Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2215–2218, 1994.
Printed in Great Britain.

Enzyme-mediated regioselective acylation of polyhydroxylated natural products

Bruno Danieli* and Sergio Riva°

* Dipartimento di Chimica Organica e Industriale, Universita' degli Studi di Milano - Centro CNR di Studio per le Sostanze Organiche Naturali, Via Venezian, 21 - 20133 Milano, Italy.
° CNR, Istituto di Chimica degli Ormoni, Via Mario Bianco, 9 - 20131 Milano, Italy.

Abstract: Several complex natural glycosides belonging to the flavonoid and terpenoid type have been regioselectively acylated by the action of the protease subtilisin Carlsberg and of the lipase from *Candida antarctica* in organic solvents.

During the last years, hydrolytic enzymes, and more specifically esterases, lipases and proteases, have become valuable tools in organic synthesis due to their large availability, low cost, wide substrate spectrum and no need of added cofactors. Recently a great impetus has been given by the finding that enzymatic catalysis in organic solvents exhibits additional advantages, e.g., increased substrate solubility, elimination of unwanted reactions, enhanced enzyme thermostability, and shift of the thermodynamic equilibrium from hydrolysis towards condensation.

Hydrolases have been mainly employed in stereoselective reactions, i.e. kinetic resolution of racemic mixtures and recognition of enantiotopic groups or faces in prochiral or meso-compounds. Less attention has been payed to exploit two other interesting characteristics of these enzymes, namely their chemo- and regioselectivity. Klibanov, together with his coworkers, has been the first to disclose the chemical utility of these properties, demonstrating that some lipases and proteases are excellent catalysts in organic solvents to get the selective acylations of diols, mono- and polysaccharides (1). Usually, lipases are active in apolar or slightly polar solvents: only the lipases from porcine pancreas and *Chromobacterium viscosum* could be used in pyridine for the acylation of the primary OH of monosaccharides. The lack of activity of these enzymes in DMF and towards di- and trisaccharides was circumvented by the use of the proteolytic enzyme subtilisin Carlsberg (2). We were aware of the potential offered by this methodology for the synthesis of acylated natural glycosides and later on we will present our results in this field.

Glycosides of various classes of natural products are widely destributed in nature, where they are often present esterified with aliphatic and aromatic acids (mainly acetic, malonic, *p*-coumaric and ferulic) at specific OH's of their sugar moieties. Many of these compounds are bioactive molecules or possess other

interesting properties. For instance, the acetylated cardioglycosides from *Digitalis* species are even nowadays powerful valuable heart stimulting agents. The *p*-coumarate of some kaempherol and quercetin disaccharide monoglycosides are active components (together with ginkolides) of the very popular medicinal extract of the leaves of *Ginko biloba*, which is used to increase peripheral and cerebral blood flow. Finally, complex *p*-coumarates of anthocyanins have been shown to play a fundamental role in determinig the colour and the colour variations of flowers.

In nature, the formation of these esters is the last step in the biosynthetic pathway and it is catalyzed by different acyltransferases. Enzymes belonging to this class show relative flexibility towards the acyl groups, but strict selectivity for the substrate to be esterified. In laboratory synthesis, acyltransferases are not very convenient, as they require stoichiometric amounts of the corresponding acyl-coenzyme A.

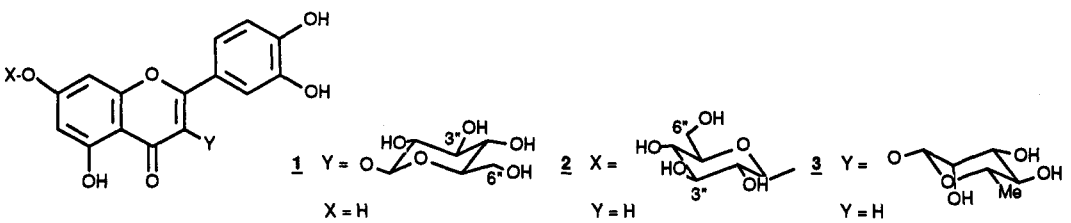
On the other hand, direct selective chemical acylation of glycosides is still a distant target because of the present lack of suitable reagents and protocols. In fact, although primary OH's are normally the most reactive towards acylation reaction, the use of an excess of acyl chlorides, anhydrides or activated esters leads invariably to a mixture of possible mono-, di- and polyesters, a clear discrimination between primary and secondary hydroxyls usually involving multistep protection and deprotection procedures.

Very recently, a simple protocol employing strictly controlled reaction conditions has been suggested to acylate the primary OH of glucosides with respect to their secondary OH's (3). However, the discrimination among primary OH's of various saccharide units present in the same molecule still remains an entangled problem and so does the regioselective acylation of one over several secondary OH's.

