ENZYME-MEDIATED ACYLATION OF FLAVONOID MONOGLYCOSIDES

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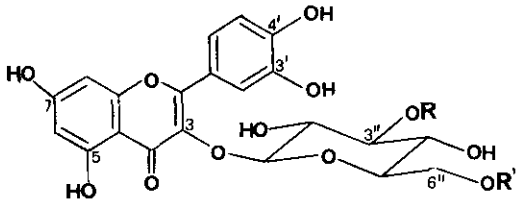
Abstract - Subtilisin catalyzes the reaction of the flavonoid glucosides 1 and 3 with trifluoroethyl butanoate ($CH_3CH_2CH_2COOCH_2CF_3$) in pyridine to afford the corresponding 3''-O-mono-, 6''-O-mono-, and 3'',6''-O-diacyl derivatives, while the rhamnoside 2 is unaffected.

Transesterification catalyzed by lipases and proteases in organic solvents[1] has proved to be a powerful methodology for the regioselective acylation of aliphatic glycols,[2] steroids,[3] mono-,[4] di- and oligosaccharides and the glycosides salicine, adenosine and uridine.[5]

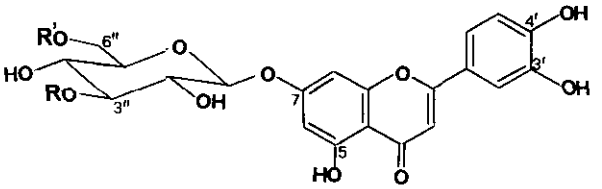
In recent years an increasing number of acyl derivatives of flavonoid glycosides has been isolated, invariably esterified at one of the sugar hydroxyls with carboxylic acids such as acetic, malonic, benzoic and, more frequently, p-coumaric, ferulic or other phenylpropanoic acids.[6] These acylglycosides can be obtained only through complex multistep synthetic procedures, and not by direct chemical esterification.[7] As a consequence, an enzyme-mediated approach to these derivatives would be of particular interest.

Here we report our results concerning subtilisin (protease Carlsberg)[5,8] catalyzed acylation of the flavonoid monoglycosides isoquercitrin (1), quercitrin (2) and luteolin-3-glucoside (3) with a "standard" activated butanoate ester and the preliminary attempts to introduce the cinnamoyl moiety.

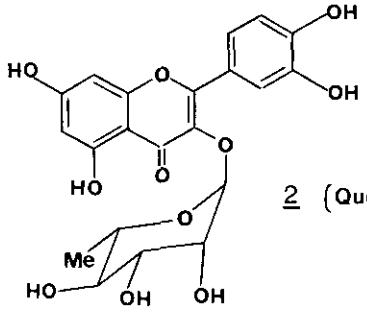
In a series of experiments, the target molecules 1-3 were treated with trifluoroethyl butanoate (TFEB) in anhydrous pyridine in the presence of subtilisin. Initial rates (Table) were comparable for glucosides 1 and 3, while the rhamnoside 2 proved to be unreactive. Subsequently the reactions were scaled up to 500 mg of substrates 1-3. Quercitrin 2 was recovered unchanged, while both 1 and 3 afforded a mixture of three products (Table). Negative FAB-ms and Daughter Ion Analysis of the products (purified by silica gel chromatography) revealed that esterification had occurred exclusively on the sugar moiety. The compounds were identified as the 6"-O-butanoyl- 1a and 3a, the 3"-O-butanoyl- 1b and 3b and the 3",6"-O-dibutanoyl- 1c and 3c derivatives from their ^1H- and ^{13}C-nmr spectra.[9]



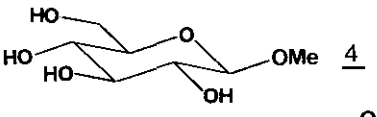
R=R'=H 1 (Isoquercitrin)
R=H R'= $COCH_2CH_2CH_3$ 1a
R= $COCH_2CH_2CH_3$ R'=H 1b
R= $COCH_2CH_2CH_3$ R= $COCH_2CH_2CH_3$ 1c



