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Note

Complete ^{1}H and ^{13}C signal assignments of 5β-cardenolides isolated from *Acokanthera spectabilis* Hook F.

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ABSTRACT: Four cardiotonic glycosides were isolated from *Acokanthera spectabilis*. Their structures and conformational behaviour were investigated by extensive application of one- and two-dimensional ^{1}H and ^{13}CNMR spectroscopy. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; stereochemistry; conformation; steroids; cardenolides; gs-COSY; ROESY; gs-HMQC; gs-HMBC; HMQC-TOCSY; 2D-INEPT

INTRODUCTION

Acokanthera spectabilis Hook F. is a highly poisonous plant containing cardiac glycosides having a digitalis-like action.[1] It has been reported also that the isolated cardenolides have an anticarcinogenic effect.[2] Many cardiac glycosides have been isolated from leaves, stems, flowers and seeds of *A. spectabilis*, such as acovenoside A,[3–6] acospectoside A,[5,7,8] acovenoside C,[4] acobioside A,[3–5,8,9] spectabiline,[10] 14-*O*-acetylacovenoside C,[9] acovenoside B,[5,8] acobioside A acetate[9] and acopieroside II.[11] The last compound was shown to be more active than digitalin and digoxin as a cardiotonic.[11] The isolation of triterpenes from various parts of *A. specatbilis* has also been reported.[7,12]

It was therefore deemed of interest to investigate the unripe fruits of *Acokanthera spectabilis* grown locally in Egypt, which has not been investigated previously, to evaluate its cardiac glycoside constituents. We managed to isolate four cardenolides, **1**–**4**, and, according to our continuing efforts to establish structures of natural products by complete signal assignments based on advanced 1D and 2D NMR methods, we identified these derivatives as acovenoside A (**1**), acovenoside B (**2**), acobioside A (**3**) and acospectoside A (**4**) (Scheme 1). There are no ^{1}H and ^{13}C NMR data for compounds **1**, **2** and **4** and there is only one reference on NMR chemical shifts of **3**.[9] Two-dimensional NMR spectroscopy now permits complete ^{1}H and ^{13}C assignments, without any need for model compounds.

EXPERIMENTAL

Isolation

Unripe fruits of *A. spectabilis* were collected in September 1996 at the experimental station, Faculty of Pharmacy, Cairo University, and identified by Dr El-Gibaly at the plant Taxonomy Department, NRC, Cairo, Egypt. Voucher specimens are deposited at the Herbarium of the National Research Centre.

The unripe fruits (2.5 kg) were ground in a mixer and exhaustively extracted with 25 l of 80% methanol at room temperature. The aqueous–methanolic extract was concentrated under vacuum to 2 l at 40 °C and defatted with light petroleum (b.p. 60–80 °C), followed by extraction with chloroform, which provided 7 g of cardiac glycoside mixture. The mixture was dissolved in 300 ml of methanol, and, after cooling, a precipitate deposited. This was recrystallized from 300 ml of methanol to give acovenoside A (**1**) (320 mg) with m.p. 210–215 °C (lit.[7] 222–223 °C), UV λ_{max} 245 nm. The mother liquor (2 g) was column chromatographed on silica gel 60, using chloroform as the eluent, with a gradual

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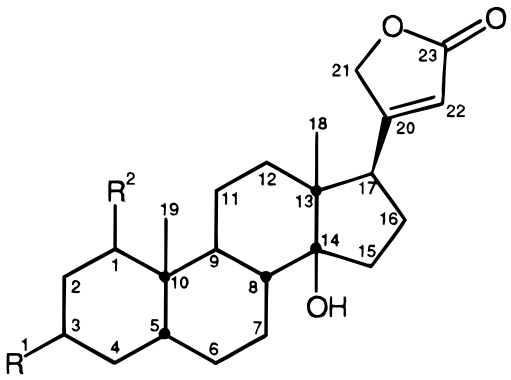
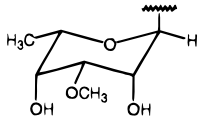


1 – 4

	1	2	3	4
R^1	OAco	OAco	Glc(1→4)-Aco-O	Glc(1→4)-Aco-O
R^2	OH	OAc	OH	OAc

[a] Ac = acetyl, Glc = β-glucosyl; Aco = α-acovenosyl: 

Scheme 1. Structures of the cardenolides 1–4.

increase in the proportion of methanol during the course of fractionation.

