TRITERPENE GLYCOSIDES OF Astragalus quisqualis.

I. THE STRUCTURE OF QUISQUAGENIN

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The hydrolysis of the glycoside quisqualoside B, isolated from the herb Astragalus quisqualis Bunge, has given the new cycloartane triterpenoid quisquagenin, which has the structure of 20(R),24(S)-epoxycycloartane-3β,16β,25-triol.

Astragalus quisqualis Bunge is a Western Pamir-Alai species with an island fragment of its area in the basin of the R. Angren (Western Tien-Shan) [1]. It grows mainly in forest groupings, and also in rose-bush and pearl-bush clumps, and on mixed-herb steppes with bushes in the height interval from 1300 to 3000 m above sea level.

Flavonoids have previously been isolated from the epigeal part of the plant [2]. We have begun a study of the triterpene compounds of this species. The raw material used was the upper part of the epigeal shoots collected in the budding-incipient flowering phase in the upper reaches of the R. Maikhura – a right-bank tributary of the R. Varzob (Tadzhikistan). The combined triterpenoids were obtained from an ethanolic extract of the plant, and these, according to TLC, contained not less than seven compounds. By column chromatography we isolated two substances which have been denoted in order of increasing polarity as A and B. In the present paper we consider the determination of the structure of the aglycon of substance B, which we have called quisquagenin (I). Glycoside B has been called quisqualoside B. The structure of the aglycon (I) was determined by analyzing the spectral characteristics of the initial substance and of its derivatives.

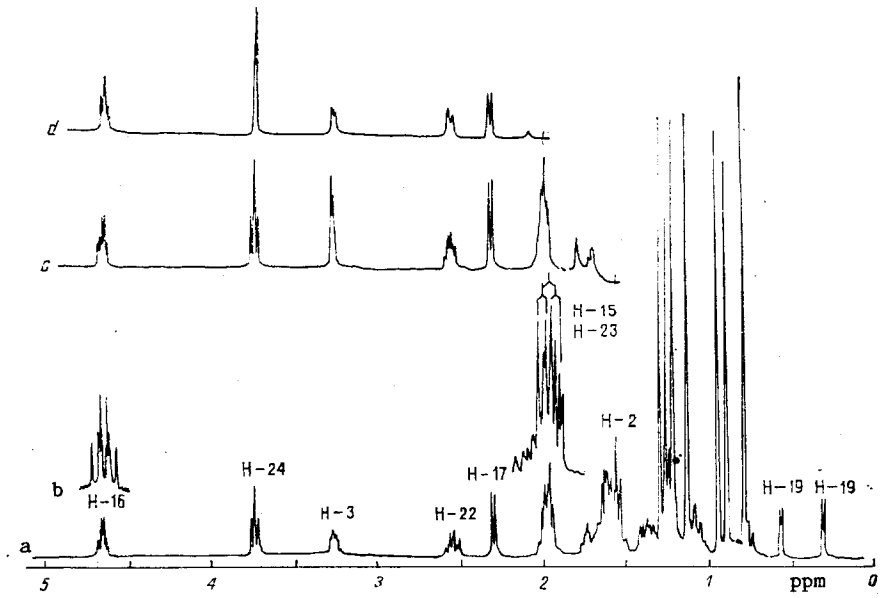

Fig. 1. ^1H NMR spectrum of quisquagenin (I) in $CDCl_3$: a) review spectrum; b) application of line-narrowing procedure; c, d) use of $^1H\{^1H\}$ double resonance.

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The combination in the PMR spectrum of the singlet signals of seven methyl groups and two one-proton doublets at 0.34 and 0.59 ppm with a SSCC of 4.39 Hz (methylene group of a cyclopropane fragment) is characteristic for triterpenoids of the cycloartane series [3, 4]. The position of the cyclopropane ring was confirmed by the ^{13}C NMR spectrum obtained with incomplete suppression of ^{13}C and ^{1}H interaction. The parameters of the C-19 signal were characteristic for the methylene group of a cyclopropane ring (t, δ 30.6 ppm [4], $^{1}J_{CH}$ = 150 Hz [5]) and differed from the parameters of the signal of a methyl group with the absence of a bond between C-9 and C-19 (q, δ 12.0 ppm [4], $^{1}J_{CH}$ = 125 Hz [7]).

