Synthesis of dinorcholane and 5β-cholane derivatives

Lutfun Nahar[a,b] and Alan B. Turner[a]

[a]*Japp Laboratory, Department of Chemistry, University of Aberdeen, Aberdeen AB24 3UE, Scotland, UK*
[b]*Present address: Division of Microbiology and Immunology, The Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB, UK*

The convenient synthesis of seven dinorcholane and 5β-cholane derivatives, 3β-acetoxy-23,24-dinorchol-5-en-22-oyl methoxy anhydride (**2**), 3β-acetoxy-23,24-dinorchol-5-en-22-oyl chloride (**3**), 3β-acetoxy-23,24-dinorchol-5-en-22-ol (**4**), 3α-acetoxy-5β-cholan-24-oic acid (**6**), 3α-acetoxy-5β-cholan-24-oic acid ethyl ester (**7**), 3-oxo-5β-cholan-24-oic acid (**8**) and 3-oxo-5β-cholan-24-oic acid ethyl ester (**9**) have been described. Full spectroscopic data for these compounds are presented.

Keywords: lithocholic acid, dinorcholane, reduction, esterification, NMR

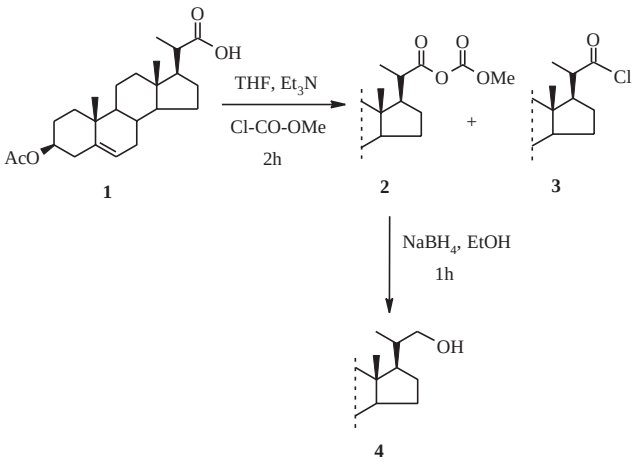
Steroids are a group of biologically active molecules widely found in both plant and animal kingdoms. The fact that small changes in the functionalities attached to the steroid nucleus can render significant changes in their biological activity has prompted synthetic chemists to carry out structural modifications of steroids.[1–3] Both 3β-acetoxy-23,24-dinorchol-5-en-22-oic acid (**1**) and lithocholic acid (**5**) have one functional group at each end of the molecule, and can easily be modified to derivatives of pharmaceutical importance. As part of our on-going studies on the synthesis of various steroidal monomers and dimers,[4–6] we report here on the synthesis of seven steroid molecules (**2**–**4**, **6**–**9**) including the novel 3β-acetoxy-23,24-dinorchol-5-en-22-oyl methoxy anhydride (**2**) and their full spectroscopic data.

Mixed anhydrides are prepared from corresponding acids using methyl chloroformate or ethyl chloroformate. The reaction of 3β-acetoxy-23,24-dinorchol-5-en-22-oic acid (**1**) in THF with Et_3N and methyl chloroformate yielded a mixture (2:1) of the 3β-acetoxy methoxy anhydride **2** and the 3β-acetoxy chloride **3** (Scheme 1) which were separated by VLC (15% EtOAc in pet-ether). The IR spectrum of **2** showed a strong absorption band at 1809 cm^{-1} which was characteristic of an anhydride carbonyl. The ^1H NMR spectrum displayed a 3H singlet at δ 3.85 assignable to the carbomethoxy group. In the ^{13}C NMR spectrum (Table 1), apart from the signals associated with the carbons of **1**, there were two signals at δ 55.8 and 150.0 were, respectively, for the methyl and the carbonyl carbons of the carbomethoxy group at C-22. The HRFABMS spectrum revealed the $[M+H]^+$ ion at *m/z* 447.2746 which confirmed the molecular formula $C_{26}H_{38}O_6$ for **2**, which is a novel synthetic steroid.

