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SYNTHESIS OF PENNOGENYL SAPONINS USING THREE METHODS

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*The synthesis of pennogenyl saponins using three important methods of glycosylation is reported in this article. Six correlative compounds (**7–12**) were first synthesized. As donors (**1–6**), glycosyl halide, trichloroimidate, and thioglycoside were chosen to study their reaction with the acceptor pennogenin. In these reactions the difference in steric hindrance between 3-OH and 17-OH of pennogenin was utilized skillfully and only the 3-hydroxyl group of pennogenin could be connected with each kind of donors selectively. There was no reaction at the 17-hydroxyl group, which had no protection. The characteristic above makes it convenient to synthesize compounds of pennogenyl saponins.*

Key words: pennogenyl saponin, pennogenin, synthesis, glycosylation, selectivity.

Steroid saponins are a structurally and biologically diverse class of glycosides that are distributed in many kinds of plants and have a good deal of biologic activities. The structural diversity of saponins lies mainly in their sapogenins and sugar moieties. Among these saponins there are some compounds called pennogenyl saponins. As medicament they have been used as hemostatic agents and promoter for the shrinkage of uterus in the clinic [1]. They also exhibit antibiotic and antitumor activity [2, 3]. Pennogenyl saponins are compounds of steroids where there is hydroxyl group at C-17. Probably this characteristic is responsible for their bioactivities, which can be confirmed by other steroids containing 17-hydroxy, for example, cephalostatins [4] and OSW-1 [5]. At the same time research has proved that the 17-hydroxyl group in the aglycon can enhance the protective effects against certain diseases [6].

Very few pennogenyl saponins can be obtained from nature, and it is extremely difficult to isolate them [6, 7].

The complicated existence of these saponins has hampered further investigation of their biological functions, as well as quality control of the clinically used saponin preparation. So it is important to synthesize these kinds of saponins. The artifical synthesis and biosynthesis [8] of pennogenin is now possible, and whether or not pennogenyl saponins can be synthesized is very pivotal.

Major synthetic challenges are presented by the laboratory synthesis of glycosides in general, as the glycosidic bond is required to be formed in the correct anomeric configuration, and only one reacting hydroxyl group is allowed in the reacting aglycon; protection of all other hydroxyl groups and other potential competing nucleophiles is essential. So in the process of synthesizing a sort of saponin, the glycosidic bond should be formed through the reaction of glycosylation between a certain hydroxyl group of sapogenin and the sugar donor. The essential purpose of this kind of reaction is to get rid of a molecule of water (H_2O) between the anomeric center of the donor and the acceptor subtly.

A pennogenyl saponin has the characteristic that there is a glycosidic bond between pennogenin (3-OH) and the sugar donor but there is no bond of this kind at 17-OH of pennogenin. So whether the reaction of glycosidation can happen or not at 17-OH in the synthesis of pennogenyl saponins is very important. If no reactions occur, the synthesis of pennogenyl saponins is convenient. Through observation and analysis of pennogenin, we find that 17-OH is a *t*-hydroxide and we assume that the activity of 17-OH is very low because of the steric hindrance at this site. In this article six pennogenyl saponins (**7–12**) have been synthesized based on the synthesis of pennogenin, and some monosaccharides (**1, 3, 5**) and disaccharides (**2, 4, 6**) were chosen as three kinds of donors [9].

