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A NEW STEROID AROMATIZATION REARRANGEMENT INVOLVING INVERSION OF SIDECHAIN CONFIGURATION

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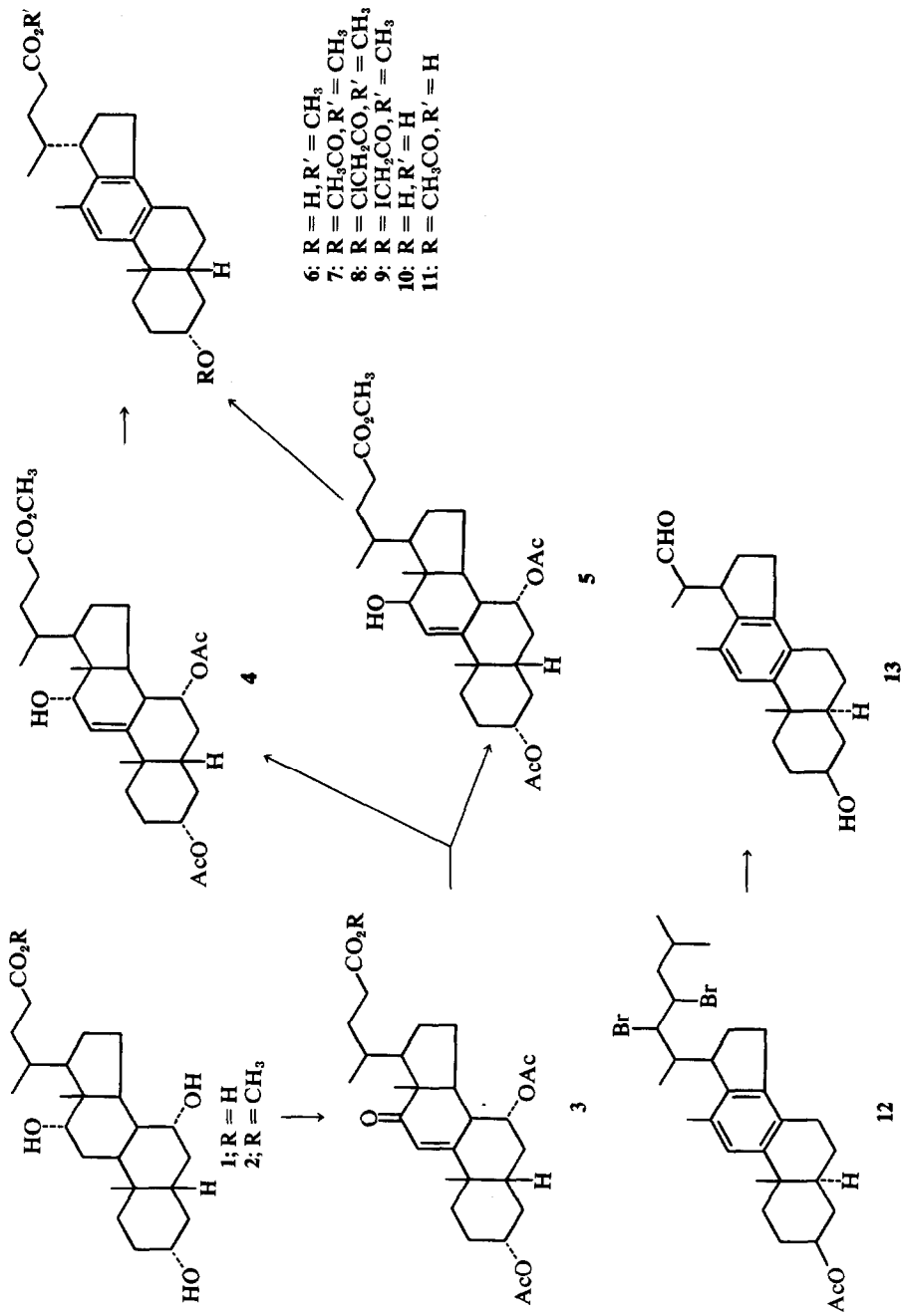
Abstract—The bile acid, cholic acid, has been transformed into 3α-hydroxy-12-methyl-18-nor-5β, 17α-chola-8,11,13-trien-24-oic acid. The constitution of this novel type of steroid (benzenoid C-ring with 17α-sidechain) has been supported by chemical degradation, and confirmed by crystal structure analysis of the methyl ester iodoacetate derivative.

