ISOLATION OF (23*R*) 3α,7α,23-TRIHYDROXY-5β-CHOLAN-24-OIC (β-PHOCAECHOLIC) ACID FROM DUCK BILE. ^{1}H NMR SPECTRA OF ITS DERIVATIVES

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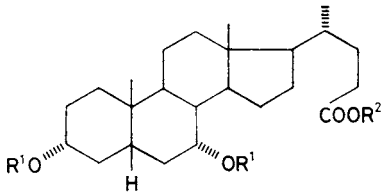
(23*R*) 3α,7α,23-Trihydroxy-5β-cholan-24-oic acid (*IV*) — a bile acid typical of some marine mammals — was now isolated from duck bile. Acid *IV* was characterized as derivatives *V*—*VIII*, *XI* and *XII* and oxidatively degraded to derivatives of 24-nor-5β-cholan-23-oic acid, *XIII*—*XVIII*. The ^{1}H NMR spectra of these compounds and (23*S*) methyl ester *X* are discussed and the effect of substitution in position 23 on the chemical shifts of the methyl groups is summarized.

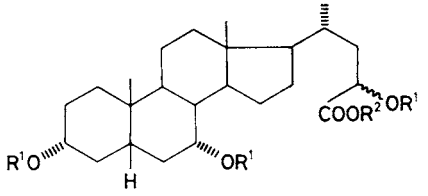
During the isolation of chenodeoxycholic acid (*I*) from the bile of various poultry according to ref.[1] we found that in the bile of ducks (*Anas platyrhynchos* f. *domestica*) from south Moravia an unusual bile acid is present in considerable amount. We separated this acid from chenodeoxycholic acid (*I*) and other bile acids by chromatography on silica gel. According to the mass and ^{1}H NMR spectra of the acid and its methyl ester it was trihydroxycholanic acid ($C_{24}H_{40}O_5$) which has only two hydroxyl groups on the steroidal skeleton, while the third hydroxyl group is on the side chain. One of the melting points of this dimorphic acid and two of the melting points of the tetramorphic methyl ester were close, but not identical, with the literature data[2-9] for β-phocaecholic acid (*IV*, (23*R*) 3α,7α,23-trihydroxy-5β-cholan-24-oic acid) and its methyl ester *V*. So far acid *IV* was isolated from the bile of some marine mammals[2-11]. In view of the mentioned discrepancy in the melting points and the optical rotation values[5,7] of acid *IV*, and in view of the lack of other physical data necessary for identification (especially spectral data; in literature we found only data on circular dichroism and brief data on the mass spectrum of methyl ester *V* (refs[9,10]) and the mass spectrum of methyl ester triacetate *VII* (ref.[12])), we confirmed the structure of the acid *IV* isolated by us independently by chemical degradation to 24-nor-5β--cholane compounds *XIII*—*XVIII* and on the basis of spectral data of derivatives *V*—*VIII*, *XI*, and *XII*.

Acid *IV* when acetylated with acetic anhydride in pyridine gave triacetate *VI* from which methyl ester triacetate *VII* was prepared. Oxidation of methyl ester *V*

with sodium dichromate in acetic acid in the presence of sodium acetate gave triketone *XI* as the main product, accompanied by a small amount of hydroxydiketone *XII*. Free acid *IV* is degraded oxidatively by losing one carbon atom: when reacting with chromium trioxide in acetic acid it gave diketo noracid *XIII* as the main product, which was also characterized as methyl ester *XIV*. When potassium permanganate in acetone was used (according to refs[4,5]) for the oxidation of acid *IV*, dihydroxy noracid *XV* was formed. This acid was converted to methyl ester *XVI*, diacetate *XVII*, and methyl ester diacetate *XVIII*. The melting points of compounds *XIII*, *XV*–*XVII* and the optical rotation of acid *XV* agree with the values given in literature[4,5,7,13,14] for norchenodeoxycholic acid (*XV*, 3α,7α-dihydroxy-24-nor-5β-cholan-23-oic acid) and its derivatives. The above mentioned facts confirm the presence of 3α,7α and 23 hydroxyl groups in acid *IV*.



