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TRITERPENE GLYCOSIDES OF *Tragacantha stipulosa* AND THEIR GENINS. STRUCTURES OF ASKENDOSIDES G AND D AND CYCLOGLOBICEPOSIDE B FROM ONE- AND TWO-DIMENSIONAL ^{1}H AND ^{13}C NMR SPECTROSCOPY

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Roots of Tragacantha stipulosa *Boriss yielded three triterpene glycosides of the cycloartane series: askendoside G (**1**), askendoside D (**2**), and cycloglobiceposide B (**3**). Glycoside **1** is 3-O-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranoside; 16-O-β-D-glucopyranoside-24R-cycloartan-3β,6α,16β,24,25-pentaol; **2** – 3-O-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranoside; 6-O-β-D-xylopyranoside-20R,24S-epoxycycloartan-3β,6α,16β,25-tetraol; **3** – 3-O-β-D-xylopyranoside; 16-O-β-D-glucopyranoside; 25-O-β-D-glucopyranoside-24R-cycloartan-3β,6α,16β,24,25-pentaol.*

Key words: cycloartanes, triterpene glycosides, two-dimensional NMR spectroscopy (^{1}H, ^{13}C, COSY, TOCSY, ROESY, HSQC, and HMBC).

In continuation of research on cycloartane triterpenoids of *Tragacantha stipulosa* Boriss (*Astragalus stipulosus* Boriss, Leguminosae) [1], we isolated from the methanol extract of its roots three known glycosides of the cycloartane series, askendosides G (**1**) and D (**2**) and cycloglobiceposide B (**3**).

The isolated glycosides were studied using ^{1}H and ^{13}C NMR spectroscopy including two-dimensional (2-D) homonuclear (H,H) COSY (Correlated Spectroscopy), TOCSY (Total Correlation Spectroscopy), and ROESY (Rotating frame Overhauser Effect Spectroscopy) and heteronuclear (H,C) HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple-Bond Correlation).

Scans and preliminary analysis of the ^{13}C NMR spectra and their one-dimensional (1-D) modifications (APT, Attached Proton Test; GD, Gated Decoupling) showed that these compounds are glycosides with three sugar residues. Analysis of the spin—spin coupling constants (SSCC) of the anomeric C atoms to anomeric protons (163-164 Hz) established that the sugars adopt the pyranose form with an axial anomeric proton. Scans and preliminary analysis of the ^{1}H NMR spectra gave additional information about the number of C-methyls in the aglycones (seven each for all glycosides) and revealed that the compounds have 1H doublets of an AB-spin system (0.13-0.61 ppm), which is characteristic of cycloartane triterpenoids.

The COSY and TOCSY spectra revealed closed spin systems belonging to sugar protons and enabled a determination of the monosaccharide composition of the carbohydrate part of each glycoside based on the magnitude of the chemical shifts and the SSCC. The COSY and TOCSY spectra also exhibited a series of closed spin systems for the protons of rings A, B, C, etc. of the aglycones and enabled their partial assignment.

The ROESY spectrum showed the bonding sequence of residues in the disaccharides or the site of attachment of the sugars to the aglycone (Agl). Thus, the ROESY spectrum of askendoside G (**1**) contains the usual correlation peaks for a pyranose with axial anomeric protons H-1/H-2, H-1/H-3, and H-1/H-5_{ax} in addition to correlation peaks H-1(α-Ara)/H-2(β-Xyl), H-1(β-Xyl)/H-3(Agl), and H-1(β-Glc)/H-16(Agl). This enables the sequence of residues in the disaccharide and the site of

