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ENZYMIC SYNTHESIS OF CHEMICALLY UNSTABLE CARDIAC GLYCOSIDES BY β-GALACTOSIDASE FROM *Aspergillus oryzae*

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Abstract: β-Galactosidase obtained from *Asp. oryzae* was found to have strong transglycosylation activity. The glycosylation of water soluble and insoluble alcohols was successfully performed under such mild conditions at pH 5 at 4-37°C by using the enzyme.

This enzymic method was applied to the one step synthesis of several cardiac glycosides difficult to obtain by ordinary chemical methods.

Numerous glycosides of terpenoids and steroids can be found in nature. The physiological activity and bioavailability of these compounds frequently depend on the type or position of sugars attached. Thus, the creation of a novel drug should be facilitated by the synthesis of the glycosides of naturally occuring genins and evaluating their physiological properties.

However, many problems still remain in the glycosylation of hydroxyl groups in terpenoids or steroids. For instance, in König-Knorr's method[1] frequently used for the synthesis of glycosides, genins with tertiary hydroxyl groups are liable to dehydration[2]. Furthermore, even if it is possible to synthesize a glycoside, little glycoside would be afforded in the case of genins having a lactone ring or aldehyde group unstable toward alkali (e.g. cardiac glycosides of genins) because of decomposition during deacetylation of the sugar moiety. But enzymatic methods seem to be promising as a means of synthesizing such unstable glycosides because they can be carried out under mind conditions accompanying no decomposition of the starting materials or products. But all enzymatic methods[3),4)] known up to the present have been performed in an aqueous solution and thus not applicable to the glycosylation of water insoluble steroids or terpenoids.

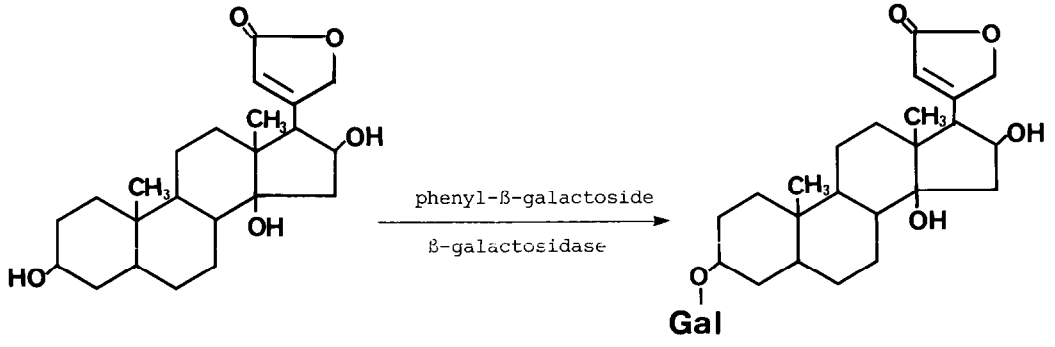
At our laboratory, several species from inexpensive industrial β-galactosidase have been found to be stable and show great transglycosylation activity even in the water-organic solvent mediums and attempt to glycosylate water insoluble genins by using the β-galactosidase obtained from *Asp. oryzae*.

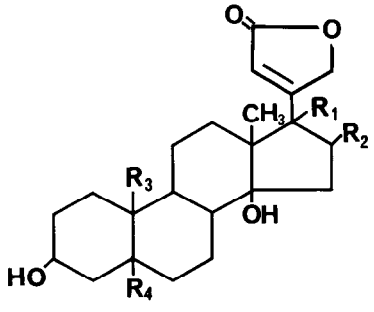
This report deals with synthesis of the glycosides of gitoxigenin and its related compounds not readily synthesized by conventional chemical methods.

Synthesis of cardic glycosides by transglycosylation in 50 % acetonitrile

Several cardic glycosides of genins unstable toward acids and bases such as gitoxigenin were glycosylated. Approximately 1.28 mmol of a genin and 5 mmol of phenyl-ß-glycosides were dissolved in 40 ml of 62.5 % acetonitrile. The enzyme dissolved in 10 ml of a 0.1 M phospate buffer (pH 5) (11400 units) was then added to the solution, and the mixture was allowed to react at 20°C for 20 min to synthesize the galactosides.

Preparation of glucosides, 40600 units of enzyme was used at 10°C for 720 min. The reaction was terminated by boiling. The resulting glycoside was isolated in a conventional manner using a Sephadex G-25 and HPLC with a NUCLEOSIL 10 C18 column. The product was confirmed as 3-O-ß-glycoside by various instrumental analyses.





I :R_1=R_4=H, R_2=OH, R_3=CH_3 Gitoxigenin 7-a)
3-O-ß-Gal 7-b)
3-O-ß-Glc 7-c)

II :R_1=R_2=R_4=H, R_3=CH_3 Digitoxigenin 8-a)
3-O-ß-Gal 8-b)
3-O-ß-Glc 8-c)

III:R_1=O, R_2= R_3=CH_3, R_4=H
16ß,17ß-Epoxy-17α-digitoxigenin 9-a)
3-O-ß-Gal 9-b)

IV :R_1=R_2=H, R_3=HCO, R_4=OH Strophanthidin 10-a)
3-O-ß-Gal 10-b)

References and Notes

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7) a. ^{13}C-NMR (C_5D_5N,90MHz) δ:28.7 (C-2), 34.0 (C-4), 44.0 (C-15), 59.4 (d,C-17), 66.1 (d,C-3), 72.4 (d,C-16), 84.3 (s,C-14).

