Reduction of α-Diazo-β-Hydroxy Esters to β-Hydroxy Esters: Application in One of Two Convergent Syntheses of a (22*S*)-22-Hydroxy Bile Acid from Fish Bile and its (22*R*)-Epimer

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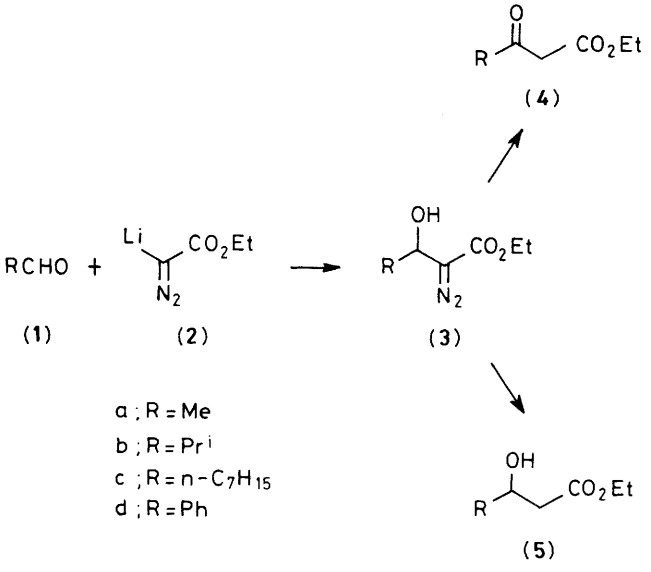
α-Diazo-β-hydroxy esters (**3**) prepared by condensation of aldehydes (**1**) with ethyl (lithio)diazoacetate (**2**) are reduced with 5% palladium over charcoal in methanol into the corresponding β-hydroxy esters (**5**) in high yield. This sequence is applied to a new synthesis of haemulcholic acid (**14c**), a (22*S*)-22-hydroxy bile acid from fish bile and its (22*R*)-epimer (**14d**). A convergent synthesis of (**14c**) and (**14d**) involves, as a key step, the dirhodium(II) tetra-acetate conversion of (**12**) into the corresponding β-keto ester (**13**).

Previous work has established the synthetic versatility of α-diazo-β-hydroxyacylmethanes, trifunctionalized compounds readily accessible by an aldol-type condensation between α-lithiated diazoacyl precursors, and aldehydes and ketones.[1a–r] In particular, the dirhodium(II) tetra-acetate catalyzed conversion of α-diazo-β-hydroxy esters (**3**), prepared by reaction of ethyl (lithio)diazoacetate (**2**) with aldehydes (**1**) followed by neutralization with acetic acid, affords the corresponding β-keto esters (**4**)[1m,p] in nearly quantitative yields (Scheme 1).



Scheme 1.

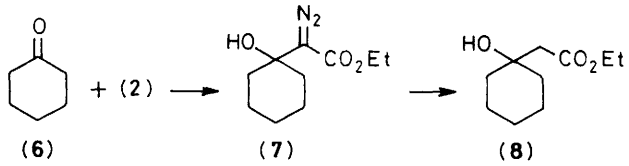
With the aim to broaden further the range of useful synthetic applications of compounds (**3**) we have studied their hydrogenolytic conversion into the important class of β-hydroxy esters (**5**).[1b,2]