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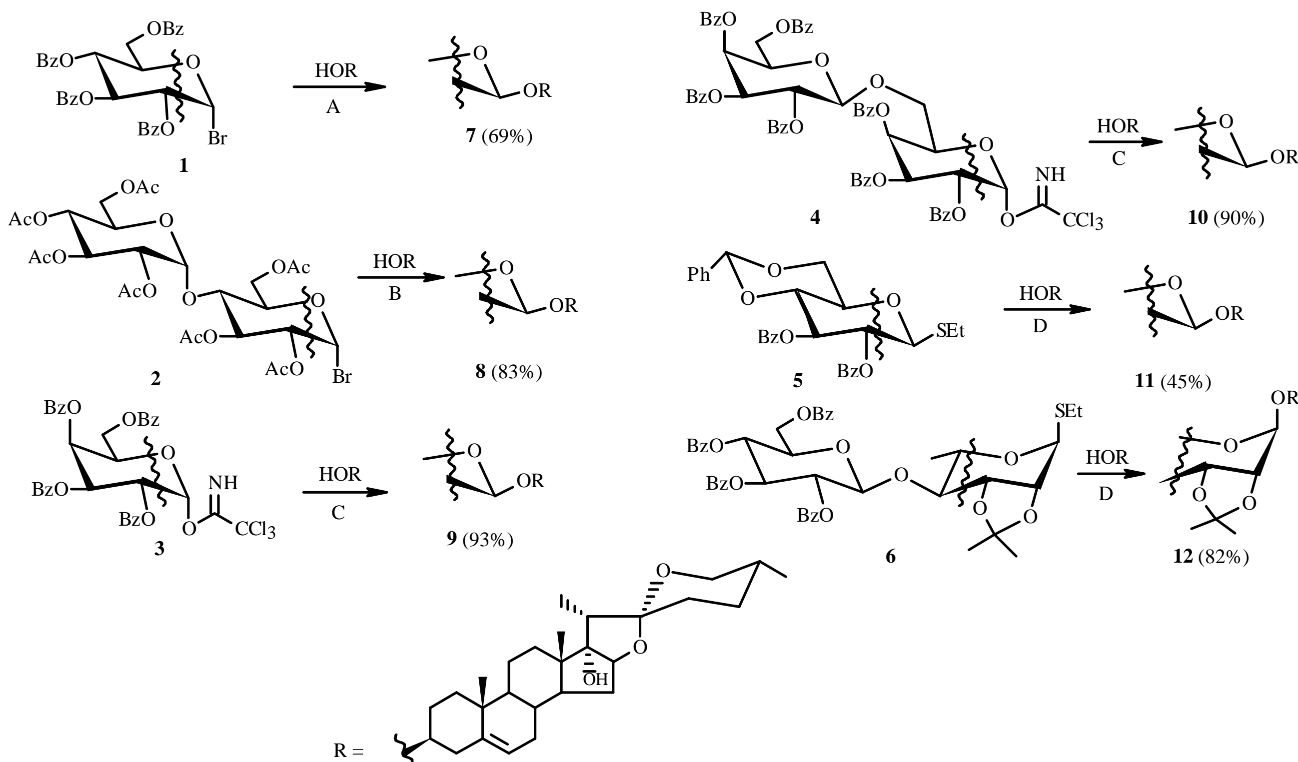

A: AgOTf (1.1 equiv.) CH_2Cl_2, -20°C → room temperature, 2h; **B:** $CdCO_3/CH_3CN$, CH_2Cl_2, 65°C;
C: TMSOTf (0.05 equiv), 4Å MS, CH_2Cl_2, ambient temperature; **D:** NIS, AgOTf, 4Å MS, CH_2Cl_2, -30°C

From the results of the reactions above, we can conclude that in the glycosylation of pennogenin only 3-OH can react with the sugar donors and no reaction occurs on 17-OH even though there is no protection at this site. The results also prove that our analysis of the reaction selectivity of two hydroxyls (3-OH, 17-OH) is correct because of the steric hindrance at 17-OH. That characteristics of pennogenin can be very convenient in the synthesis of a series of pennogenyl saponins.

We can also conclude from the reactions that in the three methods of glycosylation, the reaction between trichloroimidate and sugar donors is the best way because of moderate condition, time economy, and high yield, which accord with [10]. It is also very important to select effective conditions, including catalyzer, temperature, and so on.

EXPERIMENTAL

Solvents were purified in the usual way. Thin-layer chromatography (TLC) was performed on precoated plates of silica gel HF (0.5 mm, Qingdao, Shandong, China), with detection by charring with 10% (v/v) H_2SO_4 in EtOH or by UV detector. Column chromatography was conducted on a column (8×100 mm, or 10×240 mm) of silica gel (100–200 mesh, Qingdao, Shandong, China).Optical rotations were determined with an Ema Optical Works polarimeter. NMR spectra were recorded on a Bruker AM-300 spectrometer with Me_4Si as the internal standard. J-values are given in Hz. Mass spectra were obtained on a HP5989 or VG Quattro mass spectrometer. Elemental analyses were performed on a Perkin–Elmer Model 2400 instrument.

