TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS.

XVI. CYCLOGALEGINOSIDES A AND B FROM *Astraglus galegiformis*

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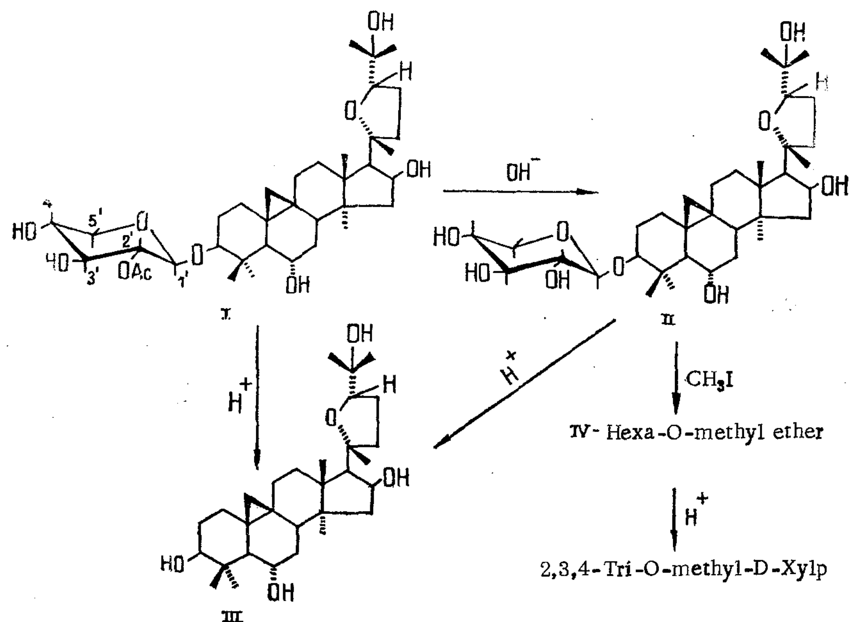
Two glycosides of the cycloartane series — cyclogaleginosides A and B — have been obtained from inflorescences of *Astragalus galegiformis* L. (Leguminosae), and their structures have been established by chemical transformations and spectral characteristics as cyclogalegigenin 3-O-(2'-O-acetyl)-β-D-xylopyranoside and cyclogalegigenin 3-O-β-D-xylopyranoside, respectively.

We have previously reported the structure of cyclogalegigenin (III) isolated from the inflorescences *Astragalus galegiformis* L. (Leguminosae) [1]. In the present paper we consider the structures of triterpenoids of glycosidic nature which we have called cyclogaleginosides A (I) and B (II) (products B and C in the total extractive substances [1]).

In the PMR spectra of glycosides (I) and (II) one-proton doublets were observed at 0.39 and 0.47 ppm, respectively, which permitted the assumption that the glycosides under consideration belonged to the cycloartane series [2]. This assumption was confirmed by the formation of cyclogalegigenin (III) [1] on the acid hydrolysis of both glycosides.

It was shown by the GLC method [3] that cyclogaleginoside A and cyclogaleginoside B each contained one D-xylose residue.



The position of the D-xylose residue in cyclogaleginoside B was established by a comparative study of the ^{13}C NMR spectra of compounds (II) and (III).

The ^{13}C NMR spectrum of cyclogalegigenin (III) was taken under the conditions of complete and partial supression of spin—spin coupling with protons. Signals exhibiting split-

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ting of a doublet nature at (ppm) 78.3 (C-3), 68.4 (C-6), and 72.9 (C-16) and a singlet at 70.3 (C-25) were assigned to carbinol carbon atoms. In the ^{13}C NMR spectrum of cyclogaleginoside B (II), the same signals were located at (ppm) 88.7 (C-3), 68.1 (C-6), 72.9 (C-16), and 70.3 (C-25). It followed from a comparison of the values given above that on passing from the genin (III) to its monoxyloside (II) the chemical shifts of the C-6, C-16, and C-25 carbon atoms remained practically unchanged, while the C-3 signals underwent a paramagnetic shift ($\Delta\delta = \delta(C\text{-}3)_{II}\ 88.7 - \delta(C\text{-}3)_{III}\ 78.3 = 10.4$ ppm). This change in the chemical shift of the C-3 atom due to the effects of glycosylation [4] unambiguously indicated that the D-xylose residue was attached to the hydroxy group at C-3.