The reduction of the diazo group has been the object of scattered studies since the pioneering work of Curtius[3] and Staudinger.[4] Birkofer[5a,b] reported that the palladium-catalyzed reduction in an acidic medium of aromatic and aliphatic or alicyclic diazoketones yields α-amino ketones and hydrazones, respectively.[5c] It was also discovered that α-diazo-β-hydroxy esters can directly be reduced to the corresponding α-amino-β-hydroxy esters by the use of platinum oxide as catalyst in an acidic medium,[6] while α-amino alcohols with a terminal amino group have been obtained by the reduction of diazo ketones with $LiAlH_4$.[7] Finally, two methods have been reported for the replacement of the diazo group in diazocarbonyl compounds by two hydrogen atoms: the palladium-catalyzed hydrogenation in the presence of CuO[5a,7] or, with more satisfactory yields, the Wolfrom method, which employs hydriodic acid for the reductive process.[8] In our work, while the latter method was excluded *a priori* in view of the known tendency of α-diazo-β-hydroxy esters (**3**) to undergo conversion into the corresponding β-keto esters in the presence of mineral acids,[1a,h] initial attempts to achieve this transformation by catalytic hydrogenation of (**3**) led to unsatisfactory results. It was discovered, in this connection, that platinum oxide catalyzes the transformation (**3**)→(**4**), although not as efficiently as dirhodium(II) tetra-acetate, and, as a consequence, β-keto esters (**4**) were the main products of hydrogenations carried out in the presence of this catalyst. On the other hand, reduction of (**3**) carried out in ethanol in the presence of palladium on charcoal catalysts resulted in highly complex mixtures comprising major amounts of the corresponding hydrazo and amino derivatives, as determined by n.m.r. and i.r. spectroscopy, along with uniformly low amounts of the desired β-hydroxy ester (**5**). A marked increase in the yield of (**5**) was obtained, however, by the replacement of ethanol by methanol as solvent in reductions carried out in the presence of 5% palladium on charcoal.

As a further improvement, when the reduction was carried out under pressure (**5**) was obtained as the only product and the reaction time sensibly reduced.

The results of the reduction by this method of α-diazo-β-hydroxy esters (**3a—d**) prepared from aldehydes (**1a—d**), are reported in the Table.

This procedure can be extended to ketonic substrates. Thus, reduction under the above conditions of the α-diazo-β-hydroxy ester (**7**) obtained by condensation of cyclohexanone (**6**) with ethyl (lithio)diazoacetate (**2**), yielded the corresponding β-hydroxy ester (**8**) in 87% isolated yield.



To further illustrate their utility and as a part of a synthetic program on bile acids we have applied the sequences (**1**)→(**5**) and (**1**)→(**4**) for two new convergent syntheses of haemulcholic acid (**14c**), a (22*S*)-22-hydroxy natural bile acid isolated in 1964

Table. Two-step conversion of aldehydes into β-hydroxy esters

Starting aldehyde	Step 1 Product	Step 1 Yield (%)[a]	Step 2 Yield (%)[b]	Final product	Overall yield (%) (1)→(5)
(1a)	(3a)	90[c]	75(82)	(5a)	67.5
(1b)	(3b)	80[c]	77(88)	(5b)	61.6
(1c)	(3c)	72	63	(5c)	45.4
(1d)	(3d)	95[d]	71(85)	(5d)	67.4

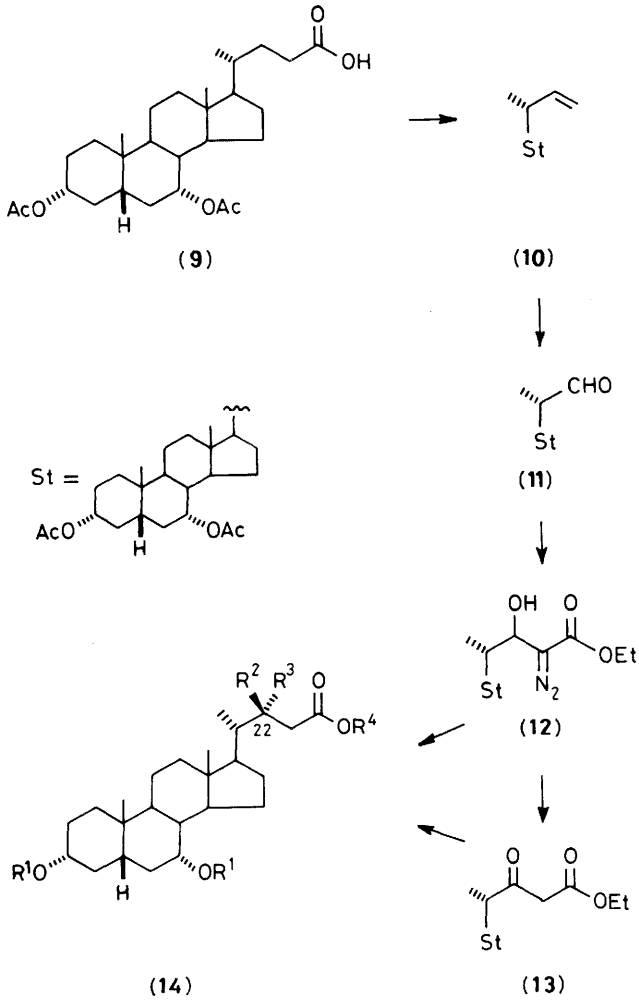
[a] The yields quoted refer to isolated samples of at least 98% purity. [b] Numbers in parentheses refer to yields obtained by g.l.c. standard methods. [c] Prepared according to ref. 1*a*. [d] Prepared according ref. 1*e*.

