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Synthesis of acetates of gomphogenin and gomphoside and evaluation of structure–activity relationships

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Summary — Acetates of gomphogenin and gomphoside have been synthesized and their structures established by NMR measurements, optical rotation, mass and infrared spectrometry. The kinetic and equilibrium parameters of the inhibitory interaction of the compounds with guinea-pig heart muscle Na^+/K^+-ATPase are presented and discussed. Acetylation of the 2α-OH group in gomphogenin or its 3β-acetate increases the binding affinity by 15- and 24-fold, respectively, whereas 3β-*O*-acetylation of gomphogenin and its 2α-acetate increases the affinity only two- and threefold. Acetylation of 4'-OH or 3',4'-OH of gomphoside, instead, reduces the high affinity of gomphoside towards Na^+/K^+-ATPase.

gomphoside / gomphogenin / 1,2-diol monoacetylation / Na^+/K^+-ATPase inhibition / structure–potency relationship

Introduction

On the basis of a large body of data, we have concluded that the potent interaction of digitalis compounds with the receptor enzyme Na^+/K^+-ATPase requires a snug fit with the digitalis receptor site [1]. The introduction of various substituents, even of a hydroxy group, at almost any position on the steroid nucleus, results in a drop of potency [1]. The most remarkable exception is the linkage of C3β-OH with any carbohydrate. Thus, it came as a surprise that the biological potency of gomphoside **1**, a C5α-H cardiac glycoside from *Asclepias fruticosa* RBr [2], in which a 4,6-dideoxyhexosulose is bilinked to the aglycone through its 2α- and 3β-hydroxy groups [3, 4], is near the maximum that seems achievable for cardiac glycosides [5–7]. In our preceding analysis of this phenomenon [8], we arrived at the conclusion that the major part of the enormous potency gain, which gomphogenin **4** undergoes through linkage of the glycosidic component to C2α-OH and C3β-OH, results from the elimination of C2α-OH as an unpaired hydrogen-bonding candidate known to effect polar repulsion [9]. This interpretation was derived from our finding that even acetylation of C2α-OH only effects a great increase in potency apparently due to formation of a derivative with a carbonyl oxygen able to accept hydrogen bonds [8]. With the aim of confirming and broadening this insight, we report here the synthesis and structural elucidation of the acetates of gomphogenin and gomphoside and their inhibitory interaction with Na^+/K^+-ATPase from guinea-pig heart muscle.

Chemistry

The two secondary equatorial hydroxy groups of gomphogenin **4** at C2α and C3β form a *trans*-1,2-diol group which is easily acetylated with acetic anhydride/pyridine at room temperature to the 2,3-diacetate **7**, as confirmed by thin-layer chromatography (TLC), infrared (IR) and mass spectrometry.

For preparing the monoacetates, the orthoester method [10] was used, which avoids diacetylation. Gomphogenin **4** reacts smoothly with triethyl orthoacetate under acid catalysis with formation of the two diastereomeric 2,3-orthoacetates. Their partial acid hydrolysis gives the monoacetates **5** and **6** in about equal amounts. The identity of **5** and **6** could be

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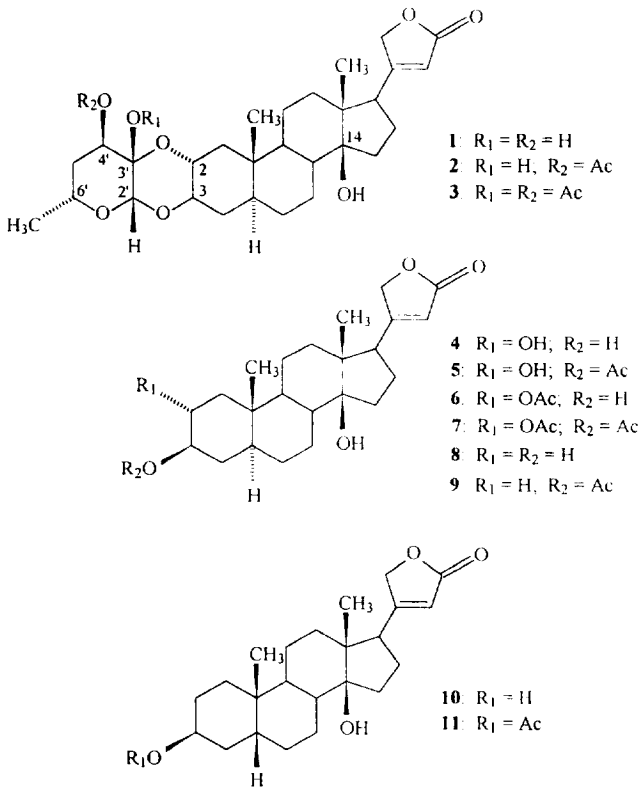
Abbreviation: Na^+/K^+-ATPase: Na^+/K^+-transporting adenosine triphosphate phosphohydrolase (EC 3.6.1.37).