As part of our general study[1-4] of routes to ring-C benzenoid steroids from readily available precursors, we recently examined the sodium borohydride reduction of conjugated unsaturated 12-ketones derived from cholic acid (**1**), since it was considered probable that dehydration of the derived epimeric allylic C-12 alcohols may yield molecular rearrangement products capable of aromatization.[5] This has now in fact been realized by a simple and novel pathway leading to a 12-methyl-18-nor-C-benzenoid derivative with inversion of configuration of the precursor bile acid sidechain.[6]

Methyl cholate (**2**) is readily converted to methyl 3α,7α-diacetoxy-12-oxochol-9(11)-enate (**3**) by partial acetylation to the 3,7-diacetate[7] followed by consecutive chromic acid and selenium dioxide oxidations.[8] Reduction of the conjugated ketone with sodium borohydride in methanol gives a mixture of the epimeric 12α- and 12β-alcohols (**4** and **5**) with a slight predominance of the former. Although these could be conveniently separated on a small scale by TLC or the 12α-epimer isolated by fractional crystallization, we intended to simplify the isolation and yield of **4** by a procedure used successfully in the deoxycholic acid series. Thus Kendall *et al.*[9] had shown that each analogue (lacking a C-7 substituent) of epimers **4** and **5** on treatment with methanol and acid catalyst yielded the same allylic 12α-methyl ether from which the 12α-alcohol could be readily re-generated. In marked contrast, we find that when hydrogen chloride is passed through a methanolic solution of the epimeric mixture of **4** and **5**, there is obtained in 80–90% yield a product which we formulate as methyl 3α-hydroxy-12-methyl-18-nor-5β,17α-chola-8,11,13-trien-24-oate (**6**) in which the original 3α-acetoxy group has been hydrolyzed, the 7-acetoxy and 12-hydroxy group have been eliminated, the tertiary (C-18) methyl group has migrated from C-13 to C-12 and the 17β-sidechain configuration has been inverted. Evidence supporting this structure comes from appropriate sepectroscopic analysis, chemical degradation, and crystal structure analysis of a suitable derivative. Each epimeric allylic alcohol (**4** and **5**) treated independently yielded the same ring-C benzenoid product (**6**).

An accurate mass measurement indicated an empirical formula $C_{25}H_{36}O_3$ for this product and the integrated NMR spectrum (Table 2) revealed the presence of a strongly shielded secondary Me group, a tertiary Me group, a benzylic Me group, a methoxycarbonyl group, and one aromatic proton. A signal attributable to an OH proton (exchanged by D_2O) and a carbinol proton (shifted downfield by 1·21 ppm on addition of trichloroacetylisocyanate[10]) was characteristic of a secondary alcohol function. Difficulties in obtaining 12-methyl-18-nor-C-benzenoid steroids in crystalline form have been previously noted;[1] from the noncrystalline alcohol (**6**), however, we have isolated crystalline acetate (**7**), chloroacetate (**8**) and iodoacetate (**9**) ester derivatives by standard techniques, and the crystalline hydroxy acid (**10**) by base hydrolysis of the acetate methyl ester (**7**). Esterification of **10** with diazomethane regenerated the noncrystalline alcohol (**6**), and acetylation of **10** gave a noncrystalline acetoxy acid (**11**).

The UV absorption spectrum of the acetate methyl ester (**7**) showed typical benzenoid absorption and, in particular, consideration of the B-band fine structure showed a close correspondence to that reported for 22,23-dibromo-12-methyl-18-norergosta-8,11,13-trien-3β-yl acetate (**12**), a ring


1; R = H
2; R = CH3
3
4
5
6: R = H, R' = CH3
7: R = CH3CO, R' = CH3
8: R = ClCH2CO, R' = CH3
9: R = ICH2CO, R' = CH3
10: R = H, R' = H
11: R = CH3CO, R' = H
12
13

C-benzenoid steroid obtained from ergosterol and of well-established constitution.[1,2] The strongly shielded secondary Me group (Tables 2–4) of the benzenoid bile acid derivatives (**6–11**) suggested however a fundamental structural difference of which the most likely, reflecting a different spatial relationship of the C-21 Me protons to the benzenoid ring, appeared to be an inversion of configuration at C-17, a transformation clearly permitted within the mechanistic context of acid-catalyzed dehydration and aromatization of **4** and **5**. To gain support for this hypothesis, it was decided to convert the 3α-hydroxy-5β-(H)-carboxylic acid (**10**) to the isomeric 3β-hydroxy-5α-(H)-carboxylic acid (**17**) and thence to the aldehyde (**26**) to permit comparison with the known aldehyde (**13**) obtained from **12** by debromination, osmium tetroxide hydroxylation and periodic acid cleavage.[1]

