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STRUCTURE–ACTIVITY RELATIONSHIPS OF SYNTHETIC CARDIAC GLYCOSIDES

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Key Word Index—Antiviral activity; cytotoxicity; anti-ATPase activity; correlation; digitoxigenin; bufalin.

Abstract—The antiviral, cytotoxic and anti-ATPase activities of 14 synthetic bufalyl glycosides were compared with each other. Among these glycosides, the activities of the gentiobioside and the melibioside were much weaker than those of the others. On the other hand, these three activities were found to be highly correlated with each other. These were parallel to the case of the digitoxigenyl glycosides in our previous paper.

INTRODUCTION

It has been reported that bufadienolides display an inhibitory activity against rhinovirus [1]. We have evaluated the anti-herpetic activity of some cardiac glycosides and steroids, and found that the former generally showed stronger activities than the latter. Hence, in our previous paper [2], we described the synthesis of some digitoxigenyl glycosides and determined that the anti-herpetic, cytotoxic and anti-ATPase activities of the gentiobioside and the melibioside were much weaker than those of the others, and that these activities were highly correlated with each other. As bufalin has shown stronger anti-herpetic activity than digitoxigenin we have now synthesized some bufalyl glycosides and compared their activities with those of the digitoxigenyl glycosides [2] in order to investigate the structure-activity relationships.

RESULTS AND DISCUSSION

Fourteen bufalyl glycosides were synthesized according to our previous method [2]. ^{13}CNMR and FAB mass spectral data of bufalin (**b-0**), bufalyl β-D-glucoside (**b-1**), β-D-galactoside (**b-2**), β-D-xyloside (**b-3**), β-L-xyloside (**b-4**), α-D-arabinoside (**b-5**), α-L-arabinoside (**b-6**), β-D-fucoside (**b-7**), β-L-fucoside (**b-8**), α-L-rhamnoside (**b-9**), β-maltoside (**b-10**), β-cellobioside (**b-11**), β-lactoside (**b-12**), β-gentiobioside (**b-13**) and β-melibioside (**b-14**) were in good agreements with the literature values [3]. The anti-herpetic, cytotoxic and anti-ATPase activities of these compounds are summarized in Table 1. The comparisons of these activities with those of **d-0**–**14** [2] are displayed in Figs 1, 2 and 3, respectively. The structures of digitoxigenin (**d-0**) and **b-0** are given in order to discuss their biological effects.

In Table 1, the anti-herpetic and cytotoxic activities of compounds **b-10**, **-11** and **-12** are as strong as **b-0**, while those of **b-13** and **-14** are much weaker than the activity of **b-0**. Thus, the (1 → 6) linkage between the sugar residues of **b-13** or **-14** lower the activity, while the (1 → 4) linkage of **b-10**, **-11** or **-12** hardly affects the activity. Similarly, the anti-ATPase activities of compounds **b-10**, **-11** and **-12** are low, but those of **b-13** and **-14** are much lower than the activity of **b-0**. Therefore, the (1 → 4)

Table 1. The anti-herpetic, cytotoxic and anti-ATPase activities of **b-0**–**14**

Compound	AHD100* (nM)	CND50† (nM)	AAD50‡ (nM)
b-0	23 ± 3.5	260 ± 43	140 ± 25
b-1	8.2 ± 1.1	82 ± 12	190 ± 28
b-2	13 ± 2.1	91 ± 13	170 ± 19
b-3	10 ± 1.5	49 ± 7.4	150 ± 20
b-4	10 ± 1.2	36 ± 5.2	88 ± 14
b-5	15 ± 2.3	34 ± 4.7	220 ± 27
b-6	14 ± 1.7	35 ± 4.9	89 ± 10
b-7	9.4 ± 1.1	67 ± 10	130 ± 15
b-8	9.4 ± 1.2	35 ± 5.1	180 ± 20
b-9	11 ± 1.3	110 ± 17	110 ± 19
b-10	11 ± 1.4	82 ± 13	660 ± 80
b-11	18 ± 2.7	190 ± 25	610 ± 75
b-12	9.4 ± 1.5	100 ± 14	270 ± 40
b-13	1600 ± 280	18000 ± 3200	8900 ± 1100
b-14	1100 ± 150	3800 ± 450	10000 ± 1500

*100% Anti-herpetic minimum dose, values are means ± s.d. ($n = 6$).