Chloroform-methanol (9.5:0.5): the factions were combined and recrystallized from methanol–diethyl ether to afford 200 mg of acovenoside B (**2**) with m.p. 275 °C (lit.[5] 230–232 °C), UV λ_{max} 225 nm.

Chloroform–methanol (8.5:1·5): the fractions were combined and recrystallized from methanol–ethyl acetate to give to 10 mg of acospectoside A (**4**) with m.p. 270–275 °C, UV λ_{max} 213 nm.

Chloroform–methanol (8:2): fractions 6–31 were combined and recrystallized several times from methanol–ethyl acetate to give 35 mg of acobioside A (**3**) with m.p. 179–181 °C (lit.[13] 248–258 °C).

Spectroscopy

NMR spectra were recorded in various solvents as indicated in Table 1 at room temperature, using a Bruker Avance DRX-500 spectrometer. Chemical shifts are given on the δ-scale and were referenced to the solvent. In the 1D measurements (^{1}H, ^{13}C, DEPT), 64K data points were used for the FID. Digital resolutions were 0.1 Hz per point for ^{1}H and 0.5 Hz per point for ^{13}C.

The pulse programs of the COSY, TOCSY, ROESY, HMQC, HMQC-TOCSY and HMBC experiments were taken from the Bruker software library and the parameters were as the same as described before.[14] In the ^{13}C coupled HMQC experiment, the accuracy of ^1J(C,H) was better then 1.4 Hz. The 1D- and 2D-INEPT experiments[15,16] were optimized for $J(^{13}C,^1H) = 7$ Hz. The digital resolution was 0.3 Hz.

RESULTS AND DISCUSSION

Signal and structural assignments

The signal assignments are based on an extensive 1D and 2D NMR investigation, utilizing the advantage of gradient selection and linear prediction (COSY, TOCSY, ROESY, HMQC, HMBC and semiselective 1D- and 2D-INEPT). The general data evaluation procedure was analogous to that described previously.[14] Therefore, we refrain from a detailed description and we restrict the discussion here to some peculiarities. The ^{1}H and ^{13}C chemical shifts of compounds **1–4** are collected in Table 1. Characteristic ^{1}H–^{1}H proximities (ROESY) and ^{13}C–^{1}H long-range correlations of **3** are summarized in Table 2.

In a few cases, the two signals of the C-20 and C-23 atoms in the lactone ring could not be differentiated by HMBC, owing to the limited resolution in F_1 (^{13}C). This problem was solved by utilizing the higher resolution in a semiselective ^{13}C-detected INEPT experiment (0.3 Hz), as depicted in Fig. 1. It can be seen that only C-20 responds when H-17 is irradiated selectively.

Despite severe overlap of ^{1}H signals in the range $\delta = 0.8$–2.6, a discrimination of diastereotopic protons within methylene groups was possible by inspecting TOCSY, ROESY and, furthermore, HMQC cross peaks

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Table 1. 1H and ^{13}C NMR chemical shifts of **1–4** (solvent as indicated)