from the bile of *Parapristipoma trilineatum*,[9] a marine teleost, and more recently from the bile of two fishes, *Polypterus senegalus* and *Mormyrus caballus*,[10] and its (22*R*)-epimer (**14d**).* The starting material was norcholene diacetate (**10**) which was prepared by oxidative decarboxylation of the diacetyl derivative of chenodeoxycholic acid (**9**) carried out using lead tetra-acetate in the presence of pyridine and copper(II) acetate.[12] Ozonolysis in chloroform–methanol at −10 °C of the olefin (**10**) thus obtained followed by zinc–acetic acid work-up afforded the unstable aldehyde (**11**) (59%) which was immediately treated with ethyl (lithio)diazoacetate (**2**) in THF at −78 °C to afford an inseparable mixture (t.l.c.) of the corresponding diastereoisomeric α-diazo-β-hydroxy esters (**12**) in 75% yield. Catalytic hydrogenation on palladium in methanol of (**12**) yielded a 3:1 mixture (g.l.c. analysis) of two epimeric β-hydroxy esters in 61% yield. Separation of these epimers by silica-gel column chromatography provided two products in the above ratio. The more polar, more abundant product (36.7% isolated yield) was considered to be the (22*S*)-22-hydroxy ester (**14a**) since its alkaline hydrolysis led to a product with properties (m.p., n.m.r. and mass spectra and $[\alpha]_D$) identical with those already reported for natural haemulcholic acid (**14c**).[11] Alkaline hydrolysis of the less polar, less abundant (22*R*)-epimeric ester (**14b**) (13.4%, isolated yield) afforded the corresponding (22*R*)-3α,7α,22-trihydroxycholanoic acid (**14d**). The predominance of the (22*S*)-isomer (**14a**) reflects a preference in the formation of the (22*S*)-epimer of α-diazo-β-hydroxy esters (**12**) as a consequence of a less hindered approach of ethyl (lithio)diazoacetate (**2**) from the α-face of the aldehyde (**11**), in agreement with the results of reactions of a variety of steroidal 20-carbaldehydes with Grignard reagents[13a–c] and with dimethylsulphonium methylide.[14]

Haemulcholic acid (**14c**) and its (22*R*)-epimer (**14d**) could be obtained from the α-diazo-β-hydroxy ester (**12**) by an alternative route. Decomposition of (**12**) in a 1,2-dimethoxyethane solution of dirhodium(II) tetra-acetate at room temperature produced the corresponding β-keto ester in nearly quantitative yield.†

The β-oxo ester (**13**) provided a useful entry into the (22*S*)-hydroxy series since it was reduced with an excess of sodium borohydride in methanol to give a 3:1 mixture of the (22*S*)-hydroxy ester (**14a**) and its (22*R*)-epimer (**14b**). The stereoselective formation of the more polar, anti Cram[16] (22*S*)-epimer (**14a**) confirms previous results of hydride reductions of a variety of 22-oxo steroidal derivatives.[17a–c]

Finally, chromatographic separation of these epimeric esters followed by alkaline hydrolysis yielded, respectively, the (22*S*)-hydroxy acid (**14c**) and its (22*R*)-epimer (**14d**).