I, $R^1 = R^2 = H$
II, $R^1 = H$; $R^2 = CH_3$
III, $R^1 = CH_3CO$; $R^2 = CH_3$

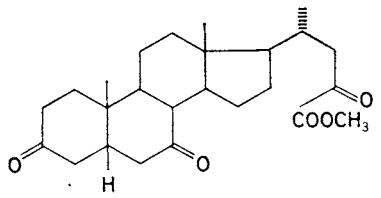


IV, $R^1 = R^2 = H$ (23*R*)
V, $R^1 = H$; $R^2 = CH_3$ (23*R*)
VI, $R^1 = CH_3CO$; $R^2 = H$ (23*R*)
VII, $R^1 = CH_3CO$; $R^2 = CH_3$ (23*R*)
VIII, $R^1 = CCl_3CONHCO$; $R^2 = CH_3$ (23*R*)
IX, $R^1 = R^2 = H$ (23*S*)
X, $R^1 = H$; $R^2 = CH_3$ (23*S*)

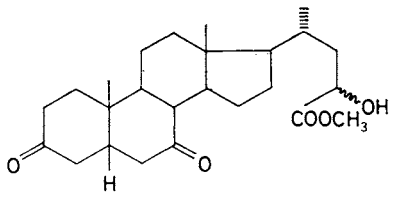
In addition to acid *IV* we also isolated a small amount of another trihydroxycholanic acid from the bile, in the form of methyl ester. The agreement of the physical constants of this methyl ester with the literature data[9] for methyl (23*S*) 3α,7α,23-trihydroxy-5β-cholan-24-oate (*X*) and the similarity of its mass and ^1H NMR spectra with those of methyl ester *V* indicate that this acid is isomeric with acid *IV* at $C_{(23)}$ and that its structure corresponds to formula *IX*. Absolute configuration at $C_{(23)}$ of acid *IV* and of its 23-isomer *IX* was derived recently by Kutner and Jaworska[9] from molecular rotations and the circular dichroism data for methyl esters *V* and *X*. In agreement with the authors of paper[9] we found a negative Cotton effect (at 211 nm) in (23*R*) methyl ester *V* and a positive Cotton effect in the (23*S*) isomer *X*. The optical rotation values of methyl esters *V* and *X* also agree with the data in paper[9].

In the mass spectra of methyl esters *V* and *X* we found all fragment ions given in literature[9,10]. In addition to them the peaks of ions *m/z* 345 appear in the spectra, characteristic of compounds with a hydroxyl group in position 23. They are formed

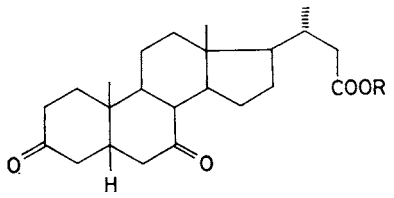
by elimination of methoxycarbonyl from ions m/z 404 ($[M - 18]^{+\bullet}$) under formation of oxonium ions. Further, the splitting off of HO—CH—$COOCH_3$ from the ion m/z 404 under formation of ion m/z 315 and (after loss of water) of ion m/z 297 is characteristic of the position of the hydroxyl on $C_{(23)}$. The isomeric methyl esters *V* and *X* can be differentiated on the basis of the ratio of the peak intensities of ions m/z 371 ($[M - 18 - 18 - 15]^+$) and m/z 404. In the case of (23*S*) methyl ester *X* this ratio is higher than 1, while in the (23*R*) isomer *V* it is lower than one. This fact was derived from the spectra measured at 70 eV, while at 15 eV the mass spectra of compounds *V* and *X* are identical. This fact does not correspond to the literature data[9] where at 15 eV the peak of the ion m/z 371 is more intensive in the (23*R*) methyl ester *V* than in the (23*S*) isomer *X*. In the spectrum of acid *IV* the peaks of the ions characterizing the hydroxyl in the position 23 do not occur. It is only evident that the hydroxyl group is bound to the side chain. A detailed discussion of the mass spectral fragmentation of the compounds presented here will be the subject of a further communication[15].



