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ENZYME-CATALYZED ALCOHOLYSIS OF FLAVONE ACETATES IN ORGANIC SOLVENT

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Abstract: *Pseudomonas* sp. lipase suspended in tetrahydrofuran was used to deacylate flavone acetates. Regioselectivity of the reaction has been observed.

In recent years regioselective protection of hydroxyl groups in polyhydric alcohols through enzyme-catalyzed transesterification has been studied to considerable extent and is now achievable applying two different concepts. The alcohol is reacted with an ester in the presence of a hydrolase[1] or, alternatively, converted into an ester which is subjected to selective enzymic hydrolysis in aqueous medium[2] or alcoholysis in organic solvent[3] to remove one of several esters. In contrast little, if any, information exists about the use of enzymes in protection-deprotection of hydroxyls in polyhydric phenols.

This lack of knowledge prompted us to examine the possibility of extending the methods for enzymic regioselective acylation of alcohols to polyhydric phenols and we chose flavones as substrates. These compounds are important natural products widespread in plants, some of pharmaceutical interest. Exploratory experiments of acylating flavones according to the lipase-catalyzed irreversible transesterification procedure using vinyl acetate as acylating reagent[4] gave unsatisfactory results. Therefore we decided to follow the alternative route of the selective cleavage of one ester group in flavone peracetates, working in an organic solvent due to the scanty solubility in water of both substrates and products. In this comunication we wish to report the results obtained using this procedure.

From a preliminary screening of seven commercially available

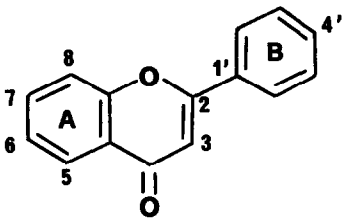
hydrolytic enzymes[5] in various organic solvents[6] on diacetylchrysin (5,7-diacetoxyflavone), *Pseudomonas* sp. lipase in tetrahydrofuran (THF) appeared best suited to our aim. Therefore, the following procedure was used: a weighed amount of flavone acetate (50-100 mg) was dissolved in dry THF (usually to a 20 mM concentration) containing *n*-butanol (5 molar equivalents) and lipase (1-30 mg/ml) was added. The suspension was shaken at 300 rpm at 42 °C and the degree of conversion monitored by HPLC (Hypersil MOS 5, acetonitrile/water mixtures as the mobile phase). After 24 hr the reaction was quenched by filtering off the enzyme, the filtrate taken to dryness *in vacuo* and the residue subjected to chromatografic separation. Identification of known products was based on comparison of their spectral data with those reported in the literature, while previously unreported compounds were unambiguously identified on the basis of their physical properties[7] and colour reactions.[8]

The results (Table) obtained with ten flavone acetates may be summarized as follows:

i) Among the four monoacetoxyflavones tested the 3-ester do not react at all, possibly due to the inaccessibility of the funtional group. The resistance to alcoholysis of the 3-ester group is observed also in 3,5,7-triacetoxyflavone.

ii) Transesterification of both 6,7- and 7,8-diacetoxyflavones was highly regioselective giving 7-acetoxy-6-hydroxyflavone (20%) and, respectively, 8-acetoxy-7-hydroxyflavone (95%). Diacetylchrysin (5,7-diacetoxyflavone) gave, in addition to 5-acetoxy-7-hydroxyflavone (47%), the product of complete alcoholysis, chrysin (42%); 7-acetoxy-5-hydroxyflavone was not detectable in the reaction mixture.

iii) Triacetylgalangin (3,5,7-triacetoxyflavone) underwent alcoholysis at position 7 and, to a slower rate, at position 5 to yield 3,5-diacetoxy-7-hydroxyflavone (48%) and 3-acetoxy-5,7-dihydroxyflavone (24%); free galangin was not detected in the reaction mixture. Triacetylapigenin (5,7,4'-triacetoxyflaflavone) gave a single product, 5,7-diacetoxy-4'-hydroxyflavone; the ester groups on the



Flavone

Table. Transesterification between flavone acetates and butanol catalysed by *Pseudomonas* sp. lipase in tetrahydrofuran.