†50% Cytotoxic dose, values are means ± s.d. ($n = 6$).

‡50% Anti-ATPase dose, values are means ± s.d. ($n = 6$).

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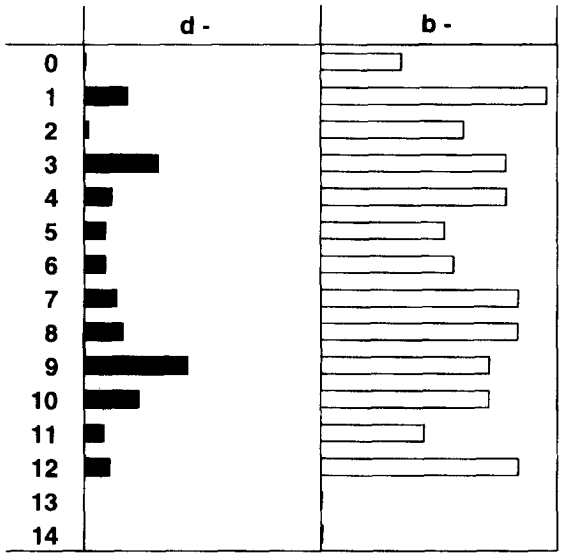


Fig. 1. Comparisons of the anti-herpetic activities between **d-** and **b-0–14**. The activities (1/AHD100) are expressed as the areas of bars. **d-**, **b-** and **0–14**: See text. Max. activity: AHD100 (**b-1**) = 8.2 nM.

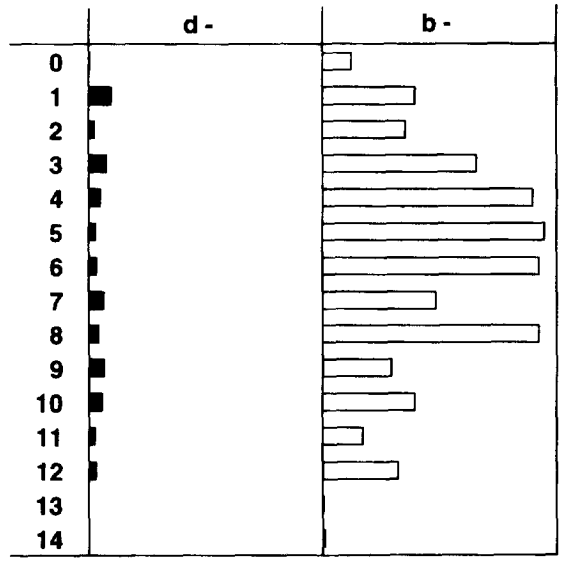
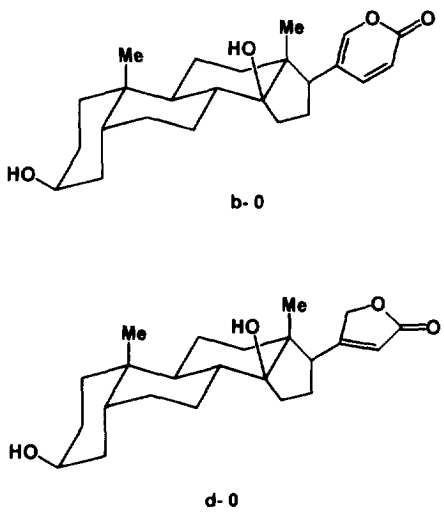


Fig. 2. Comparisons of the cytotoxic activities between **d-** and **b-0–14**. The activities (1/CND50) are expressed as the areas of bars. **d-**, **b-** and **0–14**: See text. Max. activity: CND50 (**b-5**) = 34 nM.



