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STRUCTURE–ACTIVITY RELATIONSHIPS OF SYNTHETIC SAPONINS

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Key Word Index—Haemolysis; antifungal activity; steroid; triterpenoid; glycoside; saponin.

Abstract—The haemolytic and antifungal activities of synthetic diosgenyl, tigogenyl, hecogenyl, methyl oleanolate, methyl ursolate and methyl glycyrrhetinate glycosides were compared with each other. Both activities of the steroid glycosides were generally parallel to each other, while almost all haemolytic triterpenoid glycosides showed no antifungal activity.

INTRODUCTION

In order to elucidate structure–activity relationships of saponins, we studied mainly how the sugar moieties in synthetic diosgenyl [1, 2], tigogenyl [3], methyl oleanolate [4], methyl ursolate [5] and methyl glycyrrhetinate [6] glycosides contribute to the haemolytic and antifungal activities. In this paper, we describe the synthesis of fifteen hecogenyl glycosides, and give a comparison of their activities and those of glycosides which had been synthesized previously [1–6]. By these comparisons, it should be possible to survey how the aglycones and sugar moieties in the glycosides influence the activities. On the other hand, the comparisons of activities between the compounds whose structures are different at one point from each other could give us definite information on the structure–activity relationship.

RESULTS AND DISCUSSION

The aglycones of diosgenin, tigogenin, hecogenin, methyl oleanolate, methyl ursolate and methyl glycyrrhetinate are abbreviated as **d**, **t**, **h**, **o**, **u** and **g**, respectively. Furthermore, for each aglycone **0**, **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14** and **15** refer to the aglycone, β-D-glucoside, β-D-galactoside, β-D-xyloside, β-L-xyloside, α-D-arabinoside, α-L-arabinoside, β-D-fucoside, β-L-fucoside, α-L-rhamnoside, β-maltoside, β-cellobioside, β-lactoside, β-gentiobioside, β-melibioside and β-maltotrioside, respectively.

Fifteen **h**-glycosides were synthesized according to our previous method [1]. The ^{13}CNMR and FAB mass spectral data of **h-0**, **h-1**, **h-2**, **h-3**, **h-4**, **h-5**, **h-6**, **h-7**, **h-8**, **h-9**, **h-10**, **h-11**, **h-12**, **h-13**, **h-14** and **h-15** were in good agreement with literature values [7].

The haemolytic and antifungal activities of these **h**-glycosides were too weak to discuss their structure–activity relationships.

On the other hand, the activities of these **h**-glycosides were compared with those of **d**-, **t**-, **o**-, **u**- and **g**-glycosides which had been synthesized by us [1–6]. In order to evaluate the activities of these samples equally and exactly, the haemolytic assays were carried out in 2.0% DMSO or 9.1% methanol using the same batch of erythrocytes and the antifungal activities were estimated as MIC (minimum 100% inhibitory concentration) in 1.0% DMSO or 4.8% methanol. MIC is more severe than GID_{50} or ATD_{50} in our previous assays [1–6], and the haemolytic activity in 2.0% DMSO would be generally a little weaker than that in 9.1% methanol, since 2.0% DMSO would have more protective effects on erythrocyte membranes than 9.1% methanol. The haemolytic activities of **d**-, **t**-, **h**-, **o**-, **u**- and **g-0–15** in 2.0% DMSO or 9.1% methanol are shown in Fig. 1, and the antifungal ones in 1.0% DMSO or 4.8% methanol are displayed in Fig. 2. The structures of the steroids (**d-0**, **t-0**, **h-0**) and the triterpenoids (**o-0**, **u-0**, **g-0**) are given in order to discuss the structure–activity relationships.

In Fig. 1, the activities of **h-10–15** were lower than those of the **t**-counterparts. Therefore, the C=O of the former weakens the activity. The activities of **d-0–15** were parallel to those of the **t**-compounds except that **d-12** showed much weaker activity than **t-12**. Generally, the activities of **d**-, **t**-, and **u-0–9** were weaker than those of **10–15**, and those of **h**- and **o-0–15** were not very variable with the different sugar moieties. By contrast, the activities of **g-0–15** showed varieties with the diversity of sugar moieties. Compounds **u-11**, **-12**, and **o-14** showed strong activity in 9.1% methanol, while they hardly had activity in 2.0% DMSO. This could be due to the solvent effects and they could not be considered in the structure–activity relationships.