(9) (10) (11) (12) (13)

(14)

a; R^1 = Ac R^2 = OH R^3 = H R^4 = Et
b; R^1 = Ac R^2 = H R^3 = OH R^4 = Et
c; R^1 = H R^2 = OH R^3 = H R^4 = H
d; R^1 = H R^2 = H R^3 = OH R^4 = H

Experimental

All reactions involving organometallic reagents were performed in dry apparatus under nitrogen. Ether, 1,2-dimethoxyethane, and tetrahydrofuran were distilled from $LiAlH_4$ immediately prior to use. Di-isopropylamine was distilled from calcium

* The configuration at C-22 has been established by a synthesis of (**14c**) and (**14d**) involving as a key step a Reformatsky reaction.[11]

† 3α-Acetoxy-12,22-dioxocholan-24-oic ethyl ester, the only 22-oxo derivative of a bile acid so far reported, was prepared by reaction of 3α-acetoxy-12-oxocholan-24-oic acid with the sodium salt of tetrahydropyranyl ethyl malonic ester in the presence of sodium.[15]

hydride and stored over 4 A molecular sieves. M.p.s were determined on a Kofler micro-hot-stage and are uncorrected. I.r. spectra were determined on a Beckmann Acculab 5 spectrophotometer. ^{1}H N.m.r. spectra were recorded with a Varian EM 390 spectrometer. G.l.c. was performed on a Hewlett-Packard 5839 A Chromatograph equipped with a flame ionization detector and a column (2 mm i.d. × 20 in) packed with 10% UCW-982 on Chromosorb-W-AWDMCS. The oven temperature was 70—230 °C (10%/min increase) with nitrogen as the carrier gas at the flow rate of 20 ml/min. Column chromatography was performed on Merck silica gel (0.063—0.200 mm).

α-Diazo-β-hydroxy Ester (**1c**).—A cold (−10 °C) solution of lithium di-isopropylamide [from addition of n-butyl-lithium in hexane (11.7 ml of a 1.6M-solution) to a solution of di-isopropylamine (2.92 ml) in tetrahydrofuran (30 ml)] was added over 45 min to a stirred solution of octanal (**1c**) (2.0 g, 15.6 mmol) and ethyl diazoacetate (1.96 g, 17.2 mmol) in THF (70 ml) at −78 °C. The mixture was stirred at −78 °C for 30 min after which acetic acid (3 ml) in diethyl ether (15 ml) was added during 5 min; the mixture was then allowed to warm to room temperature. Water (250 ml) was then added, and the organic layer was separated, washed with saturated aqueous sodium hydrogencarbonate and brine, dried, and evaporated under reduced pressure to give a yellow oil (2.95 g). Flash chromatography on Alumina (activity II) and elution with light petroleum–ether 5:5 (400 ml) followed by ether–ethyl acetate 9:1 (500 ml) gave ethyl 2-diazo-3-hydroxydecanoate (**3c**) as a yellow oil (2.58 g, 68.2%); $\nu_{max.}$ 3 600, 3 440 (OH), 2 100 (C=N$_2$), and 1 690 cm^{-1} (CO); δ(CDCl$_3$) 0.90 (3 H, t, Me), 1.00 (3 H, t, CH_3CH$_2$), 1.20—1.90 (12 H, m, CH$_2$), 3.30—3.60 (1 H, br m, OH), 4.2 (2 H, q, CH$_3$CH_2), and 4.65 (1 H, t, C*H*OH).

1-*Hydroxy*-1-*ethoxycarbonyldiazomethylcyclohexane* (**7**).—A cold (−10 °C) solution of lithium di-isopropylamide [from addition of n-butyl-lithium in hexane (3.5 ml of a 1.6M-solution) to a solution of di-isopropylamine (0.67 g) in tetrahydrofuran (15 ml)] was added during 25 min to a stirred solution of cyclohexanone (**6**) (0.5 g, 5.1 mmol) and ethyl diazoacetate (0.582 g, 5.1 mmol) at −78 °C. The mixture was stirred at −78 °C for 45 min after which acetic acid (0.6 g) in diethyl ether (10 ml) was added during 5 min and the mixture allowed to warm to room temperature. Work-up as above and chromatography of the oily residue (1.15 g) on Al$_2$O$_3$ (activity IV) with light petroleum as eluant gave pure (**7**) (0.89 g, 82%); $\nu_{max.}$ (CHCl$_3$) 3 620 (OH), 2 100 (C=N$_2$), and 1 700 cm^{-1} (C=O); δ(CDCl$_3$) 1.33 (3 H, t, CH_3CH$_2$), 1.43—2.10 [10 H, m, (CH$_2$)$_5$], 3.52 (1 H, s, OH), and 4.25 (2 H, q, CH$_3$CH_2).