We chose to effect the configuration changes at C-3 and C-5 (**10** → **17**) by oxidation, bromination, dehydrobromination and lithium-ammonia reduction sequence. Oxidation of the hydroxy acid (**10**) with Jones' reagent gave the keto acid (**14**) which was treated with bromine in acetic acid solution to give the 4β-bromoketo acid (**15**) which, without purification, was dehydrobrominated with lithium chloride in dimethylformamide to give the conjugated ketone acid (**16**). The 4β- (equatorial) halogen configuration of the bromo ketone is assigned on the basis of the characteristic IR absorption CO frequency shift as compared to the parent ketone[11] and the large spin-spin coupling constant associated with the 4α (axial) proton. Reduction of **16** with lithium in ammonia-ethanol gave the desired 3β-hydroxy-5α-(H)-carboxylic acid (**17**) in crystalline form which, on acetylation yielded the non-crystalline acetoxy-5α-(H)-carboxylic acid (**18**).

The conversion of the original benzenoid product, the hydroxyl methyl ester (**6**), to the same required 3β-acetoxy-5α-(H)-carboxylic acid (**18**) was also effected by an alternative pathway. Oxidation of **6** with Jones' reagent gave the keto ester (**19**), which on bromination yielded the rather labile 4β-bromoketo ester (**20**), which was dehydrobrominated as before. In this case, a more complex mixture of products was obtained from which, after re-esterification and TLC separation, there was obtained the Δ^4-3-keto methyl ester (**21**). A minor fraction also isolated from this experiment, although incompletely characterized, is considered to be the Δ^1-isomer (**22**). The lithium-ammonia reduction of the conjugated keto ester (**21**) was much less satisfactory than found previously for the corresponding acid. Thus, examination of the NMR spectrum of the total reduction product showed the absence of carbomethoxyl protons. This led to the conclusion that the principal product was the diol (**23**); acetylation of a small quantity yielded an acetylation product with NMR spectrum in agreement. The transformation of **23** to the required acetoxy acid (**18**) was then completed by oxidation to the keto acid (**24**), followed by sodium borohydride reduction and acetylation.

The assignment of the expected more stable configurations at C-3 (β equatorial) and C-5 (A/B *trans*) in compound **17** is supported (Table 3) by the chemical shift value (δ 2·03) found for the acetoxyl protons of the derived acetate (**18**), i.e., at the same value found for the 3β-acetoxy protons of the 5α-(H)-ring C benzenoid analogue obtained from ergosterol[1] and further downfield than the corresponding signal (δ 1·93) of the protons (Table 2) of 3α-acetoxy-5β-(H) compounds which are closer to the shielding influence of the benzenoid ring. The *trans* A/B ring junction of **17** is also established by obtaining the same compound by reduction of the keto acid (**24**) which differs from the keto acid (**14**) obtained unexceptionally from the original rearrangement product (**6**).

Attention was then turned to degradation of the sidechain. Treatment of the acetoxy acid (**18**) with lead tetraacetate[12,13] yielded the expected product of oxidative decarboxylation, the olefin (**25**) which on oxidation with osmium tetroxide-hydrogen peroxide followed by periodate cleavage yielded the aldehyde (**26**), further characterized as the 2,4-dinitrophenylhydrazone derivative. Since the aldehyde (**26**) so obtained (and its derivative) differ from the aldehyde (**13** and corresponding derivative) previously obtained from ergosterol and of established 3β-hydroxy-, 5α-(H), and 17β-configurations, this supports our original structural hypothesis that the aromatized bile acid product (**6**) had also undergone a sidechain configurational inversion.

Being aware that an unrecognized unpropitious epimerization[14,15] at C-20 in the isolation of either aldehyde (**13** or **26**) would have vitiated this conclusion, we sought to verify the proposed constitution by undertaking a crystal structure analysis of the iodoacetate derivative (**9**).