Pennogenin 2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranoside (7). A mixture of the glycosyl bromide **1** (99 mg, 0.15 mmol), 54 mg (0.125 mmol) pennogenin, and 33 mg 4 Å MS in dry CH_2Cl_2 (5 mL) was stirred at -20° under argon. Then a solution of AgOTf (30 mg) in anhydrous toluene (1 mL) was added and the whole kept under this condition for 4 h. Then the system was diluted by CH_2Cl_2 5 mL and filtered. The filtrate was washed with brine and saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated. Chromatography of the residue on a silica gel column (petroleum ether–EtOAc 6:1) gave 86.9 mg (69%) **7** as a white solid: $[\alpha]_D^{20}$ -10.31° (*c* 0.32, $CHCl_3$), R_f 0.28 (petroleum ether–EtOAc 4:1), $C_{61}H_{68}O_{13}$. ^1H NMR (300 MHz, $CDCl_3$, J/Hz): δ 8.01–7.27 (20H, m, 5PhH), 5.88 (1H, t, J = 9.5, gluH-3), 5.61 (1H, t, J = 9.6 Hz, gluH-4), 5.49 (1H, t, J = 9.7,

gluH-2), 5.22 (1H, s, H-6), 4.93 (1H, d, J = 7.9, gluH-1), 4.60–4.51 (2H, m, gluH-6), 4.14 (1H, m, gluH-5), 3.96 (1H, t, H-16), 3.51–3.36 (3H, m, H-26a,b,H-3), 2.15 (2H, m), 0.91 (3H, s, 19-Me), 0.89 (3H, d, J = 7.0, 21-Me), 0.78 (6H, s, 18-Me, 27-Me). ESIMS (*m/z*): 1031 (M+Na).

Pennogenin 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (8). A mixture of 37 mg (0.0868 mmol) pennogenin, dried $CdCO_3$ (37 mg, 0.217 mmol), and 2 mL anhydrous acetonitrile was refluxed under argon to ensure there was no water in the system. When the temperature fell to room temperature, the sugar donor **2** (122 mg, 0.174 mmol) was added quickly. The mixture was stirred at 65° under argon for 2–3 h until all pennogenin disappeared, as judged by TLC. Then the system was diluted by 5 mL CH_2Cl_2 and fitered through diatomite. The filtrate was concentrated and purified by silica gel column chromatography (petroleum ether–acetone 4:1) to give **8** as a white solid (75 mg 83%): $[\alpha]_D^{20}$ +91.3° (*c* 0.28, $CHCl_3$), R_f 0.25 (petroleum ether–acetone 3:1), $C_{53}H_{76}O_{21}$. ^1H NMR (300 MHz, $CDCl_3$, J/Hz): δ 5.34 (2H, m, gluH-4″, H-6), 5.19 (1H, d, 9.6, gluH-3′), 5.11 (1H, dd, J = 10.5, 8.4, gluH-2″), 4.94 (1H, dd, J = 10.5, 3.6, gluH-3″), 4.86 (1H, dd, J = 9.6, 7.8, gluH-2′), 4.52 (1H, d, J = 8.4, gluH-1″), 4.47 (1H, d, J = 7.8, gluH-1′), 4.43–4.38 (1H, m, gluH-6″a), 4.17–3.70 (6H, m, gluH-5″, gluH-6″b, gluH-4′, gluH-6′a, b, H-16), 3.59 (1H, m, gluH-5′), 3.51–3.36 (3H, m, H-3, H-26 a,b), 2.15, 2.11, 2.06, 2.04, 2.03, 1.98, 1.88 (3H, s each, each, H-OAc), 0.98 (3H, s, 19-Me), 0.96 (3H, d, J = 7.0, 21-Me), 0.79 (6H, s, 18-Me, 27-Me). ESIMS (*m/z*): 1071 (M+Na).

