiary hydroxy group in the side chain. The fifth oxygen atom is included in a tetrahydrofuran ring.

As mentioned above, one of the three signals of protons geminally located in relation to secondary hydroxy groups resonates at 3.24 ppm and has the form of a quartet (^3J = 4.8 and 11.2 Hz). The chemical shift of this signal coincides with that of H-3 of cyclosiversigenin (III) [5]. This gave us grounds for placing one of the secondary hydroxy groups of the cyclogalegigenin (I) molecule at C-3. The values of the spin–spin coupling constants of the signal under consideration indicates the α orientation of H-3. Consequently, the hydroxy group itself at C-3 is β-oriented.

As has been shown previously [5, 6, 12], for cycloartanes having an α-hydroxy function at C-6 a considerable paramagnetic shift of the signal of one of the methyl groups at C-4 is shown when the PMR spectra are taken in pyridine. It must be mentioned that in the PMR spectrum of cyclogalegigenin (I) taken in C_5D_5N, among the singlet signals one stands out particularly by its position in a distinct weak field at 1.78 ppm. This characteristic shift permitted the conclusion that in the molecule of the genin (I) there is likewise a hydroxy group at C-6. Both the chemical shift of the signal at 3.48 ppm ($CDCl_3$) which we have assigned to H-6 and also the sextet nature of its splitting with ^3J = 3.8, 9.8, and 9.8 Hz [5, 12] agree with this. The values of the spin–spin coupling constants given determine the β orientation of H-6 and, consequently, the α orientation of the hydroxy group at C-6.

The Jones oxidation [13] of cyclogalegigenin (I) led to compounds (II) and (VI) with R_f 0.15 and 0.26, respectively (system 3). The structure of substance (II) will be considered below.

The oxidized derivative (VI) forms a triketone (M^+ 484) the mass spectrum of which contains a fragment with m/z 143, showing that the side chain has undergone no changes. This is also confirmed by the PMR spectrum of the compound under discussion, where the signals of seven methyl groups can clearly be traced. In the IR spectrum of the triketone (VI) there are absorption bands at 1715 and 1735 cm^{-1}. The first of them must be assigned to the carbonyl groups formed on the oxidation of the 3β- and 6α-hydroxy functions. The band with a frequency of 1735 cm^{-1} shows that in compound (VI) the oxo group is likewise in the five-membered ring. Consequently, in the initial genin (I) a hydroxy function must be present in ring D.

Further information on the position and configuration of this hydroxy group was obtained in the following way.

In the PMR spectrum of the acetate (IV) the signals of the protons at C-3 and C-6 are shifted downfield compared with those for the tetraol (I) and appear at 4.5 and 4.6 ppm, respectively, while the signal of the pro-

ton resonating at 4.54 ppm has undergone practically no changes. This indicates that compound (IV) is the 3,6-diacetate with a free secondary hydroxy group in ring D.

The Jones oxidation [13] of the diacetate (IV) gave the monoketo derivative (VIII) (M^+ 572), the PMR spectrum of which lacks the signal of a proton located geminally to an unidentified hydroxy group.

The CD curve of compound (VIII) shows a negative Cotton effect at 305 nm ($\Delta\varepsilon = -5.29$). This fact shows the position of the keto function in compound (VIII), which means also that of the corresponding hydroxy group in the diacetate (IV), at C-16 [14]. As was to be expected, in the PMR spectrum of the triacetate (V) the resonance lines of the proton at C-16 are shifted downfield and appear at 5.37 ppm. In the PMR spectrum of the genin (I) ($CDCl_3$), the sextet signal at 4.54 ppm mentioned above corresponds to the same proton. The sum of the vicinal coupling constants (21 Hz) is one more confirmation of the position of this proton and, consequently, of the hydroxy group geminal to it at C-16.

The increment of the molecular rotations between the triacetate (V) and the diacetate (IV) [$[M]_{D-V} = +493°$, $[M]_{D-IV} = +383°$, $\Delta[M] = +110°$] determines the β configuration of the hydroxy group at C-16 [15].