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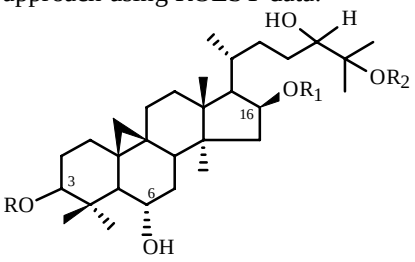
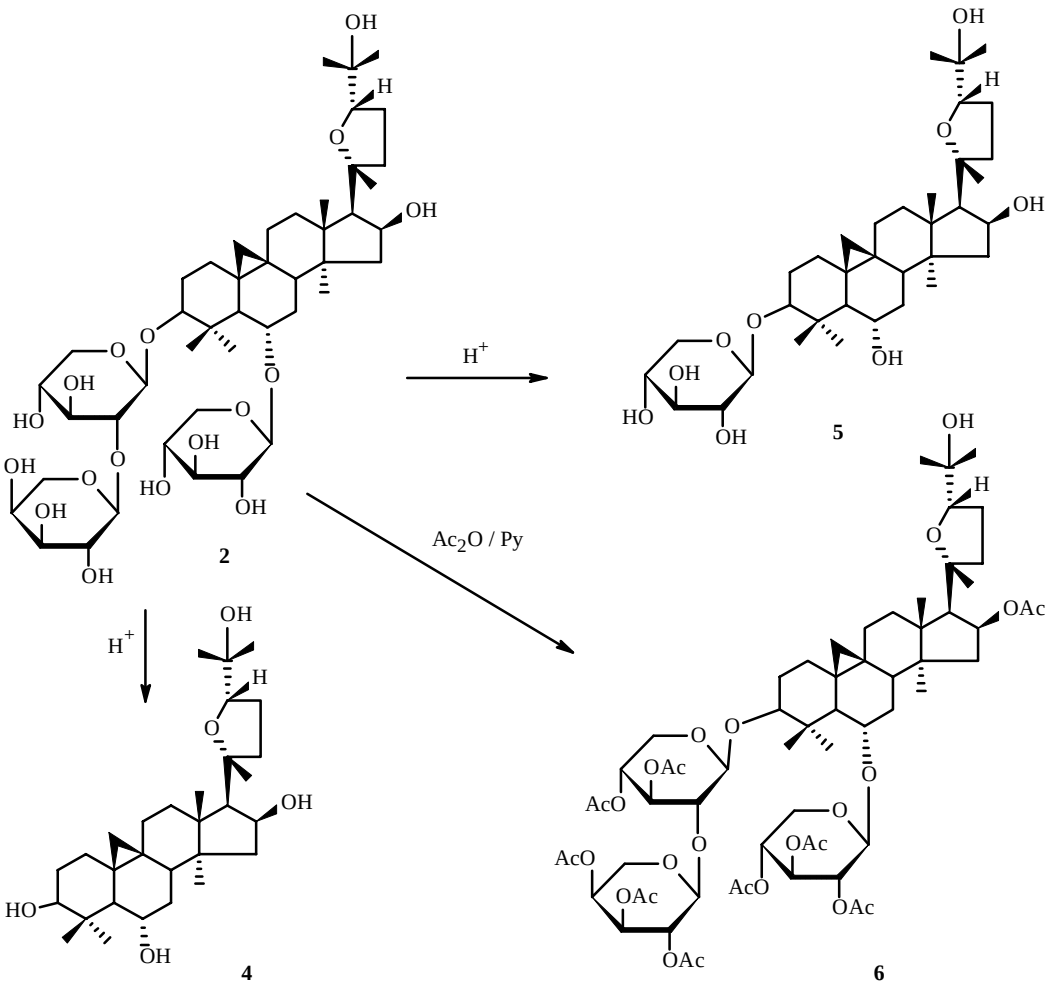
attachment of the disaccharide and the glucopyranose to the aglycone to be determined. The site of the sugar residues in the remaining compounds was determined by an analogous approach using ROESY data.



1: R = α-L-Ara*p*-(1→2)-β-D-Xyl*p*, R_1 = β-D-Glc*p*, R_2 = H;
3: β-D-Xyl*p*, R_1 = R_2 = β-D-Glc*p*



2 **4** **5** **6**

We assigned completely the proton signals of the aglycone by analyzing the ROESY spectrum and established the bonding type of all rings in the aglycone. Thus, the presence of through-space contacts for H-3 and H-5 and for each of these with H-29 indicates that rings A, B, and C and the cyclopropane fragment C-9, C-10, and C-19 are fused. According to cross-peaks in the ROESY spectrum, the H-30 protons are close to H-29, H-2_{ax}, H-6, and one of the protons of the aforementioned AB-system on C-19, which has a chemical shift at weaker field (0.13-0.61 ppm, H-19_{endo}).