The Hakomori methylation [5] of cyclogaleginoside B gave the hexa-O-methyl ether (IV). In an acid hydrolysate of the permethylate (IV) 2,3,4-tri-O-methyl-D-xylopyranose was detected by GLC [6] and by TLC in the presence of an authentic sample.

In the PMR spectrum of the hexa-O-methyl ether (IV), the anomeric proton of the D-xylopyranoside residue resonated at 4.19 ppm in the form of a doublet with a spin–spin coupling constant (SSCC) $^3J = 7.5$ Hz. The SSCC value of the anomeric proton showed the C1 conformation of the D-xylopyranoside residue, and, consequently, the β configuration of the glycosidic center [7]. A calculation of molecular rotation differences [8] agreed well with the β configuration of anomeric carbon atom of the D-xylopyranose. Thus, cyclogaleginoside B (II) is cyclogalegigenin 3-O-β-D-xylopyranoside.

The IR spectrum of cyclogaleginoside A (I) had absorption bands at 1755 and 1245 cm^{-1}, which are characteristic for an ester grouping. The presence in the PMR spectrum of the same glycoside of a three-proton singlet at 1.97 ppm showed that cyclogaleginoside A (I) contained one acetyl group. The presence of an acetyl residue in cyclogaleginoside A (I) was confirmed by resonance lines in the ^{13}C NMR spectrum at 169.9 and 21.1 ppm.

The alkaline hydrolysis of glycoside (I) led to the formation of cyclogaleginoside B (II).

The position of the acetyl group in compound (I) was established by an intercomparison of the characteristics of the ^{13}C NMR spectra of cyclogaleginosides B (II) and A (I). On passing from compound (II) to (I) the chemical shifts of the carbon atoms of the aglycone moiety scarcely changed. At the same time, definite changes were observed in the positions of the resonance signals of the carbon atoms of the sugar residue. The chemical shifts of the carbon atoms of the sugar residues of compounds (II) and (I) were characterized by the following values (ppm; the figures for (I) are given in parentheses): 107.5 (104.8), C-1'; 75.5 (75.6), C-2'; 78.3 (76.2), C-3'; 71.2 (71.2), C-4'; 66.9 (67.0), C-5'. It can be seen from this that the most considerable diamagnetic shift was undergone by the signal of the anomeric carbon (C-1') [$\Delta\delta = \delta(C\text{-}1')_{II}\ 107.5 - \delta(C\text{-}1')_{I}\ 104.8 = 2.7$ ppm and by that of the C-3' carbon atom [$\Delta\delta = \delta(C\text{-}3')_{II}\ 78.3 - \delta(C\text{-}3')_{I}\ 76.2 = 2.1$ ppm]. The upfield shifts of the C-1' and C-3' signals by 2.7 and 2.1 ppm determine the position of the acetyl group at the C-2' carbon atom of the xylopyranoside residue of the cyclogaleginoside A molecule [9, 10]. Consequently, cyclogaleginoside A (I) is cyclogalegigenin 3-O-(2'-O-acetyl)-β-D-xylopyranoside.

EXPERIMENTAL

For general observations, see [1]. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) butanol–methanol–water (5:3:1); 3) chloroform–methanol–water (70:23:4); and 4) benzene–acetone (15:1).

For the GLC conditions, see [11]. The PMR spectra were recorded in deuterochloroform or deuteropyridine on a JMN-4H 100/100 MHz instrument (δ, 0 — HMDS), and ^{13}C NMR spectra on a CFT-20 instrument (Varian) in deuteropyridine (δ, 0 — TMS).

For the isolation of the triterpenoids of *Astragalus galegiformis* L., see [1]. Cyclogaleginoside A (I) — substance B in [1]: $C_{37}H_{60}O_{10}$, mp 224-226°C (from chloroform–methanol (1:1)), $[\alpha]_D^{24}$ +40 ± 2° (c 1.0; pyridine); ν_{max}^{KBr}, cm^{-1}: 3530-3300 (OH); 3050 (CH_2 of a cyclopropane ring); 1755, 1245 (ester group). PMR (C_5D_5N, ppm): 0.39 (1 H at C-19, d, $^2J = 4$ Hz), 0.86 (3 H, s, CH_3), 1.11 (3 H, s, CH_3), 1.15 (3 H, s, CH_3), 1.21 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.52 (3 H, s, (CH_3), 1.63 (3 H, s, CH_3), 1.97 (3 H, s, CH_3–COO); 4.68 (2 H, H-1', d, $^3J \approx 8$ Hz, and H-16, m), 5.41 (1 H, t, $^3J \approx 15$ Hz, H-2').