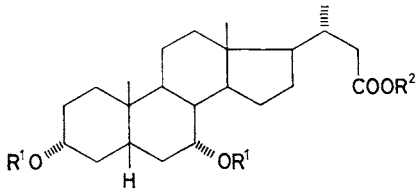
XI



XII (23 *R*)



XIII, R = H
XIV, R = CH_3



XV, $R^1 = R^2 = H$
XVI, $R^1 = H$; $R^2 = CH_3$
XVII, $R^1 = CH_3CO$; $R^2 = H$
XVIII, $R^1 = CH_3CO$; $R^2 = CH_3$

Characteristic parameters of the ^1H NMR spectra of the (23*R*) acid *IV*, its derivatives and degradation products and of the (23*S*) methyl ester *X* are listed in Table I. For comparison analogous derivatives of chenodeoxycholic acid, *II*, *III*, *XIX*, and *XX*, are also included in the table. The data found for these derivatives are in good agreement with the data in refs[16,17]. The presence of 3α- and 7α-hydroxyl groups

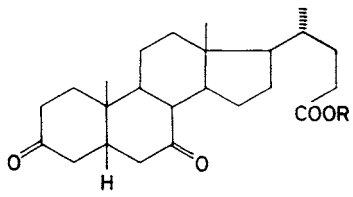
TABLE I

Parameters of the ^{1}H NMR spectra (in deuteriochloroform)

Compound	$C_{(18)}H_3$[a]	$C_{(19)}H_3$[a]	$C_{(21)}H_3$[b]	OCH_3[a]	3β-H[c]	7β-H[d]	23-H[e]
II	0·66	0·90	0·91	3·66	3·4	3·82	—
III[f]	0·65	0·93	0·93	3·65	4·6	4·88	—
IV[g]	0·69	0·89	0·97	—	[h]	[h]	4·03 dd (J = 10·5, ~2)
V	0·69	0·90	1·01	3·77	3·42	3·82	4·23 dd (J = 10·5, ~2·5)
VI[i]	0·67	0·94	0·97	—	4·58	4·88	5·08 dd (J = 10·5, ~1·5)
VII[i]	0·67	0·94	0·96	3·72	4·58	4·88	5·06 dd (J ~ 11, ~2)
VIII[j]	0·70	0·97	1·02	3·78	4·68	5·03	5·19 dd (J = 10·5, ~2)
X	0·67	0·90	1·01	3·77	3·46	3·84	4·21 bt (ΣJ ~ 11)
XI[k]	0·74	1·31	0·97	3·86	—	—	—
XII	0·73	1·30	1·02	3·78	—	—	4·23 dd (J = 10·5, ~2)
XIII	0·74	1·31	1·04	—	—	—	—
XIV	0·74	1·30	0·99	3·66	—	—	—
XVI	0·70	0·91	0·98	3·65	3·46	3·83	—
XVII[f,l]	0·69	0·93	1·03	—	4·58	4·88	—
XVIII[f]	0·69[m]	0·93[m]	0·98	3·66	4·58	4·86	—
XIX	0·70	1·30	0·95	—	—	—	—
XX	0·70	1·30	0·93	3·66	—	—	—

[a] Singlet; [b] asymmetric broadened doublet, splitting 5·3—6·3 Hz; [c] broad multiplet ($W_{1/2}$ = 20—25 Hz), approximate centre of the multiplet is given; [d] narrow multiplet ($W_{1/2}$ = 7—8 Hz); [e] dd doublet of doublets, bt broad triplet; [f] CH_3COO: 2·02 and 2·04; [g] measured in a mixture of C^2HCl_3 + $(C^2H_3)_2SO$ 2 : 7; [h] overlapped by further signals; [i] CH_3COO: 2·03, 2·05, and 2·14; [j] prepared *in situ* with trichloroacetyl isocyanate, NH: 8·23, 8·31, and 8·53; [k] 22-H_2: 2·62 dd (J = 17·0 and 9·5 Hz) and 2·89 dd (J = 17·0 and 3·1 Hz); [l] some of the signals are also given in ref.[14]; [m] ref.[18] gives for these methyl signals 0·75 and 0·96.

