Effective High-Pressure Cleavage of Sterically Hindered Steroid Esters

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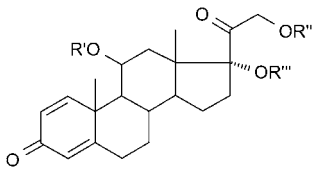
A simple and effective method to deprotect of sterically hindered steroid esters is described. Deprotection was carried out in MeOH in the presence of a catalytic amount of Et_3N under high-pressure conditions. Enzymatic, anionite, and high-pressure methods are compared.

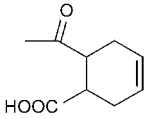
Introduction. – The problem of mild ester hydrolysis is of great importance in the chemistry of natural compounds. Corticosteroids, which are important anti-inflammatory drugs, seem to be interesting examples. Some esters are formed as superfluous by-products; in some cases, the ester group is prepared as a protection of a hydroxy group and then it should be easily removed. Starting materials are very expensive; therefore, a great need still exists for a versatile and simple process whereby esters may be hydrolyzed without heating or exposure to extreme pH values. Drastic conditions, such as the use of KO^{t}Bu, are excluded for this family of compounds because of the presence of a relatively unstable dihydroxyacetone moiety [1–3]. The usual procedure, which applies a MeOH solution of Na_2CO_3 or K_2CO_3 at low temperature [4], fails if the esters are sterically hindered.

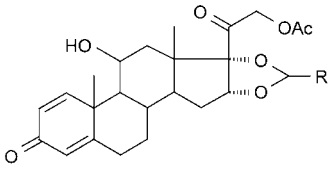
Therefore, many hindered esters of corticosteroids cannot be hydrolyzed without providing protection for the dihydroxyacetone moiety. Such an operation, followed by deprotection (*e.g.*, derivatives of vinyl ethers or dihydropyran are obtained and cleft in the presence of acids, following alkaline hydrolysis) [4], must result in a significant loss of yield. For this reason, searching for an efficient way of deprotection under neutral conditions is still justified. Various methods have been tested, such as protection of the dihydroxyacetone moiety [5] and the use of an anionite and microbiological approaches [6].

We started from plant enzymes that had been effective in certain simple ester hydrolyses [7][8]. It seemed probable that these enzymes could exhibit some selectivity. Then we used the anionite that had been recommended as a very efficient reagent [9]. Unfortunately, both of these methods failed in the case of hindered steroid esters. Yamamoto *et al.* [10] have reported a very effective hydrolysis of β-hydroxy, keto, and amino esters with aqueous MeCN under high-pressure conditions. In our case, this method was not efficient enough. Good results obtained earlier in our laboratory [11] encouraged us to apply a transesterification approach to achieve hydrolysis of sterically hindered steroid esters.

Results and Discussion. – Steroid esters are sensitive to both acidic and alkaline conditions. Therefore, the hydrolysis of such systems should be performed in neutral medium. The esters **1**–**10** of prednisolone (=(11β)-11,17,21-trihydroxypregna-1,4-diene-3,20-dione) and budesonide (=(11β,16α)-16,17-[butylidenebis(oxy)]-11,21-dihydroxypregna-1,4-diene-3,20-dione) were selected as model compounds (see *Fig.*). Unfortunately, because of steric hindrance, which makes the attack at the reaction center difficult, as well as instability of the systems having protective groups, the yields of hydrolysis of such systems by means of conventional methods are low. Attempts with aqueous-alcoholic KOH solution, methanolic ammonia, hydrazine hydrate in ethylene glycol plus KOH, KOH in a phase-transfer system in the presence of [18]crown-6 as a catalyst, KO^{t}Bu in DMSO, HCl in $CHCl_3$, AcOH in quinoline, and other reagent systems exhibited slow hydrolysis to prednisolone and simultaneous formation of decomposition products, whereas the yields of the desired products were low.



