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Cephalotoside A: a tridesmosidic cycloartane type glycoside from *Astragalus cephalotes* var. *brevicalyx*

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Abstract

A new tridesmosidic cycloartane type glycoside, cephalotoside A was isolated from the roots of *Astragalus cephalotes* var. *brevicalyx* in addition to the known glycosides cyclocanthosides A, D and E. The structure of the new compound was determined by spectral (IR, ^{1}H- and ^{13}C-NMR, and FABMS) and chemical (acetylation) methods and established as 3β-(β-D-xylopyranosyl)oxy-16β-(β-D-glucopyranosyl)oxy-24-(β-D-xylopyranosyl)oxy-cycloartane-6α,25-diol. © 1999 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

As a continuation of our work on the cycloartane type triterpene glycosides of the genus *Astragalus* (Çalis, Zor, Saracolgu, Isimer, & Rüegger, 1996; Çalis et al., 1997; Bedir, Çaliş, Zerbe, & Sticher, 1998), we have studied the roots of *A. cephalotes* var. *brevicalyx*. The water soluble part of the 80% aqueous ethanolic extract of the roots of *A. cephalotes* var. *brevicalyx* was first subjected to VLC on reversed phase silica gel and eluted with methanol–water mixtures. The fraction rich in saponins eluted with methanol was subjected to open column chromatography on normal phase silica gel using chloroform–methanol-water mixtures as eluents to yield eleven saponin containing fractions. By further chromatographic separations on these fractions five saponins, **1**–**4**, were isolated.

2. Results and discussion

Compound **1** was the most polar compound among the glycosides isolated in this study. Its IR spectrum showed only strong hydroxyl absorption bands at 3400 cm^{-1}. The FAB mass spectrum of **1** exhibited ion peaks at *m/z* 941 $[M+Na]^+$ and at 919 $[M+H]^+$, which are compatible with the molecular formula $C_{46}H_{78}O_{18}$. The ^{1}H NMR spectrum of **1** (Table 1) showed signals characteristic of cyclopropane–methylene protons as an AX system (δ 0.46 and 0.25, $J_{AX} = 3.6$ Hz; H_2-19) and six tertiary (δ 2.00, 1.51, 1.49, 1.34, 1.20 and 0.88) and a secondary methyl (δ 0.97 d, $J = 6.3$ Hz) group. Additionally, the resonances for three anomeric protons were observed at δ 5.04 (d, $J = 7.6$ Hz, anomeric proton of β-D-glucose), 4.90 and 4.80 (both d, $J = 7.3$ Hz, anomeric protons of two β-D-xylose units). Thus, compound **1** was considered to be a cycloartane-type triterpene triglycoside. This observation was supported by the ^{13}C NMR spectral data of **1** (Table 1). The ^{13}C NMR spectrum of **1** exhibited 46 lines. Thirty signals were accounted for the aglycone moiety. The remaining signals were in good accordance for the presence of one hexose and two pentose units. The resonances assigned to the aglycone moiety consist of 7 methyl, 9 methylene, 8 methine and 6 quaternary carbons. The proton and carbon NMR signals were analyzed by 2D-^{1}H–^{1}H-shift correlation spectroscopy (COSY and TOCSY) coupled with 2D-^{1}H–^{13}C-heteronuclear COSY (HMQC). Among the sugar protons, the remaining signals showed correlations to the signals at high field, indicating the presence of four geminal protons on oxygenated carbon atoms of the sapogenol moiety. These resonances were observed at δ 3.64 (2H, H-3 and H-6), 4.13 (H-24) and 4.42 (H-16). Additionally, in the ^{13}C NMR spectrum of **1**, a quaternary carbon signal at δ 72.26 indicated the presence of a tertiary hydroxyl group on the sapogenol moiety. The