The IR spectrum of **3** exhibited a strong band at 1805 cm^{-1} which was characteristic for an acyl carbonyl. The FABMS spectrum of **3** revealed the $[M+H]^+$ ion at *m/z* 407 (~75%) and 409 (~25%) due to the presence of two isotopes of chlorine, and confirmed the molecular formula $C_{24}H_{35}ClO_3$ for this compound. The m.p. and ^{13}C NMR data (Table 1) of **3** were in good agreement with the published data.[7,8] However, published ^{13}C NMR data[8] were only for selected carbons. Therefore, the complete ^{13}C NMR data of **3** are presented here.

3β-Acetoxy alcohol **4** was synthesised from **2** by reduction using 1 molar equivalent of $NaBH_4$ (Scheme 1) and identified by comparison of its m.p., IR and ^1H NMR data with literature data.[8,9] The identity was confirmed further from its ESIMS data where the $[M+Na]^+$ ion was observed at *m/z* 353 and also the presence of the oxymethylene at C-22 was evident from the signal at δ 68.0 in its ^{13}C NMR spectrum (Table 1).

Lithocholic acid (**5**) was acetylated to afford the 3α-acetoxy acid **6**[10,11] which was then used for the synthesis of ethyl 3α-acetoxy cholanate (**7**). Methoxy anhydride lithocholate was



Scheme 1 Synthesis of dinorcholane derivatives (Δ^5) 2–4.

synthesised from lithocholic acid using Et_3N and ethyl chloroformate.[12] Similar reaction protocol was adopted for the synthesis of **7** using methyl chloroformate and EtOH (Scheme 2). The presence of an ethyl ester group in **7** (instead of acid in **6**) was evident from the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 447 and 469 observed in its FABMS spectrum. The IR spectrum exhibited the absorption band at 1737 cm^{-1} owing to the ester carbonyl group in the molecule. In the ^1H NMR spectrum of **7**, in addition to the signals found in the ^1H NMR spectrum of **6**, a deshielded 2H quartet at δ 4.07 (J = 7.2 Hz) and a 3H triplet at δ 1.21 (J = 7.2 Hz) confirmed the esterification of **6** and the formation of **7**. The ^{13}C NMR spectrum (Table 1) contained signals at δ 60.2 and 14.3, for the oxymethylene and methyl carbons of the ethyl group. In the ^1H–^{13}C HMBC spectrum, a long-range (^3J) correlation from the oxymethylene protons (δ 4.07) to the ester carbonyl carbon (δ 174.6) confirmed further the formation of ethyl ester at C-24. The HRFABMS spectrum revealed the $[M+H]^+$ ion at *m/z* 447.3476 confirming the molecular formula $C_{28}H_{46}O_4$ for **7**. 3α-Acetoxy-5β-cholan-24-oic acid ethyl ester (**7**) was previously synthesised from (i) 3α-acetoxy acid in EtOH using *p*-TsOH and also using LDA and *trans*-2-(phenylsulfonyl)-2-phenyloxaziridine in THF[13] and (ii) acetylation of ethyl lithocholate[14], and identified only on the basis of direct comparison of its m.p. with published data. The full IR, MS, ^1H NMR and ^{13}C NMR data for **7** are presented here.

Secondary alcohols are easy to oxidise to corresponding ketones by the treatment of Jones' reagent. Lithocholic acid (**5**) was oxidised to 3-oxo acid **8** (Scheme 2)[15] the identity of which was confirmed by NMR (^1H and ^{13}C) and ESIMS analyses. The presence of the ketonic carbonyl (C-3) and acid carbonyl (C-24) was confirmed from the peaks, respectively,