The absence from the IR spectrum of bands characteristic for the absorption of a C=C bond, together with the presence in the PMR spectrum of the signal of a cyclopropane methylene group also showed the native nature of the aglycon, i.e., the absence of transformations under the action of acid during the hydrolysis of the glycoside with the opening of the cyclopropane ring that has been described for similar compounds [6, 7].

The elementary composition of (I), $C_{30}H_{50}O_4$, showed the presence of four oxygen functions in its molecule. The nature of two of them followed from the mass spectrum. An intense peak with m/z 143 (100%) is characteristic for the breakdown of the molecules of cycloartane derivatives containing an α-methyl-α'-(hydroxyisopropyl)tetrahydrofuran residue at C-17 [6]. The presence of an hydroxy group in this fragment was confirmed by the result of deuterium exchange: after (I) had been treated with deuteromethanol, an intense peak with m/z 144 (100%) appeared in the mass spectrum. The ether nature of the second oxygen function was determined by the presence in the PMR spectrum of (I) of a one-proton triplet at 3.77 ppm (H-24) which did not change its position when the (I) was acetylated (Table 1). The presence in the IR spectrum of (I) of intense absorption in the 3500-3300 cm^{-1} region confirmed that its molecule contained hydroxy functions. Two of the four oxygen atoms of (I) are present in two secondary hydroxy groups as was shown by the presence in the PMR spectrum of (I) of one-proton signals at 3.28 and 4.67 ppm which shifted downfield on acetylation.

The PMR spectrum of compound (II) (Table 1) contained the signal of one methyl radical of an acetoxy group at 2.03 ppm, while the signal at 3.29 ppm in the spectrum of (I) has shifted to 4.54 ppm. The parameters of the latter are characteristic for protons at C-3 geminal to hydroxy (3.29 ppm) and acetoxy (4.54 ppm) groups. The parameters of the cyclopropane signals, which depend on the nature of the substituent at C-3 likewise agree with the presence at C-3 of an OH (I) or acetoxy (II) group and of two CH_3 groups in position 4 [8].

The signal at 4.65 ppm of a second proton geminal to an hydroxy group underwent practically no changes in the passage from (I) to (II), which indicates some steric hindrance of this OH group on acetylation. To such a condition corresponds an hydroxy group at C-16 in the cis position to a substituent at C-17. The chemical shift of this signal likewise agrees with literature figures for a proton at C-16 geminal to an OH group [9, 10].

The spectrum of compound (III) shows the signals of two acetoxy groups (Table 1 and Fig. 2), while the signal of a proton geminal to an oxygen function has shifted from 4.65 to 5.38 ppm. In agreement with the presence of an OH group at C-16, the signals of the proton at C-17 and of one of the protons at C-15 have shifted downfield by 0.17-0.21 ppm in the transition from (I) and (II) to (III).

The tertiary hydroxyl of the hydroxyisopropyl grouping attached to the tetrahydrofuran ring was not acetylated under the conditions of the experiment described, as was shown by an absorption band of an OH group in the IR spectrum of (III): 3570 cm^{-1} ($\nu_{OH\ free}$) and 3480 cm^{-1} ($\nu_{OH\ bound}$). The IR spectrum of (II) measured in chloroform also showed the presence of two types of absorption bands: 3610 cm^{-1} ($\nu_{OH\ free}$) and 3445 cm^{-1} ($\nu_{OH\ bound}$) [11]. The absence of a shift of the second band on 4- to 5-fold dilution permits the assumption that the hydrogen bond is of intramolecular nature.

In the PMR spectrum of (III) a doublet of triplets appeared at 2.36 ppm which was apparently due to one of the protons at C-12. In the spectra of (I) and (II), this signal was present in a stronger field and was overlapped by other signals. It is possible that on the acetylation of the OH group at C-16, because of steric hindrance by the C-substituents at C-16 and C-17, the C-12 proton undergoes a descreening effect from the substituent at C-17.