R=R'=H 3 (Luteolin-3-glucoside)
R=H R'= $COCH_2CH_2CH_3$ 3a
R= $COCH_2CH_2CH_3$ R'=H 3b
R= $COCH_2CH_2CH_3$ R'= $COCH_2CH_2CH_3$ 3c



2 (Quercitrin)



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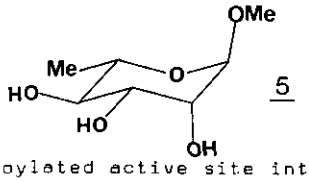

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These results also clearly indicate that the butanoylated active site intermediate is prone to receive large nucleophiles such as flavonoid glucosides, without discriminating between the positions of sugar attachment. On the other hand, the different behaviour between glucosides 1,3 and rhamnoside 2 points out the deep influence of sugar structure on reactivity. This last finding has been further confirmed by comparing the acylation of methyl-β-D-glucopyranoside (4) and methyl-α-L-rhamnoside (5): while a 0.33 M solution of 4 in pyridine containing TFEB (1 M) was completely transformed by subtilisin in 30 hours into 6-O-butanoyl- and 3,6-dibutanoyl-methyl-β-D-glucopyranoside (41 and 47 % yield respectively), 5 was acylated less than 10 % after 4 days, furnishing a mixture of the three possible regioisomers in 8 % global yields.[10]

Attempts to react 1 or 3 as well as 4 with trifluoroethyl cinnamate failed to give any products. On the other hand, butyl cinnamate was formed in 42 % yield after 7 days when trifluoroethyl cinnamate (0.10 mmol) was dissolved in pyridine (1 ml) containing n-butanol (0.30 mmol) and subtilisin (10 mg). Therefore under these conditions the cinnamoyl-enzyme intermediate is still forming. However the bulkiness of the acyl moiety inhibits the approach of large molecules, thus preventing the subsequent transfer of the cinnamoyl group.

Work is in progress to extend this acylation methodology to more complex flavonoid glycosides as well as to improve the regioselectivity and to search alternative enzymatic approaches to the phenylpropanoic derivatives.

compound	[a] initial rate µmol/h	[b] % conv	product	[c] isolated yield mg (%)
Isoquercitrin (1)	0.95	86	1a	120 (20)
			1b	76 (13)
			1c	160 (24)
Quercitrin (2)	0	0	-	-
Luteolin-3-glucoside (3)	0.52	76	3a	85 (14)
			3b	70 (12)
			3c	78 (12)

a Substrates 1-3, 50 µmol; trifluoroethyl butanoate (TFEB), 100 µmol; pyridine, 1 ml; subtilisin (prelyophilized from a water solution pH 7.8), 20 mg; 45°C; shaking at 250 rpm. Monitored by hplc : JASCO 880/PU pump; JASCO 870 UV/VIS detector; Erbasil 10 µM C_{18}/M column (250 mm x 4.6 mm). A 15 min linear gradient from 10 % to 60 % acetonitrile in water (containing 0.1 % trifluoroacetic acid) was employed. The flow rate was 1 ml/min and readings were made at 254 nm.

b Substrates 1-3, 500 mg; TFEB, 550 mg; pyridine, 5 ml; prelyophilized subtilisin, 135 mg; 45°C; 48 h. Conversion determined by hplc (see above). No appreciable conversion was detected without the enzyme.

c After silica gel chromatography, eluting with AcOEt; AcOH; H O 250;1;2.

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9. **Selected analytical and spectroscopic data:**

   1a : Hplc, t_R 12.1 min ; ^{1}H-nmr (200 MHz.,DMSO-d_6) δ 5.42 (d, J=7.5, H-1'')

4.02 (m, CH_2-6''), 1.96 (t,J=7.5) and 1.24 (sext,J=7.5) and 0.64 (t,J=7.5) aliphatic moiety; ^{13}C-nmr (50.2 MHz.,DMSO-d_6) δ sugar moiety : 100.5 (C1''), 76.2 (C3''), 74.0 (C2''), 73.8 (C5''), 70.0 (C4''), 62.8 (C6''), butanoyl moiety : 172.2, 35.1, 17.7, 13.0; negative FAB-ms : m/z (%) 533 ($M-H^-$, 100), 463 (2), 301 (52).