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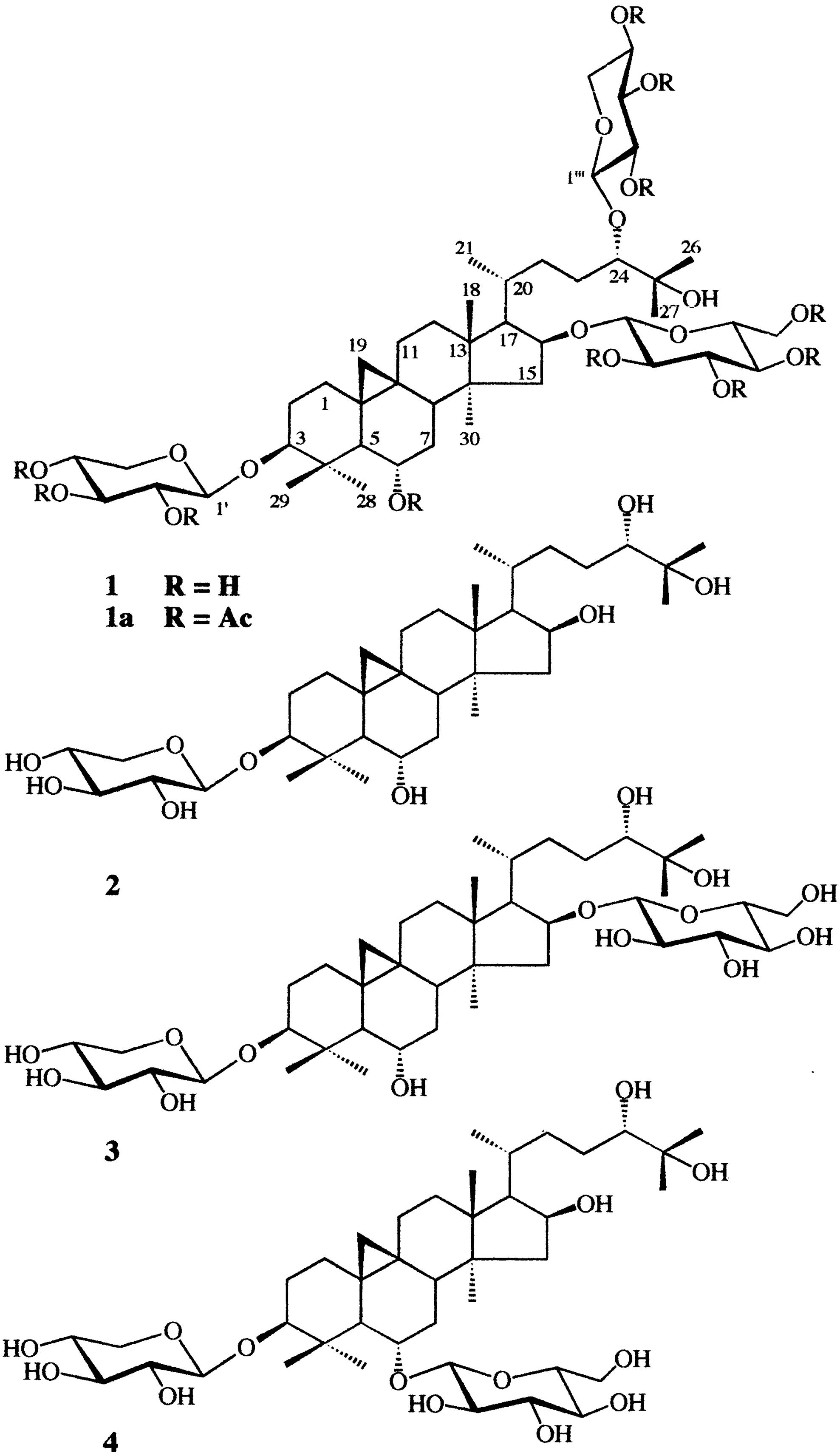

1 **R = H**

1a **R = Ac**

2

3

4

Structure 1.