Pennogenin 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranoside (9). A mixture of the trichloroimidate (**3** 110 mg, 0.148 mmol, 1.7 equiv), pennogenin (37 mg, 0.0868 mmol), and 4 Å MS (33 mg) in dry CH_2Cl_2 (3 mL) was stirred at room temperature under argon. Then a solution of TMSOTf in CH_2Cl_2 (0.1 M × 80 μL, 0.09 equiv) was added. After being stirred for 1 h, the reaction mixture was quenched with Et_3N (10 mL). The mixture was concentrated and purified by silica gel column chromatography (petroleum ether–EtOAc 6:1) to give **9** as a white solid 81 mg, 93%: $[\alpha]_D^{20}$ +41.3° (*c* 0.32, $CHCl_3$), R_f 0.29 (petroleum ether–EtOAc 4:1),$C_{61}H_{68}O_{13}$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.11–7.25 (20H, m, 5PhH), 5.96 (1H, d, J = 3.3, galH-4), 5.77 (1H, dd, J = 10.2, 8.4, galH-2), 5.58 (1H, dd, J = 10.2, 3.3, galH-3), 4.90 (1H, d, J = 8.4, galH-1), 4.65 (1H, dd, J = 11.1, 6.9, galH-6a), 4.41–4.25 (2H, m, galH-6b, galH-5), 3.95 (1H, t, H-16), 3.51–3.36 (3H, m, H-26a,b, H-3), 0.98 (3H, s, 19-Me), 0.96 (3H, d, J = 7.0, 21-Me), 0.80 (3H, d, J = 6.0, 27-Me), 0.78 (3H, s, 18-Me). ESIMS (*m/z*): 1031 (M+Na).

Pennogenin 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (10). A mixture of the trichloroimidate **4** (163 mg, 0.148 mmol, 1.7 equiv) and pennogenin (37 mg, 0.0868 mmol), and 4 Å MS (33 mg) in dry CH_2Cl_2 (3 mL) was stirred at room temperature under argon. The next procedure is similar to that for the preparation of **9**. A white solid **10** was obtained (115 mg, 90%): $[\alpha]_D^{20}$ -49.4° (*c* 0.22, $CHCl_3$), R_f 0.31 (toluene–EtOAc 6:1), $C_{88}H_{90}O_{21}$. ^1H NMR (300 MHz, $CDCl_3$, J/Hz): δ 8.07–7.23 (35H, m, 7PhH), 5.88 (1H, d, J = 3.4, galH-4′), 5.84 (d, J = 3.4, H-4), 5.72 (1H, dd, J = 8.9, 10.4, H-2′), 5.60 (1H, dd, J = 3.4, 10.4, H-3′), 5.54 (1H, dd, J = 3.4, 10.4, H-3), 5.45 (1H, dd, J = 8.0, 10.4, H-2), 5.20 (1H, s, H-6), 5.03 (1H, d, J = 8.0, H-1′), 4.90 (1H, d, J = 8.0, H-1′), 4.40 (1H, dd, J = 5.4, 11.2, H-6′), 4.27–4.08 (4H, m, 2H-6′6, 2H-5′,5), 3.98–3.96 (2H, m, H-6, H-16), 3.51–3.36 (3H, m, H-26a, b, H-3), 2.15 (2H, m), 0.97 (3H, s, 19-Me), 0.95 (3H, d, J = 7.0, 21-Me), 0.77 (3H, d, J = 6.0, 27-Me), 0.75 (3H, s, 18-Me). ESIMS (*m/z*): 1505 (M +Na).

Pennogenin 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (11). Thioglycosides 5 (73 mg, 0.14 mmol), pennogenin (50 mg, 0.117 mmol), and 4Å MS (50 mg) in dry CH_2Cl_2 (2 mL) was stirred under argon at room temperature for 1h, then cooled to –30°. NIS (40 mg, 0.176 mmol) was added, followed by immediate addition of a solution of AgOTf (12 mg, 0.046 mmol) in dry toluene. After being stirred for 0.5 h the mixture was quenched with Et_3N (0.1 mL) and filtered, then concentrated. Chromatography of the residue on a silica gel column (petroleum ether–EtOAc 6:1) afforded **11** as a white solid (46 mg, 45%): $[\alpha]_D^{20}$ 8.3° (*c* 0.24, $CHCl_3$), R_f 0.35 (petroleum ether–EtOAc 4:1), $C_{54}H_{64}O_{11}$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.00–7.30 (15H, m, 3PhH), 5.75 (1H, t, J = 9.5, 9.5, gluH-3′), 5.53 (1H, s, PhCH), 5.43 (1H, dd, J = 7.9, gluH-2′), 5.22 (1H, d, J = 5.2, H-6), 4.89 (1H, d, gluH-1′), 4.34 (1H, t, gluH-4′), 4.26 (1H, dd, J = 4.9, 12.8, gluH6′a), 3.99–3.97 (2H, m, gluH-6′b, H-16), 3.67 (1H, m, gluH-5′), 3.60–3.40 (3H, m, H-3, H-26a, H-26b), 0.97 (3H, s, 19-Me), 0.95 (3H, d, J = 7.0, 21-Me), 0.78 (3H, d, J = 6.0, 27-Me), 0.76 (s, 3H, 18- e). EIMS (*m/z*): 888, 459, 105 (base).