Thus, the cyclopropane occupies the β,β-position on C-9 and C-10. Also, contacts with H-8 are observed for H-19_{endo} in two glycosides (**1** and **3**). This unambiguously defines the fusion type of rings B and C. The through-space contacts of C-18 and C-28 methyl protons must be examined to establish the fusion type of rings C and D. Contacts with H-11_{ax}, H-12_{eq}, H-20, and H-21 were found for H-18. Cross-peaks with H-12_{ax}, H-7, H-15, H-17, and H-16 (weak) were observed in the ROESY spectrum for H-28. These data indicate not only the β-position for C-18 and the α-position for C-28 but also the β-position for the substituents on C-16.

TABLE 1. ^{1}H and ^{13}C Chemical Shifts of Cycloglobiceposide B (**3**) (C_5D_5N, 0 = TMS, δ, ppm)

Atom	Chemical shift		Atom	Chemical shift	
	^{13}C	^{1}H		^{13}C	^{1}H
1	32.55	1.63; 1.25	26	22.10	1.55
2	30.30	2.38; 2.00	27	23.55	1.52
3	88.80	3.63	28	20.20	0.96
4	42.70	-	29	28.85	1.99
5	53.90	1.76	30	16.70	1.34
6	66.90	3.67		*β*-D-Xyl*p*-(1→3-Agl)	
7	38.25	1.75; 1.61	1	107.45	4.89
8	46.90	1.73	2	75.40	4.06
9	21.25	-	3	78.20	4.17
10	29.25	-	4	71.10	4.22
11	26.25	1.91; 1.17	5	67.90	4.36; 3.72
12	32.85	1.61; 1.61		*β*-D-Glc*p*-(1→16-Agl)	
13	45.65	-	1	106.10	4.77
14	46.85	-	2	75.80	3.92
15	47.30	2.21; 1.99	3	78.60	4.20
16	82.85	4.39	4	71.40	4.20
17	57.45	1.84	5	77.90	3.88
18	19.05	1.23	6	62.75	4.41; 4.29
19	30.30	0.45; 0.25		*β*-D-Glc*p*-(1→25-Agl)	
20	31.70	2.19	1	98.60	5.14
21	18.20	1.00	2	75.15	4.00
22	34.50	2.42; 1.11	3	78.40	4.25
23	29.90	2.19; 1.64	4	71.70	4.21
24	78.30	3.87	5	78.00	3.94
25	80.85	-	6	62.70	4.49; 4.26

Complete assignment of the proton signals in the spectra of the glycosides enables the signals in the ^{13}C NMR spectra to be assigned using 2-D HSQC (for protonated C atoms) and HMBC (for quaternary C atoms). The HMBC spectra also assist resolution of the following structural issues.

Location of carbohydrate residues using the presence of SSCC ^{3}J for the anomeric sugar proton and the *trans*-glycoside C of the glycosylated residue (or aglycone) or the anomeric C and H on the *trans*-glycoside C atom. Structural information from this method is identical to that obtained by analyzing the ROESY spectrum. However, the C spectrum can in several instances become indispensible considering that it has better resolution than the H spectrum. The location of the carbohydrate residues observed in the ROESY spectra was confirmed by the presence in the HMBC spectra of correlation peaks H-1(Ara)/C-2(Xyl), H-1(Xyl)/C-3(Agl), H-1(Glc)/C-16(Agl) for **1**; H-1(Ara)/C-2(Xyl), H-1(Xyl)/C-3(Agl), and H-1(Xyl)/C-6(Agl) for **2**; and H-1(Xyl)/C-3(Agl), H-1(Glc)/C-16(Agl), and H-1(GlcII)/C-25(Agl) for **3**.

