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Lipase-catalyzed regioselective acylation of diosgenyl saponins

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Abstract—Diosgenyl saponins were regioselectively acylated by Novozyme 435 with vinyl esters as acylating agents in THF to afford the corresponding mono- or diacyl diosgenyl saponins. © 2001 Elsevier Science Ltd. All rights reserved.

Saponins are widely distributed in terrestrial plants and some lower marine animals and possess a broad range of biological activities.[1] The notable diversity of their structures lies mainly in the 'glycoforms', e.g. the number of saponins with diosgenin as the aglycone is at least more than 30.[1] As a consequence, purification of a homogeneous saponin from the natural sources is always a formidable task. Alternatively, chemical synthesis could provide a realistic way to determine the availability of homogenous saponins,[2] thus affording new opportunities for understanding and applying this important group of natural products. On the other

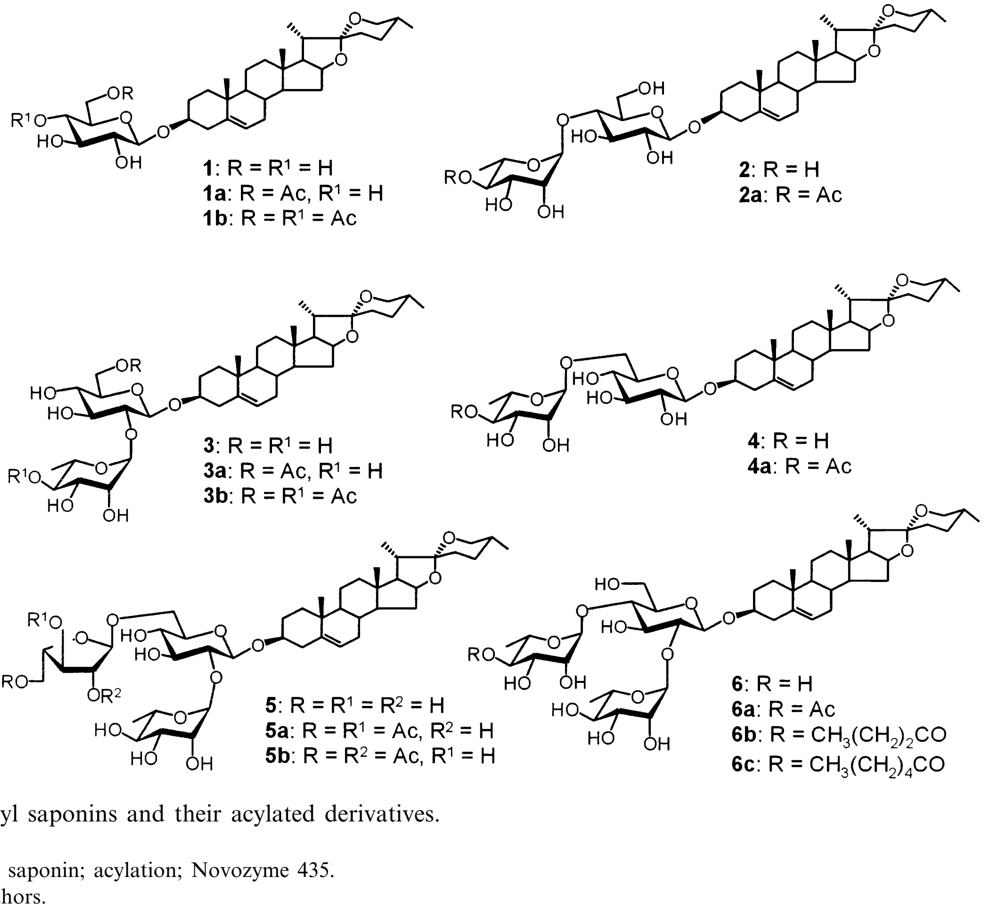

Figure 1. Diosgenyl saponins and their acylated derivatives.

Keywords: diosgenyl saponin; acylation; Novozyme 435.

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hand, decoration with acyl groups provides a further dimension for the structural diversity of the natural saponins;[1] and the acyl moieties could be essential for the bioactivity of the compounds. For example, the cholestane saponins bearing an acetylated disaccharide isolated from *Ornithogalum saudersiae* show exceptionally potent antitumor activities (with IC$_{50}$ at 0.1 nM level), while the deacylated compound is 1000 times less potent.[3] The presence of an additional acyl moiety in a saponin molecule should greatly enhance the complexity of its synthesis. A totally different synthetic strategy, especially a different protection strategy, is needed compared to the synthetic route to the mother saponin without the acyl group. Ideally, the acyl group is to be introduced onto the requisite position of the mother saponin directly; and this could be better handled by the enzymatic approach. Although lipase-catalyzed acylation is one of the most extensively studied enzymatic transformations,[4] use of saponins as substrates has rarely been reported.[5] This paper describes that regioselective acylation of diosgenyl saponins can be achieved by catalysis with Novozyme 435.