established here for the first time beyond doubt by multiple pulse NMR measurements.

The ^{1}H-NMR spectrum of the less polar monoacetate **5** shows multiplet signals at δ 3.78 for the >C*H*-OH proton and at δ 4.58 for the >C*H*-OAc proton. The decision of which is C2β-H and which is C3α-H is made possible by the two-dimensional H,H-COSY spectrum. It shows coupling of the >C*H*-OH proton with the diastereotopic CH_2 protons at δ 1.03/2.08 and the hydroxy proton at δ 2.16 (*cf* fig 1). Of these, the β-H at δ 2.08 couples only with the protons at δ 3.78 and 1.03. This indicates that the spin system ends at the CH_2 group at δ 1.03/2.08, which is consequently identified with C1-H_2. This in turn means that the >C*H*OH proton at δ 3.78 is C2β-H. Furthermore, the >C*H*OAc proton at δ 4.58 shows coupling to the CH_2 protons at δ 1.38/1.77. Regarding the well-separated β-H at δ 1.77, there is a second coupling (besides that to its geminal proton at δ 1.38) to a proton at δ 1.26. Thus, the spin system is not terminated with the CH_2-group at δ 1.38/1.77. The resulting assignment is: δ 1.26 (C5α-H), δ 1.38/1.77 (C4-H_2), δ 4.58 (C3α-H). Taken together, the signals establish that the less polar isomer is the 3β-acetate **5** and, consequently, the polar isomer is the 2α-acetate **6**.

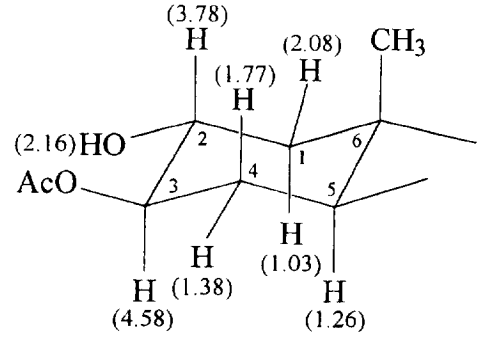


Fig 1. Chemical shifts (δ-values) of the protons in ring A of gomphogenin 3-acetate **5**.

The positions of the acetoxy groups in **5** and **6** have been further confirmed by the different molecular rotation increments for the acetylation of C2α- and C3β-OH. In accordance with reference [11] the $\Delta[M]_D$ value for C2α-OH acetylation (**4** → **6**) is with –135° greater than for C3β-OH acetylation (**4** → **5**; –83°) indicating that **6** is the 2α-acetate and **5** the 3β-acetate.

The monoacetates **5** and **6** show only small differences in melting point, mass spectra and TLC mobility. Their IR spectra differ slightly in the range of C-O-C vibrations of the acetate residues (**5**: 1270 cm^{-1}; **6**: 1245 cm^{-1}; **7**: 1220, 1270 cm^{-1}).

The hydroxy groups of the *cis*-1,2 diol group of gomphoside **1** at C3' (tertiary, equatorial) and C4' (secondary axial) show sufficient difference in steric hindrance to allow selective monoacetylation with excess acetic anhydride/pyridine during 1 h at room temperature giving the 4'-monoacetate **2** in good yield.