	1 $CDCl_3$		**2** $CDCl_3$		**3** CD_3OD		**4** C_5D_5N	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1 α	3.70	72.5	4.89	74.2	3.79	74.0	5.19	74.5
2 α	1.88	31.8	1.79	29.9	1.98	32.7	1.95	30.0
β	1.95		2.00		2.09		2.25	
3 α	4.23	72.3	4.04	68.7	4.26	73.9	4.15	69.6
4 α	1.82	28.3	1.81	27.8	2.02	29.4	1.86	28.2
β	1.62		1.61		1.67		1.55	
5 β	1.87	30.4	2.03	31.1	2.01	32.0	2.07	31.5
6 α	1.37	26.0	1.35	25.8	1.45	27.4	1.25	26.2
β	1.83		1.82		1.95		1.72	
7 α	1.74	21.0	1.73	20.6	1.91	22.1	2.13	21.0
β	1.25		1.32		1.35		1.43	
8 β	1.63	41.9	1.63	41.6	1.77	42.7	1.84	41.5
9 α	1.47	37.6	1.55	35.9	1.69	38.6	1.67	36.7
10	—	40.3	—	38.7	—	41.3	—	38.9
11 α	1.34	21.2	1.42	21.6	1.40	22.3	1.37	21.8
β	1.34		1.29		1.40		1.23	
12 α	1.32	39.9	1.36	39.7	1.55	40.7	1.36	39.5
β	1.52		1.54		1.57		1.48	
13	—	49.4	—	49.5	—	52.1	—	49.9
14	—	85.4	—	85.3	—	86.2	—	84.4
15 α	2.08	33.2	2.09	33.1	2.27	33.3	2.15	32.9
β	1.72		1.73		1.81		1.97	
16 α	2.16	26.9	2.17	26.9	2.24	28.1	2.15	27.2
β	1.88		1.89		1.95		2.00	
17 α	2.77	50.8	2.77	50.8	2.90	52.1	2.83	51.2
18	0.88	15.8	0.88	15.8	0.97	16.4	1.04	16.1
19	1.09	18.8	0.97	18.4	1.18	19.2	0.99	18.4
20	—	174.4	—	174.5	—	178.3	—	176.0
21 a	4.85	73.4	4.80	73.5	4.99	75.3	5.07	73.7
b	4.98		4.97		5.11		5.34	
22	5.88	117.9	5.88	117.8	5.97	117.8	6.17	117.6
23	—	174.2	—	174.4	—	177.2	—	174.7
1-O_2CCH_3			—	170.7			—	170.9
1-O_2CCH_3			2.00	21.4			2.21	21.4
1′	5.00	97.7	4.96	97.4	4.97	99.8	5.29	99.1
2′	3.87	68.4	3.86	68.6	3.81	70.3	4.19	69.9
3′	3.35	75.0	3.34	75.4	3.55	76.9	3.78	76.4
4′	3.85	69.8	3.80	70.0	4.26	76.5	4.51	76.4
5′	3.95	66.7	3.81	66.2	4.10	68.7	4.10	67.4
6′	1.33	16.6	1.28	16.5	1.42	17.1	1.73	17.2
3′-OCH_3	3.48	55.7	3.46	55.5	3.57	56.7	3.71	55.9
1″					4.43	105.0	5.00	105.1
2″					3.34	75.3	3.99	75.3
3″					3.44	77.7	4.25	78.2
4″					3.44	71.2	4.20	71.4
5″					3.35	78.1	3.99	78.5
6″a					3.78	62.5	4.37	62.7
b					3.94		4.57	

exhibiting characteristic patterns for axially or equatorially located hydrogens.[17,18] The H-1α and H-3α signals can be observed directly in the 1D ^1H NMR spectrum. The vicinal coupling constants are *ca.* 1.5 Hz, proving their equatorial position, i.e. the two oxygen functionalities at C-1 and C-3 are in a *cis*-diaxial position.

The ROE responses proved to be very informative, not only for the signal assignment, but also for the recognition of stereochemistry. For example, the α-position of H-17 is proved by the proximity of H-17 and H-12 protons in the case of **2**. Free rotation of the lactone ring is obvious from cross peaks H-18/H-21 and H-18/H-22 (Table 2 and Fig. 2). Moreover, the ring

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Table 2. Characteristic ^{1}H-^{1}H proximities (ROESY) and ^{13}C-^{1}H long-range correlations (HMBC, optimized to 7 Hz couplings) of **3**