Employing diosgenyl β-D-glucopyranoside (**1**, trillin), the structurally simplest saponin, as the substrate, THF as the solvent and vinyl acetate as the acylating agent, we screened several lipases purchased from Sigma and Aldrich, i.e. PPL (lipase from Porcine Pancreas), CRL (lipase from *Candida rugosa*), MJL (lipase from *Mucor javanicus*), WGL (lipase from Wheat Germ), PFL (Amano lipase from *Pseudomonas fluorencens*), and Novozyme 435 (lipase B from *Candida antarctica* immobilized on acrylic resin). Various reaction conditions were also examined, such as the water content in THF, the molar ratio of the reactants, reaction temperature and reaction time. The reactions under PPL, MJL and WGL gave no or only a trace of the acetylation products. For CRL and PFL, diosgenyl 6′-*O*-Ac-β-D-glucopyranoside **1a** was produced as the only product, but in less than 17 and 43% yields, respectively. Fortunately, the reaction catalyzed by the immobilized lipase Novozyme 435 proceeded very well, affording **1a** and 4′,6′-di-*O*-Ac-β-D-glucopyranoside **1b** in up to a 99% yield. It is noted that Novozyme 435 has recently been successfully applied in various acylation/esterification reactions, as well as in resolution and polymerization reactions (Fig. 1).[5,6]

In the Novozyme 435 catalyzed acetylation of saponin **1**, product **1a** was isolated in 75% yield and **1b** in 17% yield after a 1 day reaction, **1a** in 64% yield and **1b** in 35% yield after a 2 day reaction, and **1a** in 53% yield and **1b** in 31% yield after a 3 day reaction. These results clearly show that the 6′-OH of **1** was preferentially acetylated over the 4′-OH, and that hydrolysis becomes apparent after a long reaction time. Saponins **2**–**6** were then treated with Novozyme 435 under similar conditions. Listed in Table 1 are the results observed. Using vinyl acetate as the acylating agent, all the reactions proceeded very well to produce the corresponding mono- and di-acetylated products in good yields (entries 2–6). It was clearly shown that the acetylation took place regioselectively at the hydroxyl groups most distant from the hydrophobic aglycone. Thus, diosgenyl α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside **2** and diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**6**, dioscin) were converted into the monoacetylated derivatives **2a** and **6a** in 91 and 86% yields, respectively; only the 4-OH of the 4′-*O*-α-L-rhamnopyranosyl moiety was acetylated. For diosgenyl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside **3**, the 6′-*O*-Ac product **3a** was isolated as the major product (53% yield) and the 6′,4″-di-*O*-Ac product **3b** in 31% yield. For disaccharide saponin **4**, the 4″-*O*-Ac product **4a** was isolated in 70% yield, while trace amounts of other products were detected on TLC. For trisaccharide saponin **5** (polyphyllin D), acetylation took place only on the hydroxyl groups on the 4′-*O*-L-arabinofuranosyl moiety, producing the 3‴,5‴-di-*O*-Ac product **5a** and the 2‴,5‴-di-*O*-Ac product **5b** in 55 and 26% yields, respectively. Other vinyl esters were then examined to acylate dioscin **6** (entries 7–10). Using vinyl *n*-butyrate and vinyl caproate as acylating agents, the corresponding 4‴-*O*-acylated products **6b** and **6c** were isolated in 64 and 40% yields, respectively. No acylated products were produced when vinyl benzoate and vinyl crotonate were used. These results show that the effectiveness of the Novozyme 435 catalyzed acylation of saponins is highly dependent on the vinyl esters used; longer chain esters are less effective for the acylation, and the acylation does not take place with conjugated vinyl esters as the donors.

