# SELECTIVE ACYLATION OF HYDROXY STEROIDS WITH ACYL CYANIDES

Miroslav HAVEL, Jiří VELEK, Jan POSPÍŠEK and Milan SOUČEK

*Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

Received June 14th, 1978

A mild acylation method of steroid alcohols with acyl cyanides is described. The high sensitivity of benzoyl cyanide toward steric conditions in the neighbourhood of the hydroxyl group was made use of for the separation of epimeric hydroxy steroids.

The commonest method for the protection of hydroxyl groups in steroids is the Einhorn–Hollandt method in which the compound is allowed to react in pyridine with an anhydride of a chloride of corresponding acid. The disadvantage of this procedure is the low positional and steric selectivity of the reagents used and the formation of anhydrides in acylations of steroids hydroxy acids.

In a previous paper[1] we have described a method of quantitative acylation of sugars based on the reaction of a hydroxy compound with acyl cyanide in the presence of a tertiary base. Later we found[2] that the reactivity of the hydroxyl group on acylation decreases in the following order: primary > secondary $\gg$ tertiary. A pronounced steric effect of the neighbourhood of the hydroxyl group on the reaction with acyl cyanides led us to the idea that this method could be used for acylations of steroids. We also expected that the lower reactivity of acyl cyanides in comparison with acyl chlorides[3] would result in a proportional increase in the selectivity of the reaction.

For comparative studies we used the easily accessible benzoyl cyanide[4] as the acylation reagent and steroid compounds with one to three free hydroxy groups as the substrate (Table I). According to expectation[2] tertiary steroids alcohols did not react. Derivatives were formed only from compounds with primary or secondary hydroxy groups. Semiquantitatively determined reactivities of the hydroxy groups with benzoyl cyanide can be arranged in the following sequence: 21 > 17β > 3β, > 6α,3α $\gg$ 20α > > 20β > 7β $\gg$ 7α > 6β > 22β > 22α, which is in agreement with the potential 1,3-interactions of other conformational effects.

The differences in the rates of acylations of some epimeric alcohols could be used even for their preparative separation. Thus, for example, the mixture after benzoyla-

TABLE I

Benzoylation of Hydroxy Steroids with Benzoyl Cyanide

| Compound[a] | X[b] Time, h | M.p., °C (ref.) | Calculated/Found % C | % H |
|---|---|---|---|---|
| 3β-Hydroxy-5-cholestene | 2 | 147—149 | — | — |
| | 0·2 | 146·6 | | |
| 3β-Hydroxy-5,7,22-ergostatriene | 3 | 161—163 | — | — |
| | 48 | 168 | | |
| 17β-Hydroxy-4-androsten-3-one | 2 | 188—189 | — | — |
| | 0·1 | 191—193 | | |
| 3β,17β-Dihydroxy-17α-methyl-5-androstane | 1·1 | 200—202[c] | 79·06 | 8·74 |
| | 0·3 | | 79·37 | 8·88 |
| 3β-Acetoxy-5α-chloro-6β-hydroxyandrosten-17-one | 3 | 225—227 | 69·26 | 6·71 |
| | 0·6 | | 69·63 | 6·47 |
| 3β,17β-Dihydroxy-5α-androst-1-one | 3 | 199—200 | 79·14 | 8·05 |
| | 4 | | 79·48 | 7·68 |
| 17β-Hydroxy-5α-androst-2-ene | 2 | 142—144 | 82·81 | 9·37 |
| | 0·1 | | 83·49 | 9·05 |
| 17α,21-Dihydroxy-4-pregnene-3,20-dione | 2 | 233—235 | 74·87 | 7·61 |
| | 0·1 | | 74·64 | 7·61 |
| 3α-Hydroxy-5α-cholestane | 6 | 99—101 | — | — |
| | 0·3 | 100—103 | | |
| 3β-Hydroxy-5α-cholestane | 3 | 130—132 | — | — |
| | 0·1 | 131—136 | | |
| 6α-Hydroxy-5α-cholestane | 3 | 100—103 | — | — |
| | 0·4 | 103—105 | | |
| 6β-Hydroxy-5α-cholestane | 10 | 96·5 | 82·87 | 10·64 |
| | 4 | | 82·60 | 10·85 |
| (20*S*)-Hydroxy-3β-acetoxy-5-pregnene | 3 | 153—155 | — | — |
| | 4 | 153—154 | | |
| (20*R*)-Hydroxy-3β-acetoxy-5-pregnene | 6 | 170—171 | — | — |
| | 60 | 172—173 | | |
| (22*R*, 25*R*)-22-Hydroxy-3β-acetoxy-26-acetamido-5-cholestene | 6 | 85— 90 | 75·33 | 9·15 |
| | 24 | | 75·04 | 9·22 |
| (22*S*, 25*R*)-22-Hydroxy-3β-acetoxy-26-acetamido-5-cholestene | 6 | 221—222 | 75·33 | 9·15 |
| | 96 | | 75·11 | 9·38 |