Crystals of **9** are monoclinic, space group C_2^2-P2$_1$, with a = 11·236 ± 0·01, b = 14·35 ± 0·01, c = 8·117 ± 0·008 A, β = 90·59 ± 0·2°. With 2 molecules in the unit cell the calculated density is 1·40 gm/cm.[3] The structure was determined from 608 observed X-ray diffraction intensities by the heavy atom method, with the I atom being located from the Patterson function. Because of the centrosymmetric arrangement of the I atoms, the structure appeared "doubled" in the I-phased electron-density map. Many maps, each adding a few more light atoms, were needed to reveal the final structure. Refinement of the structure by least-squares calculations[16] to a conventional R-factor of 0·07 led to the parameters listed in Table 1. Fig 1 shows one molecule viewed along **b**. The structure confirms the 17α configuration of the side chain and provided an independent verification of the entire

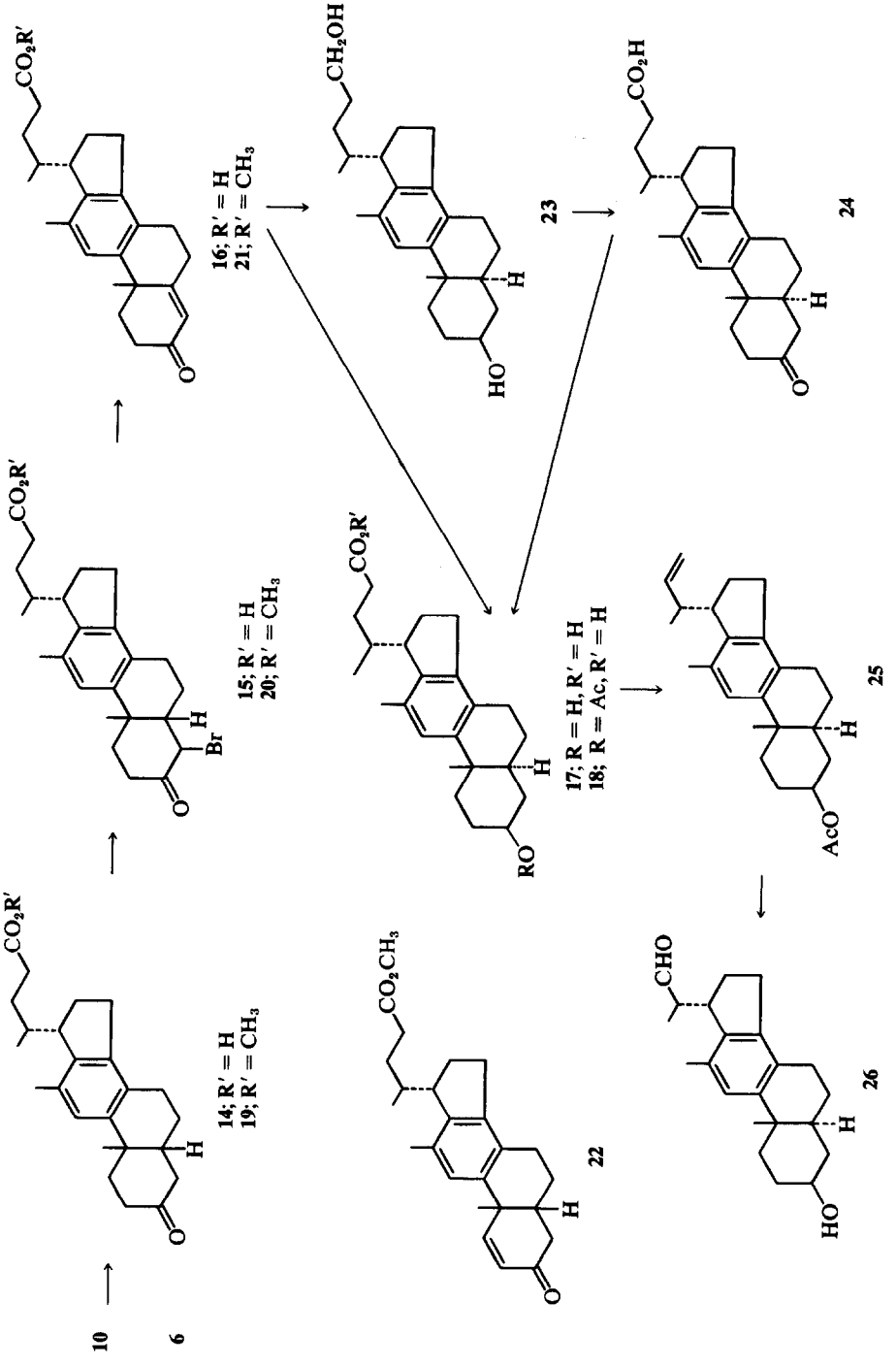

10
6
14; R′ = H
19; R′ = CH3
15; R′ = H
20; R′ = CH3
16; R′ = H
21; R′ = CH3
22
17; R = H, R′ = H
18; R = Ac, R′ = H
23
24
25
26
CO2R′
CO2CH3
CH2OH
CO2H
CHO
Br
RO
HO
AcO
H
O