Under harsher conditions (6 d at 40°C) the tertiary 3'-OH group is also acetylated, forming the 3',4'-diacetate **3**. The other tertiary OH group in the molecule, 14β-OH at the steroid skeleton, is not acetylated under these conditions.

The gomphoside acetates **2** and **3** show characteristic differences in their mass spectra: **2** (with 3'-OH) easily splits off the sugar part of the molecule (base peak: 128, sugar fragment), whereas acetylation of 3'-OH in **3** stabilizes the sugar part and therefore the base peak changes to M^+-AcOH (542, glycosidic fragment).

Biological data

In table I, the kinetic and equilibrium parameters of the inhibitory interaction of the compounds described here with guinea-pig heart muscle Na^+/K^+-ATPase are listed and compared to those of representatives of the A/B-*cis* (digitoxigenin **10**) and A/B-*trans* cardenolide

Table I. Parameters of the inhibitory interaction of A/B-*trans*-cardenolide derivatives (**1–9**) with Na^+/K^+-ATPase from guinea-pig heart muscle.

Inhibitor	K'_D (μM)	± *SD*	k_{on} ($\mu M^{-1} \cdot min^{-1}$)	± *SD*	k_{off} (min^{-1})	± *SD*
Digitoxigenin **10** (3β,14-dihydroxy-5β,14β-card-20(22)-enolide)	1.93	0.21	0.56	0.12	1.1	0.2
Digitoxigenin 3-acetate **11**	1.40	0.05	0.45	0.11	0.63	0.16
Uzarigenin **8** (3β,14-dihydroxy-5α,14β-card-20(22)-enolide)	7.4	0.3	0.075	0.015	0.56	0.12
Uzarigenin 3-acetate **9**	24	1	0.020	0.005	0.47	0.12
Gomphogenin **4** (2α-hydroxyuzarigenin)	26	2	0.031	0.020	0.82	0.55
Gomphogenin 3-acetate **5**	13.1	0.6	0.037	0.007	0.49	0.09
Gomphogenin 2-acetate **6**	1.72	0.05	0.86	0.39	1.48	0.67
Gomphogenin 2,3-diacetate **7**	0.55	0.02	1.68	0.48	0.92	0.27
Gomphoside **1** (2α,3β-di-*O*-(3'β,4'β-dihydroxy-6'α-methyl-tetrahydro-2*H*-pyran-3'α,2'α-diyl)gomphogenin)	0.184	0.017	2.4	1.1	0.45	0.21
Gomphoside 4'-acetate **2**	0.37	0.02	2.5	1.2	0.92	0.45
Gomphoside 3',4'-diacetate **3**	2.4	0.1	0.22	0.08	0.52	0.19

Measurements were made under conditions of optimal activity at 37°C, pH 7.4, as described in reference [12]. Given are the equilibrium constants, K'_D, and the rate constants for association, k_{on}, and dissociation, k_{off}, with standard deviations (SD) taken from joint calculations of 3–5 separate experiments. Digitoxigenin **10** and its 3-acetate **11** (A/B-*cis*) have been included for comparison.

series (uzarigenin **8**). As demonstrated, the transition from A/B-*cis*- to A/B-*trans*-junction (**10** → **8**) reduces Na^+/K^+-ATPase affinity fourfold essentially through decrease of the association rate constant, k_{on}. As expected from the responsiveness of other cardenolides [1], introduction of an additional OH group into the steroid structure (**8** → **4**) further reduces the binding affinity by both decreasing k_{on} and increasing the dissociation rate constant, k_{off}.

Acetylation of uzarigenin at 3β-OH (**8** → **9**) reduces the binding affinity about threefold through reduction of k_{on}. Contrariwise, 3β-O-acetylation of both gomphogenin (**4** → **5**) and gomphogenin 2-acetate (**6** → **7**) increases affinity, though only two- and threefold, respectively, by increasing k_{on} and decreasing k_{off}. Acetylation of the 2α-OH group in gomphogenin (**4** → **6**) or gomphogenin 3-acetate (**5** → **7**), however, increases binding affinity by 15- and 24-fold, respectively, in both cases by increasing k_{on}.