in acids *IV* and *IX* is evident from the comparison of hydroxy derivatives *V*, *X*, and *XVI* with methyl chenodeoxycholate (*II*) and the acetates *VI*, *VII*, *XVII*, and *XVIII* with the methyl ester diacetate *III*: The chemical shifts of 3β-H and also the shifts of 7β-H agree in the mentioned series of compounds. The signal of the axial 3β-H appears in all compounds as a broad multiplet ($W_{1/2}$ = 20–25 Hz) and the signal of the equatorial 7β-H as a narrow multiplet ($W_{1/2}$ = 7–8 Hz). Both the chemical shifts and the shapes of the signals correspond according to ref.[16] to 3α,7α--substitution and they differ from other possible positions and configurations of skeletal hydroxyl groups. The agreement may also be observed in the chemical shifts of the methyl group $C_{(19)}H_3$, while in the case of $C_{(18)}H_3$ only small differences are observable, which can be ascribed to the effect of the structural changes in the side chain. Similarly the chemical shifts of the angular methyl groups of keto derivatives *XI*–*XIV* are in agreement with the shifts in 3,7-dioxo derivatives *XIX* and *XX*. On their basis other possible combinations of the positions on the steroidal skeleton may be excluded, for example 3, 12 or 7, 12 (see ref.[16]). The shape of the spectrum in the region of the signals of the protons in α-position to the carbonyl groups (δ 2·1–3·0) is similar as in 3,7-diketones *XIX* and *XX*, but it differs from diketones with other positions of the keto groups.



XIX, R = H
XX, R = CH_3

In contrast to derivatives *II* and *III* of chenodeoxycholic acid the multiplet in the region δ 2·1–2·5, characteristic of the methylene group in α-position to the $COOCH_3$ group is not present in the spectra of compounds *V*–*VIII* and *X*. The signal 23-H is shifted downfield (with respect to 3β-H and 7β-H) in compounds *IV*–*VIII*, *X*, and *XII*, and is coupled with two protons. The hydroxyl group in the side chain of the methyl esters *V*, *VII*, *X*, and *XII* causes a downfield shift of the ester methoxyl singlet and the doublet of the methyl group on $C_{(20)}$ (see also Table II). The signal of the acetoxyl group methyl in the side chain in acetates *VI* and *VII* is also shifted downfield (δ 2·14) in comparison with 3α- and 7α-acetoxyl methyls (δ ~ 2·02 and ~ 2·04). In the spectrum of triketone *XI* the signal of the methoxy group is shifted downfield and both protons of the methylene group in position 22 display, in addition to the geminal coupling (17 Hz), vicinal coupling with one adjacent hydrogen (9·5

and 3·1 Hz). All these characteristics are in agreement with the presence of the 23-hydroxyl group in acids *IV* and *IX*.

The isomers on $C_{(23)}$ differ in the shape of the signal 23-H. In all (23*R*) derivatives *IV*–*VIII* and *XII* this signal appears as a doublet of doublets with $J_{22,23} \sim 10{\cdot}5$ and ~2 Hz. The values of the vicinal coupling constants indicate that one of the conformers of the $C_{(22)}$—$C_{(23)}$ bond with antiperiplanar arrangement of 23-H and one of the 22-H is highly preferred. On the other hand, in the spectrum of (23*S*) methyl ester *X* the signal of 23-H appears as a triplet ($\sum J \sim 11$ Hz) which can be caused by a different population of conformers than in the preceding case, or rather by a degeneration of the spectrum (chemical equivalence of the protons in position 22).