Table 1. Atomic parameters for the iodoacetate[a]

Atom	x	y	z	B(Å²)	Atom	x	y	z	B(Å²)
I[b]	0·8697	0·5000	−0·0342		C(12)	0·692	−0·015	0·308	4·6
O(1)	0·658	0·436	0·262	6·0	C(13)	0·742	−0·015	0·467	4·9
O(2)	0·554	0·514	0·065	7·6	C(14)	0·712	0·041	0·590	5·4
O(3)	0·096	−0·246	0·160	13·3	C(15)	0·778	0·023	0·750	4·4
O(4)	0·930	−0·274	0·245	12·1	C(16)	0·833	−0·070	0·719	5·4
C(1)	0·462	0·228	0·196	5·7	C(17)	0·843	−0·093	0·527	6·5
C(2)	0·571	0·290	0·158	4·5	C(18)	0·724	−0·079	0·166	5·3
C(3)	0·556	0·375	0·284	5·6	C(19)	0·352	0·129	0·400	5·8
C(4)	0·570	0·336	0·465	5·5	C(20)	0·959	−0·069	0·440	4·5
C(5)	0·471	0·266	0·505	4·9	C(21)	0·006	0·032	0·492	6·3
C(6)	0·481	0·239	0·687	6·3	C(22)	0·064	−0·126	0·506	12·3
C(7)	0·603	0·181	0·706	5·1	C(23)	0·038	−0·218	0·481	8·9
C(8)	0·629	0·114	0·561	4·8	C(24)	0·040	−0·246	0·282	11·0
C(9)	0·570	0·114	0·406	4·8	C(25)	0·081	−0·276	−0·034	12·8
C(10)	0·462	0·186	0·376	4·4	C(26)	0·647	0·500	0·137	6·4
C(11)	0·601	0·054	0·283	3·6	C(27)	0·751	0·562	0·131	5·0

[a]Estimated standard deviations are about 0·003 in x, y, and z of the light atoms.
[b]The anisotropic temp factor for exp $[-(0{\cdot}021h^2 + 0{\cdot}013k^2 + 0{\cdot}032l^2 - 0{\cdot}005hk + 0{\cdot}010kl + 0{\cdot}004hl)]$.

Table 2. Proton resonance signals (δ) for 18-nor-5β,17α-chola-8,11,13-trien-24-oic acid derivatives

	C-21	C-19	C-18	H-17	3β-H (Eq.)	H-11	Other
3α-Hydroxy methyl ester **6**	0·59d. ($J = 6$)	1·17s.	2·23s.	3·33m.	*ca.* 3·67m.	6·94s.	3·68 (CO_2CH_3) 1·79 br. (OH)
3α-Acetoxy methyl ester **7**	0·61d. ($J = 6$)	1·19s.	2·24s.	3·33m.	4·83m.	6·91s.	3·68 (CO_2CH_3) 1·93 ($OCOCH_3$)
3α-Chloroacetoxy methyl ester **8**	0·62d. ($J = 6{\cdot}5$)	1·19s.	2·24s.	3·33m.	4·93m.	6·91s.	3·67 (CO_2CH_3) 3·93 ($OCOCH_2Cl$)
3α-Iodoacetoxy methyl ester **9**	0·62d. ($J = 6{\cdot}5$)	1·19s.	2·25s.	3·33m.	4·93m.	6·92s.	3·67 (CO_2CH_3) 3·58 ($OCOCH_2I$)
3α-Hydroxy acid **10**	0·60d. ($J = 6$)	1·18s.	2·23s.	3·33m.	3·67m.	6·90s.	6·53 (-OH and $-CO_2H$)
3α-Acetoxy acid **11**	0·62d. ($J = 6$)	1·18s.	2·23s.	3·33m.	4·78m.	6·84s.	1·93 ($OCOCH_3$) 9·48 (CO_2H)
3-Keto acid **14**	0·61d. ($J = 6$)	1·38s.	2·26s.	3·33m.		6·98s.	
4β-Bromo-3-keto acid **15**	0·61d. ($J = 6$)	1·35s.	2·27s.	3·33m.		6·96s.	4·57d. ($J = 12$) H-4 9·92 (CO_2H)
3-Keto methyl ester **19**	0·59d. ($J = 6$)	1·37s.	2·27s.	3·33m.		6·98s.	
4β-Bromo-3-keto methyl ester **20**	0·59d. ($J = 6{\cdot}5$)	1·35s.	2·27s.	3·33m.		6·95s.	4·57d. ($J = 11{\cdot}5$) H-4 3·66 (CO_2CH_3)

