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BIOLOGICAL ACTIVITIES OF SYNTHETIC TRITERPENOID AND STEROID β-D-XYLOPYRANOSYL-(1→6)-β-D-GLUCOPYRANOSIDES*

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Key Word Index—haemolysis; antifungal activity; anti-ATPase activity; antiviral activity; saponin; cardiac glycoside.

Abstract—Comparisons of the activities of diosgenyl, methyl glycyrrhetinate or digitoxigenyl 3-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside with those of our previous glycosides supported our assumptions that both haemolytic and antifungal activities of steroid saponins are generally parallel to each other, while almost all haemolytic triterpenoid saponins show no antifungal activity, and that both antiviral and anti-ATPase activities of cardiac glycosides having a (1→6) sugar linkage are much lower than those of the others. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

We have studied the structure–activity relationships of diosgenyl [1, 2], tigogenyl [3], hecogenyl [4], methyl oleanolate [5], methyl ursolate [6], methyl glycyrrhetinate [7], digitoxigenyl [8] and bufalyl glycosides [9], and supposed that both haemolytic and antifungal activities of steroid saponins are generally parallel to each other, while almost all haemolytic triterpenoid saponins show no antifungal activity [4] and that both antiviral and anti-ATPase activities of cardiac glycosides having a (1→6) sugar linkage are much weaker than those of the others, e.g. containing a (1→4) linkage [9]. In order to confirm these suppositions, diosgenyl, methyl glycyrrhetinate and digitoxigenyl diglycosides have been selected as representative steroid, triterpenoid and cardiac glycosides, respectively, and synthesized to contain D-glucose as an inner residue of the sugar moiety for strict comparisons with each other and with our previous diglycosides [2–9] whose inner sugar residues were D-glucose. These compounds allow us to investigate how differences in the outer sugar residues in these diglycosides affect the activities. For this report, we have synthesized diosgenyl, methyl glycyrrhetinate and digitoxigenyl 3-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranosides and compared their activities with those of our previous glycosides [4, 9].

RESULTS AND DISCUSSION

For this report, diosgenin, methyl glycyrrhetinate, digitoxigenin, β-D-glucoside, β-gentiobioside and β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside are abbreviated as DIO, GAM, DIG, Glc, Gen and 6DX, respectively. The ^{13}C NMR spectral data for DIO–6DX, GAM–6DX and DIG–6DX are shown in Table 1; the assignments were deduced from the literature values [10–12].

The haemolytic and antifungal activities of DIO–6DX and GAM–6DX are compared in Fig. 1 with those of DIO, DIO–Glc, DIO–Gen, GAM, GAM–Glc and GAM–Gen. Both haemolytic and antifungal activities of DIO–6DX were high, while GAM–6DX had strong haemolytic, but no antifungal activity. These facts supported our previous assumption [4] that both haemolytic and antifungal activities of steroid saponins are generally parallel to each other, while almost all haemolytic triterpenoid saponins show no antifungal activity. Both the haemolytic and antifungal activities of DIO–6DX were as strong as those of DIO–Gen, and so the CH_2OH-6″ of the latter scarcely contributes to these activities. However, the CH_2OH-6″ group of GAM–Gen is harmful to the haemolytic activity, since GAM–Gen showed weaker activity than GAM–6DX.

The anti-ATPase and anti-herpetic activities of DIG–6DX are compared in Fig. 2 with those of DIG, DIG–Glc and DIG–Gen. Both activities of DIG–6DX were as weak as those of DIG–Gen and much lower than those of DIG or DIG–Glc, which were as strong as those of DIG-maltoside, -cellobioside or -lactoside [9]. Therefore, these results confirmed our assumption [9] that the cardiac glycosides having a (1→6) sugar linkage show much lower anti-ATPase and anti-herpetic activities than the other compounds containing a (1→4) linkage. In order to confirm further the above assumptions and also to investigate how differences in

*Part 1 in the series 'Biological Activities of Synthetic Diglycosides'.