Table 1
^{1}H and ^{13}C NMR spectral data for cephalotoside A (**1**) and cephalotoside A undecaacetate (**1a**) (δ: ppm; *J*: Hz)[a]

C,H atom		**1**	**1a**[c]	**1**	**1a**[d]
1	CH_2	32.62	31.80	1.58[b], 1.14[b]	1.68[b]
2	CH_2	30.43	29.00	2.42[b], 2.06[b]	1.92[b], 1.72[b],
3	CH	88.82	88.27	3.64[b]	3.14 dd (11.5, 4.6)
4	C	42.76	41.54		
5	CH	54.08	49.82	1.72[b]	1.63 d (9.0)
6	CH	67.98	70.44	3.64[b]	4.70 ddd (4.5, 9.0, 9.0)
7	CH_2	38.51	34.40	1.69[b], 1.58[b]	1.53[b], 1.28[b]
8	CH	47.01	44.90	1.65[b]	1.65[b]
9	C	21.26	21.00		
10	C	29.30	27.95		
11	CH_2	26.26	25.80	1.91[b], 1.13[b]	1.85[b], 1.25[b]
12	CH_2	32.76	32.30	1.58[b]	1.50[b].
13	C	45.67	45.53		
14	C	46.83	46.66		
15	CH_2	48.18	48.80	2.19[b], 1.94[b]	1.88[b], 1.62[b]
16	CH	82.75	80.92	4.42[b]	4.19[b]
17	CH	57.40	56.31	1.90[b]	1.71[b]
18	CH_3	19.15	17.78	1.20 s	1.06 s
19	CH_2	30.55	28.30	0.46 and 0.25 ($J_{AX}=3.6$)	0.55 and 0.33 ($J_{AX}=4.8$)
20	CH	31.32	30.12	2.23[b]	1.75[b]
21	CH_3	17.83	17.73	0.97 d (6.3)	0.87 d (6.3)
22	CH_2	34.43	32.90	2.20[b], 2.05[b]	1.55[b], 1,25[b]
23	CH_2	30.00	28.90	1.95[b], 1.32[b]	1.53[b], 1.49[b]
24	CH	90.67	91.00	4.13[b]	3.37 dd (9.0, 2.0)
25	C	72.26	72.10		
26	CH_3	25.83	26.18	1.51 s	0.90 s
27	CH_3	26.85	24.10	1.49 s	1.15 s
28	CH_3	16.78	15.98	1.34 s	0.88 s
29	CH_3	28.99	26.29	2.00 s	1.18 s
30	CH_3	20.34	20.95	0.88 s	0.87 s
1′	CH	107.68	101.30	4.90 d (7.3)	4.62 d (7.2)
2′	CH	75.68	71.61	4.07[b]	5.00[b]
3′	CH	78.58	71.67	4.26–4.10[b]	5.21 t (9.0)
4′	CH	71.29	68.68	4.22[b]	5.00[b]
5′	CH_2	67.10	62.11	4.34[b], 3.72[b]	4.18 dd (11.7, 5.3), 3.40 dd (11.7, 9.5)
1″	CH	106.23	101.08	4.80 d (7.3)	4.46 d (7.7)
2″	CH	75.81	71.33	3.97[b]	4.98 dd (7.7, 9.4)
3′	CH	78.62	71.87	4.26–4.10[b]	5.16 t (9.4)
4″	CH	71.82	68.77	4.22[b]	5.09 dd (9.4, 9.7)
5″	CH	77.95	71.65	3.88 m	3.69 m
6″	CH_2	62.97	61.84	4.38[b], 4.30[b]	4.23 dd (12.3, 2.6), 4.20[b]
1‴	CH	106.97	102.91	5.04 d (7.6)	4.50 d (7.1)
2‴	CH	75.36	72.26	4.03[b]	4.94[b]
3‴	CH	78.50	72.26	4.26–4.10[b]	5.15 t (9.4)
4‴	CH	71.03	69.11	4.17[b]	4.94[b]
5‴	CH_2	67.25	62.29	4.20[b], 3.65[b]	4.10 dd (11.8, 5.2), 3.31 dd (11.8, 9.2)

[a] For **1**: ^{13}C: 75.5 MHz; ^{1}H: 300 MHz; pyridine-d_5. For **1a**: ^{13}C: 125 MHz; ^{1}H: 500 MHz; $CDCl_3$. All assignments are based on 2D NMR (COSY, TOCSY, HMQC and HMBC).