The positions of the OH groups at C-3 and C-16 were confirmed by the parameters of the ^{13}C NMR spectrum (Table 2).

The β-configuration and equatorial orientation of the OH group at C-3 follows from the difference in the molecular rotations of (II) and (I), which is +139.2° [12] and the value

TABLE 1. Parameters of the PMR Spectra of Quisquagenin (I) and Its Derivatives (II) and (III) (chemical shifts, δ, ppm, O-TMS, solvent $CDCl_3$, multiplicity, J, Hz)

Compound	Compound											
	H-2	H-3	H-12	H-15	H-16	H-17	21H-19	H-22	H-23	H-24	CH_3 group	CH_3COO
I	1,59** m	3,29 bq		1,98** dd ^2J=13,67 $^3J_{15,16}$ = =8,06	4,67dt * $J_{1\,(16,15)} = J_{16,17}$ = =8,06 $J_{2\,(16,15)}$=6,35	2,31 d J=8,06	0,59 d 0,34 d ^2J = 4,39	2,57 bq ^2J = 11,7	1,98** m	3,77 t $J^1 = J^2$ = 7,32	1,31; 1,28; 1,25; 1,16; 0,97; 0,92; 0,81	
II	1,58** m	4,54 dd J_{aa} = 11,47 J_{ae}=4,39		1,95** m ^2J=13,67 $^3J_{15,16}$ = 8,06	4,65 dt* $J_{1\,(16,15)} = J_{16,17}$ = = 8,06 $J_{2\,(16,15)}$ =6,35	2,29 d J =8,06	0,59 d 0,33 d ^2J = 4,39	2,57 bq ^2J = 11,7	1,95** m	3,73 t $J_1 = J_2$=7,32	1,28; 1,25; 1,21; 1,13; 0,99; 0,86; 0,83	2,03
III	1,58 m	4,53 dd J_{aa} = 11,47 J_{ae} = 4,39	2,36 dt $^2J = {}^3J_{aa}$ = 11,47 $^3J_{ae}$ = 5,39	2,19 dd ^2J = 13,67 $^3J_{15,16}$ = 8,06	5,38dt $J_{1\,(16,15)} = J_{16,17}$ = = 8,06 $J_{2\,(16,15)}$ = 6,35	2,49 d J = 8,06	0,59 d 0,34 d ^2J = 4,39			3,67 dd J^1 = 8,54 J^2 = 7,08	1,26; 1,27; 1,17; 1,06; 0,93; 0,86; 0,82	2,03; 2,03

Symbols: s, singlet; d, doublet; t, triplet; q, quartet; bq, broadened quartet; dt, doublet of triplets; dd, doublet of doublets; m, multiplet.

*Determined with line narrowing.

**Partial overlapping with other signals; position of the signal determined by the double line resonance method.

TABLE 2. Parameters of the ^{13}C NMR Spectra of Quisquagenin in Comparison with Literature Figures (δ, ppm, O − TMS, Multiplicity)

Compound	Solvent	Number of the carbon atom				$\Delta\delta_{20-24}$	Literature
		3	16	20	24		
Quisquagenin (20(R), 24(S)	$CDCl_3$	78,77d 146,1 Hz	73,48d 146,1 Hz	87,14s	81,56 d 141,49 Hz	5,58	
Cyclosiversigenin (20(R), 24(S))	C_5D_5N	78,2	73,3	87,1	81,6	5,5	[9, 13, 14]
Cyclogalegigenin (20(S), 24(R))	C_5D_5N	78,3	72,9	86,7	85,0	1,7	[9, 13]
Astramembrangenin (20(R), 24(S))	$(CD_3)_2SO$	77,8d	73,2 d	87,2 s	81,6d	5,6	[13, 14]