The structures of the acylation products were unambiguously determined by extensive 2D NMR analysis, including ^{1}H–^{1}H COSY, HSQC and TOCSY, and were further confirmed by ESI-MS and elemental analysis.[7] In comparison to the ^{1}H and ^{13}C NMR signals of the mother saponins without acyl groups, of which the NMR signals have previously been fully assigned,[8] the acylation induced shifts (AIS)[9] were diagnostic of the

Table 1. Acylation of diosgenyl saponins catalyzed by Novozyme 435[a]

Entry	Substrate	Acyl donor	Products (yield)
1	**1**	CH_2=CHOAc	**1a** (64%), **1b** (35%)
2	**2**	CH_2=CHOAc	**2a** (91%)
3	**3**	CH_2=CHOAc	**3a** (53%), **3b** (31%)
4	**4**	CH_2=CHOAc	**4a** (70%)
5	**5**	CH_2=CHOAc	**5a** (55%), **5b** (26%)
6	**6**	CH_2=CHOAc	**6a** (86%)
7	**6**	CH_2=CHOC(O)$(CH_2)_2CH_3$	**6b** (63%)
8	**6**	CH_2=CHOC(O)$(CH_2)_4CH_3$	**6c** (40%)
9	**6**	CH_2=CHOC(O)CH=CHCH_3	Trace
10	**6**	CH_2=CHOC(O)Ph	Trace

[a] Reaction conditions: Saponin substrate (~30 mg), vinyl ester (0.5 mL), Novozyme 435 (100 mg), THF (2 mL), shaking at 40°C, 2 days (3 days for entry 5).

Table 2. Cytotoxic activities (IC$_{50}$, μM) of some diosgenyl saponin acetates

	3^a	**3a**	**3b**	**5a**	**5b**	**6**^{a}	**6a**
P388	2.24	3.2	0.8	0.3	1.4	0.46	0.5
A-549		9.6	9.4	0.5	7.9		8.2

^{a} Calculated from the corresponding literature GI$_{50}$ values.[10]

acylation position. We observed that acetylation caused deshielding of the α-carbon by 1.6–3.3 ppm, deshielding of the α-methine hydrogen by ∼1.50 ppm, and deshielding of the α-methylene hydrogen by ∼0.35 ppm.

The cytotoxic activities of some diosgenyl saponin acetates were tested against P388 (mouse leukemia) and A-549 (human pulmonary adenocarcinoma). The results are listed in Table 2. These compounds showed moderate activities. The 6′-*O*-acetylated saponin **3a**, 6′,4″-*O*-diacetylated **3b**, and 4‴-*O*-acetylated dioscin **6a** showed similar IC$_{50}$ results against P388 as that of the corresponding saponins without acetyl groups (**3** and dioscin **6**, respectively).[10]