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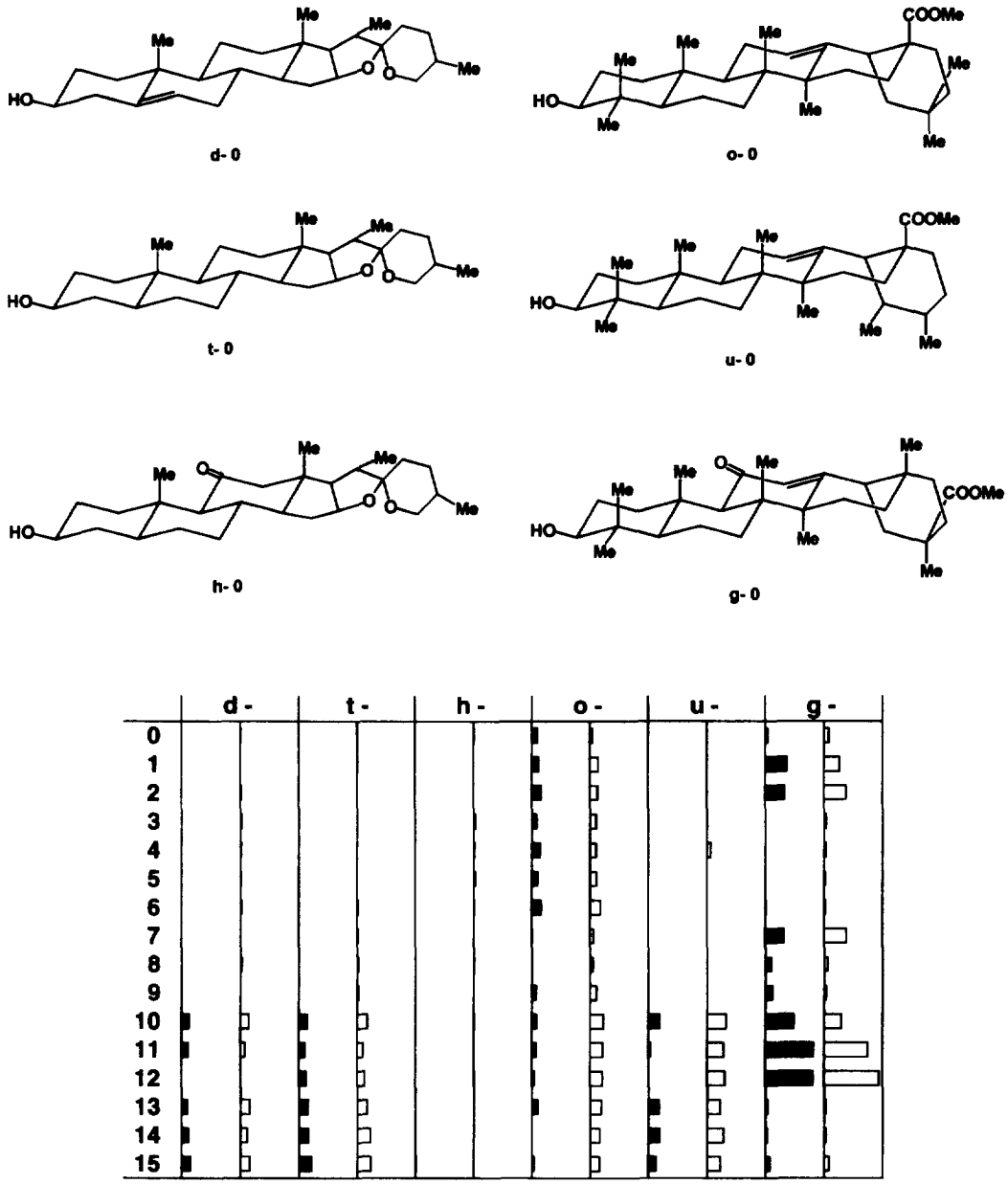

Fig. 1. Comparisons of the haemolytic activities between **d-**, **t-**, **h-**, **o-**, **u-** and **g-o–15**. The HD_{50} values are means of 5 assays, and their s.d. values are ± 5–20% of each mean value, and the activities ($1/HD_{50}$) are expressed as the areas of bars. **d-**, **t-**, **h-**, **o-**, **u-**, **g-** and **0–15**: See text. Black bar: in 2.0% DMSO; White bar: in 9.1% MeOH. Max. activity: HD_{50} (**g-12** in 9.1% MeOH) = 1.3 μM.

