ALLIOGENIN AND ALLIOGENIN β-D-GLUCOPYRANOSIDE FROM Allium giganteum

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In a study of plants of the genus Allium, we isolated from the bulbs of A. giganteum Rgl. (family Alliaceae) a crystalline compound giving a positive reaction for steriod sapogenins [1] and called it alliogenin [2].

The IR spectrum of alliogenin (VI) has absorption bands at (cm^{-1}) 825, 900 (strong), 920 (weak), and 985, which permits the assignment of (VI) to the steriod sapogenins of the iso (25R) series [3, 4]. A broad band at 3200-3500 cm^{-1}, the absence of selective absorption in the IR and UV spectra in the regions of double bonds and carbonyl groups, the elementary analysis, and the molecular weight of 464 for alliogenin determined by mass spectrometry show that (VI) is a tetrahydroxy sapogenin with the composition $C_{27}H_{44}O_6$.

Under the action of electron impact, the genin of (VI) undergoes the decomposition characteristic for steroid sapogenins with a spiroketal structure of the side chain. In addition, ions with m/e 115, 139, and 321 show the localization of the hydroxy groups in the androstane part of the molecule [5].

The acetylation of (VI) with acetic anhydride in pyridine gave the triacetate (VII) (mol. wt. 590), the IR spectrum of which showed absorption in the hydroxy group region. The hydroxy group that had remained unacetylated was resistant to the action of chromic anhydride in pyridine. This permits the assumption that it is tertiary.

The broad multiplet in the 5.20-ppm region (with an intensity of two proton units) and the broadened singlet at 4.78 ppm (with an intensity of one proton unit) observed in the NMR spectrum of (VI) (Fig. 1) can be assigned to protons geminal to acetyl groups. A signal at 2.84 ppm corresponds to the proton of a tertiary hydroxy group; it shifts on the addition of CD_3OD. Multiplets in the region of 4.40 ppm (with an intensity of one proton unit) and 3.40 ppm (with an intensity of two proton units) relate, respectively, to the protons at C_{16} and C_{26} [6, 7]. Signals at 1.96 and 2.03 ppm with a total intensity of nine proton units are due to three acetyl groups. Thus, the NMR spectrum confirms the structure of (VII) as a 25R-spirostane with three secondary acetyl groups and one tertiary hydroxy group.

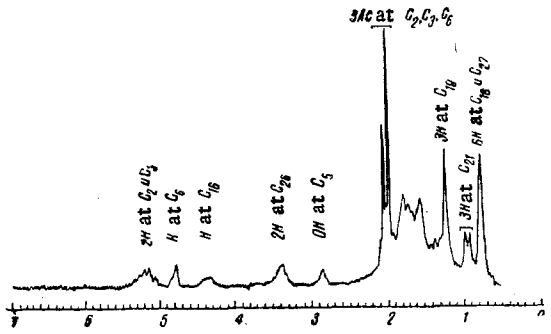

Fig. 1. NMR spectrum of alliogenin acetate (VII).

The tertiary hydroxy group was readily eliminated by treating the triacetate (VII) with thionyl chloride in pyridine, which formed an anhydro derivative (mol. wt. 572), the IR spectrum of which lacked absorption in the OH-group region. The signal of an olefinic proton at 5.42 ppm in the NMR spectrum of the Δ-acetate (XI) shows that the double bond formed cannot be ditertiary.

Characteristic for the mass spectrum of

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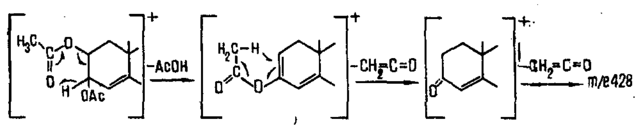
(XI) are strong peaks of ions with m/e 512 (M−60), 470 (M−60−42), and 428 (M−50−2 · 42). The 512 → 470 and 470 → 428 transitions are shown by metastable peaks. Such a decomposition can be explained rationally if it is assumed that the molecule of compound (XI) has a homoannular vicinal diol grouping with the allyl arrangement of the double bond. Since it follows from the NMR spectrum that the double bond in (XI) is not ditertiary, this type of combination is possible only in ring A.