* Correspondence. E-mail: L.Nahar@rowett.ac.uk

Table 1 ^{13}C NMR data of compounds **2**–**4** and **6**–**9**

Carbon no.	Chemical shifts (δ) in ppm						
	2	**3**	**4**	**6**	**7**	**8**	**9**
1	36.7	37.0	37.0	35.0	35.3	37.5	37.3
2	27.7	27.8	27.8	26.3	26.6	37.3	37.0
3	73.9	73.9	74.0	74.4	74.7	203.8	213.5
4	31.7	36.6	36.6	32.2	32.5	42.6	42.4
5	139.6	139.6	139.7	40.4	40.7	44.6	44.4
6	122.4	122.5	122.5	26.6	27.3	26.1	25.8
7	31.8	31.8	31.9	27.0	26.9	26.9	26.6
8	65.5	31.9	31.9	35.8	36.1	35.6	35.5
9	52.3	52.4	52.4	41.9	42.2	41.1	40.7
10	38.1	38.1	38.1	34.6	34.9	35.2	34.9
11	20.9	21.0	21.0	21.5	21.1	21.5	21.2
12	42.6	42.7	39.7	40.1	40.4	40.3	40.1
13	42.9	43.9	42.4	42.7	43.0	43.1	42.8
14	56.1	56.1	56.4	56.5	56.8	56.7	56.4
15	24.3	24.4	24.4	24.2	24.5	24.4	24.2
16	27.1	27.3	27.7	28.2	28.5	28.4	28.2
17	49.9	49.9	50.0	56.0	56.3	56.3	56.0
18	16.5	16.6	16.8	12.0	12.3	12.4	12.1
19	19.3	19.3	19.3	23.3	23.6	22.9	22.7
20	39.4	39.5	38.8	35.3	35.7	35.8	35.4
21	12.1	12.1	11.9	18.2	18.6	18.5	18.3
22	170.5	172.2	68.0	30.9	31.6	31.2	31.3
23	–	–	–	30.8	31.3	31.0	31.0
24	–	–	–	170.7	174.6	180.1	174.4
3–CO	170.5	170.6	170.5	170.7	171.0	–	–
3–CO–Me	21.4	21.5	21.4	21.5	21.8	–	–
22–**C**O–O–**C**O–OMe	150.0	–	–	–	–	–	–
22–CO–O–CO–O**Me**	55.8	–	–	–	–	–	–
24–O**C**H$_2$CH$_3$	–	–	–	–	60.2	–	60.1
24–OCH$_2$**C**H$_3$	–	–	–	–	14.3	–	14.0

Spectra obtained in $CDCl_3$, 100 MHz

at δ 203.8 and 180.1 in its ^{13}C NMR spectrum (Table 1). The downfield shift (0.10 ppm) of the protons of C-19 methyl group (δ 1.01) compared to that of **5** (δ 0.91) also supported the presence of an oxo group at C-3. The ESIMS spectrum of **8** exhibited $[M+Na]^+$ ion at *m/z* 397.

The reaction protocol, which was carried out for the synthesis of **7**, was employed for the synthesis of ethyl 3-ketochlolanate (**9**) from **8** (Scheme 2)[15]. The presence of an ethyl ester group in **9** (instead of acid in **8**) was evident from the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 403 and 425 observed in its FABMS spectrum. The IR spectrum exhibited the absorption band at 1736 cm^{-1} assigned to the ester carbonyl group in the molecule. In the ^{1}H NMR spectrum of **9** in addition to the signals associated with compound **8**, a deshielded 2H quartet at δ 4.08 (J = 7.2 Hz) and a 3H triplet at δ 1.20 (J = 7.2 Hz) confirmed the formation of **9**. The ^{13}C NMR spectrum (Table 1) had signals at δ 60.1 and 14.0, for the oxymethylene and methyl carbons of the ethyl group. In its ^{1}H-^{13}C HMBC spectrum, a long-range (^3J) correlation from the oxymethylene protons (δ 4.08) to the ester carbonyl carbon (δ 174.4) confirmed further the formation of ethyl ester at C-24. The HRFABMS spectrum of **9** showed $[M+H]^+$ ion at *m/z* 403.3212 which confirmed the molecular formula $C_{26}H_{42}O_3$. Compound **9** was synthesised previously by esterification of 3-ketocholanic acid and identified by m.p.[14] The possible reaction mechanism for the formation of **7** and **9** is shown in Scheme 3. It is assumed that in the intermediate step, methyl anhydride was formed and then EtOH attacked the carbonyl centre to yield solely ethyl ester compound.