Pennogenin 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-*iso*-propylidene-α-L-rhamnopyranoside (12). A procedure similar to that for the preparation of **11** was employed. Thioglycosides **6** (217 mg, 0.1 mmol) and pennogenin (37 mg, 0.0868 mmol) were treated with NIS (40 mg, 0.176 mmol) and AgOTf (12 mg, 0.046 mmol) to afford **12** as a white solid (85 mg, 82%): $[\alpha]_D^{20}$ -39.2° (*c* 0.33, $CHCl_3$), R_f 0.38 (petroleum ether–EtOAc 4:1), $C_{70}H_{82}O_{17}$. ^1H NMR (300 MHz, $CDCl_3$, J/Hz): δ 8.06–7.26 (20, m, Ph-H), 5.89 (1H, t, J = 9.63, gluH-3), 5.63 (1H, t, J = 9.75, gluH-4), 5.46 (1H, dd, J = 8.03, 9.52, gluH-2), 5.34–5.27(2H, m, H-6, gluH-1), 4.85 (1H, s, rhaH-1), 4.61–4.50 (2H, m, gluH-6), 4.05 (1H, m, gluH-5), 3.97 (1H, t, H-16), 3.89–3.80 (3H, m, rhaH-2, rhaH-3, rhaH-5), 3.69–3.37 (4H, m, rhaH-4, H-26a,b, H-3), 1.45–1.16 (m, 9H,

$C(CH_3)_2$, $2CH_3$), 0.98 (3H, s, 19-Me), 0.96 (3H, d, J = 7.0, 21-Me), 0.79 (6H, s, 18-Me, 27-Me). ESIMS (*m/z*): 1217 (M+Na), 1195 (M +1).

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REFERENCES

1. J. Zhou, *Pure Appl. Chem.*, 61, 457 (1989).
2. Editor in chief: H. Li, *The Genus Paris (Trilliaceae)*, Beijing: Science Press, 1998, p. 193–194.
3. C. O. Okunji, C. N. Okeke, H .C. Guguani, and M. M. Iwu, *Int. J. Crude Drug Res.*, **28**, 193 (1990).
4. W. S. Tian, *Org. Chem.*, **20**, 11 (2000).
5. C. X. Guo, T. G. LaCour, and P. L. Fuchs, *Bioorg. Med. Chem. Lett.*, **9**, 419 (1999).
6. M. Hisashi, P. Yutana, and M. Toshio, *Bioorg. Med. Chem. Lett.*, 13, 1101 (2003).
7. a) C. X. Chen, Y. T. Zhang, and J. Zhou, *Acta Botanica Yunnanica*, **5**, 91 (1983); b) C. X. Chen, J. Zhou, *Acta Botanica Yunnanica*, 3, 89 (1981); c) C. X. Chen, and J. Zhou, *Acta Botanica Yunnanica*, **5**, 219 (1983).
8. a) W. S. Tian, Q. H. Xu, and L. Chen, *Sci. China Ser. B Chem.*, **47**, 142 (2004); b) J. Y. Li, P. Zhou, *Microbiological Transformation of Pennogenin*, http://prep.istic.ac. cn/eprint/Upload//2004/ 1101949685258. doc.
9. K. Toshima and K. Tatsuba, *Chem. Rev.,* 93, 1503 (1993).
10. S. J. Deng, B. Yu, and J. M. Xie, *J. Org. Chem.*, **64**, 7265 (1999).
11. H. G. Fletcher, *Carbohydr. Chem.*, **2**, 226 (1963).
12. M. P. DeNinno and P. A. McCarthy, *J. Med. Chem.*, **40**, 2547 (1997).
13. R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, **50**, 21 (1994).
14. A. X. Li, Y. Zeng, and F. Z. Kong, *Carbohydr. Res.*, 339, 673 (2004).
15. a) A. F. Bochkov and A. C. Jain, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1**, 179 (1969); b) *Chem. Abstr*, **69**, 52437s (1968).
16. H. Yu, B. Yu, and X. Y. Wu, *J. Chem. Soc., Perkin Trans. 1*, 1445 (2000).