a m/e 572 b m/e 572 c m/e 470

It may be assumed that on mass spectrometric decomposition the molecular ion a loses a molecule of acetic acid and forms the acetate of the enol b which, in its turn, readily splits out a $-CH_2 = C = O$ group, being converted into the α,β-unsaturated ketone c. It is well known [8] that ketones of this type decompose under the action of electron impact with the loss of 42 amu. In this case, two of the three acetylated groups of alliogenin must be present in positions 2 and 3 of ring A and the nonacetylated tertiary hydroxy group in position 5.

A vicinal diol system was confirmed by periodate oxidation; alliogenin absorbed one mole of periodate. The molecular weight of the compound (IX) isolated after oxidation was 2 units less (mol. wt. 462) than that of the initial alliogenin, which also corresponds to the cleavage of one diol grouping. The structure of ring A permits the expectation that the product of the oxidation of (VIII) should exhibit aldehydic properties. However, no absorption characteristic of an aldehyde grouping could be detected in either the IR or the NMR spectrum. This is explicable by the assumption that the product of the oxidation of alliogenin exists in the cyclic lactol form, i.e. the two aldehyde groups arising as a result of the periodate cleavage form semiacetals with the two remaining hydroxyls. A proof of this is the fact that in the 5.29-ppm region of the NMR spectrum of (IX) there is a two-proton multiplet relating to the new grouping

HO\ /CH$_2$–
C
H / \O–

(protons at C_2 and C_3). In the NMR spectrum of the diacetate (X), the corresponding signals are shifted downfield and appear in the 6.05-ppm region. The IR spectrum of (X) lacks absorption in the OH-group region.

On considering the formation of semiacetal groupings with molecular models, it can be seen that the aldehyde group at C_2 readily forms a furanose ring with a hydroxy group at C_5, while the aldehyde group at C_3 gives a furanose semiacetal with a hydroxy group at C_6 or a pyranose semiacetal with an OH at C_7. It follows from this that alliogenin is 2,3,5,6- or 2,3,5,7-tetrahydroxysapogenin. The final choice between these structures was made on the basis of the following transformations. After the oxidation of alliogenin (VI) with chromic anhydride in glacial acetic acid, the lactone (XII) was isolated from the reaction mixture. The reduction of (XII) with zinc in acetic acid led to chlorogenonic acid (XIII), the methylation of which gave the dimethyl ester (XIV). Compounds (XII–XIV) proved to be known: their physicochemical constants corresponded to those given in the literature [9, 10]. The production of compounds (XII), (XIII), and (XIV) unambiguously shows that the choice must be made in favor of 2,3,5,6-tetrahydroxyapogenin.

Marker obtained a compound with the same arrangement of OH groups by the oxidation of yuccagenin [9]. However, the 2,3,5,6-tetrahydroxyapogenin that he synthesized differs considerably from alliogenin in its melting point (356° C and 321–325° C, respectively) and may be its isomer.

The orientation of the hydroxy groups in alliogenin (VI) was determined in the following way. From a methanolic extract of A. giganteum we isolated the alliogenin glycoside (I). The glycoside was hydrolyzed with 5% HCl, which gave (VI) and a sugar identified by paper and gas-liquid chromatography [11] as D-glucose. Elementary analysis, leading to the formula $C_{33}H_{54}O_{11}$, showed that (I) is a monoside of alliogenin.

The Hakomori methylation [12] of the glycoside (I) with subsequent separation of the reaction products by chromatography on Al_2O_3 gave the hexamethyl derivative (II). The products of the hydrolysis of (II) contained 2,3,4,6-tetra-O-methyl-D-glucose and a dimethoxy derivative (III) of alliogenin.