Substrate	Enzyme (mg/ml)	Product (yield %)[a]
3-Acetoxyflavone	25	No reaction
5-Acetoxyflavone	10	5-Hydroxyflavone (94)
6-Acetoxyflavone	1	6-Hydroxyflavone (98)
7-Acetoxyflavone	10	7-Hydroxyflavone (82)
5,7-Diacetoxyflavone	30	5-Acetoxy-7-hydroxyflavone (47)
		5,7-Dihydroxyflavone (42)
6,7-Diacetoxyflavone	25	7-Acetoxy-6-hydroxyflavone (20)
7,8-Diacetoxyflavone	1	8-Acetoxy-7-hydroxyflavone (95)
3,5,7-Triacetoxyflavone	30	3,5-Diacetoxy-7-hydroxyflavone (48)
		3-Acetoxy-5,7-dihydroxyflavone (24)
4',5,7-Triacetoxyflavone	1	5,7-Diacetoxy-4'-hydroxyflavone (98)
5,7-Diacetoxy-4'-methoxyflavone	25	No reaction

[a] Determined by HPLC and/or ^{1}H NMR analysis

A-ring resisted cleavage. The closely related diacetylacacetin (5,7-diacetoxy-4'-methoxyflavone) was not affected. The resistance to cleavage of the ester groups on the A-ring in the last two compounds can find a rationalization in the following observation. Conjugation between the hydroxyl (or methoxyl) on the B-ring and the carbonyl at C-4 increases the π character of the C-2—C-1' bond

thereby favouring a planar conformation of the molecule which is possibly unsuitable for the enzyme to carry out its catalytic function.

References and notes

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5. Lipases from *Candida cylindracea* and porcine pancreas were purchased from Sigma Chemical Co.; *Chromobacterium viscosum* lipase was from FinnSugar Biochemicals; lipases from *Aspergillus niger* (AP6), *Rhizopus javanicus* (FAP-15), *Mucor javanicus* (M-10), *Pseudomonas* sp. (PS) were a gift from Amano International Enzyme Co. Four of these enzymes (*C. cylindracea, C. viscosum, A. niger* and porcine pancreatic lipases) gave no appreciable conversion (TLC and HPLC) within 24 hr.
6. Due to the solubility properties of substrates and products very hydrophobic solvents, that often afford highest enzymatic activity [A. Zaks and A. M. Klibanov, *J. Biol. Chem.* **263**, 3194 (1988)], could not be used and the choice was limited to the following: diethylether, dichlorometane, tetrahydrofuran, acetone, acetonitrile, *tert*-amyl alcohol.
7. ^{1}H-NMR data (250 MHz, $CDCl_3$):
   5-Acetoxy-7-hydroxyflavone: δ 2.45 (s, Ac-5), 6.48 (d, J=2 Hz, H-6), 6.62 (s, H-3), 6.87 (d, J=2 Hz, H-8), 7.53 (bm, H-3', H-4', H-5'), 7.85 (bm, H-2', H-6').
   7-Acetoxy-6-hydroxyflavone: δ 2.39 (s, Ac-7), 6.75 (s, H-3), 7.11 (s, H-8), 7.52 (bm, H-3', H-4', H-5'), 7.89 (bm, H-2', H-6'), 7.92 (s, H-5).
   8-Acetoxy-7-hydroxyflavone: δ 2.52 (s, Ac-8), 6.77 (s, H-3), 7.10 and 8.01 (H-5, H-6, AB-System, J=9 Hz), 7.55 (bm, H-3', H-4', H-5'), 7.78 (bm, H-2', H-6').
   3-5-Diacetoxy-7-hydroxyflavone: δ 2.31 (s, Ac-3), 2.42 (s, Ac-5); 6.55 and 6.76 (AB-System, J = 2Hz, H-6, H-8), 7.52 bm, H-3', H-4', H-5'), 7.81 (bm, H-2', H-6').
   3-Acetoxy-5,7-dihydroxyflavone: δ 2.35 (s, Ac-3), 6.31 and 6.42 (AB-System, J = 2Hz, H-6, H-8), 7.52 (bm, H-3', H-4', H-5'), 7.81 (bm H-2', H-6'), 12.18 (s, OH-5).
   5,7-Diacetoxy-4'-hydroxyflavone; δ 2.37 (s, Ac-7), 2.45 (s, Ac-5), 6.83 and 7.33 (AB-System, J = 2Hz, H-6, H-8), 6.94 (d, J = 8Hz, H-3', H-5'), 7.75 (d, J = 8Hz, H-2', H-6').
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