Type of Ring Fusion. Information about the type of ring fusion is based on the dependence of the SSCC ^{3}J on the dihedral angle between the ^{1}H–X and Y–^{13}C bonds in an H–X–Y–^{13}C fragment, where X and Y can be, in particular, C atoms. According to the Karplus equation, the largest SSCC ^{3}J values correspond to dihedral angles 0° and 180°; the smallest, 90°. Correlations of *trans*-oriented protons and C atoms separated by three bonds (angle close to 180°) in addition to protons and C atoms in an eclipsed configuration or one close to it (angle close to 0°) appear in the HMBC spectrum owing to large SSCC (5-10 Hz) if the experiment is optimized for detection of cross-peaks. Thus, HMBC spectra of the studied compounds contain correlation peaks H-5/C-30 (but not H-5/C-29). This indicates that C-30 and H-5 are axial in ring A. On the other hand, the presence of H-19_{exo}/C-1 and H-19_{exo}/C-11 cross peaks correspond to a configuration close to eclipsed for the H-19_{exo}–C-19 and C-10–C-11 (or C-9–C-11) bonds.

The NMR results enabled the structures of the three glycosides to be determined. As it turned out, they were identical to some that had been isolated previously from other sources. Thus, glycosides **1** and **2** are identical to askendosides G (**1**) and

D (**2**), respectively [2-4] whereas **3** corresponds with cycloglobiceposide B [6] (Table 1). Comparison of spectral data established that the previously isolated cyclostipuloside B [1] corresponds with **1**, which is askendoside G.

According to acid hydrolysis, the carbohydrates of **1**-**3** are various combinations of the sugars xylose, arabinose, and glucose. The aglycone of askendoside D (**2**) is cyclosiversigenin (**4**) [5, 7]. The genins of cycloglobiceposide B and askendoside G were identified as cycloasgenin C [8]. Progenin 5, which was identified as cyclosiversigenin 3-O-*β*-xylopyranoside, was obtained from glycoside **2** [9]. Acetylation of askendoside D (**2**) by acetic anhydride in pyridine produced the nonaacetate (**6**).

Comparison of our ^{13}C NMR data and those in the literature [2, 3, 6] showed that only a thorough approach guarantees the correct assignment of signals in the ^{13}C spectrum and enables a complete assignment of signals in the proton spectrum. However, the principal value of this approach is that the structure of the polycyclic part of the genin can be established in fine detail without requiring x-ray structure analysis, which is not always feasible (e.g, see [10]). As concerns the flexible parts of the genin (in this instance, C-21—C-27), the proposed approach can be useful for comparing experimental data for the SSCC and the Overhauser effect with data obtained by calculations using molecular mechanics methods and averaged over the rotamer population.

EXPERIMENTAL

General comments have been published [1]. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500.13 and 125.27 MHz, respectively, in C_5D_5N at 30°C with TMS-standard. Standard Bruker methods were used to record 2-D spectra. The pulse delay for recording TOCSY and ROESY spectra was 0.2 sec. The accuracy of the ^1H and ^{13}C chemical shift measurements was ±0.01 ppm; $^1H/^1H$ SSCC, 0.2 Hz.

Askendoside G (1). Yield 26 mg (0.00074%), $C_{46}H_{78}O_{18}$, mp 273-275°C (methanol).

IR spectrum (KBr, ν, cm^{-1}): 3348 (OH), 3015 (cyclopropane).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 1.61, 1.24 (H-1), 2.32, 1.99 (H-2), 3.56 (H-3), 1.71 (H-5), 3.68 (H-6), 1.73, 1.60 (H-7), 1.71 (H-8), 1.90, 1.17 (H-11), 1.61, 1.61 (H-12), 2.24, 1.93 (H-15), 4.37 (H-16), 1.86 (H-17), 1.23 (H-18), 0.29, 0.51 (H-19), 2.19 (H-20), 1.01 (H-21), 2.42, 1.11 (H-22), 2.29, 1.66 (H-23), 3.71 (H-24), 1.52 (H-26), 1.46 (H-27), 0.97 (H-28), 1.96 (H-29), 1.42 (H-30), 5.22 (H-1′-Ara*p*), 4.59 (H-2′), 4.23 (H-3′), 4.33 (H-4′), 4.41, 3.81 (H-5′), 4.91 (H-1″-Xyl*p*), 4.11 (H-2″), 4.19 (H-3″), 4.20 (H-4″), 4.29, 3.63 (H-5″), 4.82 (H-1‴-Glc*p*), 3.97 (H-2‴), 4.21 (H-3‴), 4.21 (H-4‴), 3.89 (H-5‴), 4.42, 4.31 (H-6‴).