Enzymatic acylations of flavonoid glycosides.

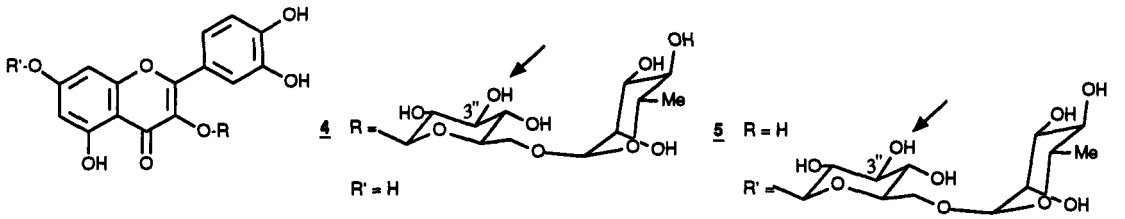
Flavonol glycosides and their esters are an important group of natural compounds widely distributed in the plant kingdom. Due to the presence of several reactive groups even on their aglycon moieties, these compounds seemed to be particularly challenging and interesting substrates for enzymatic esterification.

In a first report (4), the usual protocol (subtilisin suspended in a pyridine solution of the substrate and of the activated ester trifluoroethyl butanoate) was applied to three flavonol monoglycosides: isoquercitrin **1**, luteolin-7-glucoside **2**, and quercitrin **3**. The two glucosides **1** and **2** were acylated to afford mainly the corresponding 6"-O-butanoates, accompained by the 3"-O-mono- and 3",6"-O-dibutanoyl derivatives. By contrast, the rhamnoside **3** was recovered unaffected.

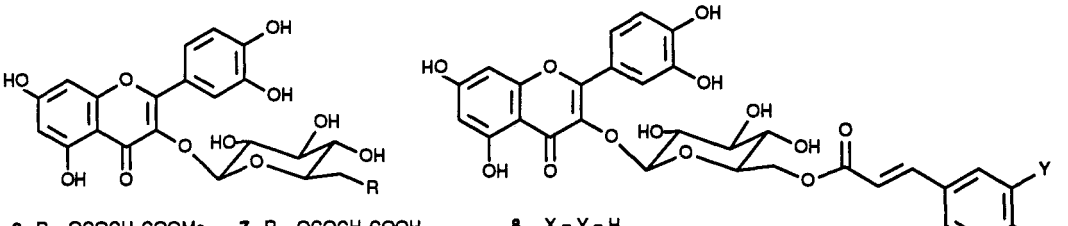


More complex flavonol disaccharide monoglycosides were then considered (5), and the results obtained showed a remarkable behaviour of subtilisin with respect of the regioselectivity of acylation. The selectivity was particularly impressive with rutin **4** and hesperidin **5**, giving single monoesters at 3" of their glucose moieties (only one hydroxyl was acylated over six secondary and four phenolic OH's).

Finally, a chemo-enzymatic approach to some 6"-O-(3-arylprop-2-enoyl) derivatives (cinnamate,



p-coumarate, feruloate) of isoquercitrin **1** was explored, to overcome the inability to directly introduce these acyl moieties by an enzyme-catalyzed reaction of **1** with the corresponding activated ester (6). This approach was based on the regioselective introduction of a methyl malonate residue at the CH_2OH of the sugar moiety by subtilisin catalysis. The thus formed mixed diester **6** was then subjected to an enzymatic chemoselective hydrolysis with biophine esterase and, finally, the malonic monoester **7** was made react in a *Knoevenagel*-type condensation with the appropriate aromatic aldehydes to afford the esters **8**, **9** and **10**.



6 R = $OCOCH_2COOMe$ **7** R = $OCOCH_2COOH$

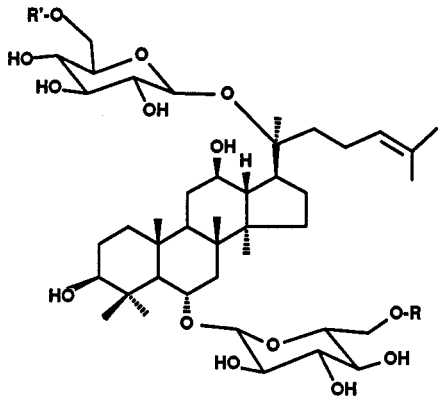
8 X = Y = H
9 X = OH Y = Y
10 X = OH Y = OMe

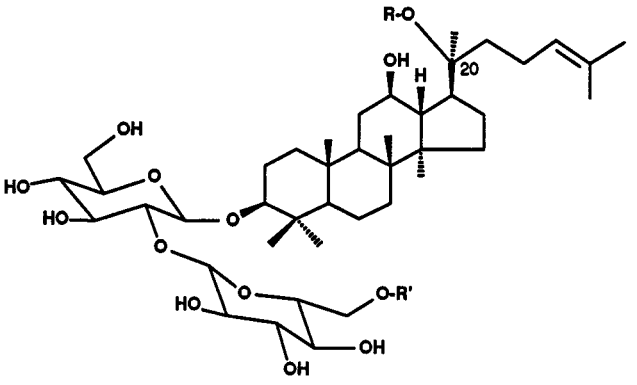
Enzymatic acylation of terpene glycosides .