Acknowledgements

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7. Selected analytical data: **1a**: $[\alpha]_D^{17}=-94.6$ (*c* 0.56, THF); ESI-MS: 641.6 (M+Na$^+$), 657.6 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 171.0, 141.1, 121.9, 109.4, 103.0, 81.3, 78.8, 78.5, 75.4, 75.3, 71.6, 67.0, 65.0, 63.1, 56.8, 50.4, 42.1, 40.6, 40.0, 39.5, 37.7, 37.2, 32.4 (2×C), 32.0, 31.8, 30.8, 30.4, 29.4, 21.3, 21.0, 19.6, 17.5, 16.6, 15.2. **1b**: $[\alpha]_D^{17}=-64.1$ (*c* 0.30, THF); ESI-MS: 661.6 (M+1), 683.6 (M+Na$^+$), 699.6 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 171.0 (2×C), 141.0, 122.0, 109.4, 102.7, 81.3, 79.2, 78.9, 75.0, 73.2, 69.7, 67.0, 64.5, 63.1, 56.8, 50.4, 42.1, 40.6, 40.0, 39.3, 37.6, 37.2, 32.4 (2×C), 32.0, 31.8, 30.8, 30.3, 29.4, 21.4, 21.3, 20.9, 19.5, 17.5, 16.5, 15.2. **2a**: $[\alpha]_D^{17}=-95.8$ (*c* 0.16, THF); ESI-MS: 765.7 (M+1), 787.7 (M+Na$^+$), 803.6 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 170.9, 141.0, 122.0, 109.4, 102.7, 102.3, 81.3, 78.4, 77.6, 77.4, 76.8, 76.2, 75.9, 72.7, 70.5, 67.6, 67.0, 63.0, 61.5, 56.8, 50.4, 42.1, 40.6, 40.0, 39.5, 37.6, 37.2, 32.4 (2×C), 32.0, 31.8, 30.8, 30.4, 29.4, 21.3 (2×C), 19.6, 18.1, 17.5. 16.5, 15.2. **3a**: $[\alpha]_D^{17}=-99.8$ (*c* 0.29, THF); ESI-MS: 765.7 (M+1), 787.7 (M+Na$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 171.0, 141.0, 121.9, 109.4, 102.3, 100.8, 81.3, 79.5, 78.7, 77.8, 75.0, 74.3, 73.0, 72.7, 71.7, 69.8, 67.0, 64.7, 63.1, 56.8, 50.4, 42.1, 40.6, 40.0, 39.2, 37.7, 37.3, 32.5, 32.4, 32.0, 31.9, 30.8, 30.4, 29.4, 21.3, 20.9, 19.6, 18.9, 17.5, 16.5, 15.2. **3b**: $[\alpha]_D^{17}=-80.4$ (*c* 0.28, THF); ESI-MS: 807.7 (M+1), 829.7 (M+Na$^+$), 845.7 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 171.0, 170.9, 140.9, 122.2, 109.5, 101.7, 100.2, 81.3, 79.4, 78.2, 76.9, 76.3, 75.1, 72.6, 71.6, 70.4, 67.0 (2×C), 64.6, 63.1, 56.8, 50.5, 42.1, 40.7, 40.0, 39.2, 37.6, 37.3, 32.5, 32.4, 32.0, 31.9, 30.8, 30.3, 29.4, 21.4, 21.3, 20.9, 19.6, 18.2, 17.5, 16.6, 15.2. **4a**: $[\alpha]_D^{17}=-75.3$ (*c* 0.24, THF); ESI-MS: 765.7 (M+1), 787.6 (M+Na$^+$), 803.6 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 170.9, 141.2, 121.8, 109.5, 103.2, 102.5, 81.3, 79.1, 78.7, 76.9, 75.9, 75.4, 72.4, 71.9, 70.4, 68.5, 67.2, 67.0, 63.0, 56.8, 50.4, 42.1, 40.6, 40.0, 39.7, 37.7, 37.2, 32.4, 32.3, 32.0, 31.8, 30.8, 30.6, 29.4, 21.3 (2×C), 19.6, 18.3, 17.5, 16.5, 15.2. **5a**: $[\alpha]_D^{17}=-92.7$ (*c* 0.19, THF); ESI-MS: 961.8 (M+Na$^+$), 977.8 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 170.8 (2×C), 140.9, 122.0, 109.8, 109.4, 102.2, 100.4, 81.4, 81.3 (2×C), 80.4, 78.2, 77.9 (2×C), 76.8 (2×C), 74.3, 73.0, 72.7, 69.7, 67.0, 64.7, 63.1, 61.2, 56.8, 50.5, 42.1, 40.6, 40.0, 39.1, 37.7, 37.3, 32.5, 32.4, 32.0, 31.8, 30.8, 30.3, 29.4, 21.3, 20.9, 20.8, 19.6, 18.8, 17.5, 16.5, 15.2. **5b**: $[\alpha]_D^{17}=-90.7$ (*c* 0.18, THF); ESI-MS: 961.8 (M+Na$^+$), 977.8 (M+K$^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 170.8, 170.6, 140.9, 122.0, 109.4, 106.6, 102.1, 100.5, 85.8, 82.5, 81.3, 78.2, 77.7 (2×C), 76.9, 76.1 (2×C), 74.3, 73.0, 72.7, 69.7, 67.0, 64.3, 63.0, 61.2, 56.8, 50.4, 42.1, 40.6, 40.0, 39.1, 37.6, 37.3,

32.5, 32.4, 32.0, 31.8, 30.8, 30.2, 29.4, 23.1, 20.8 (2×C), 19.6, 18.8, 17.5, 16.5, 15.2. **6a**: $[\alpha]_D^{17} = -100.8$ (*c* 0.39, THF); ESI-MS: 911.9 (M+1), 933.8 ($M+Na^+$), 949.8 ($M+K^+$); ^{13}C NMR (100 MHz, pyridine-d_5): 170.9, 140.9, 122.0, 109.4, 102.5, 102.2, 100.5, 81.3, 78.2, 78.1 (3×C), 77.2, 76.0, 74.3, 73.0, 72.8, 72.6, 70.4, 69.7, 67.7, 67.0, 63.0, 61.3, 56.8, 50.4, 42.1, 40.6, 40.0, 39.1, 37.7, 37.3, 32.5, 32.4, 32.0, 31.9, 30.8, 30.3, 29.4, 21.3 (2×C), 19.6, 18.8, 18.0, 17.5, 16.5, 15.3.

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