In Table II the effects of the substituents in position 23 are summarized as well as the effect of the shortening of the side chain (in 24-nor derivatives) on the chemical shifts of the methyl groups in the side chain and in its vicinity (the signal $C_{(19)}H_3$ is practically unaffected by the changes in the side chain). The values given in Table II complete the tables published by Iida and Chang[16] and they may have a diagnostic value for the identification of bile acids with a modified side chain. For example 23-hydroxy-24-cholanoic acids can be differentiated easily from other bile acids on the basis of the chemical shifts of the methoxy group and the methyl group on $C_{(20)}$ in their methyl esters.

(23*S*) 3α,7α,23-Trihydroxy-5β-cholan-24-oic acid (*IX*) has not yet been found in natural material. In our case it evidently represents an artifact, formed from (23*R*) acid *IV* during the isolation, probably during the alkaline hydrolysis of the conjugated bile acids. We found that on heating (23*R*) methyl ester *V* in boiling 10% potassium hydroxide in water (28 h) partial isomerization takes place in position 23 and a small amount of acid *IX* is formed which we have identified as methyl ester *X* by thin-layer chromatography.

TABLE II

Effect of the changes in the side chain on the chemical shift of the methyl groups protons

Compound	$\Delta\delta^a$		
	$C_{(18)}H_3$	$C_{(21)}H_3$	$COOCH_3$
(23*R*)-OH	0·03	0·09—0·10	0·11—0·12
(23*S*)-OH	0·01	0·10	0·11
(23*R*)-$OCOCH_3$	0·02	0·03	0·07
23-oxo	0·04	0·04	0·20
24-nor	0·04	0·05—0·09	~0

[a] $\Delta\delta = \delta$ (23-substituted or 24-nor derivative) − δ (corresponding derivative of acid *I*).

β-Phocaecholic acid (*IV*) was found in the bile of marine mammals of the order *Pinnipedia* (seals, sea lions, and walruses[2-11]) exclusively and has not been identified so far in other natural sources. Its presence in duck bile is surprising. To the knowledge of the authors neither acid *IV* nor any other bile acid with a hydroxyl group in the side chain has been found in the bile of poultry.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. Optical rotations were measured on an ETL–NPL polarimeter (Bendix–Ericsson) with a $\pm 2°$ accuracy. The infrared spectra were measured in chloroform on a PE 684 (Perkin–Elmer) instrument, the ^{1}H NMR spectra on a Tesla BS 487A spectrometer at 80 MHz, in deuteriochloroform solutions with hexamethyldisiloxane as internal standard. The chemical shifts were converted with respect to tetramethylsilane as a reference ($\delta_{HMDS} + 0{\cdot}06$) and they are given in δ-scale (in ppm). The coupling constants were obtained by first order analysis. The mass spectra were measured on an AEI MS 902 spectrometer at electron energy of 70 or 15 eV and temperature of the ion source 120–130°C; the composition of individual ions was determined by measurement at a resolving power of 10 000. The circular dichroism absorption was measured on Dichrographe II (Roussel–Jouan).

For column chromatography silica gel Silpearl (Kavalier, Votice) and alumina (Reanal, activity II) were used. The purity of the preparations was checked by thin-layer chromatography on silica gel according to Stahl (Merck G 60, detection with 10% sulfuric acid and heating) and on Silufol UV-254 foils (Kavalier, Votice; detection with a 10% phosphomolybdic acid solution and heating). The samples for analysis were dried under reduced pressure at 100°C over phosphorus pentoxide. Methyl esters were prepared on reaction of the acids with an ethereal diazomethane solution. The solution of the ester was filtered through a layer of alumina. The duck bile used was obtained from Drůbežářský průmysl in Prague.