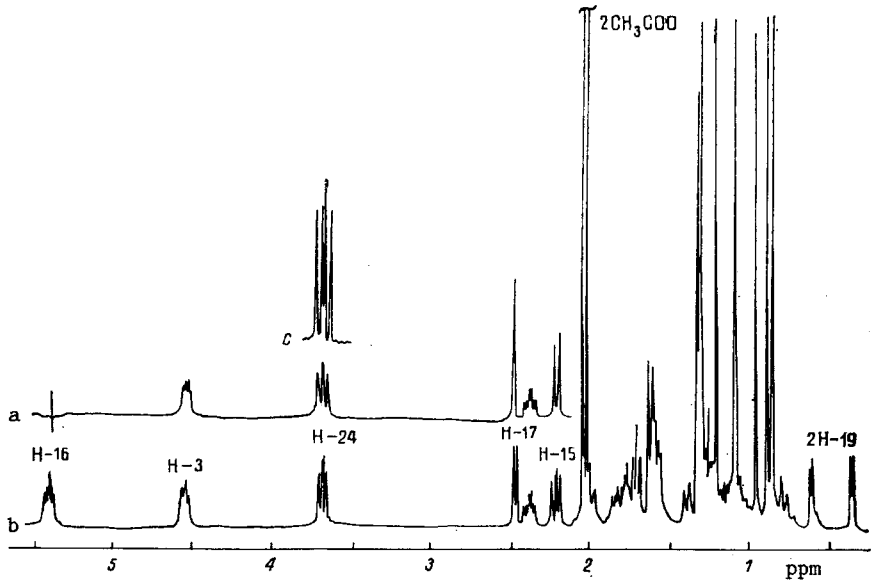


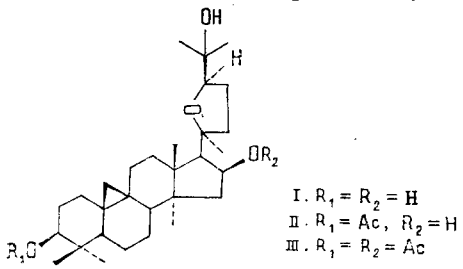
Fig. 2. NMR spectrum of quisquagenin diacetate (III) in $CDCl_3$: a) review spectrum; b) application of $^1H\{^1H\}$ double resonance; c) application of the line-narrowing procedure.

of the SSCC between the H-3 and two H-2 protons (Table 1). The hydroxy group at C-16 also has the β-configuration, since the difference between the molecular rotations of (III) and (II) amounts to +178.3° [12].

The absolute configurations of the C-20 and C-24 asymmetric centers (R and S, respectively) were determined by comparing the ^{13}C NMR spectrum of quisquagenin and those of known compounds (Table 2).

It must be mentioned that not only the ^{13}C chemical shifts but also the difference between them ($\Delta\delta$) can be regarded as characteristic parameters in the determination of absolute configurations of C-20 and C-24. In the case of the 20(S),24(R) configuration, $\Delta\delta_{20-24}$ am amounts to 1.7 ppm, while for the 20(R),24(S) configuration this magnitude is 5.5-5.6 ppm (Table 2).

Thus, the aglycon of quisqualoside B – quisquagenin – is represented by structure (I), and its mono- and diacetates by (II) and (III), respectively



I. $R_1 = R_2 = H$
II. $R_1 = Ac$, $R_2 = H$
III. $R_1 = R_2 = Ac$

EXPERIMENTAL

Thin-layer chormatography was performed on Silufol activated plates. The spots of the triterpenoids were revealed by spraying the plates with a 10% ethanolic solution of tungstophosphoric acid followed by heating at 110°C for 5-10 min. Silica gel L 40/100 (Chemapol) was used for column chromatography. IR spectra were obtained on a UR-20 spectrophotometer in KBr tablets and in chloroform, and mass spectra on a MS-30 Kratos instrument (United Kingdom) fitted with a MS-50 data-collecting and processing system, with direct introduction into the ion source. The temperature of the ionization chamber was 200°C and the ionizing energy 70 eV. The temperature of heating the samples was varied according to their volatility within the range of 170-200°C. PMR and ^{13}C NMR spectra were recorded on a Bruker WM-400 spectrometer (^1H at 400.13 MHz, and ^{13}C at 100.61 MHz) in deuterochloroform (δ, 0 – TMS).