Although diacetylation of gomphogenin (**4** → **7**) increases binding affinity near 50-fold by an increase in k_{on}, this is still surpassed by glycosidic bonding of both OH groups (**4** → **1**) which increases affinity 140-fold almost entirely by increasing k_{on}. This additional increase appears to be brought about essentially by introduction of two OH groups. Acetylation of the 3'-OH group in gomphoside 4'-acetate (**2** → **3**) greatly diminishes the above affinity increase (**4** → **1**), whereas acetylation of the 4'-OH group (**1** → **2**) contributes only a little to the overall effect (**1** → **3**).

Discussion

The formation of the orthoacetates from the 2,3-diol group of gomphogenin **4** is remarkable, as the comparable *trans*-1,2 diol group (3'-OH, 4'-OH; both equatorial) of the rhamnosyl residue of proscillaridin A does not react under these conditions. However, the *cis*-1,2 diol group of the rhamnosyl residue (2'-OH, axial; 3'-OH, equatorial) reacts smoothly [13], reflecting differences in the reactivity of *cis*- and *trans*-1,2-diols in orthoacetate formation which proceeds strongly in favour of the former.

On splitting the orthoacetates of diols with different steric hindrance of the involved OH groups, the acetate of the more hindered OH group is predominantly or exclusively formed as shown in the literature

[10, 13]. The formation of the 2α- and 3β-monoacetates **6** and **5** of gomphogenin **4** in about equal amounts appears to reflect the absence of greater differences in steric hindrance between 2α- and 3β-OH.

Investigation of the inhibition of the Na^+/K^+-ATPase from guinea-pig heart by the synthesized and related compounds (table I) reveals (among other facts) that, as already observed with other cardenolides [1], the addition of OH groups to the steroid nucleus invariably leads to a decrease in inhibitory effectivity. The fact that this is more than compensated for by acetylation especially of the 2α-OH group, demonstrates that the adverse effect of its introduction does not originate from steric hindrance, but from the presence of an unpaired hydrogen bond candidate, which evokes strong repulsion forces [9]. Moreover, this invites other derivatizations at C2α-OH as a possible way to novel highly potent derivatives.

The over 100-fold increase in effectivity by attaching a carbohydrate-like ring to the 2α-OH and the 3β-OH groups in **4** makes gomphoside one of the pharmacologically most potent cardenolide glycosides [14]. As deduced above, this increase in effectivity is mainly related to the presence of two OH-groups, of which especially the 3'-OH group seems to participate in hydrogen bonding. Thus, the 3'-OH group in gomphoside seems to take the place of the 4'-OH group of the proximate carbohydrate residue in other cardenolide glycosides, which has been shown to provide the increase in binding affinity effected by glycosylation [1].

Summarizing, the detailed examination of the inhibitory activity of the synthesized derivatives shows that the kinetic parameters of interaction can provide additional information on the molecular mechanism of the cardenolide-Na^+/K^+-ATPase interaction. Opposing earlier views [15], the major changes in affinity are mainly effected, at least in this series of compounds, by changes in the association rate constant, which varies about 150-fold, whereas the dissociation rate constant varies, if at all, about threefold at maximum. This adds another facet to the overall picture of structure–activity relationships of steroidal Na^+/K^+-ATPase inhibitors as reviewed previously [1].

Experimental protocols

General

Melting points were determined with a Boëtius micro hot-stage apparatus (VEB Analytik Dresden) and are corrected. Reactions were monitored by TLC using TLC aluminium sheets silica gel 60 F_{254} (E Merck, Darmstadt); plates were developed twice in *n*-butyl acetate (front: 10 cm) and spots were visualized under UV light after spraying with 15% phosphoric acid in methanol, followed by heating at 140°C. Column chromatography was performed on silica gel 60 (70-230 mesh; E Merck, Darmstadt). Optical rotations were measured in chloroform with a Zeiss Polamat A polarimeter. ^{1}H-NMR and two-dimensional H,H-COSY spectra were recorded in $CDCl_3$ at 600 MHz with a Bruker AMX-600 spectrometer using tetramethylsilane as an internal standard. IR spectra were measured in KBr tablet with an ATI Mattson type Genesis FTIR spectrophotometer (compounds **2** and **4**) or a Nicolet FTIR 205 instrument (**3** and **5–7**). Electron ionization mass spectra (EI-MS) were obtained with a Varian MAT model MAT 7 (**2, 3** and **5–7**) or a VG Analytical AutoSpecQ (**4** and **7**) mass spectrometer. Fast atom bombardment mass spectra (FAB-MS) were obtained with a glycerol matrix on a Varian MAT CH5 (**2**) or a MAT 212 mass spectrometer (**4**).