β-*Hydroxy Esters* (**5**).—Ethyl 2-diazo-3-hydroxybutyrate (**3a**) (1.96 g, 12.4 mmol) was treated in an autoclave (277 ml capacity) for 1 h at room temperature under hydrogen (4 atm) in methanol (80 ml) in the presence of 5% Pd–C catalyst (0.3 g). The catalyst was filtered off and the filtrate concentrated under reduced pressure. Ethyl 3-hydroxybutyrate (**5a**) was purified by distillation, b.p. 70—72 °C/13 Torr (lit.,[18] 80—84 °C/18 Torr); $\nu_{max.}$ 3 530 (OH) and 1 710 cm^{-1} (CO); δ(CDCl$_3$) 1.20 (3 H, d, CH$_3$), 1.30 (3 H, t, CH_3CH$_2$), 2.45 (2 H, d, CH_2CO$_2$Et), 3.35 (1 H, br m, OH), and 4.0—4.3 (3 H, m, CH$_3$CH_2 and C*H*OH). Hydrogenation as above of ethyl 2-diazo-3-hydroxy-4-methylpentanoate (**3b**) (0.98 g, 5.27 mmol) in methanol (80 ml) in the presence of 5% Pd–C (0.1 g) and filtration of the oily residue on silica gel (elution with ether) yielded ethyl 3-hydroxy-4-methylpentanoate (**5b**)[19] (0.64 g, 77%), b.p. 88—89 °C/10 Torr; (Found: C, 60.4; H, 10.2. C$_8$H$_{16}$O$_3$ requires C, 59.95; H, 10.07%); $\nu_{max.}$ 1 730 cm^{-1} (CO); δ(CDCl$_3$) 0.90, 1.0 (6 H, 2 × d, *Me*$_2$CH), 1.25 (3 H, t, CH_3CH$_2$), 1.50—1.80 (1 H, m, Me$_2$C*H*), 2.40 (2 H, m, CH_2CO$_2$Et), 2.70—3.00 (1 H, br m, C*H*OH), and 4.15 (2 H, q, CH$_3$CH_2).

Hydrogenation as above of ethyl 2-diazo-3-hydroxydecanoate (**3c**) (1.02 g, 4.2 mmol) in methanol (40 ml) in the presence of 5% Pd–C (0.2 g) and purification of the residue by chromatography on silica gel with ether as eluant afforded pure ethyl 3-hydroxydecanoate (**5c**)* (0.57 g, 63%), b.p. 129—131 °C/1 Torr (Found: C, 66.5; H, 11.22. C$_{12}$H$_{24}$O$_3$ requires C, 66.61; H, 11.19%); $\nu_{max.}$ 3 540 (OH) and 1 720 cm^{-1} (CO); δ(CDCl$_3$) 0.90 (3 H, t, CH$_3$), 1.10—1.60 [15 H, m, (CH$_2$)$_6$, CH_3CH$_2$], 2.40 (2 H, m, CH$_2$CO$_2$Et), 3.10 (1 H, br m, OH), 4.00 (1 H, br m, C*H*OH), and 4.2 (2 H, q, CH$_3$CH_2).

Hydrogenation of ethyl 2-diazo-3-hydroxy-3-phenylpropionate (**3d**) (0.49 g, 2.23 mmol) in methanol (30 ml) in the presence of 5% Pd–C (0.1 g) and purification of the residue (0.415 g) by chromatography on silica gel with light petroleum–ether (8:2) as eluant gave ethyl 3-hydroxy-3-phenylpropionate (**5d**) (0.3 g, 71%), b.p. 90—91 °C/0.1 Torr (lit.,[20] b.p. 154—156 °C/12 Torr).

Hydrogenation as above of 1-hydroxy-1-ethoxycarbonyldiazomethylcyclohexane (**7**) (1.0 g, 4.72 mmol) in methanol (6 ml) in the presence of 5% Pd–C (0.2 g) and chromatography of the residue (0.81 g) on silica gel with ether gave pure ethyl 1-hydroxycyclohexylacetate (**8**) (0.77 g, 87%) identical (n.m.r., i.r., b.p.) with an authentic sample.