Table 1. ^{13}C NMR chemical shifts (δ) of DIO-6DX, GAM-6DX and DIG-6DX

C	DIO-6DX*	GAM-6DX*	DIG-6DX†
1	37.3	39.4	30.0
2	30.3	26.4	26.1
3	78.4	88.3	73.2‡
4	39.3	39.8	29.6
5	140.9	55.2	35.7
6	121.5	17.5	26.6‡
7	32.1‡	32.7	21.1
8	31.5	43.3	41.0
9	50.0	62.0	35.1
10	36.9	37.1	34.9
11	21.0	199.3	20.9
12	39.7	128.6	39.8
13	40.3	168.8	49.5
14	56.5	45.4	83.9
15	31.7	26.6‡	32.3
16	81.0	26.7‡	26.5‡
17	62.8	31.9	50.5
18	16.2	48.5	15.7
19	19.3	41.1	23.5
20	41.8	44.1	175.9
21	14.9	31.1	73.1‡
22	109.1	38.0	116.4
23	32.1‡	28.1	173.8
24	29.1	16.9	
25	30.5	16.7	
26	66.7	18.6	
27	17.2	23.4	
28		28.4‡	
29		28.5‡	
30		176.8	
-COO $\underline{C}H_3$		51.6	
1′	102.8	105.9	101.3
2′	74.8	74.7	73.2‡
3′	78.1‡	78.0	76.4
4′	71.5	71.6	71.9
5′	77.0	76.7	75.6
6′	69.7	70.2	69.6
1″	105.7	106.8	103.9
2″	75.0	75.5	73.5
3″	78.1‡	78.5	76.8
4″	71.0	71.0	70.0
5″	67.1	67.0	67.1

*In ppm downfield from TMS in pyridine-d_5.
†In ppm downfield from TMS in $CDCl_3$–DMSO-d_6 (1:2).
‡The near values (<±0.1 ppm) within a vertical column are interchangeable.

the linkages or sugar species affect these activities, it is desirable to evaluate DIO-, GAM- or DIG-diglycosides whose inner D-glucose residue have a (1→2), (1→3), (1→4) or (1→6) linkage to various monosaccharides.

EXPERIMENTAL

General. ^{13}C NMR: 126 MHz in $CDCl_3$, pyridine-d_5 or $CDCl_3$–DMSO-d_6 (1:2), TMS as int. standard. DIO was purchased from Sigma. GAM and DIG were prepd according to our previous papers [5, 8]. DIO–Glc, DIO–Gen, GAM–Glc, GAM–Gen, DIG–Glc and DIG–Gen were our previous samples [1, 2, 7, 8].

Syntheses of DIO–6DX, GAM–6DX and DIG–6DX. These syntheses are summarized in Scheme 1. The synthesis of **1** was performed as described in ref. [13]. D-glucose (18 g) was tritylated and acetylated, and then detritylated to yield 24 g of **1** (69% yield from D-glucose). Identification of **1** was based on its ^{13}C NMR spectrum measured in $CDCl_3$. The synthesis of **2** was performed according to the method of ref. [14]. D-Xylose (10 g) was acetylated and then thioethylated with 5.4 ml EtSH and 1.1 ml $SnCl_4$ to yield 18 g **2** (84% yield from D-xylose). Compound 3 was synthesized with 10 g *N*-iodosuccinimide and 0.40 ml CF_3SO_3H from 13 g **1** and 18 g **2** according to the method of ref. [14], and purified by silica gel CC using hexane–Et_2O to yield 10 g **3** (44% yield from **1**). The

