ONE-POT ESTERIFICATION AND SELECTIVE 3α-ACETYLATION OF CHOLIC AND DEOXYCHOLIC ACID

Ksenija KUHAJDA, Julijan KANDRAC, Vera CIRIN-NOVTA and Du¿an MILJKOVIC

Institute of Chemistry, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 3, 21000 Novi Sad, Yugoslavia

Received September 20, 1995
Accepted March 19, 1996

A simple one-pot esterification and selective 3α-acetylation of cholic and deoxycholic acid with selected esters of acetic acid (ethyl, propyl and butyl) in the presence of catalytic amount of 4-toluenesulfonic acid and water has been described. The yields of the obtained esters varied in range of 74–95%.

Key words: Bile acids; Selective 3α-acetylation; Esterification.

Cholic and deoxycholic acids (**1a** and **1b**) represent important precursors for the commercial production of corticosteroids[1–3] and litocholic acid[4]. In the reported procedures, esterification of carboxyl group as well as selective acylation of 3α-hydroxyl function represent the first two steps of the synthesis. Conventional methods for esterification of bile acids involve their reaction with the corresponding absolute alcohol (usually methanol or ethanol) in the presence of catalytic amount of concentrated mineral acid[5] (hydrochloric, sulfuric or perchloric) or 4-toluenesulfonic acid[6]. The acetyl protecting group has been more widely used in transformation of bile acids, mainly due to the ease of its preparation, stability in various reaction conditions, while at the same time it can be easily removed by hydrolysis. Generally there are two procedures for the preparation of 3α-acetates: the acetic anhydride–pyridine method[7–10] and the acetic anhydride–acetic acid–perchloric acid method[11–12].

In this work we wish to report a novel procedure for one-pot esterification and 3α-acetylation of cholic (**1a**) and deoxycholic (**1b**) acid with suitable esters of acetic acid (ethyl acetate, propyl acetate and butyl acetate). The reactions were carried out at reflux temperature, in the presence of a catalytic amount of 4-toluenesulfonic acid and water (Scheme 1).

Under these reaction conditions, all the mentioned esters of acetic acid reacted with cholic (**1a**) and deoxycholic (**1b**) acid with high regioselectivity, whereupon the corresponding 3α-acetoxy derivatives **2** were obtained in high yield. The reaction of cholic acid with ethyl acetate gave 85% yield of ethyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholanate (**2a**). The similar reaction with propyl acetate gave 95% yield of the correspond-

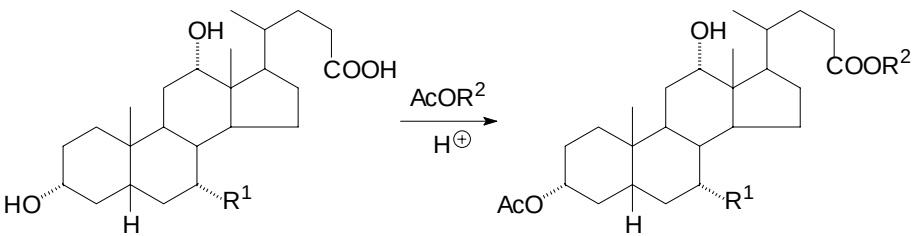
ing propyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholanate (**2b**), since the esterification with butyl acetate afforded butyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholanate (**2c**) in a yield of 86%. Reaction of the same esters (ethyl acetate, propyl acetate and butyl acetate) with deoxycholic acid (**1b**) gave the corresponding 3α-acetoxy esters (**2d–2f**) in shorted reaction time but in slightly lower yields (74–79%). This reaction might be useful for further functionalization of cholic acid at C-7 and C-12 i.e. of deoxycholic acid at C-12.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker AC 250 E instrument (250 MHz) in deuteriochloroform with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ-scale) and the coupling constants (*J*) in Hz. Mass spectra were obtained using a Kratas MS 80 spectrometer. The IR spectra were recorded in chloroform on a Perkin–Elmer 457 spectrometer, wavenumbers are given in cm^{-1}. Thin-layer chromatography (TLC) was performed on DC-Alufolien Kieselgel 60 F_{254} (Merck). Column chromatography was carried out on Kieselgel 60.