3α,7α-*Diacetoxy*-5β-*bisnorcholan*-22-*al* (**11**).—Ozone was bubbled at −10 °C through a solution of 3α,7α-diacetoxy-5β-norchol-22-ene (**10**) (5.2 g, 12.1 mmol) in chloroform–methanol (1:1) (40 ml) until a light blue colour appeared. Zinc dust (24 g, 0.37 mmol) and 30% acetic acid (50 ml) were added to the cold solution, and the mixture was stirred for 2 h at room temperature. The zinc dust was filtered off and washed with chloroform (3 × 15 ml) and the aqueous layer separated and extracted with chloroform (3 × 40 ml). The combined organic phases were washed with saturated solutions of aqueous sodium hydrogen carbonate and sodium chloride, dried (Na$_2$SO$_4$), and evaporated under reduced pressure. The residue (4.8 g) was crystallized from hexane (160 ml) to give the aldehyde (**11**) (3.05 g, 59%), m.p. 131—132 °C; $\nu_{max.}$ (Nujol) 1 731 cm^{-1} (CO); δ(CDCl$_3$) 0.67 (3 H, s, 18-Me), 0.90 (3 H, s, 19-Me), 1.99 (3 H, s, 7-OCOMe), 2.01 (3 H, s, 3-OCOMe), 4.20—4.63 (1 H, br m, C*H*OAc), 4.67—4.80 (1 H, m, 7-C*H*OAc), and 9.30 (1 H, d, *J* 3 Hz, CHO) (Found: C, 72.4; H, 9.55. C$_{26}$H$_{40}$O$_5$ requires C, 72.19; H, 9.32%).

Ethyl 3α,7α-*Diacetoxy*-22-*hydroxy*-23-*diazo*-5β-*cholan*-24-*oate* (**12**).—A cold (−78 °C) solution of lithium di-isopropylamide [from addition of n-butyl-lithium in hexane (12 ml of a 1.4M-solution) to a solution of di-isopropylamine (2.43 ml, 17.36 mmol) in anhydrous THF (20 ml)] was added during 30 min to a stirred solution of the aldehyde (**11**) (5.8 g, 14.96 mmol) at −78 °C. After the mixture had been stirred at −78 °C for 45 min, a solution of acetic acid (0.97 g, 16.1 mmol) in ether (10 ml) was added during 5 min and the reaction mixture was allowed to warm to room temperature. Water (200 ml) was then added, the organic layer was separated, and the aqueous layer was extracted with ether (2 × 50 ml). The combined organic phases were washed with saturated aqueous sodium hydrogen carbonate, dried (MgSO$_4$), and evaporated under reduced pressure. Chromatography of the residue (8.9 g) on Al$_2$O$_3$ (activity IV)

* Alkaline hydrolysis of (**5c**) yielded racemic 3-hydroxydecanoic acid, with analytical properties in agreement with the literature data: K. Serck-Hanssen and E. Stenhagen, *Acta Chem. Scand.*, 1955, **9**, 866; optically active 3-hydroxydecanoic acid (myrmicacin) is an insect herbicide: H. Schildknecht and K. Koob, *Agnew. Chem., Int. Ed. Engl.*, 1971, **10**, 124. See also M. Nakahata, M. Imaida, H. Ozaki, T. Harada, A. Tai, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2186.

and elution with light petroleum–ethyl acetate (from 5:1 to 1:1) afforded in 75% yield a mixture of the (22*S*)- and (22*R*)-α-diazo-β-hydroxy ester (**12**) (5.49 g, 75%); $\nu_{max.}$ (Nujol) 3 440 (OH), 2 100 (C=N_2), and 1 730 and 1 655 cm^{-1} (CO); δ($CDCl_3$) 0.65 and 0.67 (3 H, br s, 18-Me), 0.90 (3 H, s, 19-Me), 1.23 (3 H, t, *J* 7.5, CH_3CH_2), 1.95 (3 H, s, 7-OCOMe), 1.98 (3 H, s, 3-OCOMe), 4.07 (2 H, q, *J* 7.5, CH_3CH_2), 4.32—4.63 (2 H, br m, 3-CHOAc and 22-CHOH), and 4.72 (1 H, m, 7-CHOAc).