Cyclogalegigenin (III) from (I). Glycoside (I) (120 mg) was dissolved in 20 ml of methanol containing 0.5% of sulfuric acid, and the solution was boiled for 30 min. Then it was diluted with water and the methanol was distilled off. The precipitate that deposited was filtered off, washed with water, and chromatographed on a column with elution by system 1. This led to the isolation of 45 mg of the genin (III) with mp 195-196°C (from methanol), $[\alpha]_D^{22}$ +29 ± 2° (c 1.2; methanol), $[\alpha]_D^{23}$ +63 ± 2° (c 1.26; pyridine). The genin (III) was identified as cyclogalegigenin [1] also from the characteristics of its IR spectrum.

The aqueous solution was evaporated to a volume of 10 ml and was boiled for 3 h. After neutralization with ARA-8p anion-exchange resin, D-xylose was detected in the filtrate by the TLC method in comparison with an authentic sample. GLC [3] showed the presence of a single D-xylose residue.

Cyclogaleginoside B (II) — substance C in [1]: $C_{35}H_{58}O_9$, mp 252-254°C (from chloroform–methanol (1:1)), $[\alpha]_D^{24}$ +32 ± 2° (c 1.0; pyridine. ν_{max}^{KBr}, cm^{-1}: 3600-3200 (OH), 3040 (CH_2 of a cyclopropane ring). PMR (C_5D_5N, ppm): 0.47 (1 H at C-19, d, ^2J = 4 Hz), 0.87 (3 H, s, CH_3), 1.16 (3 H, s, CH_3), 1.18 (3 H, s, CH_3), 1.21 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.52 (3 H, s, CH_3), 1.83 (3 H, s, CH_3), 4.70 (2 H, m, H-1' and H-16).

Cyclogalegigenin (III) from (II). Glycoside (II) (70 mg) was hydrolyzed under the conditions similar to those for (I). As the genin 23 mg of cyclogalegigenin (III) with mp 195-196°C (from methanol) was isolated. In the hydrolysate, after the usual working up, D-xylose was detected by TLC. GLC showed the presence of one D-xylose residue.

Cyclogaleginoside B (II) from (I). Cyclogaleginoside A (I) (100 mg) was hydrolyzed with 10 ml of a 2% methanolic solution of potassium hydroxide at room temperature for 10 min. The reaction mixture was diluted with a twofold volume of water and was extracted with ethyl acetate. The ethyl acetate extract was washed with water to neutrality, dried, and evaporated. The residue was chromatographed on a column with elution with system 3. This led to the isolation of 68 mg of a glycoside with mp 249-253°C [from chloroform–methanol (1:1)], $[\alpha]_D^{24}$ +38 ± 2° (c 1.0; pyridine), which was identified as cyclogaleginoside B (II) also from the characteristics of its IR spectrum and its chromatographic mobility on TLC.

The Hexa-O-methyl Ether (IV) from (II). With stirring, 1.2 g of sodium hydride was added in small portions to a solution of 1.2 g of the glycoside (II) in 120 ml of dry dimethyl sulfoxide. After the addition of the whole amount of sodium hydride, stirring was continued for another 1 h. Then 10 ml of methyl iodide was added dropwise to the reaction mixture and stirring was continued for another 3 h. The reaction products were poured into 500 ml of water containing 6 g of sodium hyposulfite and exhaustively extracted with chloroform. The chloroform extract was washed with water, dried with anhydrous sodium sulfate, and evaporated. The residue was dried in vacuum and was remethylated under similar conditions once more. From the reaction products by elution from a column with system 4 we isolated 159 mg of the amorphous hexa-O-methyl ether (IV), $[\alpha]_D^{24}$ +28 ± 2° (c 1.0; pyridine). The IR spectrum of (IV) lacked absorption due to hydroxy groups. M^+ 706. PMR ($CDCl_3$, ppm): 0.42 (1 H at C-19, d, ^2J = 4 Hz), 0.88 (3 H, s, CH_3), 0.91 (3 H, s, CH_3), 1.07 (3 H, s, CH_3), 1.09 (3 H, s, CH_3), 1.11 (3 H, s, CH_3), 1.20 (3 H, s, CH_3), 1.23 (3 H, s, CH_3), 3.11 (3 H, s, OCH_3), 3.17 (6 H, s, 2 × OCH_3), 3.39 (3 H, s, OCH_3), 3.55 (6 H, s, 2 × OCH_3), 3.82 (1 H, m, H-16) 4.19 (1 H, d, ^3J = 7.5 Hz, H-1').