General Procedure for Preparation of 3α-Acetoxy Esters of Bile Acids **2a–2f**

A solution of bile acid (0.5 g; ca 1.3 mmol), the corresponding ester of acetic acid (15 ml), 4-toluenesulfonic acid (0.05 g; 0.3 mmol) and water (0.15 ml) was stirred at reflux temperature. The course of the reaction was followed by TLC using chloroform–acetic acid–acetone (6 : 0.5 : 3.5) as developing solvent. The reaction mixture was cooled to room temperature, poured into saturated aqueous $NaHCO_3$ and extracted with chloroform. The extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated in vacuo to afford the crude product in a form of pale yellow oil. After



1	R^1
a	OH
b	H

2	R^1	R^2
a	OH	CH_2CH_3
b	OH	$(CH_2)_2CH_3$
c	OH	$(CH_2)_3CH_3$
d	H	CH_2CH_3
e	H	$(CH_2)_2CH_3$
f	H	$(CH_2)_3CH_3$

SCHEME 1

chromatographic purification on a column of silica gel (50 g; chloroform–acetone, 9 : 1) the pure 3α-acetoxy ester of bile acid was obtained as a colourless oil.

Ethyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholanate (**2a**). A solution of cholic acid (0.2 g; 0.49 mmol), ethyl acetate (6 ml), 4-toluenesulfonic acid (0.02 g; 0.1 mmol) and water (0.06 ml) was stirred at reflux temperature for 48 h. Yield 199 mg (85%) of title compound, the crude product contains a small amount of ethyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholanate. IR spectrum: 3 440 (OH); 1 740 and 1 255 (OAc); 1 720 (C=O ester). ^{1}H NMR spectrum: 0.69 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 0.98 d, 3 H, *J*(21,20) = 6.2 (3 × H-21); 1.28 t, 3 H, *J* = 7.2 ($OCH_2\mathbf{CH}_3$); 2.02 s, 3 H (OAc); 3.86 bs, 1 H (H-12β); 3.99 bs, 1 H (H-7β); 4.12 q, 2 H, *J* = 7.2 ($O\mathbf{CH}_2CH_3$); 4.57 m, 1 H (H-3β). High resolution mass spectrum, *m/z*: found 478.3273, for $C_{28}H_{46}O_6$ calculated *m/z* 478.3294. Mass spectrum, *m/z* (%): 478 (4), 460 (40), 442 (59), 382 (100).

Propyl 3α-acetoxy 7α,12α-dihydroxy-5β-cholanate (**2b**). Reaction time 6 h, yield 95%. IR spectrum: 3 480 (OH), 1 740 and 1 250 (OAc), 1 720 (C=O ester). ^{1}H NMR spectrum: 0.69 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 0.94 d, 3 H, *J*(21,20) = 6.0 (3 × H-21); 1.00 t, 3 H, *J* = 6.9 ($OCH_2CH_2\mathbf{CH}_3$); 1.66 m, 2 H, *J* = 6.9 ($OCH_2\mathbf{CH}_2CH_3$); 2.00 s, 3 H (OAc); 3.98 bs, 1 H (H-12β); 4.00 bs, 1 H (H-7β); 4.02 t, 2 H, *J* = 6.7 ($O\mathbf{CH}_2CH_2CH_3$); 4.5 m, 1 H (H-3β). High resolution mass spectrum, *m/z*: found 492.3491, for $C_{29}H_{48}O_6$ calculated *m/z* 492.3450. Mass spectrum, *m/z* (%): 492 (5), 474 (20), 456 (30), 396 (100).

Butyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholanate (**2c**). Reaction time 4.5 h, yield 86%. IR spectrum: 3 480 (OH), 1 730 and 1 250 (OAc), 1 720 (C=O ester). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19); 0.95 d, 3 H, *J*(21,20) = 6.2 (3 × H-21); 0.98 t, 3 H, *J* = 7.0 ($OCH_2CH_2CH_2\mathbf{CH}_3$); 1.40 m, 2 H, *J* = 7.2 ($OCH_2CH_2\mathbf{CH}_2CH_3$); 1.60 m, 2 H, *J* = 6.6 ($OCH_2\mathbf{CH}_2CH_2CH_3$); 2.05 s, 3 H (OAc); 3.88 bs, 1 H (H-12β); 4.02 bs, 1 H (H-7β); 4.05 t, 2 H, *J* = 6.8 ($O\mathbf{CH}_2CH_2CH_2CH_3$); 4.58 m, 1 H (H-3β). High resolution mass spectrum, *m/z*: found 506.3597, for $C_{30}H_{50}O_6$ calculated 506.3606. Mass spectrum *m/z* (%): 506 (13), 488 (45), 470 (70), 410 (100).