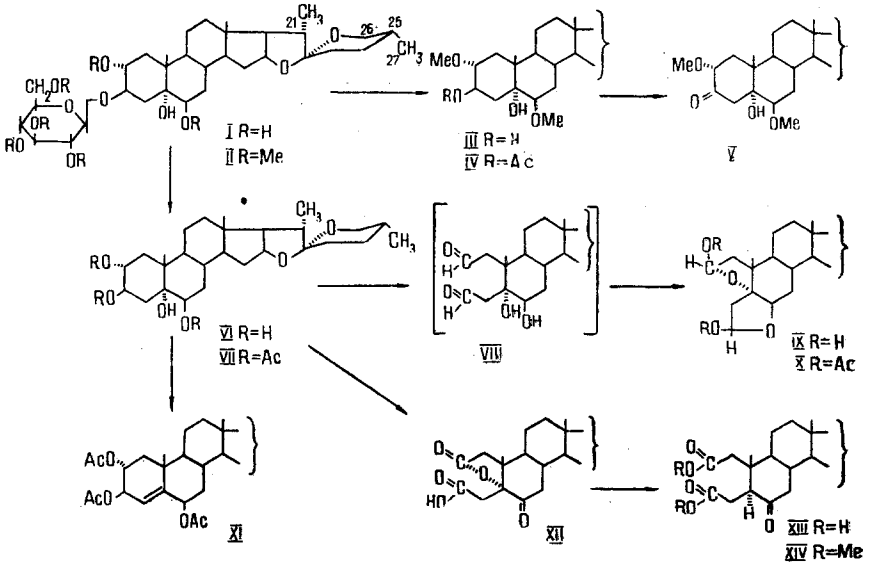
Compound (III) was oxidized by chromic anhydride in pyridine to the ketone (V). The optical rotatory dispersion curve of the dimethoxy ketone (V), with a positive Cotton effect, had the form characteristic for 2-oxo- or 3-oxo-5α-steroids [13]. However, when the optical rotatory dispersion of substance (V) was determined in methanol with the addition of hydrochloric acid, a sharp decrease in amplitude was observed, showing that (V) must be a 3-oxo-5α-steroid [14].

According to the NMR spectrum of the acetate (IV), the proton at C_3 occupies the axial position, since the half-width of its signal at 4.97 ppm is ~20 Hz. This, in its turn, determines the equatorial arrangement of the acetyl group at C_3. Consequently, taking into account the trans linkage of rings A/B, the hydroxy group at C_3 in alliogenin must have the β orientation.

In view of the fact that alliogenin does not form an acetonide, we ascribed the α orientation to the OH group at C_2 [15].

The configuration of the hydroxyl at C_6 follows from a consideration of the NMR spectrum of alliogenin acetate (VII). In this, the broad multiplet in the 5.20-ppm region corresponds to axial protons at C_2 and C_3, the signals of which overlap one another. The signal of the proton at C_6 is obviously represented by a broadened singlet at 4.78 ppm with a half-width of 5 Hz, which shows the equatorial position of the proton. In this case, the acetyl group at C_6 is axial with the β orientation.

Thus, alliogenin has the structure of 5α,25R-spirostane-2α,3β,5α,6β-tetraol.



As was stated above, the glycoside (I) contains one molecule of glucose. The hydrolysis of the methylated glycoside (II) formed 2,3,4,6-tetra-O-methyl-D-glucose and the 3,5-dihydroxy-2,6-dimethoxy genin (III). Consequently, the sugar residue must be attached to the hydroxyl at C_3 and, according to Klyne's rule [16], this bond is β-glycosidic (M_D of (I) 484°; M_D of (VI) 331°; ΔM_D 153°). Consequently, compound (I) is alliogenin 3-β-D-glucopyranoside.

EXPERIMENTAL

The mass spectra were obtained on a MKh-1303 instrument fitted with a system for the direct introduction of the substance into the ion source at an ionizing voltage of 40 V and a temperature of 110-160° C; the IR spectra were obtained on a UR-20 spectrometer in KBr; and the NMR spectra were obtained on a JNM-4H-100 instrument with HMDS as internal standard (δ scale). The solutions were dried in anhydrous Na_2SO_4 and distilled in vacuum. The molecular weights were determined mass spectrometrically. The amounts of C and H found corresponded to the calculated figures.