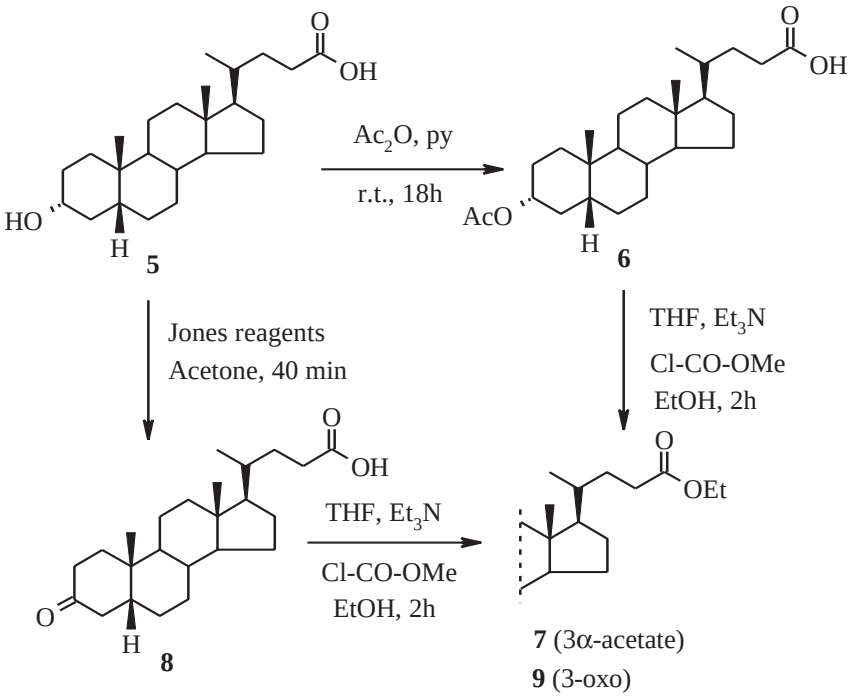
Experimental

The steroid starting materials [3β-acetoxy-23,24-dinorchol-5-en-22-oic acid (**1**) and lithocholic acid (**5**)], $NaBH_4$ and methyl chloroformate were purchased from Aldrich and used as received. All chemicals and solvents were used throughout without further purification. The reactions were monitored and the purity of the products was assessed by thin-layer chromatography (TLC) performed on silica gel (Merck type 60) and visualised under UV illumination and/or by I_2 vapour. Melting points of the products were determined on a Gallenkamp melting point apparatus. Infrared spectra (wavenumbers in cm^{-1}) were recorded on an ATI Mattson Genesis FTIR spectrophotometer as KBr pellets. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from TMS, using the middle resonance of $CDCl_3$ (7.25 ppm for ^{1}H and 77.23 ppm for ^{13}C) as an internal standard and coupling constants (J) in Hz. Mass spectroscopic analyses were performed at the EPSRC Mass Spectrometry Service at Swansea.