Ginsenosides are an important class of dammarane-type triterpene oligoglycosides which are isolated from the water soluble portion of the dried roots and leaves of *Panax ginseng C.A. Meyer*, a plant widely used in the traditional Chinese medicine. Recently, a carefull examination of white Ginseng extracts has revealed that some ginsenosides are present as monoesters of malonic acid, the acylation site occurring invariably at one of the primary OH's of the sugar moiety. Therefore, we decided to start a research program directed to examine the behaviour of some ginsenosides towards enzymatic acylation. We first attempted to use subtilisin in pyridine solution to acylate ginsenoside Rg_1 **11**, but our efforts were unsuccessfull because a slow and non-selective acylation reaction occurred. More suitable conditions were investigated and the best results were obtained with the lipase from *Candida antarctica* in *t*-amyl alcohol using vinylacetate as acyl donor. A complete conversion to only two products in a 22:1 ratio took place. The two products were identified as 6'-O-acetyl- and 6',6"-O-diacetyl-ginsenoside Rg_1 **12** and **13**, respectively (7). The structure of **12** was unequivocally attributed on the basis of an extensive analysis of the ^{1}H-NMR spectrum at 600 MHz, which allowed to correlare the signals of the acetylated oxymethylene to the anomeric proton of the glucose moiety at C-6 OH.

Excellent results were also obtained with the more complex ginsenodides Rb_1 **16** and Rg_3 **18** (20S + 20R mixture) which gave with good selectivity the 6"-O-acetyl derivatives **17** and **19**, respectively (7).

In an attempt to introduce the malonyl residue into Rg_1, the ginsenoside was made react with





11 R = R' = H

12 R = COMe R' = H

13 R = R' = COMe

14 R = $COCH_2COOBz$ R' = H

15 R = $COCH_2COOH$ R' = H

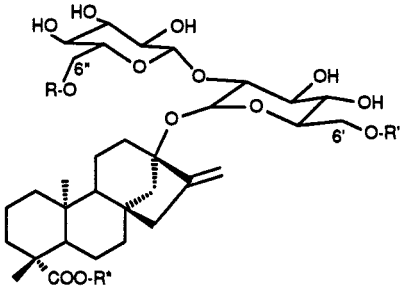
16 R = β-glc^6-β-glc R' = H

17 R = β-glc^6-β-glc R' = COMe

18 R = H R' = H (20R + 20S)

19 R = H R' = COMe (20R + 20S)

dibenzylmalonate under the usual catalytic conditions. The 6"-O-benzylmalonate **14** was obtained in very high yield (85%) and its transformation into the target malonate **15** is under current investigation. Finally, the sweet diterpene glucoside stevioside **20** gave a neat acetylation at the outer glucose to form the 6"-O-acetyl derivative **21**. Surprisingly, the steviol bioside **22** underwent acetylation at the primary OH of the inner glucose to afford **23** (7).



20 R = R' = H R* = β-glc

21 R = COMe R' = H R* = β-glc

22 R = R' = R* = H

23 R = R* = H R' = COMe

In conclusion, we have shown that complex polyhydroxylated natural compounds can be selectively esterified under the catalysis of the enzymes subtilisin and *Candida antarctica* lipase.

REFERENCES

1. A.M. Klibanov. *Acc.Chem.Res.* **23**, 114, (1990).
2. S. Riva, J. Chopineau, A.P.G. Kieboom, A.M. Klibanov. *J.Am.Chem.Soc.* **110**, 584, (1988).
3. K. Ishihara, H. Hurihara, H. Yamamoto. *J.Org.Chem.* **58**, 3791, (1993).
4. B. Danieli, P.DeBellis, G. Carrea, S. Riva. *Heterocycles* **29**, 2061, (1989).
5. B. Danieli, P.DeBellis, G. Carrea, S. Riva. *Helv.Chim.Acta* **73**, 1837, (1990).
6. B. Danieli, A. Bertario, G. Carrea, B. Redigolo, S. Riva. *Helv.Chim.Acta* **76**, 2981, (1993).
7. B. Danieli, S. Riva. Unpublished results.