Syntheses

2α,3β,14-Trihydroxy-5α,14β-card-20(22)enolide (gomphogenin) 4
To a solution of gomphoside **1** (200 mg) in methanol (30 ml), were added hydrogen peroxide (30%, v/v, 30 ml) and 1 N HCl (1.5 ml) [16]. The mixture was stirred at room temperature for 8 d, diluted with water (100 ml) and extracted with chloroform/ethyl acetate (1:1; 3 x 100 ml). The organic layers were combined, washed with 10% $K_2S_2O_5$ solution and water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue obtained (185 mg) was chromatographed on silica gel (10 g). Elution with chloroform/ethyl acetate (1:1) provided **4** (124 mg, 82%) which crystallized from methanol/ether as colorless leaflets; mp 231–233°C (lit [17]: 234–236°C), $[\alpha]_D^{20}$ +18° (*c* = 0.65), $[M]_D$ +70°, identical with an authentic specimen in TLC, mixed melting point and comparison of the IR spectrum. TLC: R_f = 0.08. IR: ν_{max} 3507, 3382 (OH), 1794, 1727 (butenolide C=O), 1626 (C=C), 1076, 1039, 1029 cm^{-1} (C-O). EI (70 eV) MS: *m/z* (relative intensity) 390 (M^+; 10), 372 ($M^+ - H_2O$; 97), 354 ($M^+ - 2H_2O$; 25), 201 ($C_{15}H_{21}$; 88), 111 (butenolide ring + C17 + C16; 99). FAB-MS: *m/z* 391.2475 (MH^+). Calcd for $C_{23}H_{35}O_5$: 391.2484.

Gomphogenin 2,3-diacetate 7
Acetylation of **4** (50 mg) at room temperature (overnight) with acetic anhydride/pyridine (4 ml; 1:1, v/v) and usual work-up gave gomphogenin 2,3-diacetate **7** (38 mg, 62%) as woolly needles (from acetone/ether), mp 282–284°C (lit [17]: 272–278°C), $[\alpha]_D^{20}$ –21° (*c* = 0.60), $[M]_D$ – 100°. TLC: R_f = 0.55. IR: ν_{max} 3480 (OH), 1770, 1730, 1705 (butenolide and acetyl C=O), 1605 (C=C), 1250, 1220 (acetoxy C-O-C), 1170, 1020 cm^{-1} (C-O). EI (80 eV) MS: *m/z* (relative intensity) 474 (M^+; 4.6), 456 ($M^+ - H_2O$; 1.6), 432 ($M^+ - CH_2=C=O$; 2.2), 414 ($M^+ - CH_3COOH$; 31), 372 (414 – $CH_2=C=O$; 100), 354 ($M^+ - 2\ CH_3COOH$; 20), 201 ($C_{15}H_{21}$; 82), 111 ($C_6H_7O_2$, butenolide ring + C17 + C16; 31).

Gomphogenin 3-acetate 5; gomphogenin 2-acetate 6
A solution prepared from gomphogenin **4** (164 mg), tetrahydrofuran (20 ml), triethyl orthoacetate (1 ml) and *p*-toluenesulfonic acid (20 mg) was stirred at room temperature for 60 min. The solvent was removed under reduced pressure and the residue containing the two diastereomeric 2,3-orthoacetates (R_f = 0.39 and 0.45) was dissolved in chloroform (50 ml), and shaken with 0.1 N sulfuric acid (10 ml) for 10 min. The organic layer was separated, washed with water (2 x 25 ml), dried (Na_2SO_4) and evaporated to dryness. The oily product (190 mg) was chromatographed on silica gel (10 g) using chloroform/