^{1}H	ROESY ^{1}H	HMBC ^{13}C
1 α	2α, 2β, 19, 5′	3, 5, 10, 19
2 α	1α, 2β, 3α	3
β	1α, 3α, 5′	5
3 α	2α, 2β, 4α, 4β, 1′, 2′, 5′	1, 5, 1′
4 α	3α, 4β	
β	3α, 4α, 1′	
5 β	19	6, 7, 9, 10
6 α		7, 8
β	19	7
7 α	7β	5
β	7α	6
8 β	11β, 18, 19	7, 9, 14, 15
9 α	11α, 15α, 19	8, 10, 12
11 α	9α	8, 12
β	8β, 18, 19	10, 12
12 α	15α, 17α	11, 18
β	18, 17α	9, 11, 13, 18
15 α	9α, 12α, 15β	13, 16
β	15α	13, 16, 17
16 α	16β	13, 15, 17, 20
β	16α, 21a	15, 20
17 α	12α, 12β, 15α, 16α, 18, 21b, 22	12, 13, 14, 16, 20, 22
18	8β, 11β, 12β, 17α, 21a, 21b, 22	12, 13, 14, 17
19	1α, 5β, 6β, 8β, 11β,	1, 5, 9, 10
21 a	16β, 17α, 18, 21b	20, 22, 23
b	17α, 18, 21a	20, 22, 23
22	16β, 17α, 18	17, 20, 21, 23
1′	4β, 2′, 3α	3, 2′, 3′, 5′
2′	3α, 1′, 4′, 5′	3′, 4′
3′	1′, 4′, 3′-O-CH_3, 5″	4′
4′	3′, 5′, 6′, 3′-O-CH_3, 1″	2′, 3′, 1″
5′	1α, 2β, 3α, 2′, 3′, 4′, 6′	4′
6′	1α, 4′, 5′, 1″	4′, 5′
3′-OCH_3	3′, 4′	3′
1″	4′, 6′, 3″, 5″	4′
2″		1″, 3″, 4″
3″	1″	2″, 4″
4″		2″, 3″
5″	1″	1″, 3″, 4″
6″ a	6″b	5″
b	6″a	4″, 5″

junction A/B can be discerned as shown in Fig. 2: only in a *cis*-configuration (5β) will cross peaks be observed between H-19 and H-5 (one of the few protons attached to a methine carbon). The ROESY cross peaks H-12α/H-15α, H-12α/H-16α, H-12α/H-17*a* and H-9α/H-15α gave evidence for the C/D *cis* ring junction.

Further evidence for the type of the ring junctions was provided by the ^{13}C chemical shifts. It is known that 5α- and 5β-cardenolides exhibit characteristically different chemical shifts. In the *cis*-decalin arrangement, the δ-values of C-5, C-7 and C-9 are much lower than in analogous *trans* derivatives.[9,19] This is due to the well known diamagnetic γ-effect.[20] The characteristic ^{13}C chemical shifts are indicated in Fig. 2.

The structures of the sugar moieties in the monogylcosides **1** and **2** and the diglycosides **3** and **4** were determined, using the same methodology as described for the aglycones. As evidenced by the HMBC cross peaks, all of them are attached to C-3 of the respective

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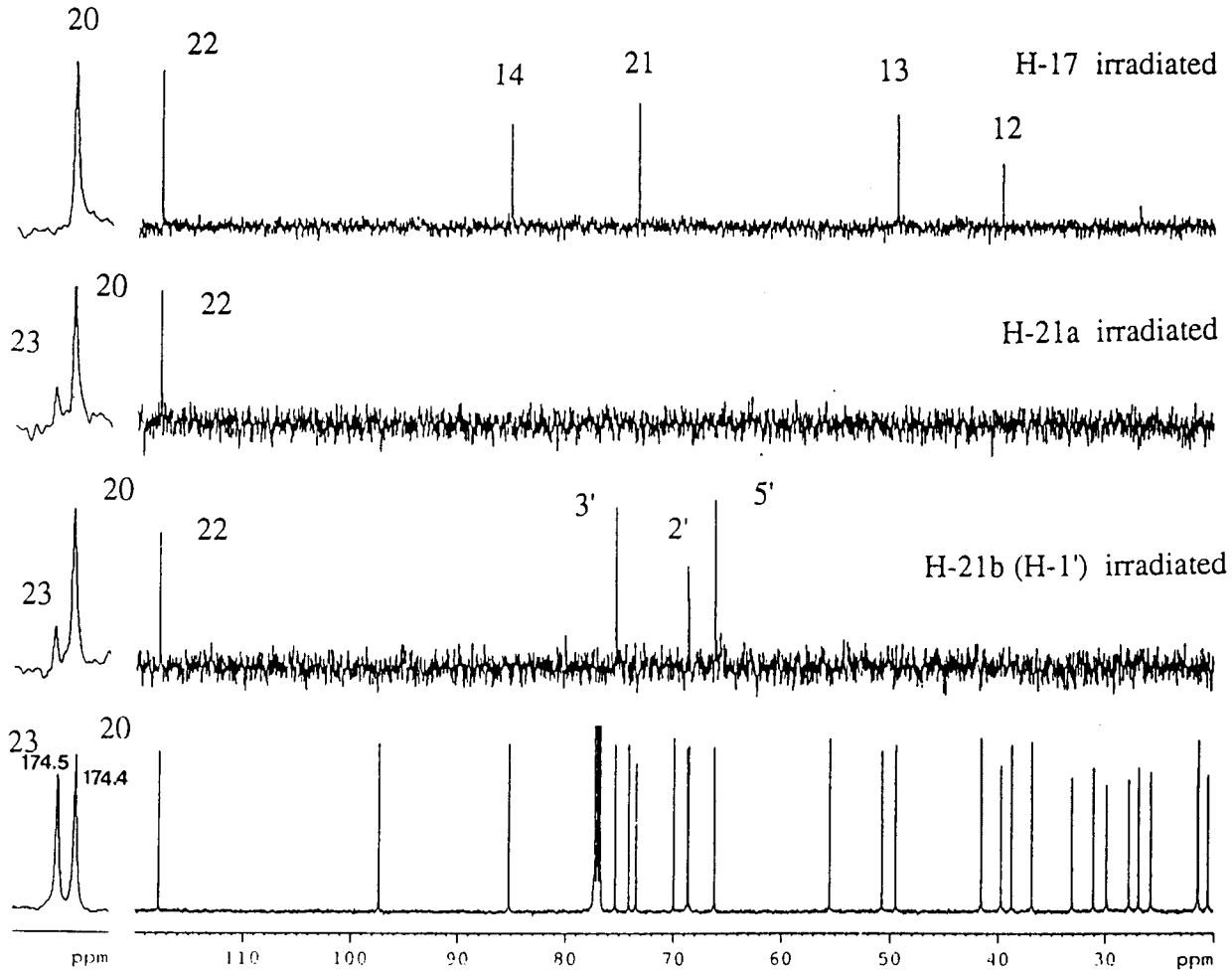