In the ^{13}C NMR spectra of cyclogalegigenin (I) and cyclosiversigenin (III), the chemical shifts of the signals of the C-3, C-6, and C-16 carbonyl carbon atoms are very close, and have the following values: (I) 78.3, 68.4, and 72.9 ppm, and (III) 78.2, 68.3, and 73.3 ppm. These figures are also evidence in favor of the identity of the positions and configurations of the secondary hydroxy groups in the pentacyclic nuclei of genins (I) and (II).

Thus, cyclogalegigenin (I), just like cyclosiversigenin (III) [5], contains hydroxy groups in the 3β, 6α, and 16β positions. Consequently, the only difference in the structures of the genins is in the side chains.

The configuration of the C-24 asymmetric center of cyclogalegigenin (I) was determined by comparative study of the ^{13}C NMR spectra of the isoprenoids (I) and (III). In the ^{13}C NMR spectrum of cyclosiversigenin (III), which has the 24R configuration [5], the chemical shift of the asymmetric C-24 carbon atom is 81.6 ppm, while in the spectrum of cyclogalegigenin (I) the signal of the C-24 atom is shifted downfield by 3.4 ppm and is located at 85.0 ppm. It is known [16] that the difference in the values of the chemical shifts of the C-24 carbon atoms in the ^{13}C NMR spectra of dammarane triterpenoids belonging to the 24R and 24S series amounts to 2.7-3.3 ppm, the C-24S atom resonating in the weak field. This fact permits us to conclude that the C-24 asymmetric center of cyclogaligigenin in (I) has the S configuration.

Thus, if the difference in the structures of the side chains of cyclogalegigenin (I) and cyclosiversigenin (III) is due only to the C-24 chiral center, the oxidation of these compounds to the 24,20-lactone should apparently give identical substances. For this purpose, cyclogalegigenin (I) was oxidized with chromium trioxide in glacial acetic acid. From the reaction products was isolated substance (II) (M^+ 440), the mass spectrum of which lacked the peak of an ion with m/z 143 but showed as the 100% peak that of an ion with m/z 99. In the IR spectrum of the oxidized derivative (II) the absorption bands of a γ-lactone (1760 cm^{-1}), of a five-membered cyclic ketone (1740 cm^{-1}), and of six-membered cyclic ketones (1718 and 1695 cm^{-1}) were clearly traced. In the PMR spectrum of product (II) signals were observed from five methyl groups. The facts given permitted substance (II) to be determined as a 3,6,16-triketo-24,20-lactone. According to its spectral characteristics and physicochemical constants, the substance isolated proved to be identical with a product having Rf 0.15 obtained by the Jones oxidation of cyclogalegigenin (I).

A comparison of the physicochemical constants and the IR and PMR spectra of compound (II) with those for the 3,6,16-triketo-24,20-lactone (VII) [6] obtained from cyclosiversigenin (III) showed that derivatives (II) and (VII) were not identical. The difference must obviously consist in the stereochemistry of the C-20 chiral center of the substances being compared. Since the 20S configuration has been found for cyclosiversigenin (III) [5], cyclogalegigenin (I) must have the 20R configuration.

We may note that these differences in stereochemistry are weakly reflected on the chemical shift of the C-20 carbon atom. In the ^{13}C NMR spectrum of the genin (I), the signal of this atom appears at 86.7 ppm, and in the spectrum of the genin (III) at 87.1 ppm.

Thus, summing the experimental facts given we may conclude that cyclogalegigenin (I) is 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol.

EXPERIMENTAL

General Observations. Thin-layer chromatography (TLC) was performed on plates prepared from type L silica gel (grain size < 50 μ) containing 7% of gypsum and on Silufol. The isoprenoids were revealed on TLC by spraying with a 25% methanolic solution of tungstophosphoric acid followed by heating at 100-150°C for 2-5 min. Silica gel of the same type with a grain size of 50-100 μ was used for column chromatography, together with the following solvent systems: 1) chloroform–methanol (15:1); chloroform–methanol–water (70:23:4); 3) benzene–ethyl acetate (2:1); and 4) benzene–ethyl acetate (1:1).

Mass spectra were obtained on a MKh-1310 instrument at an ionizing voltage of 50 V and a temperature of 130-170°C. IR spectra were taken on a UR-20 spectrophotometer in KBr, PMR spectra on JNM-4H-100 and XL-200 spectrometers in deuteropyridine and deuterochloroform (δ, 0 – HMDS), and ^{13}C NMR spectra on a Varian CFT-20 instrument in deuteropyridine (δ, 0 – TMS).