ethyl acetate solutions of gradually increasing ethyl acetate content to develop the column. Fractions of 25 ml each were collected with the following eluents: 1–4, $CHCl_3$; 5–8, 10%; 9–20, 15%; 21–32, 20%; 33–44, 30% ethyl acetate in $CHCl_3$. Fractions 6–20 (109 mg) contained the monoacetates **5** and **6**; fractions 23–40 provided reformed gomphogenin (73 mg), which was treated in the above manner to give a further monoacetate fraction (52 mg) and gomphogenin (23 mg). Repeated chromatography of the combined monoacetate fractions on silica gel (10 g) as above gave first gomphogenin 3-acetate **5** (60 mg; 38%) which crystallized from acetone/ether as woolly needles; mp 250–253°C, $[\alpha]_D^{20}$ – 3° (c = 1.25), $[M]_D$ –13°. TLC: R_f = 0.29. IR: ν_{max} 3495, 3440 (OH), 1730, 1710 (butenolide and acetyl C=O), 1615 (C=C), 1270 (acetoxy C-O-C), 1185, 1050, 1025 cm^{-1} (C-O). EI (80 eV) MS: *m/z* (relative intensity) 432 (M^+; 13), 414 ($M^+ - H_2O$; 6.4), 372 ($M^+ - CH_3COOH$; 100), 201 (69), 111 (40). ^{1}H-NMR: δ 0.85 (s, 19-H_3), 0.88 (s, 18-H_3), 1.01 (m, 9α-H), 1.03 (dd, $J_{1\alpha1\beta}$ = 12.4, 1α-H), 1.10 (m, 7α-H), 1.20 (m, 6α-H), 1.26 (m, 5α-H), 1.29 (m, 11α-H), 1.38 (m, 4α-H), 1.40 (m, 12α-H), 1.43 (m, 6β-H), 1.49 (m, 8β-H), 1.53 (m, 12β-H), 1.55 (m, 11β-H), 1.67 (dd, $J_{15\alpha15\beta}$ = 13.2, $J_{15\alpha16\alpha}$ = 9.4, 15α-H), 1.77 (ddd, $J_{4\beta4\alpha}$ = 12.9, $J_{4\beta3\alpha}$ = 5.2, $J_{4\beta5\alpha}$ = 3.0, 4β-H), 1.86 (m, 16α-H), 1.96 (m, 7β-H), 2.08 (m, 1β-H and 15β-H), 2.09 (s, CH_3COO), 2.16 (m, 16β-H and 2-OH), 2.78 (dd, $J_{17\alpha16\alpha}$ = 9.6, $J_{17\alpha16\beta}$ = 5.7, 17α-H), 3.78 (m, 2β-H), 4.58 (m, 3α-H), 4.80 (dd, $J_{21\alpha21\beta}$ = 18.0, $J_{21\alpha22}$ = 1.5, 21α-H), 4.98 (dd, $J_{21\beta21\alpha}$ = 18.0, $J_{21\beta22}$ = 1.3, 21β-H), 5.88 ppm (s, 22-H).