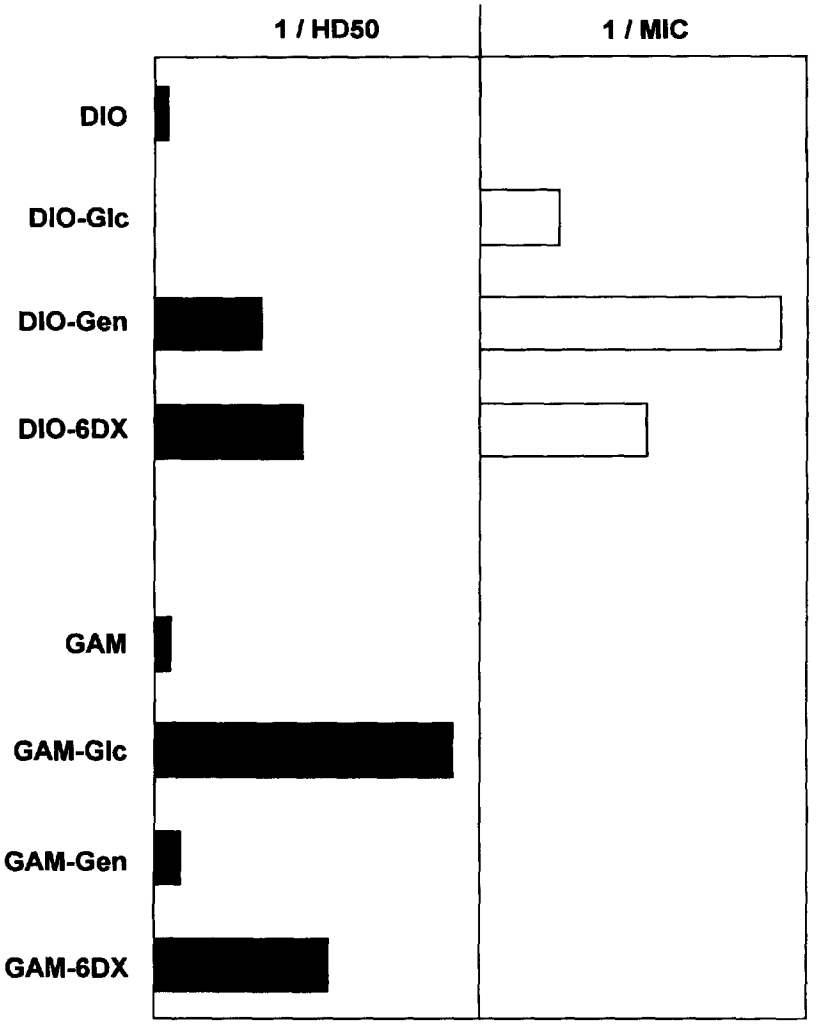


Fig. 1. The haemolytic and antifungal activities of DIO–6DX and GAM–6DX. The HD50 or MIC values are means of 5 assays, and their s.d. values are ±2.7–16% or ±7.7–13% of each mean value. The haemolytic (1/HD50) or antifungal (1/MIC) activities are expressed as the areas of black or white bars, respectively. Max. activity: HD50 (GAM–Glc) = 3.1 μM, MIC (DIO–Gen) = 6.6 μM; min. activity: HD50 (DIO–Glc) = >160 μM, MIC (GAM) = >62 μM.