In Fig. 2, the activities of **h-1**, **-2**, **-10**, and **-13–15** were weaker than those of the **t**-counterparts. Thus, the C=O of the former reduces the activities, analogous to the above haemolytic case. The activities of **d-0–15** were parallel to those of the **t**-ones except that **d-11** showed much stronger activity than **t-11**. Some of the steroid glycosides exhibited strong activity, but all the triterpenoids showed no activity except **g-10**. It might be thought that the former would have higher affinity for ergosterol in fungal cell membranes than the latter, which had been assumed in our previous papers [8, 9] reporting that steroid haemolytic saponins showed faster haemolysis than triterpenoid ones. Further, it might be due to the lack of steroids in bacterial cell membranes that these steroid and triterpenoid saponins scarcely exhibit the anti-bacterial activity against *Staphylococcus aureus* or *Bacillus subtilis* (data not shown). Compound **d-2** displayed strong antifungal activity in 4.8% methanol, while it had no activity in 1.0% DMSO. It could also be caused by the solvent effects.

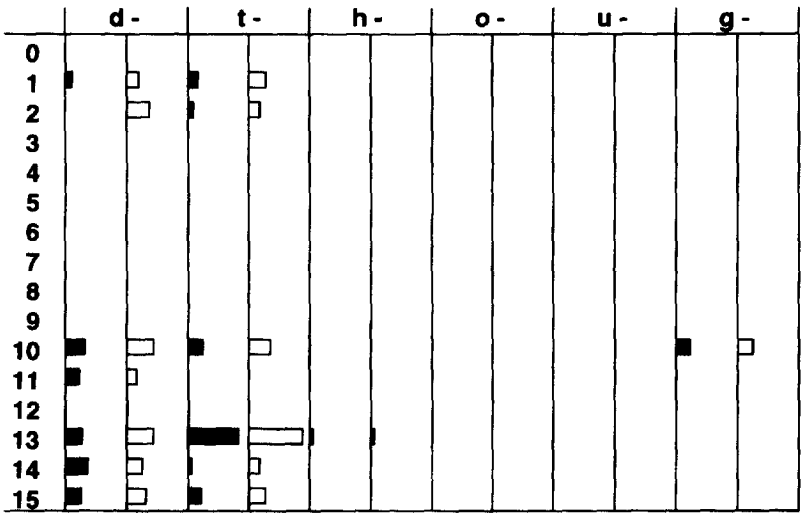

Fig. 2. Comparisons of the antifungal activities against *Trichophyton mentagrophytes* between **d-**, **t-**, **h-**, **o-**, **u-** and **g–0–15**. The MIC values are means of 5 assays, and their s.d. values are ± 10–30% of each mean value, and the activities (1/MIC) are expressed as the areas of bars. **d-**, **t-**, **h-**, **o-**, **u-**, **g-** and **0–15**: See text. Black bar: in 1.0% DMSO; White bar: in 4.8% MeOH. Max. activity: MIC (**t-13** in 4.8% MeOH) = 3.8 μM.

Comparing Figs. 1 and 2, the haemolytic and antifungal activities of the steroid saponins were parallel to each other except **d-1**, **t-1**, **-2**, **-11** and **-12**. On the other hand, all the haemolytic triterpenoid saponins displayed no antifungal activity except **g-10**. It might be assumed that most of the steroid haemolytic saponins could penetrate into fungal cell walls to combine with ergosterol in the cell membranes, while the triterpenoid ones were scarcely able to do this. Compounds **d-1**, **t-1** and **-2** would be useful as antifungal drugs since they might have higher affinity for ergosterol in fungi than for cholesterol in erythrocytes, and it is interesting to elucidate why only **g-10** shows the antifungal activity among the many haemolytic triterpenoid saponins.

EXPERIMENTAL

General. Conditions are the same as those described in our previous paper [3].

Syntheses of **h-***glycosides.* In general, the syntheses were carried out according to our previous method [1].

Haemolytic and antifungal activities. These activities of **h-0–15** and our previous samples [1–6] were measured in 2.0% DMSO or 9.1% MeOH and 1.0% DMSO or 4.8% MeOH, respectively. The haemolytic activities were measured using the same batch of sheep erythrocytes. MIC against *Trichophyton mentagrophytes* is the minimum concentration of sample which causes no hypha visible by microscope (× 40) after 7 days incubation. The other conditions are the same as those described in our previous paper [10].

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