Isolation of (23*R*) 3α,7α,23-Trihydroxy-5β-cholan-24-oic Acid (*IV*)

Hydrolysis of the conjugates and the isolation of bile acids were carried out according to ref.[1]. From 100 ml of duck bile 9·1 g of a raw mixture of bile acids were obtained, which was suspended in a mixture of chloroform and acetone (1 : 1, 50 ml) and introduced onto a silica gel column (700 g). Using benzene and mixtures of benzene and acetone (10 : 1 to 4 : 1) the elution proceeded as follows: mixtures of unidentified non-polar substances (1·3 g) were eluted first, followed by chenodeoxycholic acid (*I*; 3·5 g), a mixture of acids *I* and *IV* (0·6 g) and crude acid *IV* (1·8 g). The crude acid *IV* was extracted three times with 5 ml of boiling acetone. The solid residue was crystallized from ethyl acetate. Yield, 1·2 g of acid *IV*, m.p. 164–166°C and, after resolidification, 233–235°C, $[\alpha]_D$ +11° (ethanol, *c* 0·6). Refs[2-8] give m.p. in the 218–224°C interval. Ref.[5] gives $[\alpha]_D$ +18°, ref.[7] +10·8°. High resolution mass spectrum: $M^{+\cdot}$ 408 ($C_{24}H_{40}O_5$).

Methyl ester *V* was obtained in four crystal modifications: m.p. 94–96°C (ethyl acetate–hexane), when further heated slowly it crystallizes and melts at 155–160°C. According to ^{1}H NMR spectrum it contains 0·5 mol of ethyl acetate; m.p. 159–162°C (ethyl acetate–hexane), according to the ^{1}H NMR spectrum it does not contain a solvent of crystallization; m.p. 103–110°C (ether or ether–hexane), according to ^{1}H NMR spectrum it contains ether; m.p. 104–125°C (chloroform or chloroform–heptane). Ref.[5] gives m.p. 105°C, ref.[8] 115–116°C, ref.[9] 110–112°C. For spectral measurements a modification free of solvent was used. $[\alpha]_D$ +6°

(chloroform, *c* 0·5), ref.[9] gives +8°. Circular dichroism (in methanol): Δε −2·2 (211 nm), ref.[9] gives −2·38. IR spectrum: 3 610, 3 535, 1 732, 1 076, and 976 cm^{-1}. High resolution mass spectrum: $M^{+\cdot}$ 422 ($C_{25}H_{42}O_5$).

Triacetate *VI* was prepared from acid *IV* by reaction with acetic anhydride in pyridine (1 : 1) at room temperature (5 days). M.p. 218–220°C (chloroform–heptane). $[\alpha]_D$ +17° (chloroform, *c* 0·7). For $C_{30}H_{46}O_8$ (534·7) calculated: 67·39% C, 8·67% H; found: 67·01% C, 8·89% H.

Methyl ester triacetate *VII*. M.p. 195–196°C (chloroform–heptane). $[\alpha]_D$ +19·5° (chloroform, *c* 0·7). Ref.[8] gives m.p. 190–191°C.

Isolation of Methyl (23*S*) 3α,7α,23-Trihydroxy-5β-cholan-24-oate (*X*)

The acetone extracts and the mother liquors obtained during the purification of crude acid *IV* were combined, evaporated and the residue (0·60 g) treated with an ethereal diazomethane solution. The mixture of the methyl esters formed was separated on a silica gel column. Chloroform and a mixture of chloroform and acetone (10 : 1) eluted first methyl ester *V* (0·53 g) and then methyl ester *X* (30 mg), which after crystallization from chloroform–heptane had m.p. 198–200°C. $[\alpha]_D$ +22° (chloroform, *c* 0·6). Ref.[9] gives m.p. 197–199°C, $[\alpha]_D$ +20°. Circular dichroism (in methanol): Δε +1·4 (211 nm), ref.[9] gives +1·50. High resolution mass spectrum: $M^{+\cdot}$ 422 ($C_{25}H_{42}O_5$).