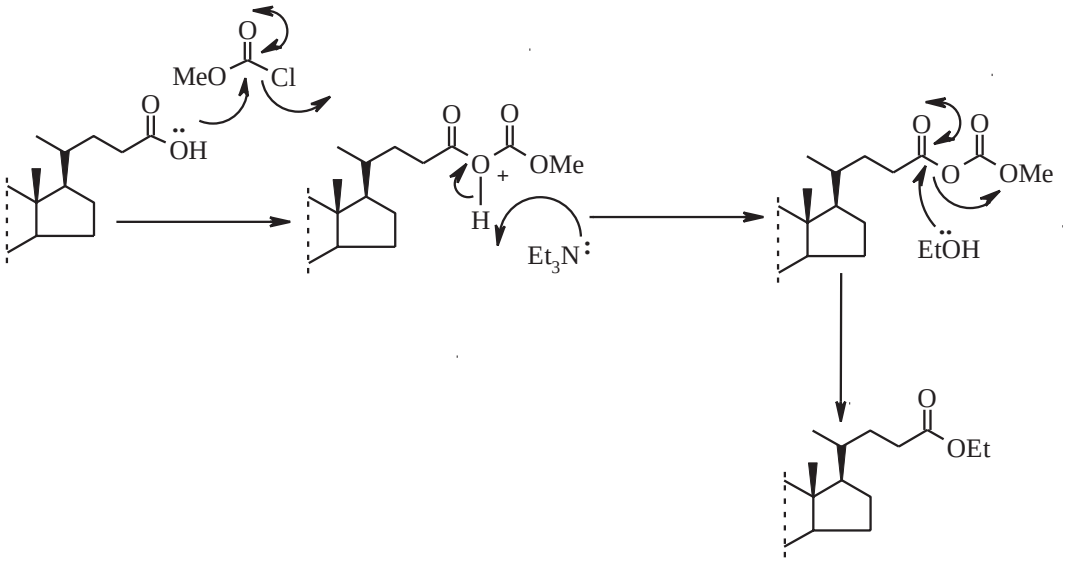
*Synthesis of 3*β*-acetoxy-23,24-dinorchol-5-en-22-oyl methoxy anhydride* (**2**) *and 3*β*-acetoxy-23,24-dinorchol-5-en-22-oyl chloride* (**3**): To a stirred solution of *3*β-acetoxy-23,24-dinorchol-5-en-22-oic acid (**1**, 200 mg, 0.52 mmol) in THF (20 ml) sequentially equal amount of Et_3N (0.5 ml) and methyl chlorofomate were added at 4°C. After 2h the reaction was quenched with H_2O, extracted with DCM and washed subsequently with dil. HCl, H_2O, $NaHCO_3$ and brine. The washed DCM extract was dried and concentrated at reduced pressure to obtain a mixture of 3β-acetoxy-23,24-dinorchol-5-en-22-oic acid methoxy anhydride (**2**) and 3β-acetoxy-23,24-dinorchol-5-en-22-oyl chloride (**3**). Compounds **2** and **3** were purified by VLC (15% EtOAc in pet-ether).

Compound **2**: colourless solid, 105 mg, 45%, m.p. 188–190 °C. IR (KBr): ν_{max} cm^{-1} 2966s (C–H), 2870s (C–H), 1809vs (anhydride C=O), 1732s (acetate C=O), 1440m, 1374m, 1247m (acetate C–O), 1157m, 1076m, 1034m, 941w and 758m. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.66 (s, 3H, 18-Me), 0.97 (s, 3H, 19-Me), 1.24 (d, J = 6.8 Hz, 3H, 21-Me), 1.98 (s, 3H, 3β-OCO–Me), 3.85 (s, 3H, 22-CO–OMe), 4.55 (m, 1H, 3α-OCH), 5.32 (m, 1H, 6-CH) and ^{13}C NMR (Table 1). FABMS *m/z*: 447 $[M+H]^+$; 469 $[M+Na]^+$. HRFABMS: Found: 447.2746; calc 447.2746 for $C_{26}H_{39}O_6$.

Compound **3**: white amorphous solid, 38 mg, 18%, m.p. 124–125 °C (*lit.*[7] m.p. 122–125 °C). IR (KBr): ν_{max} cm^{-1} 2945s (C–H), 2883s (C–H), 1805s (acetyl chloride C=O), 1732vs (acetate C=O), 1458m, 1375m, 1254s (acetate C–O), 1169m, 1038s (alcoholic C–O), 1018s. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.69 (s, 3H, 18-Me), 1.00 (s, 3H, 19-Me), 1.13 (d, J = 5.8 Hz, 3H, 21-Me), 1.99 (s, 3H, 3β-



Scheme 2 Synthesis of 5β-cholane derivatives **6**–**9**.



Scheme 3 Possible reaction mechanism for the formation of **7** and **9**.

OCO–Me), 4.56 (br m, 1H, 3α-O–CH), 5.36 (m, 1H, 6-CH) and ^{13}C NMR[8] (Table 1). FABMS *m/z*: 407/409 $[M+H]^+$, 429/431 $[M+Na]^+$.