Ethyl 3α,7α-*Diacetoxy*-22-*hydroxy*-5β-*cholan*-24-*oate from* (**12**).—A solution of the mixture of the (22*S*)- and (22*R*)-α-diazo-β-hydroxy ester (0.5 g, 0.91 mmol) in methanol (60 ml) was hydrogenated with 5% Pd–C under hydrogen (4 atm), at room temperature for 1 h. The catalyst was removed by filtration of the mixture through Celite and the solvent was evaporated under reduced pressure to afford a mixture of the two (22*S*)- and (22*R*)-hydroxy compounds (**14a,b**). These two epimers could be separated by chromatography on silica gel. Elution with light petroleum–ether (8:2) afforded the less polar (22*R*)-hydroxy ester (**14b**) (0.064 g, 13.4%), m.p. 110—112 °C; $\nu_{max.}$ (Nujol) 3 530 (OH) and 1 730 cm^{-1} (CO); δ($CDCl_3$) 0.67 (3 H, s, 18-Me), 0.90 (3 H, s, 19-Me), 1.23 (3 H, t, *J* 6 Hz, CH_3CH_2), 1.94 (3 H, s, 7-OCOMe), 1.97 (3 H, s, 3-OCOMe), 2.13—2.33 (2 H, m, 23-CH_2), 2.50 (1 H, br s, 22-OH), 3.83—4.17 (1 H, m, 22-CHOH), 4.02 (2 H, q, *J* 6 Hz, CH_3CH_2), 4.20—4.60 (1 H, br m, 3-CHOAc), and 4.60—4.77 (1 H, m, 7-CHOAc).

Elution with light petroleum–ether (7:3) gave a mixture (0.052 mg, 10%) of the two epimers and further elution with the same solvents afforded the more polar (22*S*)-hydroxy ester (**14a**) (0.175 mg, 36.7%), m.p. 143—145 °C; $\nu_{max.}$ (Nujol) 3 530 (OH) and 1 730 cm^{-1} (CO); δ($CDCl_3$), 0.63 (3 H, s, 18-Me), 0.90 (3 H, s, 19-Me), 1.21 (3 H, t, *J* 6 Hz, CH_3CH_2), 1.94 (6 H, s, 3- and 7-OCOMe), 2.13—2.67 (2 H, m, 23-CH_2), 2.50 (1 H, s, 22-OH), 3.83—4.10 (1 H, m, 22-CHOH), 3.95 (2 H, q, *J* 6 Hz, CH_3CH_2), 4.14—4.57 (1 H, br m, 3-CHOAc), and 4.57—4.73 (1 H, m, CHOAc).

(22R)-3α,7α,22-*Trihydroxy*-5β-*cholan*-24-*oic Acid* (**14d**).—The (22*R*)-hydroxy ester (**14b**) (0.1 g, 0.19 mmol) was treated with 10% KOH–MeOH (10 ml) at reflux under stirring for 8 h. The reaction mixture was then poured into water, acidified with concentrated hydrochloric acid, and extracted with ethyl acetate–methanol 95:5 (3 × 20 ml). The organic phases were combined and evaporated under reduced pressure to give a residue (0.065 g) which was dissolved in $CHCl_3$–MeOH 7:3 and filtered through silica gel to give, after evaporation of the solvent, pure (22*R*)-3α,7α,22-trihydroxycholan-24-oic acid (**14d**) (0.06 g, 76%), m.p. 133—136 °C (lit.,[11] 141—142 °C); δ(CD_3OD), 0.72 (3 H, s, 18-Me), 0.92 (3 H, s, 19-Me), 0.97 (3 H, d, 21-Me), 3.20—3.50 (1 H, br m, 3-CHOH), 3.70—3.85 (1 H, m, 7-CHOH), 4.00—4.30 (1 H, br m, 22-CHOH), 4.76 (4 H, s, 3-, 7-, and 22-OH and 24-CO_2H).