Figure 1. Semiselective ^{13}C-detected INEPT experiment on **2**.

aglycones. The J(H-1″,H-2″) ≈ 8 Hz couplings in **3** and **4** corroborate the β-configuration of the glucose moiety. Owing to the axial OH group at C-2′ in the acovenose moiety, the magnitude of ^{3}J(H-1′,H-2′) < 1 Hz coupling is not indicative of the α- or β-configuration. To overcome this uncertainty, the ^{1}J(C-1′,H-1′) couplings were utilized, which were detected by the ^{13}C-coupled HMQC experiments. It is well known that, owing to the lone-pair effect, an antiperiplanar arrangement of the lone pair on the ring oxygen atom with respect to the anomeric C—H bond (β-configuration), the characteristic value is about 160 Hz, i.e. 10 Hz less than in the α-configuration.[21] For the acovenose moiety in compounds **1–4**, the magnitude of ^{1}J(C-1′,H-1′) was measured between 169 and 171 Hz, whereas the corresponding values for the β-D-glucose in **3** and **4** were 159 and 161 Hz, respectively. These data unequivocally prove the α-configuration of the acovenose moiety.

Some conclusions concerning a preferred conformation of the sugars can be drawn from the ROESY spectra. These conformations, as depicted in Fig. 3, are in accordance with the well known *exo*-anomeric effect[22,23] and are further confirmed by the determination of the three-bond coupling of 3.5 Hz between C-3 and the anomeric proton H-1′ in **2**, a value that is typical of a *gauche* orientation of the atoms involved.[24] In **3** and **4**, the preferred conformation around the glycosidic C-4′—O—C-1″ bond is also in agreement with the *exo*-anomeric effect, i.e. along with C-4′—O—C-1″—C-2″ bonds the C-4′—O and C-1″—C-2″ bonds are antiperiplanar. It is noteworthy that in **2** and **4** the 1-OAc group is located above the A ring, allowing spatial proximities between CH_3CO/H-3′ and CH_3CO/3′-OCH_3 protons, respectively.

In conclusion, we were able to obtain a complete NMR data set of four cardenolides (**1–4**), allowing an unequivocal structural assignment of all compounds.