structure. The packing of the L-shaped molecules is shown in Fig 2. With the exception of the side chain at C-17, bond lengths agree with expected values[17] to within 0·1 A. The side chain is poorly resolved in the final electron-density map and has higher temperature factors than the other atoms, indicating some disorder. The sidechain bond lengths are consequently not accurate, but are within 0·2 A of expected values. Ring A is in a chair and ring B in a half-chair conformation. Ring C is planar within experimental error, no atom deviating more than 0·04 A from the least-squares plane. Ring D is puckered with C-16 being 0·4 A above the plane of C-13, C-14, C-15, and C-17.

Concerning the chronology of the steps involved in the rearrangement transformation (**4** or **5** → **6**), the fact that the configuration of the sidechain has been inverted implicates a probable intermediate dehydration product (e.g. **B** formed from **A**) which is subsequently re-protonated from the front β side. We consider that this probably occurs prior to elimination of the 7α-substituent whose hindering influence on the rear side of the C-13, C-17 region is significant. In this connection, a ring-C benzenoid bile acid derivative previously described, and isomeric with **7**, was formulated

Table 3. Proton resonance signals (δ) for 18-nor-5α,17α-chola-8,11,13-trien-24-oic acid derivatives

		C-21	C-19	C-18	H-17	3α-H (ax.)	H-11	Other
3β-Hydroxy acid	**17**	0·61d. ($J = 6$)	1·28s.	2·26s.	3·33m	3·72m.	6·95s.	7·03 (-OH and -CO_2H)
3β-Acetoxy acid	**18**	0·61d. ($J = 6$)	1·12s.	2·24s.	3·33m.	4·78m.	6·90s.	2·03 ($OCOCH_3$) 11·12 (CO_2H)
3-Keto-5α acid	**24**	0·62d. ($J = 6$)	1·30s.	2·27s.	3·33m.		6·95s.	9·95 (CO_2H)
3β-Acetoxy olefin	**25**	0·73d. ($J = 7$)	1·12s.	2·31s.	3·43m.	4·83m.	7·03s.	2·03 ($OCOCH_3$) 4·99m. and 5·23m. (H-23) 5·80–6·37m. (H-22)
3β-Hydroxy aldehyde	**26**	0·83d. ($J = 6·5$)	1·08s.	2·26s.	*ca.* 3·67m.	*ca.* 3·75m.	6·97s.	9·84 br. s. (CHO)

Table 4. Proton resonance signals (δ) for conjugated 3-ketones of 18-norchol-8,11,13-triene derivatives

Δ^4 3-Keto acid	**16**	0·60d. ($J = 6$)	1·57s.	2·28s.	3·33m.	6·92s.	5·93s. H-4 10·47 (CO_2H)
Δ^4 3-Keto methyl ester	**21**	0·59d. ($J = 6$)	1·57s.	2·28s.	3·33m.	6·92s.	5·92s. H-4 3·68s. (CO_2CH_3)
Δ^1 3-Keto methyl ester	**22**	0·58d. ($J = 6$)	1·55s.	2·25s.	3·33m.	6·93s.	5·90d. ($J = 10$) H-2 7·07d. ($J = 10$) H-1

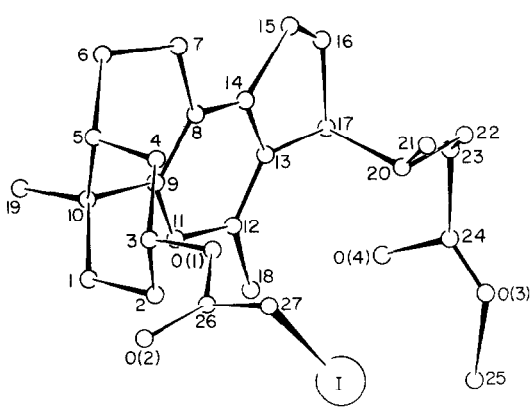


Fig 1. The iodoacetate molecule viewed along **b**. Atoms not marked with an element symbol are carbon.