Synthesis of 3β-acetoxy-23,24-dinorchol-5-en-22-ol (**4**)*:* To a stirred solution of **2** (100 mg, 0.22 mmol) in EtOH (5 ml) was added dropwise a solution of $NaBH_4$ (1 molar equiv.) in EtOH (5 ml). After 1h, the ethanolic solution was concentrated under reduced pressure. The residue was taken up in EtOAc, washed with dil HCl and H_2O. The organic solvent was separated, dried ($MgSO_4$) and evaporated to dryness. After purification by PTLC (100% $CHCl_3$) the title compound was obtained as a white solid (**4**, 28 mg, 34%), m.p. 151–153 °C (*lit.* m.p.[8] 154–155 °C, IR[9], MS and ^{1}H NMR[8]) and ^{13}C NMR (Table 1). ESIMS *m/z*: 353 $[M+Na]^+$.

Synthesis of 3α-acetoxy-5β-cholan-24-oic acid (**6**)*:* A stirred solution of lithocholic acid (**5**, 200 mg, 0.53 mmol) in dry pyridine (10 ml) was added Ac_2O (3 ml) and stirred for 18h at r.t. Then the reaction mixture was quenched with ice-H_2O and a white precipitate was formed. The precipitate was collected, taken into ether, dried over $MgSO_4$ and ether was evaporated to afford the title compound **6** (133 mg, 60%), m.p. 168–169 °C (*lit.*[10] m.p. 168–170 °C and IR). ^{1}H NMR[11] (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.88 (d, *J* = 6.2 Hz, 3H, 21-Me), 1.99 (s, 3H, 3α-OCO–Me), 4.68 (m, 1H, 3β-OCH) and ^{13}C NMR[11] (Table 1). ESIMS *m/z*: 441 $[M+Na]^+$.

Synthesis of 3α-acetoxy-5β-cholan-24-oic acid ethyl ester (**7**)*:* To a solution of **6** (100 mg, 0.24 mmol) in THF (5 ml), Et_3N (5 drops) and methyl chloroformate (5 drops) were added sequentially. The mixture was stirred at 4 °C for 2h. Then the reaction was quenched with EtOH and the reaction mixture was rotary evaporated to dryness. The compound was purified by PTLC (100% $CHCl_3$) to obtain the title compound (**7**, 40 mg, 37%), m.p. 94–96 °C (*lit.*[14] m.p. 95–98 °C). IR (KBr): ν_{max} cm^{-1} 2937s (C–H), 2870s (C–H), 1737vs (acetate and ethyl ester C=O), 1449m, 1378m, 1363m, 1243s (acetate C–O), 1165s (ester C–O), 1028m and 734m. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (s, 3H, 18-Me), 0.91 (s, 3H, 19-Me), 0.90 (d, *J* = 6.7 Hz, 3H, 21-Me), 1.21 (t, *J* = 7.2 Hz, 3H, 24-OCH$_2$**CH$_3$**), 2.01 (s, 3H, 3α-OCO-Me), 4.07 (q, *J* = 7.2 Hz, 2H, 24-O**CH$_2$**CH$_3$), 4.70 (m, 1H, 3β-OCH) and ^{13}C NMR (Table 1). FABMS *m/z*: 447 $[M+H]^+$, 469 $[M+Na]^+$. HRFABMS: Found: 447.3476; calc 447.3474 for $C_{28}H_{47}O_4$.

Synthesis of 3-oxo-5β-cholan-24-oic acid (**8**): To a solution of lithocholic acid (**5**, 500 mg, 1.33 mmol) in acetone (10 ml) was added Jones' reagent (1 ml) at 4 °C and the mixture was allowed to stand for 40 min. After addition of EtOH to decompose the excess reagent, the resulting solution was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and H_2O, dried ($MgSO_4$) and evaporated. The crude dark green product was dissolved in DCM and filtered through a TLC grade silica pad to obtain the title compound (**8**, 463 mg, 93%), m.p. 122–123 °C (*lit.*[15] m.p. 121–122 °C and IR). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, 18-Me), 0.92 (d, *J* = 6.1 Hz, 3H, 21-Me), 1.01 (s, 3H, 19-Me) and ^{13}C NMR (Table 1). ESIMS *m/z*: 397 $[M+Na]^+$.