Ethyl 3α-acetoxy-12α-hydroxy-5β-cholanate (**2d**). Reaction time 24 h, yield 75%. IR spectrum: 3 420 (OH), 1 735 and 1 250 (OAc), 1 720 (C=O ester), 1 250 (C–O). ^{1}H NMR spectrum: 0.68 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 0.97 d, 3 H, *J*(21,20) = 6.2 (3 × H-21); 1.26 t, 3 H, *J* = 7.1 ($OCH_2\mathbf{CH}_3$); 2.02 s, 3 H (OAc); 3.99 bs, 1 H (H-12β); 4.12 q, 2 H, *J* = 7.1 ($O\mathbf{CH}_2CH_3$); 4.71 m, 1 H (H-3β). High resolution mass spectrum, *m/z*: found 402.3119, for $C_{26}H_{42}O_3$ calculated 402.3133; found 444.3238, for $C_{28}H_{44}O_4$ calculated 444.3239. Mass spectrum, *m/z* (%): 444 (38), 402 (60), 385 (70), 255 (100).

Propyl 3α-acetoxy-12α-hydroxy-5β-cholanate (**2e**). Reaction time 3.5 h, yield 74%. IR spectrum: 3 520 (O–H); 1 730 and 1 250 (OAc), 1 715 (C=O ester). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 0.92 d, 3 H, *J*(21,20) = 6.0 (3 × H-21); 1.00 t, 3 H, *J* = 6.9 ($OCH_2CH_2\mathbf{CH}_3$); 1.64 m, 2 H, *J* = 6.9 ($OCH_2\mathbf{CH}_2CH_3$); 2.08 s, 3 H (OAc); 3.98 bs, 1 H (H-12β); 4.02 t, 2 H, *J* = 6.8 ($O\mathbf{CH}_2CH_2CH_3$); 4.68 m, 1 H (H-3β). High resolution mass spectrum, *m/z*: found 458.3381, for $C_{29}H_{46}O_4$ calculated 458.3396. Mass spectrum, *m/z* (%): 458 (40), 416 (65), 255 (100).

Butyl 3α-acetoxy-12α-hydroxy-5β-cholanate (**2f**). Reaction time 3 h, yield 79%. IR spectrum: 3 520 (OH), 1 735 and 1 250 (OAc), 1 720 (C=O ester). ^{1}H NMR spectrum: 0.68 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 0.93 d, 3 H, *J*(21,20) = 6.2 (3 × H-21); 0.96 t, 3 H, *J* = 7.2 ($OCH_2CH_2CH_2\mathbf{CH}_3$); 1.40 m, 2 H, *J* = 7.1 ($OCH_2CH_2\mathbf{CH}_2CH_3$); 1.63 m, 2 H, *J* = 6.6 ($OCH_2\mathbf{CH}_2CH_2CH_3$); 2.02 s, 3 H (OAc); 3.99 bs, 1 H (H-12β); 4.06 t, 2 H, *J* = 6.6 ($O\mathbf{CH}_2CH_2CH_2CH_3$); 4.71 m, 1 H (H-3β). High resolution mass spectrum, *m/z*: found 490.3688, for $C_{30}H_{50}O_5$ calculated *m/z* 490.3657. Mass spectrum, *m/z* (%): 490 (2), 472 (60), 2 545 (100).

The authors thank Mr S. Addison, Department of Chemistry, University of Newcastle upon Tyne, for the mass measurements. This work was supported by the Serbian Ministry of Science and Technology.

REFERENCES

1. Sarett L. H.: J. Biol. Chem. *162*, 601 (1946).
2. Turner R. B., Mattox V. R., Engel L. L., McKenzie B. F., Kendall E. C.: J. Biol. Chem. *166*, 345 (1946).
3. Sarett L. H.: J. Am. Chem. Soc. *70*, 1454 (1948); *71*, 2443 (1949).
4. Sarel S., Yanika Y.: J. Org. Chem. *24*, 2018 (1959).
5. Harano T., Fugita C., Harano K., Yamosaki K.: Steroids *30*, 393 (1977).
6. Dayal B., Speck J., Bagan E., Tint G. S., Salen G.: Steroids *37*, 239 (1981).
7. Plattner P. A., Heusser H.: Helv. Chim. Acta *27*, 748 (1944).
8. Fieser L. F.: J. Am. Chem. Soc. *71*, 3935 (1949).
9. Fieser L. F., Rajagapalan S.: J. Am. Chem. Soc. *72*, 5530 (1950).
10. Schwartz V., Pihera P., Halaskova J.: Cesk. Farm. *33*, 327 (1984).
11. Wieland H., Dane E., Scholz E.: Z. Physiol. Chem. *211*, 261 (1932).
12. Miljkovic D., Kuhajda K., Hranisavljevic J.: Review of Research, Faculty of Sciences, University of Novi Sad *12*, 39 (1982).