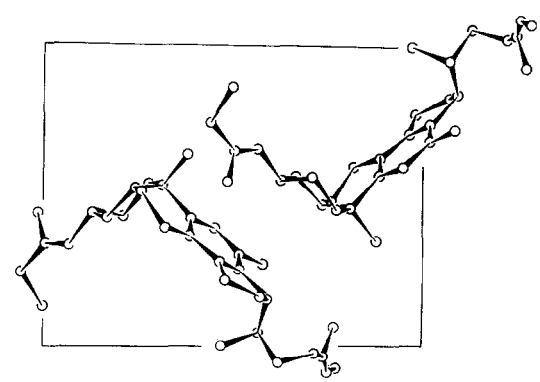
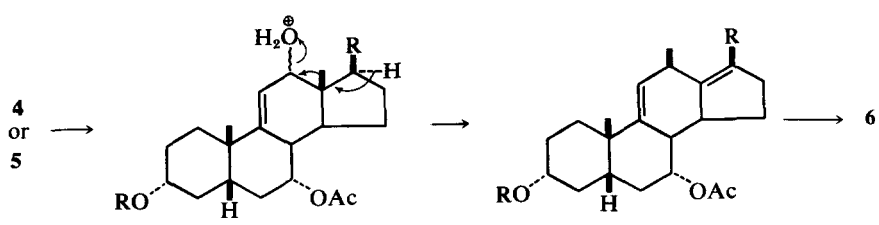


Fig 2. The unit cell viewed along **c**. Origin is at the lower left-hand corner with **a** to the right and **b** up.

with unassigned configuration at C-17.[3] It may now be concluded that this compound has the normal 17β-sidechain; consonant with the above mechanistic implication, it was formed from precursors lacking substituents at C-7. The most characteristic feature distinguishing the 17α and 17β-sidechain isomers in the NMR spectra lies in the chemical shift of the sidechain secondary Me group. With the 17α-sidechain, the value (typically, δ 0·60) indicates strong shielding; an examination of models indicates that, with this configuration at C-17 and the sterically most favorable conforma-



tion of the sidechain, the C-21 Me group indeed lies within the shielding region of the benzenoid ring.

Within the last few years, interest has been apparent in the preparation of ring-C benzenoid steroids by total synthesis, and three independent different procedures have been communicated.[18-20]

EXPERIMENTAL

M.ps were determined with either a Gallenkamp or Fisher-Johns apparatus. Specific rotations were determined for solns in $CHCl_3$. NMR spectra were determined for solns in $CDCl_3$ with TMS as internal reference by means of a Varian A60 spectrometer.

For preparative TLC, silica gel PF (Merck; 1 mm thick) was used with benzene-diethyl ether mixtures as developing solvents.

UV spectra were recorded for solns in 95% EtOH (unless otherwise specified) with a Cary spectrophotometer and IR spectra were determined using a Perkin-Elmer Infrared spectrophotometer.

Methyl 3α-*hydroxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**6**)

The epimeric mixture, methyl 3α,7α-diacetoxy-12αβ-hydroxychol-9(11)-enate[5] (1·935 g) obtained by $NaBH_4$ reduction of methyl 3α,7α-diacetoxy-12-oxochol-9(11)-enate, was dissolved in MeOH (180 ml) and cooled at 0°. HCl gas was bubbled through for 4 hr, the flask then stoppered and kept at room temp for 4 hr. The soln was then concentrated (to *ca* 50 ml) under reduced pressure, water (300 ml) added and the mixture extracted with ether. The dried neutral extract on evaporation yielded *methyl* 3α-*hydroxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**6**) as a colorless gum (1·85 g) which could not be obtained crystalline, $[\alpha]_D + 64°$ (*c*, 0·54): high resolution mass spectrum, M^+, *m/e* 384·2655 ($C_{25}H_{36}O_3$ requires: 384·2664); λ(CCl_4) 2·75 (OH), 5·74 (CO_2CH_3) and 11·46 μ (Ar-H). The 3β-H proton signal of the NMR spectrum, (Table 2), after addition of trichloroacetylisocyanate, was at δ 4·88. Mass spectrum: *m/e* 384 (M^+), 366 (M-H_2O), 353 (M-OCH_3), 297 (M-$CH_2CH_2CO_2CH_3$), 269 (M-sidechain), 251 (M-H_2O-sidechain).

The same product was isolated by treatment of each epimer independently in the same way. TLC analysis indicated that the 12β-epimer reacted more rapidly.