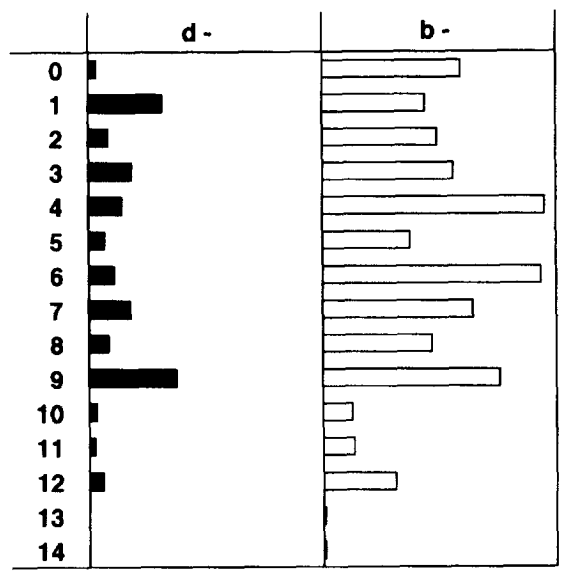


Fig. 3. Comparisons of the anti-ATPase activities between **d-** and **b-0–14**. The activities (1/AAD50) are expressed as the areas of bars. **d-**, **b-** and **0–14**: See text. Max. activity: AAD50 (**b-4**) = 88 nM.

linkage between the sugar residues of **b-10**, **-11** or **-12** would slightly decrease, while the (1 → 6) linkage of **b-13** or **-14** destroy the activity.

Comparing the anti-herpetic activities of **b-0–14** with those of the **d**-counterparts [2] in Fig. 1, the former are stronger than the latter, however the activity of **d-0** is generally more strengthened than that of **b-0** by the glycosidation except for **13** and **14**. Therefore, the δ-lactone ring of **b-0** would contribute more to the activity than the γ-lactone ring of **d-0** whose contribution would be more enhanced by the glycosidations, except for **13** and **14**, than the former. The comparisons of the cytotoxic and anti-ATPase activities of **b-0–14** with those of the **d**-counterparts [2] shown in Figs 2 and 3 are analogous to the case of Fig. 1. The activities of **d-** and **b-10–12** (diglycosides) are a little lower than those of **1–9** (monoglycosides).

On the other hand, the correlation coefficient between anti-herpetic and anti-ATPase, cytotoxic and anti-herpetic, or anti-ATPase and cytotoxic activities of **b-0–14** was 0.92, 0.95 or 0.90, respectively. These three activities were found to be highly correlated with each other and

similarly in the case of **d-0**–**14** [2]. Therefore, the discussions on the mechanisms would be similar to those in our previous paper [2].

It has been reported that the cytotoxicity of **d**-β-gentiobiosyl-α-L-cymaroside was much weaker than that of the α-L-cymaroside [4]. It might be universal that such cardiac glycosides having the (1 → 6) linkage as gentiobioside or melibioside hardly show these activities.

EXPERIMENTAL

General. They are the same as those described in our previous paper [2].

Syntheses of bufalyl glycosides. Compound **b-0** was purified from the $CHCl_3$ extract of Ch'an Su (circle cake) by silica gel CC using $CHCl_3$–MeOH as solvent (yield: ~ 1%). The glycosides were synthesized and purified according to our previous method [2]. The yields of **b-1**–**14** were 28, 20, 30, 13, 26, 21, 15, 13, 5.8, 3.6, 3.6, 4.0, 5.2, 5.1% of starting material (**b-0**), respectively. Their purities were checked and the structures were identified by our previous methods [2].

Anti-herpetic, cytotoxic and anti-ATPase activities. These assays were carried out according to our previous paper [2].

Correlation between anti-herpetic, cytotoxic and anti-ATPase activities. The correlation coefficients were calculated as described in our previous paper [2].

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