The circular dichroism curves were measured on a Jasco-J-20 spectropolarimeter.

Isolation of the Isoprenoids. The air-dry comminuted flowers of A. galagiformis (2 kg) collected in the environs of the village of Bakuriani, Georgian SSR, were extracted with 80% ethanol. After the solvents had been distilled off, from the combined extractive substances the purified total isoprenoids and the glycosides were obtained [6], and these were separated by column chromatography. When the column was eluted with system 1, a mixture of feebly polar compounds was isolated, the rechromatography of which gave substance A – cyclogalegigenin.

Elution of the column with system 2 led to the individual compounds B, C, and D.

Cyclogalegigenin (I), $C_{30}H_{50}O_5$, mp 195-196°C (from methanol) $[\alpha]_D^{31}$ +28.7 ± 2° (c 1.15; methanol). ν_{max}^{KBr}, cm^{-1}: 3460-3380 (OH); 3040 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 490 (1.8), 475 (8.6), 472 (17.2), 457 (15.6), 454 (23.4), 439 (14.1), 431 (4.7), 421 (8.6), 413 (23.4), 395 (37.5), 377 (14.8), 289 (17.2), 271 (56.3), 143 (100), 125 (87.5).

Cyclogalegigenin 3,6-Diacetate (IV) and 3,6,16-Triacetate (V) from (I). The genin (I) (318 mg) was acetylated with 3 ml of acetic anhydride in 6 ml of pyridine at room temperature for 3 days. The reaction mixture was poured into ice water, and the precipitate that deposited was filtered off. The products were separated on a column with elution by system 3. This gave 130 mg of the amorphous triacetate (V), $C_{36}H_{56}O_8$. $[\alpha]_D^{23}$ +80 ± 2° (c 1.1; methanol). ν_{max}^{KBr}, cm^{-1}: 3545-3510 (OH); 1740, 1250 (ester group). Mass spectrum, m/z (%): $(M-15)^+$ 601 (7.8), 598 (0.5), 556 (16.9), 541 (5.2), 538 (1.1), 497 (26.0), 496 (58.4), 481 (20.8), 478 (3.9), 463 (2.6), 454 (16.9), 438 (48.1), 437 (48.1), 421 (19.5), 403 (4.5), 395 (27.3), 378 (48.1), 377 (41.6), 363 (14.3), 335 (27.2), 289 (45.5), 255 (32.5), 185 (68.8), 143 (100), 125 (90.9).

Continuing the elution of the column with the same solvent system, we isolated 150 mg of the noncrystalline diacetate (IV), $C_{34}H_{57}O_7$, $[\alpha]_D^{13}$ +66.7 ± 2° (c 0.97; methanol). ν_{max}^{KBr}, cm^{-1}; 3450-3360 (OH); 3040 (CH_2 of a cyclopropane ring); 1742, 1252 (ester group). Mass spectrum, m/z (%): $(M-15)^+$ 559 (1.3), 556 (0.5), 541 (0.8), 530 (0.9), 514 (4.7), 499 (2.3), 497 (2.3), 496 (2.4), 481 (1.6), 454 (10.2), 438 (6.8), 437 (6.7), 436 (4.7), 421 (4.4), 413 (4.1), 395 (21.6), 378 (6.8), 377 (8.0), 353 (5.8), 289 (11.4), 271 (9.1), 269 (8.0), 253 (12.5), 243 (12.5), 201 (17.0), 200 (11.4), 199 (17.0), 185 (45.5), 143 (100), 125 (86.4).