Synthesis of 3-oxo-5β-cholan-24-oic acid ethyl ester (**9**)*:* 3-Oxo-5β-cholan-24-oic acid ethyl ester (**9**) was obtained from **8** following the procedure described for compound **7**. The crude solid was purified by PTLC (100% $CHCl_3$) to yield the title compound (**9**, 30 mg, 36%), m.p. 95–96 °C (*lit.*[14] m.p. 94.5–95.5 °C). IR (KBr): ν_{max} cm^{-1} 2940s (C–H), 2867s (C–H), 1736s (acetate C=O), 1716vs (ketonic C=O), 1446m, 1378m, 1263m, 1174s (ester C–O), 1096w and 732w. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (s, 3H, 18-Me), 0.88 (s, 3H, 19-Me), 0.90 (d, *J* = 6.4 Hz, 3H, 21-Me), 1.20 (t, *J* = 7.2 Hz, 3H, 24-$OCH_2\mathbf{CH_3}$), 4.08 (q, *J* = 7.2 Hz, 2H, 24-$O\mathbf{CH_2}CH_3$) and ^{13}C NMR (Table 1). FABMS *m/z*: 403 $[M+H]^+$, 425 $[M+Na]^+$. HRFABMS: Found: 403.3212; calc 403.3212 for $C_{26}H_{43}O_3$.

The authors thank the funding body, the University of Aberdeen Development Trust, for a PhD studentship (LN) and EPSRC National Mass Spectrometry Service Centre (Department of Chemistry, University of Wales Swansea, Swansea, UK) for MS and HRMS analysis.

Received 6 January 2004; accepted 12 February 2004
Paper 04/2297

References

1 R.F. Witzmann, *Steroids – Key to Life*, Van Nostrum Reinhold, New York, 1981.
2 Y.X. Li and J.R. Dias, *Chem. Rev.*, 1997, **97**, 283.
3 P. Fernandes, A. Cruz, B. Angelova, H.M. Pinheiro and J.M.S. Cabral, *Enzyme and Microb. Tech.*, 2003, **32**, 688.
4 P.J. Cox, L. Nahar and A.B. Turner, *J. Chem. Res. (S)*, 2001, 162.
5 L. Nahar and A.B. Turner, *Tetrahedron*, 2003, **59**, 8623.
6 L. Nahar and A.B. Turner, *Steroids*, 2003, **68**, 1157.
7 R. Hayatsu, *Chem. Pharm. Bull.*, 1957, **5**, 452.
8 D.J. Simpson, C.J. Unkefer, T.W. Whaley and B.L. Morrone, *J. Org. Chem.*, 1991, **56**, 5391.
9 G.M. Segal and I.V. Torgov, *Sov. J. Bioorg. Chem.*, 1979, **5**, 1236.
10 Y.X. Li and J.R. Dias, *Org. Prep. Proc. Int.*, 1996, **28**, 203.
11 C. Ollivier, P. Panchaud and P. Renaud, *Synthesis*, 2001, **10**, 1573.
12 K. Baburao, A.M. Costello, R.C. Petterson and G.E. Sander, *J. Chem. Soc. C.*, 1968, 1779.
13 H. Schneider, H. Fiander, K.M. Harrison, M. Watson, G.W. Burton and P. Arya, *Bioorg. Med. Chem. Lett., 1996*, **6**, 637.
14 F.C. Chang, R.T. Blickenstaff, A. Feldstein, J.R. Gray, G.S. M^{c}Calab and D.H. Sprunt, *J. Am. Chem. Soc.*, 1957, **79**, 2164.
15 A.U. Siddiqui, A.H. Siddiqui, and T.S. Ramaiah, *J. Ind. Chem. Soc.*, 1993, **70**, 255.