Extraction of the Plant. The bulbs of A.giganteum (3.2 kg) collected in the flowering stage (Turkmen SSR, region of the central Kopet-Dagh) was extracted with chloroform (4 × 5 liters) and then with the same amount of methanol.

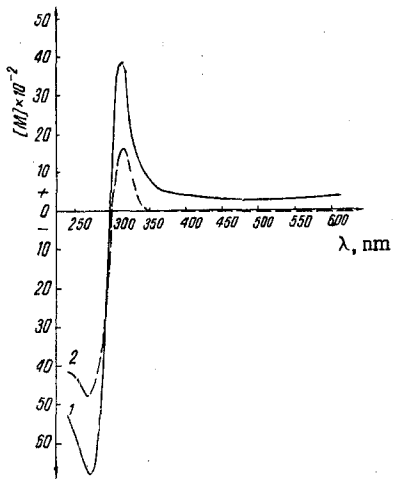


Fig. 2. Optical rotatory dispersion curves of compound (V) in methanol (1) and in methanol with the addition of hydrochloric acid (2).

Alliogenin (VI). The chloroform extract was evaporated to dryness, and the residue was dissolved in methanol and precipitated with ether. The viscous resinous mass so formed was again dissolved in methanol and the solution was filtered through a small layer of Al_2O_3. This yielded 30.0 g of resinous substances giving a positive reaction for steroid sapogenins [1]. Of this material, 15.0 g was heated with 300 ml of 50% aqueous methanol containing 5% of HCl in the boiling water bath for 4 h. The reaction mixture was diluted with a twofold volume of water. The precipitate that deposited was recrystallized from a mixture of chloroform and methanol, giving 2.0 g of (VI), $C_{27}H_{44}O_6$, mp 321-325° C, $[\alpha]_D^{18}$ – 71.4° (c 1.12; pyridine). IR spectrum, cm^{-1}: 3200-3500 (OH), 865, 900> 920, 985 (spiroketal chain; 25R configuration). Mass spectrum: M^+ 464 (2.8%); 405 (1.3%); 3.95 (1.9%), 392 (5.6%), 350 (2.06%); 335 (2.4%); 332 (7.9%), 321 (4.5%), 139 (100%), 115 (11.5%).

Alliogenin β-D-Glucopyranoside (I). The methanolic extract from the plant was evaporated to 3 liters and diluted with an equal volume of water, and the methanol was distilled off as completely as possible. The residual aqueous solution was extracted with butanol. The butanol was evaporated off in vacuum and the residue was dissolved in methanol. After brief standing, the solution deposited 1.6 g of compound (I), $C_{33}H_{54}O_{11}$, mp 263-265° C (from methanol – acetone), $[\alpha]_D^{18}$ – 76.2° (c 1.39; pyridine). IR spectrum, cm^{-1}: 3200-3500 (OH), 870, 905> 930, 985 (spiroketal chain).

A solution of 500 mg of the glycoside (I) in 200 ml of 50% aqueous methanol containing 5% of HCl was heated in the boiling water bath for 3 h. Then the hydrolysate was diluted with a twofold amount of water, and the precipitate that deposited was separated off. The genin obtained had mp 320-323° C (from methanol –chloroform), $[\alpha]_D^{20}$ – 73.0° (c 0.52; pyridine). The IR spectrum of the sapogenin isolated could be superposed on the spectrum of alliogenin (VI). In the aqueous part of the hydrolysate, paper chromatography in the butan-1-ol–acetic acid–water (4:1:5) system in the presence of markers, and also the GLC method [11], showed the presence of D-glucose.