The 20R-3,6,16-Triketo-24,20-lactone (II) from (I). A solution of 100 mg of cyclogalegigenin (I) in 5 ml of glacial acetic acid was treated with 200 mg of chromium trioxide in 6 ml of the same solvent, and the mixture was left at room temperature for 14 h. Then it was poured into 30 ml of water containing 5 ml of isopropanol in order to decompose the oxidizing agent and exhaustive extraction was carried out with chloroform. The chloroform extract was washed with a dilute solution of sulfuric acid and with water and was dehydrated with anhydrous sodium sulfate. After evaporation of the solvent, the reaction products were separated on a column by repeated rechromatography with elution by system 4. This gave 20 mg of the triketolactone (II), $C_{27}H_{36}O_5$, mp 187-189°C (from ethanol), $[\alpha]_D^{22}$ 0 ± 3° (c 0.4; chloroform). ν_{max}^{KBr}, cm^{-1}; 1760 (C=O of a γ-lactone ring); 1740 (C=O at C-16); 1718, 1695, (C=O at C-3 and C-6). Mass spectrum, m/z (%): M^+ 440 (30.8), 425 (15.4), 422 (3.3), 412 (19.2), 407 (4.4), 398 (15.9), 383 (30.8), 379 (4.4), 369 (6.0), 356 (8.8), 342 (9.9), 341 (9.3), 327 (12.1), 285 (19.2), 99 (100).

25-Hydroxy-20R,24S-epoxycycloartane-3,6,16-trione (VI) and the 20R-3,6,16-Triketo-24,20-lactone (II) from (I). A solution of 163 mg of cyclogalegigenin in 30 ml of acetone cooled to −5°C was treated with 0.3 ml of the Jones reagent [13] and the mixture was stirred for 35 min. To decompose the excess of oxidizing agent, the reaction mixture was poured into 200 ml of water containing 5 ml of isopropanol. The oxidation products

were extracted with chloroform, the extract was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated off. The residue was separated on a column with elution by system 3. This gave 67 mg of triketone (VI), $C_{30}H_{44}O_5$, mp 152-153°C (from ethanol), $[\alpha]_D^{20}$ +17.7 ± 2° (c 0.9; chloroform). ν_{max}^{KBr}, cm^{-1}: 3520-3495 (OH); 1735 (C=O at C-16); 1715 (C=O at C-3 and C-6). Mass spectrum, m/z (%); M^+ 484 (0.7), 469 (13.5), 466 (1.6), 451 (15.8), 433 (2.5), 425 (100), 411 (73.7), 407 (13.5), 395 (3.8) 383 (78.9), 369 (12.0), 367 (6.8), 365 (12.8), 341 (7.5), 327 (7.1), 286 (24.8), 285 (21.1), 283 (4.5), 143 (31.6), 125 (47.4).

The further washing of the column with the same solvent system led to 38 mg of a substance with mp 187-189°C (from ethanol). This substance was identified as the 3,6,16-ketolactone (II) likewise by its R_f value on TLC (systems 3 and 4) and by the characteristics of its IR and mass spectra.

The 3,5-Diacetoxy-25-hydroxy-20R,24S-epoxycycloartan-16-one (VIII) from (IV). A solution of 95 mg of the diacetate (IV) in 20 ml of acetone at −8°C was treated with 0.1 ml of the Jones reagent, and the mixture was stirred for 20 min. Then it was poured into 20 ml of water containing 300 mg of sodium sulfate. The reaction products were extracted with chloroform, the extract was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated off. The residue was chromatographed on a column with elution by system 3. This gave 45 mg of substance (VIII), $C_{34}H_{52}O_7$, $[\alpha]_D^{24}$ +32.3 ± 2 (c 0.47; methanol). ν_{max}^{KBr}, cm^{-1}: 3530-3470 (OH); 1740 (C=O at C-16); 1740, 1250 (ester group). CD (c 0.1; methanol); $\Delta\varepsilon = -5.29$ (305 nm). Mass spectrum, m/z (%): M^+ 572 (2.1), 557 (27.4), 554 (3.4), 539 (21.4), 523 (4.8), 521 (4.2), 513 (53.6), 499 (20.4), 495 (4.8), 483 (8.3), 471 (15.5), 453 (34.5), 419 (18.5), 411 (21.4), 394 (66.7), 393 (37.5), 379 (29.8), 375 (9.5), 351 (50.0), 335 (15.5), 314 (44.0), 313 (41.7), 295 (11.9), 269 (21.4), 253 (66.7), 247 (25.0), 239 (16.7), 227 (19.6), 201 (36.9), 185 (75.0), 143 (100), 125 (91.7).