[b] Signal patterns are unclear due to overlapping.

[c] Additional signals: $COCH_3$ δ 20.66–21.9 q; $COCH_3$: δ 170.75–168.80.

[d] Additional signals: δ 2.10, 2.07, 2.06, 2.05, 2.042, 2.041, 2.036, 2.02, 2.018, 2.00, 1.99 (each 3H, *s*, aliphatic acetoxy × 11).

carbon resonances attributed to the aglycone moiety supported the presence of cyclocanthogenin [(24*S*)-cycloartane-3β,6α,16β,24,25-pentaol] as the sapogenol moiety (Fadeev et al., 1987). The carbon resonances assigned to C-3 (δ 88.7), C-16 (δ 82.8) and C-24 (δ 90.7) were found +6–8 ppm down-field shifted in comparison to those of cyclocanthogenin, indicating the three sites of glycosidations on the sapogenol moiety. A HMBC experiment made clear the intermolecular connectivities (Fig. 1). The carbon resonances of C-3 and C-24 of the sapogenol moiety showed the long-range correlations to the anomeric protons of two xylose units (δ 4.90 and 4.80, respec-

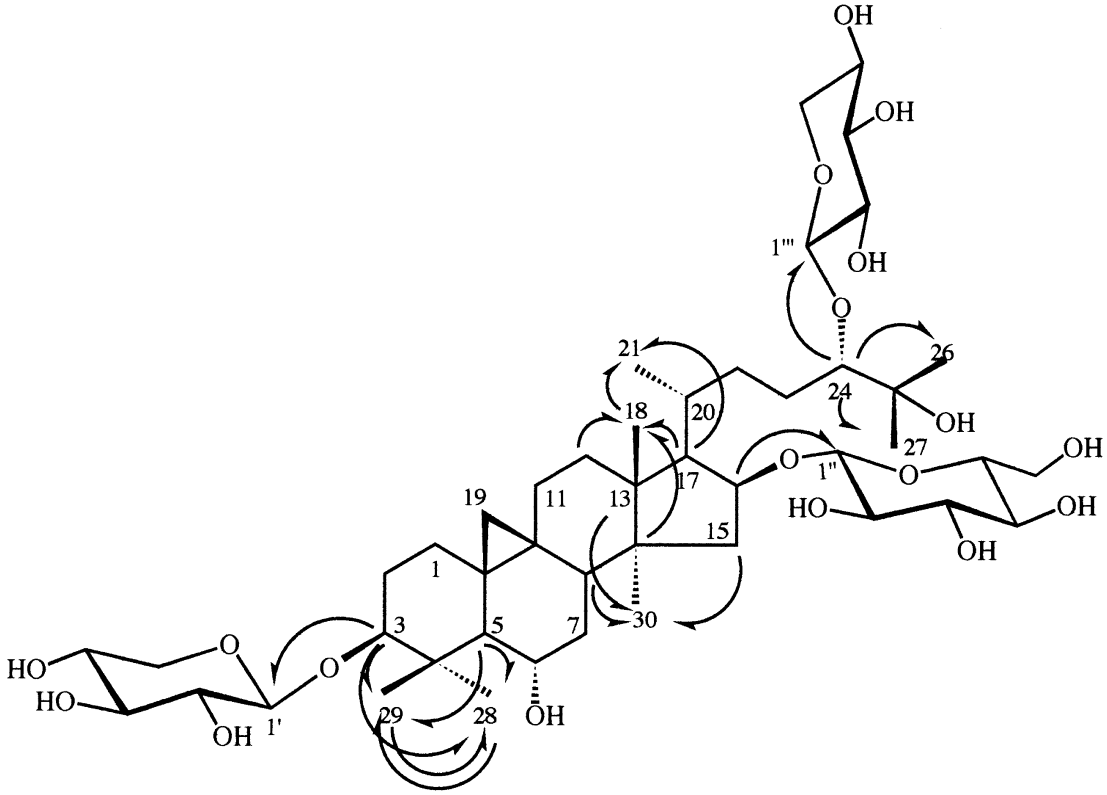

Fig. 1. Heteronuclear Multiple Bond Correlations (HMBC) for compound **1**. *Arrows point from carbon to proton.*