(22S)-3α,7α,22-*Trihydroxy*-5β-*cholan*-24-*oic Acid* (*Haemulcholic Acid*) (**14c**).—The (22*S*)-hydroxy ester (**14a**) (0.78 g, 1.5 mmol) and 10% KOH–MeOH was stirred and treated under reflux for 7 h. The mixture was then poured into water (50 ml), acidified with concentrated hydrochloric acid, extracted with ethyl acetate–methanol 95:5 (4 × 30 ml) and the combined organic layers were evaporated and dried under reduced pressure. Flash filtration of the residue through silica gel and elution with $CHCl_3$–MeOH 7:3 gave pure haemulcholic acid (**14c**) (0.53 g, 87%), m.p. 248—251 °C (lit.,[11] 247—250 °C); $[\alpha]_D$ +2.5 (*c* 1.5, MeOH) [lit.,[11] +2.4 (*c* 1.3, MeOH)]; $\nu_{max.}$ (Nujol) 3 400 (OH) and 1 710 cm^{-1} (CO); δ($CDCl_3$–CD_3OD), 0.68 (3 H, s, 18-Me), 0.92 (3 H, s, 19-Me), 0.95 (3 H, d, 21-Me), 3.17—3.50 (1 H, br m, 3-CHOH), 3.68—3.87 (1 H, m, 7-CHOH), 3.97—4.27 (1 H, m, 22-CHOH), 4.57 (4 H, s, 3-, 7-, and 22-OH, 24-CO_2H).

Ethyl 3α,7α-*Diacetoxy*-22-*oxo*-5β-*cholan*-24-*oate* (**13**).—A mixture of the α-diazo-β-hydroxy ester (**12**) (1.0 g, 1.83 mmol) and dirhodium(II) tetra-acetate (0.005 g, 0.011 mmol) in 1,2-dimethoxyethane (DME) (10 ml) was stirred at room temperature for 15 min and the solvent was then evaporated off. Filtration of the residue through silica gel with 85:15 benzene–ethyl acetate as eluant afforded the β-keto ester (**13**) (0.93 g, 98%), m.p. 49—51 °C; $\nu_{max.}$ (Nujol) 1 740, 1 640, and 1 620 cm^{-1} (CO); δ($CDCl_3$), 0.72 (3 H, s, 18-Me), 0.98 (3 H, s, 19-Me), 1.27 (3 H, t, *J* 6 Hz, CH_3CH_2), 1.95 (3 H, s, 7-OCOMe), 2.00 (3 H, s, 3-OCOMe), 3.38 (3/4 2 H, s, 23-CH_2), 4.11 (2 H, q, *J* 6 Hz, CH_3CH_2), 4.33—4.93 (2 H, br m, 3- and 7-CHOAc), and 11.87 (1/4 H, s, 22-enolic OH) (Found: C, 68.95; H, 8.95. $C_{30}H_{46}O_7$ requires C, 69.47; H, 8.94).

Ethyl 3α,7α-*Diacetoxy*-22-*hydroxy*-5β-*cholan*-24-*oate from* (**13**).—To a solution of the β-keto ester (**13**) (0.28 g, 0.54 mmol) in methanol (20 ml) was added to sodium borohydride (0.115 g, 2.96 mmol) in small portions, during 1 h. Once the addition was completed, the reaction mixture was acidified with 3M-hydrochloric acid, diluted with water (50 ml), and extracted with chloroform (3 × 40 ml). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated. Chromatography of the residue (0.28 g) on silica gel column and elution with light petroleum and then light petroleum–ether mixtures (up to 7:3) afforded, sequentially: the (22*R*)-hydroxy ester (**14b**) (0.05 g, 18%), a mixture of the (22*R*)- and (22*S*)-epimers (0.04 g) and, finally, the (22*S*)-hydroxy ester (**14a**) (0.15 g, 54%). The spectroscopic data and analytical properties of (22*S*)- and (22*R*)-22-hydroxy esters obtained from (**13**) were identical, respectively, with those of (**14a**) and (**14b**) obtained from (**12**). Analogously, alkaline hydrolysis of (**14a**) and (**14b**) obtained from (**13**) under the conditions above reported, yielded the (22*R*)- and (22*S*)-hydroxy acids with properties identical in all respects to the corresponding acids (**14c**) and (**14d**) obtained by the catalytic hydrogenation route.

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