The 20S-3,6,16-Triketo-24,20-lactone (VII) from (III). At room temperature, 150 mg of cyclosiversigenin in 6 ml of glacial acetic acid was oxidized for 14 h with 300 mg of chromium trioxide dissolved in 10 ml of the same solvent. Then the reaction mixture was poured into 50 ml of water containing 8 ml of isopropanol. After the usual working up and chromatography on a column (system 3), 30 mg of the triketolactone (VII) was obtained with mp 207-208°C (from methanol), $[\alpha]_D^{23}$ 0 ± 3° (c 1.5; chloroform). For spectral details, see [6].

Smith Degradation of the Total Glycosides. A solution of 1.2 g of purified total isoprenoids obtained from A. galegiformis, in 50 ml of methanol, was treated with 4 g of periodic acid in 30 ml of water, and the mixture was stirred at room temperature for about 1 h. To decompose the excess of oxidizing agent, 5 ml of ethylene glycol was added and the solution was poured into 200 ml of water. The reaction products were exhaustively extracted with chloroform. After the usual working up and distillation of the solvent, the residue was dissolved in 20 ml of methanol, and 1.7 g of sodium tetrahydroborate was added in small portions, after which the mixture was left at room temperature for 2 h. Then it was acidified with dilute silfuric acid to pH ~ 2 and, after 4 h, it was extracted with chloroform. The chloroform extract was washed with water to neutrality and dried over anhydrous sodium sulfate. The residue obtained after the solvent had been distilled off was purified on a column with elution by system 1. This gave 110 mg of a substance with mp 195-196°C (from methanol), $[\alpha]_D^{24}$ +29.5 ± 2° (c 1.2; methanol), identical with cyclogalegigenin (I).

SUMMARY

The inflorescences of the plant Astragalus galegiformis L. (family Leguminosae) have yielded a new isoprenoid – cyclogalegigenin, which is 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol.

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INTERACTION OF CARDENOLIDES OF THE STROPHANTHIDIN SERIES WITH FUNCTIONALLY IMPORTANT SECTIONS OF TRANSPORT Na,K-ATPase

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On the basis of an analysis of the structure–activity interrelationship of cardiac glycosides and their analogs, a four-center model of the binding of the compounds with Na,K-ATPase has been proposed. These centers are represented by the steroid nucleus with a polar group at C-3 and a hydroxy group at C-14, and also by a lactone ring with a double bond. It has been shown that the hydrophobic binding section of the glycoside must be in contact with a hydrophobic region of the enzyme having a fairly large volume.

The directed search for cardiotonic agents with given properties is possible only on the basis of a knowledge of the concrete mechanism of the action of this type of substances at the cell level. This knowledge is important both for those who are engaged in a search for active agents in the plant world and for synthetic chemists working on the complete or partial synthesis of cardiotonic compounds. In the latter case, of course, hope is placed on the production of more active drugs. In view of this, the finding of functionally important structural elements of cardiosteroids and the investigation of features of their interaction with Na,K-ATPase still remains urgent.

According to Barnett [1], these loci in the enzyme are necessary for the binding of a cardiotonic glycoside with Na,K-ATPase. The first binds with the carbohydrate moiety of the glycosides, the second interacts with the hydroxy group in the C-14 position of the steroid molecule, and the third is attached to the lactone ring of the glycoside.

According to a hypothesis due to Yoda and Yoda [2], the interaction of cardiac glycosides with Na,K-ATPase is first effected by a steroid-specific site through which a sugar-binding center is activated, and then the addition of the glycosidic moiety of the inhibitor takes place.

Facts are known which indicate that the glycoside binds to the enzyme through hydrogen bonds [3, 4] or through a stronger chemical interaction [5]. A hypothesis has been put forward that on the binding of cardiosteroids with the receptor site of the transport enzyme, the formation of a covalent bond of the unsaturated lactone ring at C-17 with the nucleophilic center of the enzyme takes place simultaneously with the formation of a hydrogen bond through the hydroxy group at C-14 [6-8]. The combination of these facts leads us to the conclusion that the interaction of cardiac glycosides is effected not with the whole enormous molecule of the enzyme but only with a definite center, the so-called digitalis receptor. No few hypotheses have been expressed on the nature of the binding of cardiosteroids with Na,K-ATPase but hitherto there have been no sufficiently convincing proofs of the mechanism of the interaction of the group of compounds under discussion with the enzyme. The structure–activity relationship has been investigated mainly of cardiac glycosides of the digitalis series, which possess an appreciable capability for cumulation and which exhibit, in the main, a healing effect

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