Isolation of Quisquagenin (I). A solution of 2.0 g of quisqualoside B in 200 ml of 0.5% methanolic sulfuric acid was heated in the water bath at 70°C for 1.5 h. Then the reaction

mixture was diluted with water and was treated with chloroform. The chloroform extracts were washed with water and evaporated to dryness. By column chromatography using chloroform–methanol (15:1) as eluent, 310 mg of the crystalline aglycon (I), $C_{30}H_{50}O_4$, was isolated, with mp 232.5-234.5°C (from methanol), $[\alpha]_D^{25}$ +36.6 ± 4° (c 0.745; chloroform), $[M]_D$ +173.5°. ν_{max}^{KBr}, cm^{-1}: 3300-3550 (OH), 2860-3050 (–CH_3, –CH_2–, –CH).

Mass spectrum, m/z (%): M^+ 474 (0.9), 456 (2.0), 441 (1.8), 423 (1.7), 415 (2.1), 397 (2.5), 255 (3.3), 217 (2.4), 191 (4.6), 187 (5.7), 175 (5.2), 174 (1.7), 173 (7.7), 171 (8.5), 161 (7.0), 159 (6.5), 147 (8.9), 145 (9.2), 144 (11.6), 143 (100.0).

Quisquagenin 3-Monoacetate (II) and 3,16-Diacetate (III). A solution of 140 mg of quisquagenin in 6 ml of pyridine was treated with 3 ml of acetic anhydride and the mixture was heated at 90°C for 1 h. It was then poured into ice water, and the precipitate that deposited was filtered off. The mixture of acetates (152 mg) was separated by column chromatography with elution by benzene–ethyl acetate (3:2). This gave 48 mg of the crystalline diacetate (III), $C_{34}H_{54}O_6$, mp 163-166°C (from methanol), $[\alpha]_D^{25}$ +88.0 ± 6° (c 0.997; chloroform), $[M]_D$ +491°. ν_{max}^{KBr} cm^{-1}: 3570, 3550 (OH_{free}), 3480 (OH_{bound}), 3035 (–CH_2– cyclopropane), 2980, 2950, 2885, 2870, 2850, 2830 (–CH_3, –CH_2–, –CH), 1733, 1250 (ester groups).

Mass spectrum, m/z (%): M^+ 558 (1.4), 499 (4.5), 498 (3.4), 441 (0.7), 423 (0.9), 397 (1.2), 379 (1.8), 255 (3.0), 219 (9.4), 217 (4.7), 197 (6.2), 191 (1.9), 187 (3.1), 181 (8.1), 175 (1.4), 174 (2.1), 173 (3.8), 169 (10.3), 161 (6.0), 159 (5.5), 149 (10.4), 147 (6.5), 145 (9.2), 144 (11.0), 143 (100.0).

Further elution gave 53 mg of the crystalline monoacetate (II), $C_{32}H_{52}O_5$, mp 258-261°C [from chloroform–methanol (1:2)]. $[\alpha]_D^{25}$ +60.6 ± 6° (c 0.95; chloroform), $[M]_D$ +312.7°. ν_{max}^{KBr}, cm^{-1}: 3420, 3320 (OH), 3040 (–CH_2 cyclopropane), 2975, 2945, 2887, 2870, 2860, 2850 (–CH_3, –CH_2–, –CH), 1736, 1250 (ester group). $\nu_{max}^{CDCl_3}$ cm^{-1}: 3610 (OH_{free}), 3445 (OH_{bound}).

Mass spectrum, m/z (%): M^+ 516 (0.7), 456 (1.5), 441 (0.7), 423 (2.0), 415 (0.7), 397 (6.8), 379 (3.6), 255 (4.5), 217 (2.2), 191 (1.1), 187 (8.1), 175 (5.9), 174 (2.3), 173 (4.8), 161 (7.2), 149 (5.8), 147 (8.2), 145 (9.3), 144 (12.0), 143 (100.0).

CONCLUSION

The hydrolysis of the glycoside quisqualoside B, isolated from the herb Astragalus quisqualis Bunge has given the new cycloartane triterpenoid quisquagenin, which has the structure of 20(R),24(S)-epoxycycloartane-3β,16β,25-triol.

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