tively), while C-16 showed correlation to the anomeric proton of the glucose unit (δ 5.04), indicating the tridesmosidic structure of **1**. These observations were confirmed by ROESY of **1** showing ROE between the protons of H-3 of sapogenol and H-1′ (δ 4.90) of one of the two xylose units, H-24 of aglycone and H-1‴ (δ 5.04) of the second xylose unit and H-16 of the aglycone and the H-1″ (δ 4.80) of the glucose units. In order to prove the proposed structure, compound **1** was acetylated, giving an undecaacetate (**1a**). In the ^{1}H NMR spectrum of **1a**, the assignments were based on a COSY experiment Table 1. All sugar protons showed down-field shifts in comparison to those of **1**, indicating that each sugar unit was glycosylated at a different site of the sapogenol. The proton resonances assigned to H-3 (δ 3.14 dd, $J = 11.5$, 4.6 Hz), H-16 (δ 4.19) and H-24 (δ 3.37 dd, $J = 9.0$, 2.0 Hz) showed no down-field shifts in comparison to those of **1**, supporting the sites of glycosidations. Finally, the FAB mass spectrum of **1a** exhibited a quasimolecular ion peak $[M+Na]^+$ at m/z 1403. Additionally, the fragmentation peaks observed at m/z 259 and 331 corresponding to the triacetyl-xylose oxonium and tetraacetyl-glucose oxonium ions, respectively, verified their terminal positions. Consequently, the structure of **1** was established as 3β-(β-D-xylopyranosyl)oxy-16β-(β-D-glucopyranosyl)oxy-24-(β-D-xylopyranosyl)oxycycloartane-6α,25-diol, for which cephalotoside A is proposed as trivial name.

The ^{1}H NMR and ^{13}C NMR spectral data of the compounds **2**, **3** and **4** were identical with those of cyclocanthosides A (Fadeev et al., 1988), D (Isaev, Imomnazarov, Fadeev, & Kintya, 1992) and E (Isaev et al., 1992), respectively.

3. Experimental

3.1. General experimental procedures

For the general experimental procedures see Çalis et al. (1996).

3.2. Plant material

Astragalus cephalotes Banks and Sol. var *brevicalyx* Eig. was collected from Borgaç village, 12 km west of Hilvan, Şanliurfa, S.E. of Anatolia in June 1995. Voucher specimens (95-004) have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

3.3. Extraction and isolation

The air-dried powdered roots (375 g) were extracted with 80% aqueous EtOH under reflux. The solvent was removed by rotary evaporation yielding 72.5 g of extract (yield 19.3%). The water soluble part of the ethanolic extract (15 g) was subjected to VLC using reversed phase silica gel (Sepralyte 40 µm, 150 g), employing H_2O (500 ml), MeOH–H_2O mixtures with an increasing amount of MeOH (MeOH–H_2O: 1:9, 200 ml, 2:8, 100 ml, 3:7, 100 ml, 4:6, 200 ml) and MeOH (200 ml). Fractions eluted with MeOH (10.0 g) were rich in saponins. An aliquot of this fraction (6 g) was further subjected to an open column chromatography (silica gel 60; 200 g) using $CHCl_3$–MeOH–H_2O mixtures with increasing polarity (80:20:2, 1000 ml; 70:30:3, 500 ml and 60:40:4, 500 ml) yielding eleven fractions (frs. A–K); fr. A (1240 mg), fr. B (92 mg), fr. C (55 mg), fr. D (145 mg), fr. E (830 mg), fr. F (1466 mg), fr. G (542 mg), fr. H (636 mg), fr. I (511 mg), fr. J (445 mg) and fr. K (338 mg). Fr. I (511 mg) was subjected to MPLC (column dimensions: 352 × 18.5 mm), using LiChroprep C-18 as stationary phase and eluted with MeOH–H_2O mixtures (50–70% MeOH in H_2O; fraction volume 12–14 ml) and 40 fractions were collected. Frs. 29–32 (145 mg) were rich in compound **1** which was further purified on normal phase silica gel (30 g) CC using $CHCl_3$–MeOH–H_2O mixtures with increasing polarity (80:20:1; 80:20:2 and 70:30:3) to yield the compound **1** (68 mg). Fr. H (636 mg) was first subjected to normal phase silica gel (70 g) column chromatography using $CHCl_3$–MeOH–H_2O mixtures with increasing polarity (80:20:1; 80:20:2 and 70:25:2.5) yielding three main fractions (H1: 70 mg, H2: 250 mg and H3: 207 mg). Fr. H1 was subjected to MPLC (column dimensions: 352 × 18.5 mm), using LiChroprep C-18 as stationary phase and eluted with MeOH–H_2O mixtures (50–70% MeOH in H_2O; fraction volume 12–14 ml) affording compounds **3** (frs. 51–54; 39 mg) and **4** (frs. 69–74; 20 mg). Fr. C (55 mg) was subjected to normal phase silica gel (10 g) CC using $CHCl_3$–MeOH (9:1) and $CHCl_3$–MeOH–H_2O mixtures (90:10:0.5 and 90:10:1) yielding compound **2** (frs. 9–15; 36.5 mg).