TABLE 1

Compound	Chemical shifts, ppm									
	C_2–H	C_3–H	C_6–H	C_{16}–H	C_{26}–2H	C_{21}–3H	C_{27}–3H*	C_{18}–3H	C_{19}–3H	Other protons
III	3,62 m	4,22 m	2,90 s†	4,22 m	3,40 m	0,85 d; J=6 Hz	0,70	0,70 s	1,02 s	3,17 s; 3,30 s –OCH_3 at C_2 and C_6
IV	3,75 m	4,97 m	2,98 s†	4,33 m	3,40 m	0,92 d; J=6 Hz	0,74	0,74 s	1.10 s	3,20 s; 3,33 s–OCH_3 at C_2 and C_6; 2,00 s–Ac at C_3
VII	5,20 m	5,20 m	4,78 s†	4,40 m	3,40 m	0,92 d; J=6 Hz	0,74	0,74 s	1,19 s	1,96 s; 2,03 s –Ac at C_2, C_3 and C_6; 2,84–OH at C_5
IX	5,29 m	5,29 m	4,15 s†	4,25 m	3,37 m	0,90 d; J=6 Hz	0,72	0,72 s	0,99 s	–
X	6,05 m	6,05 m	4,15 s†	4,25 m	3,36 m	0,92 d; J=6 Hz	0,72	0,72 s	1,07 s	2,00 s; 2,03 s–Ac at C_2 and C_3
XI	5,00 m	5,20 m	5,22 s†	4,30 m	3,36 m	0,93 d; J=6 Hz	0,76	0,76 s	1,22 s	5,42† –H at C_4; 1,94 s; 2,00 s –Ac at C_2, C_3 and C_6

*The signals of the protons at C_{18} and C_{27} overlap.

†Broadened singlet; m) multiplet; d) doublet; s) singlet.

Tri-O-acetate of Alliogenin (VII) from (VI). Alliogenin (100 mg) in 100 ml of pyridine was acetylated with 5 ml of acetic anhydride at room temperature for 3 days. This gave 70 mg of the acetate (VII), $C_{33}H_{50}O_9$, mp 250-253° C (from ether–petroleum ether), $[\alpha]_D^{20}$ 109.5° (c 1.16; chloroform). IR spectrum, cm^{-1}: 3470-3500 (OH), 1740 (C = O of an acetyl group), 870, 905>930, 1005 (spiroketal chain). (For NMR, see Table 1.) Mol. wt. 590.

25R-Spirost-5-ene-2α,3β,6β-triol 2,3,6-triacetate (XI) from (VII). At – 10° C, 1 ml of thionyl chloride was added to a solution of 500 mg of the acetate (VII) in 15 ml of dry pyridine. The mixture was left for 10 min, after which it was neutralized with a solution of $KHCO_3$ and extracted with chloroform, and the chloroform extract was washed first with dilute H_2SO_4 and then with water. After the solvent had been distilled off, 250 mg of compound (XI) was obtained; $C_{33}H_{48}O_8$, mp 156-159° C (from methanol), $[\alpha]_D^{18}$ – 163.1° (c 1.30; chloroform). IR spectrum, cm^{-1}: 1740 (C = O of an acetyl group), 870, 900>925, 990 (spiroketal chain). There was no absorption in the OH group region. (For the NMR spectrum, see Table 1.) Mass spectrum: M^+ 572 (1.6%); 513 (4.1%), 512 (3.9%), 500 (2.9%), 471 (10.2%), 470 (31.5%), 453 (2.2%), 452 (2.4%), 428 (8.8%), 398 (11.5%), 356 (27.5%), 338 (16.7%), 314 (9.1%), 296 (10.3%), 139 (100%), 115 (4.8%).

Periodate Oxidation of Alliogenin (IX from VI). A solution of 400 mg of alliogenin (VI) in 200 ml of methanol was treated with 2.2 g of $NaIO_4$ in 50 ml of water, and the reaction mixture was left at room temperature for 20 h. After this, the mixture was diluted with water and the bulk of the methanol was distilled off in vacuum. The aqueous solution was extracted with chloroform. Distillation of the solvent yielded 320 mg of (IX), $C_{27}H_{42}O_6$, mp 191-193° C (from methanol), $[\alpha]_D^{20}$ – 22.8 (c 1.05; chloroform). IR spectrum, cm^{-1}: 3350-3450 (OH), 870, 900>925, 990 (spiroketal chain). There was no absorption in the C=O group region. (For the NMR spectrum, see Table 1.) Mol. wt. 462.