Hydrolysis of the Hexa-O-methyl Ether (IV). A solution of 85 mg of substance (IV) in 10 ml of 0.5% methanolic sulfuric acid was boiled for 3 h. Then the reaction mixture was diluted with water, the methanol was evaporated off, and the precipitate that deposited was filtered off and washed with water. It consisted of a mixture of products difficult to separate.

The aqueous solution was evaporated to a volume of 10 ml and was boiled for 3 h. After cooling, the reaction mixture was neutralized with ARA-8p anion-exchange resin and was then filtered and evaporated. 2,3,4-Tri-O-methyl-D-xylopyranose was detected in the residue by GLC [6] and by TLC in the presence of an authentic sample.

SUMMARY

Two new glycosides of cycloartane series — cyclogaleginosides A and B — have been isolated from inflorescences of *Astragalus galegiformis* L. Cyclogaleginoside A has the structure of cyclogalegigenin 3-O-(2'-O-acetyl)-β-D-xylopyranoside, and cyclogaleginoside B is cyclogalegigenin 3-O-β-D-xylopyranoside.

LITERATURE CITED

1. M. D. Alaniya, M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, E. P. Kemertelidze, and N. K. Abubakirov, Khim. Prir. Soedin., 332 (1983).
2. R. B. Boar and C. R. Romer, Phytochemistry, 14, 143 (1975).
3. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).
4. R. Kasai, M. Suzuo, I. Asakawa, and O. Tanaka, Tetrahedron Lett., 175 (1977).
5. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
6. Y. O. Aspinall, J. Chem. Soc., 1676 (1963).
7. J. M. Van der Veen, J. Org. Chem., 28, 564 (1963).
8. W. Klyne, Biochem. J., 47, xli (1950).
9. K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nanaka, M. Tsuboi, and I. Nishioka, Tetrahedron Lett., 1231 (1971).
10. A. N. Svechnikova, R. U. Umarova, N. D. Abdullaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 629 (1982).
11. M. I. Isaev, M. B. Gorovits, T. T. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, Khim. Prir. Soedin., 173 (1983).

USE OF THE METHOD OF METASTABLE DEFOCUSING FOR THE ANALYSIS OF THE PROCESSES INVOLVED IN THE FRAGMENTATION OF LYCOCTONINE BASES

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The metastable transitions in the mass spectra of 21 lycoctonine bases have been studied by the method of metastable defocusing (MD). It has been established that as a criterion of the monotypicity of single-stage fragmentation reactions it is possible to use a linear dependence of the relative intensities of the metastable peaks (MPs) on the stability of the daughter ion. In the MD spectra of the ions formed in several stages, to characterize the generality of the breakdown reactions, in addition to the intensities of the MPs, the accurate positions of their maxima and the general shape of the curve are important.

The mass spectra of diterpene bases with the lycoctonine skeleton give important information on the structures of these compounds, thanks to the characteristic redistribution of the intensities of the peaks of the ions M^+, $(M - CH_3)^+$, and $(M - OR)^+$ in the spectra of the alkaloids of various subgroups. In the majority of cases, the nature of the fragment OR split off is known, while the processes involved in the formation of the ions $(M - CH_3)^+$ are ambiguous. On studying the spectra of delcosine (VII) and its derivatives, Waller et al. [1] suggested that a CH_3 group was split off from the CH_3O at C-6. Previously Pelletier and Aneja [2] had come to the conclusion that the $(M - 15)^+$ ions arose on the fragmentation of the N-ethyl group. These authors [2] considered the spectra of compounds containing no methoxyl at C-6.

On comparing the spectrum of neoline (III) and its N-nor analogue we detected a sharp decrease in the height of the peak of $(M - CH_3)^+$ ion. Two conclusions followed from this: 1) M^+ of the neoline ion breaks down with the elimination of CH_3 predominantly from N–Et; and 2) in the absence of an OH group at C-7 a methoxyl at C-6 does not take an appreciable part in the formation of the $(M - CH_3)^+$ ions.

Having these facts available, in the present paper we shall consider these processes from the point of view of the parameters of the metastable ions obtained by the method of metastable defocusing (MD). How sensitive these parameters are to the various methods of splitting out identical fragments and what are the criteria for the monotypicity of the

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