^{13}C NMR spectrum (C_5D_5N, δ, ppm, TMS): 32.60 (C-1), 30.35 (C-2), 88.60 (C-3), 42.80 (C-4), 54.10 (C-5), 68.00 (C-6), 38.40 (C-7), 46.85 (C-8), 21.30 (C-9), 29.30 (C-10), 26.30 (C-11), 32.90 (C-12), 45.65 (C-13), 46.85 (C-14), 47.80 (C-15), 83.10 (C-16), 57.60 (C-17), 19.10 (C-18), 30.40 (C-19), 32.80 (C-20), 18.10 (C-21), 34.45 (C-22), 30.30 (C-23), 80.00 (C-24), 72.75 (C-25), 26.20 (C-26), 25.50 (C-27), 20.20 (C-28), 28.70 (C-29), 16.35 (C-30), 105.70 (C-1′), 83.60 (C-2′), 77.70 (C-3′), 71.05 (C-4′), 66.65 (C-5′), 106.60 (C-1″), 73.70 (C-2″), 74.35 (C-3″), 69.15 (C-4″), 67.05 (C-5″), 106.60 (C-1‴), 75.80 (C-2‴), 78.85 (C-3‴), 71.90 (C-4‴), 78.10 (C-5‴), 63.00 (C-6‴).

Acid Hydrolysis. Askendoside G (**1**, 15 mg) was hydrolyzed by methanolic H_2SO_4 (10 mL, 0,15%) with heating on a boiling-water bath for 6 h. The reaction mixture was cooled and treated with water (10 mL). The methanol was evaporated. The precipitate was filtered off, washed with water, neutralized with $BaCO_3$, and evaporated. Paper chromatography (PC) using butan-1-ol—pyridine—water (6:4:3) detected D-xylose, D-glucose, and L-arabinose by comparison with authentic samples.

Based on the above physicochemical constants and spectral data in addition to a comparison with literature data, **1** was identified as askendoside G [2, 3].

Askendoside D (2). Yield 4 g (0.11%), $C_{45}H_{74}O_{17}$, mp 235-236°C (methanol).

Based on the above physicochemical constants and spectral data in addition to a comparison with literature data, **2** was identified as askendoside D [3, 4].

IR spectrum (KBr, ν, cm^{-1}): 3450-3285 (OH), 3040 (cyclopropane).

PMR spectrum (C_5D_5N, δ, ppm): 1.53, 1.28 (H-1), 2.25, 1.95 (H-2), 3.37 (H-3), 1.83 (H-5), 3.76 (H-6), 2.05, 2.05 (H-7), 2.07 (H-8), 1.74, 1.46 (H-11), 1.66, 1.53 (H-12), 2.33, 1.82 (H-15), 5.04 (H-16), 2.58 (H-17), 1.39 (H-18), 0.13, 0.61 (H-19), 1.31 (H-21), 3.09, 1.65 (H-22), 2.32, 2.07 (H-23), 3.88 (H-24), 1.58 (H-26), 1.31 (H-27), 1.14 (H-28), 1.77 (H-29), 1.34 (H-30), 5.19 (H-1′-Ara*p*), 4.58 (H-2′), 4.21 (H-3′), 4.30 (H-4′), 4.42, 3.80 (H-5′), 4.79 (H-1″-Xyl*p*), 4.09 (H-2″), 4.18 (H-3″), 4.19 (H-4″), 4.29, 3.60 (H-5″), 4.81 (H-1‴-Xyl*p*), 3.98 (H-2‴), 4.16 (H-3‴), 4.18 (H-4‴), 4.30, 3.68 (H-5‴).

^{13}C NMR spectrum (C_5D_5N, δ, ppm, TMS): 31.85 (C-1), 30.00 (C-2), 87.75 (C-3), 42.60 (C-4), 51.95 (C-5), 77.25 (C-6), 33.10 (C-7), 43.45 (C-8), 21.30 (C-9), 27.95 (C-10), 26.30 (C-11), 33.50 (C-12), 45.25 (C-13), 46.20 (C-14), 45.55

(C-15), 73.35 (C-16), 58.05 (C-17), 20.20 (C-18), 25.90 (C-19), 87.35 (C-20), 28.60 (C-21), 34.90 (C-22), 26.45 (C-23), 81.70 (C-24), 71.25 (C-25), 28.05 (C-26), 27.00 (C-27), 19.60 (C-28), 27.65 (C-29), 16.35 (C-30), 105.25 (C-1′), 83.40 (C-2′), 77.40 (C-3′), 70.95 (C-4′), 66.45 (C-5′), 106.50 (C-1″), 73.40 (C-2″), 74.10 (C-3″), 68.95 (C-4″), 66.90 (C-5″), 105.55 (C-1‴), 75.20 (C-2‴), 78.00 (C-3‴), 70.80 (C-4‴), 66.75 (C-5‴).