The Diacetate (X) from (IX). The acetylation of 120 mg of compound (IX) was performed in 3 ml of pyridine by means of 1 ml of acetic anhydride at room temperature for 18 h. Distillation of the solvent yielded 70 mg of the diacetate (X), $C_{31}H_{46}O_8$, mp 204-206° C (from methanol); $[\alpha]_D^{20}$ – 26.1° (c 1.30; chloroform). IR spectrum, cm^{-1}: 1725, 1750 (C = O of an acetyl group), 870, 900, 925, 990 (spiroketal chain). There was no absorption in the hydroxy group region. (For the NMR spectrum, see Table 1). Mol. wt. 546.

Oxidation of Alliogenin with Chromic Anhydride (XII) from (VI). To 350 mg of alliogenin (VI) in 100 ml of acetic anhydride was added 350 mg of CrO_3 in 80% acetic acid. The reaction mixture was left at 25° C for 1 h. Then the excess of chromic anhydride was decomposed with a solution of $NaHSO_3$, and the solvent was distilled off to dryness. The residue was dissolved in ether and the ethereal solution was washed with water and then the acid fraction was extracted from it with a solution of Na_2CO_3. The alkaline solution was acidified with H_2SO_4 and extracted with ether, and the extract was evaporated to dryness.

This gave 250 mg of compound (XII), $C_{27}H_{38}O_7$, mp 220-223° C (from methanol), $[\alpha]_D^{18}$ – 10.9° (c 0.91; chloroform). IR spectrum, cm^{-1}: 3480-3500 (OH), 1798 (C = O of a lactone), 1730 (C = O), 880, 910, 925, 995 (spiroketal chain). Literature data for (XII): mp 225-226° C [9].

Chlorogenonic Acid (XIII) from (XII). To 250 mg of (XII) in 15 ml of acetic acid were added 0.5 ml of water and 500 mg of zinc dust. The reaction mixture was heated in the boiling water bath for 4 h. Then the solvent was distilled off and the residue was treated with water and ether. The ethereal extract was washed with a small amount of water and distilled to dryness. This gave 120 mg of compound (XIII), $C_{27}H_{40}O_7$, mp 232-234° C (from aqueous acetic acid), $[\alpha]_D^{18}$ – 41.1°(c 1.50; dioxane). IR spectrum, cm^{-1}: 3200-3600 (OH), 2640-2680 (OH of the dimer), 1708 (C = O), 870, 905, 925, 985 (spiroketal chain). Literature data for (XIII): mp 235-237° C, $[\alpha]_D$ – 42.8° (dioxane) [10], mp 233-234° C [9].

Dimethyl Chlorogenonate (XIV) from (XIII). The acid (XIII) (110 mg) was methylated with an ethereal solution of diazomethane for 18 h. Distillation of the ether yielded the dimethyl ester (XIV), $C_{29}H_{44}O_7$, mp 161-164° C, $[\alpha]_D^{20}$ – 38.2° (c 1.36; dioxane). IR spectrum, cm^{-1}: 1720, 1750 (C = O) 1180 (C = C – OCH_3), 880, 910, 930, 995 (spiroketal chain). There was no absorption in the OH group region. Mol. wt. 504. Literature data for (XIV): mp 158-159° C, $[\alpha]_D$ – 39.1° [10], mp 162-163° C [9].