As a more polar component gomphogenin 2-acetate **6** (51 mg; 33%; yields of **5** and **6** considering the recovery of starting material) was eluted. Prisms from acetone/ether; mp 238–240°C, $[\alpha]_D^{20}$ –15° (c = 1.20), $[M]_D$ –65°. TLC: R_f = 0.25. IR: ν_{max} 3520, 3440 (OH), 3145 (=C-H), 1775, 1730 (butenolide and acetyl C=O), 1615 (C=C), 1245 (acetoxy C-O-C), 1130, 1065, 1030 cm^{-1} (C-O). EI (80 eV) MS: *m/z* (relative intensity) 432 (M^+; 6.6), 414 ($M^+ - H_2O$; 6.6), 372 ($M^+ - CH_3COOH$; 100), 201 (67), 111 (52). ^{1}H-NMR: δ 0.88 (s, 18-H_3), 0.89 (s, 19-H_3), 0.98 (m, 9α-H), 0.99 (t, J ~ 12, 1α-H), 1.11 (m, 7α-H), 1.21 (m, 5α-H), 1.23 (m, 6α-H), 1.28 (m, 11α-H), 1.38 (m, 12α-H), 1.44 (m, 4α-H), 1.45 (m, 6β-H), 1.49 (m, 11β-H), 1.49 (m, 12β-H), 1.51 (m, 8β-H), 1.69 (dd, $J_{15\alpha15\beta}$ = 13.0, $J_{15\alpha16\alpha}$ = 9.3, 15α-H), 1.75 (ddd, $J_{4\beta4\alpha}$ = 12.9, $J_{4\beta3\alpha}$ = 5.2, $J_{4\beta5\alpha}$ = 2.8, 4β-H), 1.86 (m, 16α-H), 1.97 (m, 7β-H), 2.05 (m, 1β-H and 15β-H), 2.08 (s, CH_3COO), 2.14 (m, 16β-H), 2.31 (br s, 3-OH), 2.77 (dd, $J_{17\alpha16\alpha}$ = 9.6, $J_{17\alpha16\beta}$ = 5.6, 17α-H), 3.60 (m, 3α-H), 4.80 (m, 2β-H), 4.81 (dd, $J_{21\alpha21\beta}$ = 18.0, $J_{21\alpha22}$ = 1.3, 21α-H), 4.98 (dd, $J_{21\beta21\alpha}$ = 18.0, $J_{21\beta22}$ = 1.1, 21β-H), 5.87 ppm (s, 22-H).

14-Hydroxy-2α,3β-(4'β-acetoxy-3'β-hydroxy-6'α-methyltetrahydro-2H-pyran-3'α,2'α-diyldioxy)-5α,14β-card-20(22)-enolide (gomphoside 4'-acetate) 2

Gomphoside **1** (52 mg) was treated at room temperature for 1 h with acetic anhydride (1.2 ml) in pyridine (0.8 ml). After usual work-up the product was purified on silica gel (10 g). Elution with chloroform/ethyl acetate (80: 20) gave **2** (40 mg, 80%) which was crystallized from aqueous methanol as fine needles; mp 202–205°C (lit [4]: 204–205°C). TLC: R_f = 0.41 (**1**: R_f = 0.30). IR: ν_{max} 3543, 3459 (OH), 1784, 1752, 1715 (butenolide and acetyl C=O), 1642 (C=C), 1270 (acetoxy C-O-C), 1064, 1044 cm^{-1} (C-O). FAB-MS: *m/z* (relative intensity) 561 (MH^+; 2.3), 483 ([M – CH_3COOH – OH]$^+$; 11). EI (80 eV) MS: 500 ($M^+ - CH_3COOH$; 0.4), 390 ($M^+ - C_8H_{10}O_4$, genin; 1.4), 372 (genin – H_2O; 21), 201 ($C_{15}H_{21}$; 14), 170 ($C_8H_{10}O_4$; 12), 128 ($C_6H_8O_3$; 100).

14-Hydroxy-2α,3β-(3'β,4'β-diacetoxy-6'α-methyltetrahydro-2H-pyran-3'α,2'α-diyldioxy)-5α,14β-card-20(22)-enolide (gomphoside 3',4'-diacetate) 3

Acetylation of **1** (54 mg) with acetic anhydride (1. 8 ml) and pyridine (1.2 ml) at 40°C (6 d), purification of the crude product (60 mg) on silica gel (10 g) and recrystallization from ethyl acetate/hexane gave the diacetate **3** as fine needles (42 mg, 67%); mp 248–252°C (lit [18]: 252–255°C). TLC: R_f = 0.51. IR: ν_{max} 3485 (OH), 3070 (=C-H), 1790, 1735, 1700 (butenolide and acetyl C=O), 1610 (C=C), 1245 (acetoxy C-O-C), 1155, 1060, 1050 cm^{-1} (C-O). EI (80 eV) MS: *m/z* (relative intensity) 602 (M^+; 0.34), 560 ($M^+ - CH_2$=C=O; 1.3), 542 ($M^+ - CH_3COOH$; 100), 524 (542 – H_2O; 18), 482 ($M^+ - 2\ CH_3COOH$; 27), 464 (482 – H_2O; 46).

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