1b : Hplc, t_R 13.2 min; ^{1}H-nmr δ 5.55 (d, J=8.4, H-1''), 4.85 (t, J= 9.25, H-3''), 2.30 (t,J=7.5) and 1.55 (sext,J=7.5) and 0.90 (t,J=7.5) aliphatic moiety; ^{13}C-nmr δ sugar moiety : 100.6 (C1''), 77.2 (C3'' and C5''), 71.9 (C2''), 67.7 (C4''), 60.5 (C6''), butanoyl moiety : 172.1, 35.6, 17.9, 13.4; negative FAB-ms : m/z (%) 533 ($M-H^-$, 100), 463 (2), 301 (52).

1c : Hplc t_R 15.7 min; ^{1}H-nmr δ 5.52 (d, J=7.5, H-1''), 4.86 (t, J=9, H-3''), 4.04 (m, CH_2-6''), 2.31 (t,J=7.5) and 1.97 (t,J=7.5) and 1.57 (sext,J=7.5) and 1.24 (sext,J=7.5) and 0.90 (t,J=7.5) and 0.64 (t,J=7.5) butanoyl moieties; ^{13}C-nmr δ sugar moiety : 100.3 (C1''), 76.9 (C3''), 73.8 (C5''), 71.7 (C2''), 67.9 (C4''), 62.4 (C6''), butanoyl moieties : 172.1, 172.0, 35.6, 35.1, 17.9, 17.7, 13.4, 13.3; negative FAB-ms : m/z (%) 603 ($M-H^-$, 100), 533 (4), 301 (70).

3a : Hplc t_R 13.1 min; ^{1}H-nmr δ 5.12 (d, J=8, H-1''), 4.20 (m, CH_2-6''), 2.26 (t,J=7.5) and 1.42 (sext,J=7.5) and 0.68 (t,J=7.5) butanoyl moiety; ^{13}C-nmr δ sugar moiety : 103.3 (C1''), 76.2 (C3''), 74.0 (C5''), 73.0 (C2''), 70.1 (C4''), 63.4 (C6''), butanoyl moiety : 172.6, 35.4, 17.9, 13.3; negative FAB-ms : m/z (%) 517 ($M-H^-$,100), 447 (2), 321 (43), 285 (43).

3b : Hplc t_R 13.1 min; ^{1}H-nmr δ 5.25 (d, J=8, H-1''), 4.94 (t, J=8, H-3''), 2.31 (t,J=7.5) and 1.56 (sext,J=7.5) and 0.90 (t,J=7.5) butanoyl moiety; ^{13}C-nmr δ sugar moiety : 103.2 (C1''), 77.1 (C3''), 76.8 (C5''), 71.1 (C2''), 67.3 (C4''), 60.2 (C6''), butanoyl moiety : 172.2, 35.6, 18.0, 13.5; negative FAB-ms : m/z (%) 517 ($M-H^-$, 100), 447 (2), 285 (68).

3c : Hplc t_R 17.0 min; ^{1}H-nmr δ 5.29 (d, J=8, H-1''), 4.96 (t, J=10, H-3''), 4.10 (m, CH_2-6''), 2.31 (t,J=7.5) and 2.25 (t,J=7.5) and 1.57 (sext,=7.5) and 1.43 (sext,J=7.5) and 0.91 (t,J=7.5) and 0.71 (t,J=7.5) butanoyl moieties; ^{13}C-nmr δ sugar moiety : 103.2 (C1''), 76.5 (C3''), 73.6 (C5''), 70.8 (C2''), 67.8 (C4''), 62.9 (C6''), butanoyl moieties : 172.5, 172.2, 35.6, 35.2, 17.9, 17.7, 13.4, 13.1; negative FAB-ms : m/z (%) 587 ($M-H^-$,94), 517 (9), 285 (100).

10. All the compounds provided ^{1}H-nmr data consistent with the proposed structure.

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