	R'	R''	R'''
1	Ac	H	H
2	Ac	H	Ac
3	Ac	Ac	H
4	Ac	Ac	Ac
5	H	Piv	H
6	H		H



	R
7	(*R*)-Pr
8	(*S*)-Pr
9	(*R*)-Furyl
10	(*S*)-Furyl

Fig. 1. *Steroid esters used for investigation of hydrolysis.* **1**, prednisolone 11β-acetate; **2**, prednisolone 11β,17-diacetate; **3**, prednisolone 11β,21-diacetate; **4**, prednisolone 11β,17,21-triacetate; **5**, prednisolone 21-pivalate (=2,2-dimethylpropanoate); **6**, prednisolone 21-tetrahydrophtalate (a 3:2 diastereoisomer mixture of hydrogen (1*R*,6*S*)- and (1*S*,6*R*)-cyclohex-3-ene-1,2-dicarboxylate; **7**, (11β,16α)-21-(acetyloxy)-16,17-[(1*R*)-butylidenebis(oxy)]-11-hydroxypregna-1,4-diene-3,20-dione; **8**, (11β,16α)-21-(acetyloxy)-16,17-[(1*S*)-butylidenebis(oxy)]-11-hydroxypregna-1,4-diene-3,20-dione; **9**, 21-(acetyloxy)-16,17-{[(1*R*)-furan-2-ylmethyl]bis(oxy)}-11-hydroxypregna-1,4-diene-3,20-dione; **10**, 21-(acetyloxy)-16,17-{[(1*S*)-furan-2-ylmethyl]bis(oxy)}-11-hydroxypregna-1,4-diene-3,20-dione.

For the study of enzymatic hydrolysis, we employed commercially available enzymatic preparations (Lipase *Sigma*, Maxatase), as well as those obtained from bacteria (*Pseudomonas aeruginosa* and *Aeromonas hydrophila*), moulds, and milled and degreased seeds of oat, sunflower, and wheat. The hydrolysis was conducted at various pH values in the range of pH 6–8. In general, the yields of enzymatic hydrolysis

of steroid esters were low. The hydrolysis of acetyl groups occurred at the 17α- and 21-positions of the steroid system but not at the 11β-position. The esters having the bulky groups (pivalate, tetrahydrophthalate) at the 21-position did not react at all.

The hydrolysis with an anionite (*Dowex*, Type I) in two solvents (MeOH and MeCN) gave low yields, irrespective of the solvent type. This protocol enabled also the hydrolysis of derivatives having a large steric hindrance, but the yield was merely 10%.

The literature and our own experience suggest that high pressure accelerates the hydrolysis of the systems having large steric hindrance. Therefore, we used a transesterification (solvolysis) reaction (*Eqn. 1*) to obtain unesterified steroid systems. Transesterification of several standard compounds was rapid and gave high yields (see *Table 1*).

$$R'COOR + MeOH \rightarrow R'COOMe + ROH \quad (1)$$

$$R = \text{steroid system}$$

Table 1. *Yields of Transesterification of Simple Carboxylic Acid Esters in 2% Et_3N/MeOH, at Room Temperature and 11 kbar Pressure for 2 h*

Ester	Yield [%]	Ester	Yield [%]
Isopropyl acetate	100	Prednisolone 21-acetate	100
tert-Butyl acetate	100	Prednisolone 21-isobutyrate	96
Isopropyl benzoate	95	Prednisolone 21-pivalate (**5**)	78
tert-Butyl benzoate	89		

Optimization of the high-pressure method was carried out by reacting **1** and **6** as steroid standards for various periods of time in the presence of Et_3N as a catalyst (see *Table 2*). The hydrolysis of prednisolone tetrahydrophthalate **6** and 11β-acetate **1** was slower than that of the standards in *Table 1*, and the yield of the product depended upon the reaction time. Unfortunately, prolongation of the reaction time favored formation of by-products, *i.e.*, of products of decomposition of the substrate and of the primary product.