Methylation of Alliogenin β-D-Glucopyranoside (II) from (I). In an atmosphere of argon, 1.8 g of sodium hydride was added to 2.1 g of the monoside (I) in 60 ml of dimethyl sulfoxide, and the mixture was stirred at room temperature for 40 min. Then 20 ml of methyl iodide was added and stirring was continued for another 1.5 h. The reaction products were poured into water and extracted with chloroform. The

chloroform extract was treated with a solution of hyposulfite, washed with water, and evaporated to dryness. The residue was methylated by the same method once more. The mixture of products obtained after the second methylation was chromatographed on a column of Al_2O_3. Elution with benzene–methanol (500 : 1) yielded 720 mg of amorphous compound (II).

5α,25R-Spirostane-2α,3β,5α,6β-tetraol 6-Dimethyl Ether (III) from (II). A solution of 500 mg of (II) in 35 ml of 3.3% methanolic HCl was boiled in the water bath for 6 h. Then 10 ml of water was added and the mixture was boiled for another 5 h. After this it was diluted with water, the hydrochloric acid was neutralized with Ag_2CO_3, the methanol was eliminated as completely as possible, and the residue was extracted with chloroform. The chloroform extract was washed with water, and the wash waters were combined with the neutral aqueous solution. The chloroform layer yielded 270 mg of substance (III), $C_{29}H_{48}O_6$, with mp 228-232° C (from acetone), $[\alpha]_D^{18}$ – 125.3° (c 1.15; chloroform). IR spectrum, cm^{-1}: 3400 – 3570 (OH), 875, 910, 930, 990 (spiroketal chain). (For the NMR spectrum, see Table 1). Mol. wt. 492.

2,3,4,6-Tetra-O-methyl-D-glucopyranose was found in the aqueous fraction by paper chromatography in the methyl ethyl ketone–water system and also by thin-layer chromatography in SiO_2 in the chloroform–methanol (9 : 1) system.

5α,25R-Spirostane-2α,3β,5α,6β-tetraol 2,6-Dimethyl Ether 3-Acetate (IV) from (III). Substance (III) (140 mg) was acetylated with 2 ml of acetic anhydride in 3 ml of pyridine at 36-38° C for 18 h. This gave 120 mg of the acetate (IV), $C_{31}H_{50}O_7$, mp 212-215° C (from methanol), $[\alpha]_D^{18}$ – 126.9° (c 1.26; chloroform). IR spectrum, cm^{-1}: 3480-3510 (OH), 1710, 1735 (C = O of an acetyl group), 870, 910, 930, 990 (spiroketal chain). (For NMR spectrum, see Table 1). Mol. wt. 534.

2α,5α,6β-Trihydroxy-5d,25R-spirostane-3-one 2,6-Dimethyl Ether (V) from (III). To 120 mg of compound (III) in 1.5 ml of pyridine was added 130 mg of CrO_3 in 2 ml of pyridine. The reaction mixture was left at room temperature for 20 h. Then it was diluted with chloroform, and the chloroform layer was washed with dilute H_2SO_4 and with water and was distilled to dryness. This gave 60 mg of (V), $C_{29}H_{46}O_6$, mp 260-262° C (from methanol), $[\alpha]_D^{18}$ – 36.6° (c 1.20; chloroform). IR spectrum, cm^{-1}: 3520 (OH), 1710 (C = O), 880, 910, 930, 990 (spiroketal chain). Optical rotatory dispersion (c 0.05; methanol): $[M]_{321}$ + 3200°, $[M]_{315}$ + 3860°, $[M]_{309}$ + 3895°, $[M]_{279}$ – 6740°, $[M]_{271}$ – 6890°, $[M]_{267}$ – 6740°; an hour after the addition of a few drops of HCl: $[M]_{321}$ + 1290°, $[M]_{315}$ + 1575°, $[M]_{309}$ + 1399°, $[M]_{267}$ – 4830°, $[M]_{271}$ – 4870°, $[M]_{260}$ – 4660°. Mol. wt. 490.

SUMMARY

Alliogenin and alliogenin 3-β-D-glucopyranoside have been isolated from the bulbs of Allium giganteum Rgl. It has been shown that alliogenin is 5α,25R-spirostane-2α,3β,5α,6β-tetraol.

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