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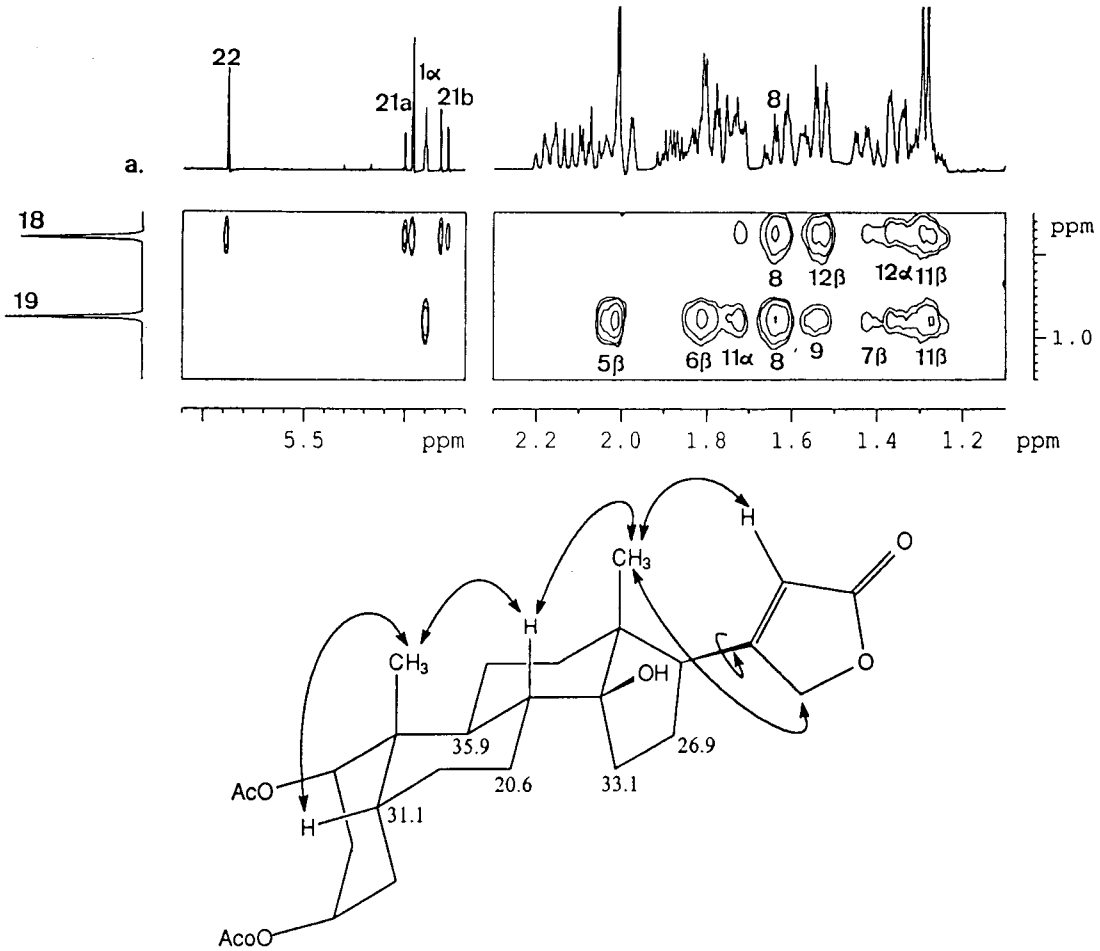

Figure 2. Section of the ROESY spectrum of **2**. Arrows on the formula indicate spatial proximities. Numbers show the characteristic ^{13}C chemical shifts.

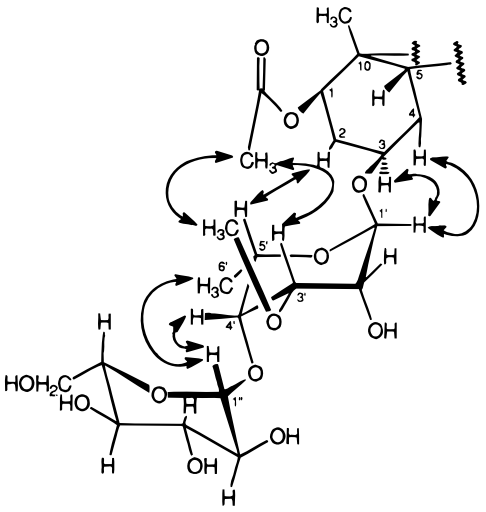

Figure 3. Relative orientation of glycone and carbohydrate moieties in **4**. Arrows indicate spatial proximities obtained from ROESY.

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