[a] All the experiments were carried out with a mixture of acetonitrile and triethylamine in a 2 : 1 ratio; the yields were practically quantitative. [b] Molar ratio of benzoyl cyanide to hydroxy steroid. [c] Monobenzoate.

tion of epimeric 3- (or 6-)hydroxycholestanes contained the 3β (or 6α) benzoate exclusively in addition to the unreacted 3α (or 6β) hydroxy compound. In contrast to this, in steroids with a hydroxyl group on the conformationally mobile side chain the differences in acylation rate of epimers are less pronounced. In spite of this it was possible to separate analogously from a mixture of (20*R*,*S*)-3β-acetoxy-20--hydroxy-5-pregnenes the less reactive 20*R* isomer, and from a mixture of (22*R*,*S*; 25*R*)-3β-acetoxy-22-hydroxy-26-acetamido-5-cholestenes the less reactive 22*S* isomer. The yields of the absolutely pure hydroxy steroids were higher than 80%.

Some other acyl cyanides also react with steroid alcohols equally well; for example acetyl cyanide, palmitoyl cyanide and ethoxycarbonyl cyanide (Table II).

## EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. Optical rotations were measured in chloroform at 24°C. Samples for analysis were dried at 80°C/0·4 Torr.

### General Procedure for Acylations of Steroids

Hydroxy steroid (0·1 mmol) was dissolved in a mixture of acetonitrile (2 ml) and triethylamine (1 ml). Acyl cyanide (0·3—1·0 mmol) was added to the solution and the reaction course was followed by thin layer chromatography on silica gel. When the hydroxy steroid had disappeared the unreacted acyl cyanide was hydrolysed by addition of water (0·1 ml) and the yellow-red mixture was evaporated. The residue was dissolved in benzene (2—5 ml) and filtered through a small column (5 × 1) of silica gel or alumina. Elution with benzene or benzene–ether mixture (depending on the polarity of the compound) gave the corresponding acyl derivative usually in quantitative yield.

TABLE II
Reaction of Hydroxy Steroids with Acyl Cyanides RCOCN

| Steroid[a] | R | M.p., °C (ref.) | |
|---|---|---|---|
| 3β-Hydroxy-5-cholestene | $CH_3$ | 112—114 | (114·5) |
| 3β-Hydroxy-5,7,22-ergostatriene | $CH_3$ | 176—177 | (181) |
| 3β-Hydroxy-5-cholestene | $CH_3(CH_2)_{14}$ | 77— 79 | (78·5) |
| 3β-Hydroxy-5-cholestene | $C_2H_5O$ | 83— 84 | (83—84) |
| 3β-Hydroxy-5-cholestene | $C_2H_5O$ | 101—103 | (105—106) |
| Methyl 3α,7α,12α-trihydroxycholate | $C_2H_5O$ | 178—178·5 | (177—178) |

[a] Molar ratio of acyl cyanide to steroid was 3 : 1; the yields of acylations were practically quantitative.

Competitive Benzoylation

*A*) Benzoyl cyanide (40 mg) was added to a solution of an equimolecular mixture of 6α- and 6β-hydroxy-5α-cholestane (78·0) mg in acetonitrile (2 ml) and triethylamine (1 ml). The acylation course was followed by thin layer chromatography of the reaction mixture analysed at 5 min intervals. Thin-layer chromatography was carried out on silica gel in benzene with 15% of ether. After 30 min (100% conversion of 6α-hydroxy steroid) the unreacted benzoyl cyanide was hydrolysed with water (0·1 ml) and the mixture evaporated.

*B*) For a 100% conversion of the equatorial 3-hydroxy steroid in an equimolecular mixture of 3α- and 3β-hydroxy-5α-cholestane, using procedure *A*), only 5 min sufficed. 3β-Benzoate and the unreacted 3α-hydroxycholestane were obtained in quantitative yields.

*C*) When an equimolecular mixture of 20α- and 20β-hydroxy-3-acetoxypregnene was submitted to reaction according to procedure *A*) the 20α-hydroxy compound was converted quantitatively in 4 h. The unreacted 20β-hydroxy derivative (83%) was separated from 20α- and 20β-benzoates chromatographically. The mixture of both benzoates was hydrolysed and separated chromatographically on silica gel. 3β,20α-Dihydroxy-5-pregnene was obtained in a 91% yield and 3β,20β--dihydroxy-5-pregnene in a 14% yield.

*D*) On benzoylation of an equimolecular mixture of 7α- and 7β-hydroxy-5α-cholestane according to procedure *A*) 7β-hydroxy steroid was acetylated completely within 16 h. Chromatography afforded 7β-benzoate (98%) and the unreacted 7α-hydroxycholestane (96%).

*Our thanks are due to the analytical department of our Institute (head Dr J. Horáček) for carefully performed elemental analyses.*

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Translated by Ž. Procházka.