Oxidation of Methyl Ester *V*

A suspension of methyl ester *V* (0·12 g), anhydrous sodium acetate (0·12 g) and sodium dichromate dihydrate (0·18 g) in acetic acid (20 ml) was stirred at room temperature. After 10 min all the components were dissolved. The solution was allowed to stand for 4·5 h, the excess of the oxidant was reduced with methanol, the mixture was diluted with water and extracted with ether. The extract was washed with water and dried over sodium sulfate. Ether was distilled off and the residue chromatographed on a silica gel column (10 g). Light petroleum–acetone mixture (10 : 1) eluted 80 mg of methyl 3,7,23-trioxo-5β-cholan-24-oate (*XI*), m.p. 156–158°C (chloroform–light petroleum). $[\alpha]_D$ −36° (chloroform, *c* 0·6). IR spectrum: 1 721, 1 709, 1 435 cm^{-1}. For $C_{25}H_{36}O_5$ (416·5) calculated: 72·08% C, 8·71% H; found: 72·03% C, 8·74% H.

Using the same solvent mixture methyl (23*R*) 23-hydroxy-3,7-dioxo-5β-cholan-24-oate (*XII*; 30 mg) was eluted, m.p. 160–161°C (chloroform–heptane), $[\alpha]_D$ −40° (chloroform, *c* 0·5). IR spectrum: 3 530, 1 728, 1 709, 1 435 cm^{-1}. For $C_{25}H_{38}O_5$ (418·5) calculated: 71·74% C, 9·15% H; found: 71·52% C, 9·43% H.

Oxidation of Acid *IV*

a) With chromium trioxide. CrO_3 (0·1 g) was added to a suspension of acid *IV* (0·1 g) in a mixture of acetic acid (25 ml) and water (1 ml) and the mixture was allowed to stand at room temperature for 2 h under occasional stirring. It was worked up as in the preceding experiment. Crystallization of the residue from ether–hexane gave 3,7-dioxo-24-nor-5β-cholan-23-oic acid (*XIII*; 60 mg). M.p. 200–202°C, $[\alpha]_D$ −37° (chloroform, *c* 0·8). Refs[4,13] give m.p. in the 200–202°C range.

Methyl ester *XIV*. M.p. 167–170°C (chloroform–heptane). $[\alpha]_D$ −36° (chloroform, *c* 0·7). For $C_{24}H_{36}O_4$ (388·5) calculated: 74·19% C, 9·34% H; found: 74·02% C, 9·54% H.

b) With potassium permanganate. A 1% aqueous potassium permanganate solution (24 ml) was added gradually to a solution of acid *IV* (0·30 g) in acetone (50 ml) at room temperature over 0·5 h and after 5 h standing the mixture was diluted with a solution of sodium bisulfite and acidified with hydrochloric acid. The product was extracted with ether, the ethereal layer washed

with water and dried over sodium sulfate. After evaporation of the ether the residue (0·25 g) was chromatographed on a silica gel column. Crystallization from a mixture of methanol and acetone 3α,7α-dihydroxy-24-nor-5β-cholan-23-oic acid (*XV*; 0·17 g) was obtained, m.p. 205—206°C, $[\alpha]_D$ +12° (ethanol, *c* 0·6). Refs[4,5,13] give m.p. 197—198°C, ref.[7] 199—202°C. Ref.[13] gives $[\alpha]_D$ +8°.

Methyl ester *XVI*, m.p. 84—86°C (ether–light petroleum), $[\alpha]_D$ +11° (chloroform, *c* 0·7). Ref.[4] gives m.p. 78°C, refs[5,13] 85—87°C.

Diacetate *XVII* was prepared from acid *XV* in the same manner as triacetate *VI* (see above). M.p. 184—186°C (ether–hexane), $[\alpha]_D$ +18° (chloroform, *c* 0·8). Ref.[13] gives m.p. 213—214°C, ref.[14] 184—186°C.

Methyl ester diacetate *XVIII*, m.p. 131—133°C (ether–hexane), $[\alpha]_D$ +19·5° (chloroform, *c* 0·6). IR spectrum: 1 727, 1 436, 1 377, 1 252 cm^{-1}. For $C_{28}H_{44}O_6$ (476·6) calculated: 70·55% C, 9·31% H; found: 70·38% C, 9·21% H.

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