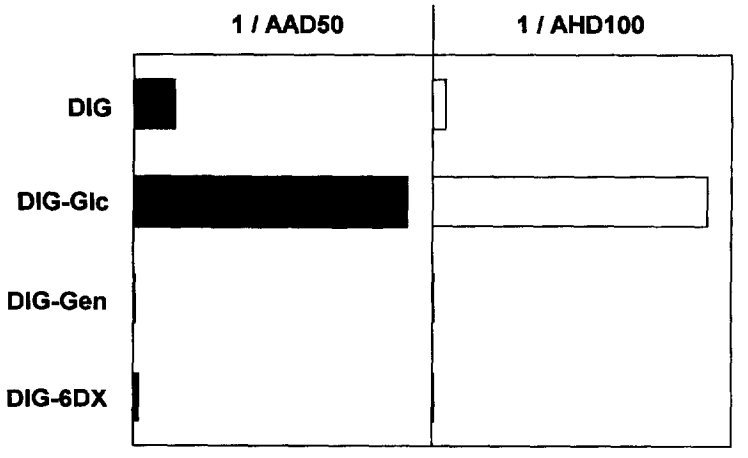
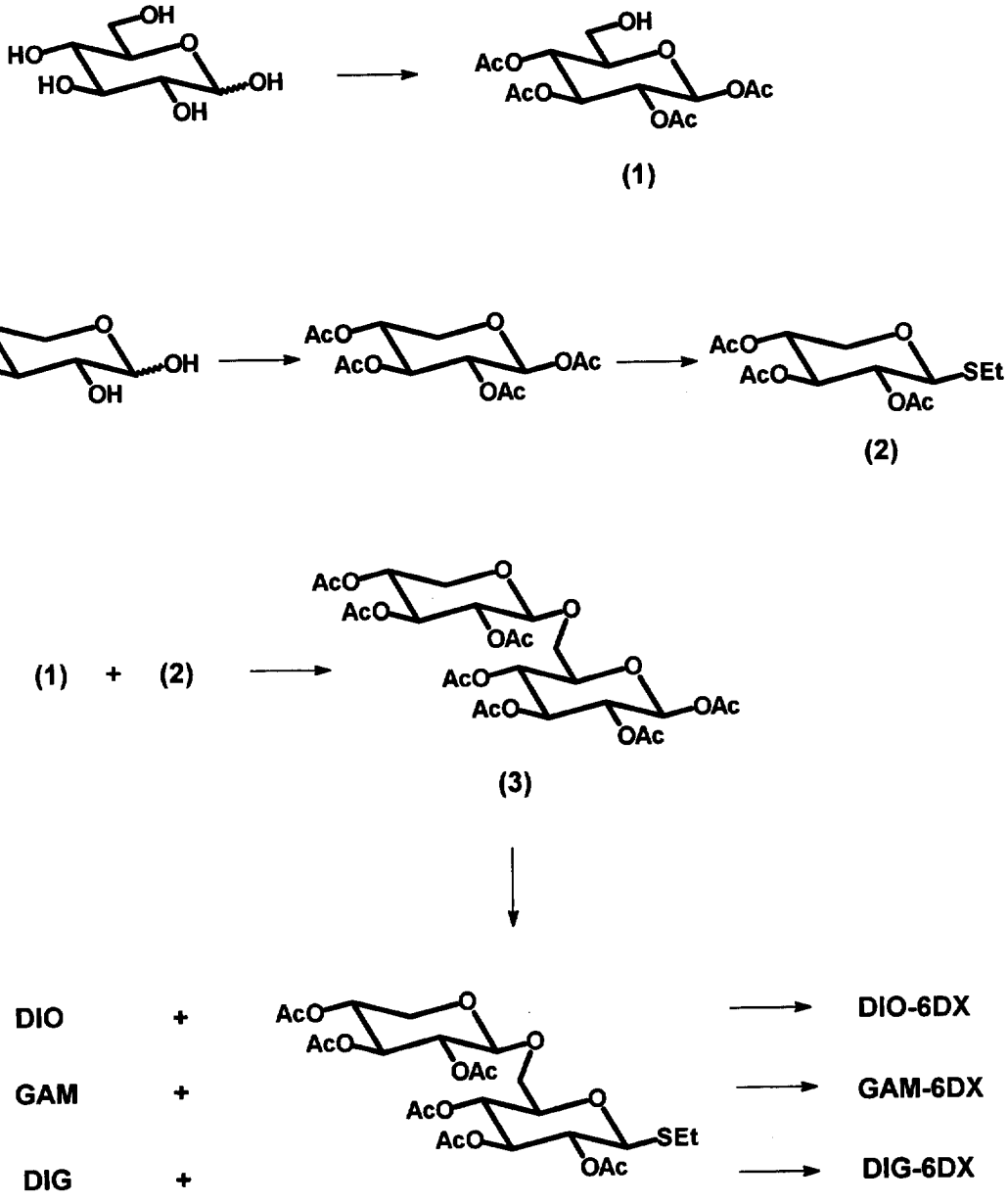


Fig. 2. The anti-ATPase and anti-herpetic activities of DIG–6DX. The AAD50 or AHD100 values are means of 5 assays, and their s.d. values are ±13–23% or ±21–25% of each mean value. The anti-ATPase (1/AAD50) or anti-herpetic (1/AHD100) activities are expressed as the areas of black or white bars, respectively. Max. activity: AAD50 (DIG–Glc) = 0.21 μM, AHD100 (DIG–Glc) = 0.030 μM; min. activity: AAD50 (DIG–Gen) = 30 μM, AHD100 (DIG–Gen) = 12 μM.



Scheme 1.

identification of **3** was based on its ^{13}C NMR spectrum measured in $CDCl_3$. Each 1.0 g of DIO, GAM or DIG was glycosylated with 1.0 g thioethylated **3** according to the method of ref. [14], and then deacetylated with 3% NaOMe [15] for DIO–6DX and GAM–6DX or with 50% Et_3N [16] for DIG–6DX. The compounds were subjected to silica gel CC and prep. TLC using $CHCl_3$–MeOH to yield 190, 90 and 110 mg (16, 7.0 and 10% yield from the thioethylated **3**), respectively. The compounds were identified from their ^{13}C NMR spectra (Table 1).

Haemolytic and antifungal activities. These activities were measured in 9.1 and 4.8% MeOH, respectively. MIC against *Trichophyton mentagrophytes* was the minimum concn of sample which caused no hyphae visible to the naked eye after 3 days incubation. The other conditions were the same as those described in ref. [4].

Anti-ATPase and anti-herpetic activities. These assays were carried out in 1% MeOH according to ref. [9].

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