b. UV λ_{max}^{MeOH} nm (log ε) :219 (4.37). IR (KBr) :3400, 1750, 1725 cm^{-1}. ^{13}C-NMR (C_5D_5N,90MHz) δ:27.1 (C-2), 30.7 (C-4), 44.0 (C-15), 59.4 (d,C-17), 72.4 (d.C-16), 84.3 (s,C-14). ORD(c=0.100, methanol) $[\alpha]^{21.5}$ (nm):+126°(300). Anal.Calcd for $C_{29}H_{44}O_{10}\cdot\frac{1}{2}H_2O$:C,62.02;H,8.08. Found: C,61.54;H,8.12. mp 226-228°C. yield 25.7%. conversion rate 2.6%.

c. UV λ_{max}^{MeOH} nm (log ε) :219 (4.37). IR (KBr) :3450, 1760, 1720 cm^{-1}. ^{13}C-NMR (C_5D_5N,90MHz) δ:27.1 (C-2), 30.7 (C-4), 44.0 (C-15), 59.4 (d,C-17), 71.9 (d,C-3), 72.4 (d,C-16), 84.3 (s,C-14). ORD(c=0.100, methanol) $[\alpha]^{21.5}$(nm):+110.3°(300). Anal.Calcd for $C_{29}H_{44}O_{10}\cdot H_2O$: C,61.04;H,8.12. Found:C,61.00;H,7.95. mp 217-219°C. yield 43.1% conversion rate 2.0%.

8) a. ^{13}C-NMR (C_5D_5N,90MHz) δ:24.1 (C-16), 28.7 (C-2), 33.2 (C-15), 34.2 (C-4), 51.4 (d,C-17), 66.1 (d,C-2), 84.8 (s,C-14).

b. UV λ_{max}^{MeOH}nm (log ε) :219 (4.23). IR (KBr) :3400, 1750, 1725 cm^{-1}. ^{13}C-NMR (C_5D_5N,90MHz) δ:27.3 (C-2), 27.3 (C-16), 30.7 (C-4), 33.1 (C-15), 51.4 (d,C-17), 72.6 (d,C-3), 84.7(s,C-14), ORD(c=0.101, mehtanol) $[\alpha]^{24}$(nm):+119.2°(300). Anal.Calcd for $C_{29}H_{44}O_9\cdot\frac{1}{2}H_2O$:

C,63.83;H,8.31. Found:C,64.21;H,8.53. mp 227-229°C. yield 38.4%. conversion rate 2.8%.

c. UV λ_{max}^{MeOH} nm (log ε) :219 (4.05). IR (KBr) :3400, 1750, 1720 cm^{-1}. ^{13}C-NMR (C_5D_5N,90MHz) δ:27.2 (C-2), 27.3 (C-16), 30.8 (C-4), 33.2 (C-15), 51.4 (d,C-17), 71.8 (d,C-3), 84.8 (s,C-14). ORD(c=0.100, methanol)$[\alpha]^{24}$(nm):+99.4°(300). Anal Calcd for $C_{29}H_{44}O_9 \cdot H_2O$:C,62.80; H,8.36. Found:C,62.68;H,8.13. mp 231-233°C. yield 74.1%. conversion rate 2.2%.

9) a. ^{13}C-NMR (C_5D_5N,90MHz) δ:28.7 (C-2), 34.0 (C-4), 34.2 (C-15), 64.2 (d,C-16), 66.0 (d,C-3), 70.4 (s,C-17), 80.9 (s,C-14).

b. UV λ_{max}^{MeOH} nm (log ε) :219 (4.39). IR (KBr) :3450, 2950, 1750, 1730 cm^{-1}. ^{13}C-NMR (C_5D_5N,90MHz) δ:26.9 (C-2), 30.6 (C-4), 34.1 (C-15), 64.2 (d,C-16), 70.3 (s,C-17), 72.4 (d,C-3), 80.8 (s,C-14). ORD(c=0.100, methanol)$[\alpha]^{21.5}$(nm):+96.5°(300). Anal Calcd for $C_{29}H_{42}O_{10}$:C,63.26; H,7.69. Found:C,63.12;H,7.93. dec. 243-246°C. yield 63.9%. conversion rate 2.3%.

10) a. ^{13}C-NMR (C_5D_5N,90MHz) δ:27.6 (C-2), 28.0 (C-16), 32.5 (C-15), 38.6 (C-4), 49.4 (d,C-17), 68.0 (d,C-3), 75.8 (d,C-5), 85.9 (s,C-14), 210.2 (d,C-19).

b. UV λ_{max}^{MeOH} nm (log ε) :219 (4.48). IR (KBr) :3450, 2900, 1740, 1710 cm^{-1}. ^{13}C-NMR (C_5D_5N,90MHz) δ:25.6 (C-2), 28.0 (C-16), 32.7 (C-15), 35.6 (C-4), 49.4 (d,C-17), 75.0 (d,C-3), 77.0 (s,C-5), 86.1 (s,C-14), 210.3 (d,C-19). ORD(c=0.107,methanol)$[\alpha]^{21.5}$ nm:+297°(300). Anal Calcd for $C_{29}H_{42}O_{11} \cdot 2H_2O$:C,57.80;H,7.69. Found:C,58.12;H,7.51. mp 164-166°C. yield 40.0%. conversion rate 3.0%.

11) The β-galactosidase from *Asp. oryzae* is crude preparation whose specific activity is 36 units/mg. This crude preparation was kindly supplied from KOHJIN Co.*. (Lot No.KG-570820).

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