3.4. Cephalotoside A (*1*)

$[\alpha]^{20}_D$ +20.9° (*c* 0.53, MeOH); IR $\upsilon^{KBr}{}_{max}$ cm^{-1} 3400 (OH), 2927 (CH), 1170 and 1044 (C–O–C); ^{1}H NMR and ^{13}C NMR see Table 1 (^{1}H: 300 MHz; ^{13}C: 75.5 MHz, pyridine-d_5. FABMS *m/z* 919 [M + H], 941 $[M+Na]^+$ (calc. for $C_{46}H_{78}O_{18}$).

3.5. Acetylation of *1*

Treatment of **1** (15 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temp. overnight followed by the usual workup yielded compound **1a**.

3.6. Cephalotoside A undecaacetate (*1a*)

IR υ^{KBr}_{max} cm^{-1} 3400 [C(25)–OH], 1758 (ester). ^{1}H NMR (see Table 1) ($CDCl_3$, 500 MHz); ^{13}C NMR (see Table 1) ($CDCl_3$, 125 MHz); FABMS *m/z* 1403 $[M+Na]^+$ (calc. for $C_{68}H_{100}O_{29}$), 331 [tetraacetyl-glucose oxonium]$^+$, 259 [triacetyl-xylose oxonium]$^+$.

3.7. Cyclocanthoside A (*2*)

IR υ^{KBr}_{max} cm^{-1} 3400 (OH), 1045 (C–O–C). ^{1}H- and ^{13}C NMR (^{1}H: 300 MHz; ^{13}C: 75.5 MHz, pyridine-d_5) data were identical to those reported in the literature (Fadeev et al., 1988).

3.8. Cyclocanthoside D (*3*)

$[\alpha]^{20}_D$ +34.3° (*c* 0.57, MeOH); IR υ^{KBr}_{max} cm^{-1} 3400 (OH), 1077 and 1045 (C–O–C). FABMS *m/z* 787 [M + H], 809 $[M+Na]^+$ (calc. for $C_{46}H_{78}O_{18}$). ^{1}H NMR and ^{13}C NMR (^{1}H: 300 MHz; ^{13}C: 75.5 MHz, pyridine-d_5) data were identical to those reported in the literature (Isaev et al., 1992).

3.9. Cyclocanthoside E (*4*)

$[\alpha]^{20}_D$ +29.2° (*c* 0.18, MeOH); IR υ^{KBr}_{max} cm^{-1} 3400 (OH), 1045 (C–O–C). ^{1}H- and ^{13}C NMR (^{1}H: 300 MHz; ^{13}C: 75.5 MHz, pyridine-d_5) data were identical to those reported in the literature (Isaev et al., 1992).

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