Acid Hydrolysis. Askendoside D (**2**, 100 mg) was hydrolyzed in methanolic H_2SO_4 (20 mL, 0,15%) for 4 h. The hydrolysate was worked up as above. The precipitate was chromatographed over a silica-gel column with elution by $CHCl_3$—CH_3OH—H_2O (40:7.5:1) to give cyclosiversigenin (**4**, 28 mg). PC of the aqueous part of the hydrolysate using butan-1-ol—pyridine—water (6:4:3) detected D-xylose and L-arabinose. Cyclosiversigenin (**4**), $C_{30}H_{50}O_5$, mp 240-242°C (methanol), $[\alpha]_D$ +49.0±2° (*c* 1.37, methanol).

Based on the physicochemical constants and spectral data in addition to a comparison with an authentic sample [TLC, $CHCl_3$—CH_3OH—H_2O (40:7.5:1)], **4** was identified as cyclosiversigenin [5, 7].

Further elution of the column by the same solvent system afforded monoside **5** (15 mg), $C_{35}H_{58}O_9$, mp 240-242°C (methanol), identified as cyclosiversigenin 3-O-β-D-xylopyranoside [9].

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.30 and 0.58 (1H each, d, ^2J = 4.2 and 4.0, 2H-19), 1.01, 1.30, 1.32, 1.43, 1.57, 1.59, 2.02 (s, 3H each, tertiary methyls), 2.55 (1H, d, ^3J = 7.9, H-17), 3.66 (1H, dd, ^3J = 11.8 and 4.4, H-3), 3.89 (1H, dd, ^3J = 9.9 and 5.2, H-24), 4.91 (1H, d, ^3J = 7.4, H-1′ of xylose).

Acetylation of Askendoside D (2). Askendoside D (**2**, 70 mg) in pyridine (2 mL) was acetylated by acetic anhydride (2 mL) at room temperature for 24 h. Solvent was removed. The solid was chromatographed over a silica-gel column with elution by $CHCl_3$—CH_3OH (60:1) to give the nonaacetate (**6**, 80 mg), $C_{63}H_{92}O_{26}$, mp 228-231°C (methanol).

IR spectrum (KBr, ν, cm^{-1}): 3580-3560 (OH), 1740, 1250 (ester), 3050 (cyclopropane).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.20 and 0.50 (1H each, d, ^2J = 4.0, 2H-19), 1.04, 1.28, 1.31, 1.32, 1.41, 1.60, 1.61 (s, 3H each, tertiary methyls), 2.00 (3×CH_3), 2.01, 2.02, 2.13, 2.20, 2.32, 2.48 (9×OAc), 4.86, 5.02, 5.14 (1H each, d, ^3J = 7.0, 7.2, 7.0; H-1′, H-1″, H-1‴, respectively).

The data indicate that **2** is askendoside D [3, 4].

Cycloglobiceposide B (3). Yield 20 mg (0.00057%), $C_{47}H_{80}O_{19}$, mp 285-287°C (methanol).

IR spectrum (KBr, ν, cm^{-1}): 3394 (OH), 3010 (cyclopropane).

Table 1 contains the ^1H and ^{13}C NMR spectra.

Acid Hydrolysis of 3. Cycloglobiceposide B (**3**, 10 mg) was hydrolyzed as above. PC of the aqueous part of the hydrolysate detected D-xylose and D-glucose.

Based on the physicochemical constants and spectral data in addition to a comparison with an authentic sample [TLC, $CHCl_3$—CH_3OH—H_2O (70:23:3)], **3** was identified as cycloglobiceposide B [6].

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