Table 2. *Yield of Prednisolone and Secondary Products from Prednisolone 11β-Acetate* (**1**) *and Prednisolone 21-Tetrahydrophthalate* **6** *in 2% Et_3N/MeOH at Room Temperature and 11 kbar*

	22 h		64 h		168 h	
	prednisolone	secondary products	prednisolone	secondary products	prednisolone	secondary products
6	31	–	49 28[a])	6	81	16
1	23	traces	45 < 5[a])	9	76	19

[a]) Yield of the reaction in MeOH without Et_3N.

A comparison of yields of the hydrolysis of the steroid esters **1**–**10** by various methods is summarized in *Table 3*. For compounds **1** and **6**, the yield of prednisolone is

Table 3. *Comparison of Yields* [%][a]) *of Various Methods of Deprotection of* **1**–**10**

	Enzymatic method	Anionite method	High-pressure method	Conditions of high-pressure method[b])
1	0	5	23	26 h, *A*
			76	168 h, *A*
2	0	< 5	25	22 h, *A*
3	0	< 5	33	22 h, *A*
4	0	< 5	20	22 h, *A*
5	traces	30	75	22 h, *A*
6	traces	28	31	22 h, *A*
			81	168 h, *A*
7	30	2	76	27 h, *B*
8	25	traces	40	6 h, *B*
9	0	0	54	6 h, *B*
10	0	0	84	6 h, *B*

[a]) For the hydrolysis products, see text. [b]) *A*, 2% Et_3N/MeOH, 11 kbar; *B*, 1% Et_3N/MeCN and 10% MeOH/MeCN, 6 kbar.

given. For compounds **2**–**4**, the main product was prednisolone 11β-acetate. The epimer ratio of recovered **6** remained close to 3:2 after application of all methods.

The presented data show that the high-pressure reaction is the best method for hydrolysis of steroid esters having large steric hindrance. This method allows hydrolysis of compounds that otherwise do not undergo hydrolysis at all (enzymatically) or hydrolyze only with difficulty (using anionites).

Experimental Part

General. Prednisolone (*Schering*) and MeCN (*Merck*, *Lichrosolv* grade) were commercially available. The steroid derivatives were kindly provided by the Pharmaceutical Research Institute in Warsaw. HPLC: Product samples were transferred to calibrated vessels and diluted with MeOH to a concentration of 0.1–0.5 mg/ml prior to quant. analysis; *Shimadzu LC-6-AV* system, *Rheodyne 7125* sample injector with a 20µl sample loop, and *CR-6* data processor; detection at 242 nm, 250 × 4 mm column packed with *Lichrosorb RP 18* (10 µm); mobile phase, 45% MeOH/H_2O for determination of prednisolone, 40% EtOH/H_2O for determination of budesonide, 60% H_2O/MeOH for determination of products from **9** and **10**; flow rate 1 ml/min.

Enzymatic Hydrolysis. Hydrolysis of the steroid ester (5 mg) was carried out in phosphate buffer soln. (1 ml) at pH 6, in the presence of oat seeds or sunflower seeds (10 mg) finely ground and degreased by Et_2O extraction at r.t. After 48 h, the mixture was filtered, the solid washed with MeOH, and the filtrate transferred to a 25-ml volumetric flask.

Anionite-Catalyzed Hydrolysis. A sample of steroid ester (5 mg) in 90% MeOH/H_2O (5 ml) was stirred under reflux with *Dowex 1* × *8*, *100/200* (OH form). After 8 h, the soln. was diluted with MeOH, filtered, and transferred to a volumetric flask.

High-Pressure Transesterification. The high-pressure apparatus [12] was charged with the *Teflon* ampoule containing a soln. of 10 mg of the steroid ester in an appropriate solvent. After closing the vessel with a mobile piston, the apparatus was placed between the pistons of a hydraulic press and the pressure was raised to 11 kbar. The mixture was kept under this pressure at r.t. for several hours and then decompressed. After decompression, the product was diluted with MeOH, filtered, and transferred to a volumetric flask.

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