Methyl 3α-*acetoxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**7**)

The alcohol methyl ester (gum, 1·2 g) was dissolved in pyridine (4 ml) and Ac_2O (2 ml), allowed to stand at room temp for 18 hr, then worked up in the usual way. The product, isolated as a light brown gum which failed to crystallize from the common solvents, was dissolved in light petroleum and chromatographed on neutral alumina. The fractions eluted with this solvent and light petroleum-benzene (10:1) were combined (0·81 g) and redissolved in light petroleum. On standing, *methyl* 3α-*acetoxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* separated as prisms, m.p. 124–126°, $[\alpha]_D + 79°$ (*c*, 1·5). (Found: C, 76·30; H, 9·16; $C_{27}H_{38}O_4$ requires: C, 76·02; H, 8·98%): λ(cyclohexane) 220 sh. (13,100), 225 sh. (11,650), 261 sh. (279), 268 (353) and 275 nm. (sh.) (262): λ(CCl_4) 5·74 (esters), 8·04 (acetate) and 11·46 (Ar-H).

3α-*Hydroxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**10**)

A soln of the methyl ester acetate (1 g) in MeOH (10 ml) was added to a soln of KOH (1 g) in MeOH (10 ml), the mixture heated under reflux for 2 hr, then acidified with aqueous AcOH, diluted with water and extracted with ether. Evaporation of the washed and dried extract gave a gum which crystallized slowly from aqueous MeOH to give the *hydroxy acid* as needles (726 mg), m.p. 79–82° raised to m.p. 81–86° on recrystallization, $[\alpha]_D + 61°$ (*c*, 0·9). (Found: C, 77·63; H, 9·42; $C_{24}H_{34}O_3$ requires: C, 77·80; H, 9·25%): λ(KBr) 2·91 (OH), 5·86 (CO_2H) and 11·46 μ (Ar-H).

Esterification with diazomethane in ether soln gave pure **6** as a non-crystallizable gum, which on acetylation with pyridine and Ac_2O yielded **7**.

Methyl 3α-*chloroacetoxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**8**)

A mixture of **6** (0·92 g) and chloroacetic anhydride (3 g) was heated on the steam bath for 4 hr, cooled, diluted with water, and extracted with ether. The washed and dried extract on evaporation yielded a yellow-green gum which was recrystallized three times from MeOH to give the *chloroacetate* as needles, m.p. 111–113°. (Found: C, 69·83; H, 8·05; Cl, 7·83. $C_{27}H_{37}O_4Cl$ requires: C, 70·18; H, 8·09; Cl, 7·69%): λ(CCl_4) 5·70 (chloroacetate), 5·75 (CO_2CH_3) and 11·51 μ (Ar-H).

Methyl 3α-*iodoacetoxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**9**)

KI (1·05 g) was added to a soln of the chloroacetate (350 mg) in acetone (17·5 ml), the mixture heated under reflux for 4 hr, then taken to dryness under reduced pressure. The residue was partitioned between water and ether, and the washed and dried ether extract evaporated. The residual gum on crystallization from MeOH gave the *iodoacetate* as prisms, m.p. 77–78°. (Found: C, 58·95; H, 6·87; I, 22·98. $C_{27}H_{37}O_4I$ requires: C, 58·69; H, 6·75; I, 22·97%): λ(CCl_4) 5·75 (esters) and 11·52 μ (Ar-H).

3α-*Acetoxy*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**11**)

A soln of **10** (3·3 g) in pyridine (5 ml) and Ac_2O (2·5 ml) was allowed to stand at room temp for 18 hr and worked up in the usual way to give a colourless gum (3·5 g). This was dissolved in pyridine (15 ml) and water (15 ml), heated at 100° for 1 hr to decompose mixed anhydride, and the product isolated by ether extraction in the usual way to yield the *acetoxy acid* as a gum, $[\alpha]_D + 80·5°$ (*c*, 0·61), which could not be obtained crystalline. (Found: C, 76·22; H, 8·98. $C_{26}H_{36}O_4$ requires: C, 75·69; H, 8·80%): λ(CCl_4) 5·72 (ester), 5·82 (CO_2H), 8·02 (acetate) and 11·46 μ (Ar-H).

3-*Oxo*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**14**)

The hydroxy acid (15 g) was dissolved in acetone (300 ml), cooled to *ca* 5°, and stirred while Jones reagent (8 N, 10·2 ml) was added. The soln was then concentrated to *ca* 150 ml, diluted with water and worked up in the usual way through ether. In this manner, **14** was obtained as a pale green gum, $[\alpha]_D + 61°$ (*c*, 0·9) and was used in this form without further purification: high resolution mass spectrum, M^+, 326·2246 ($C_{24}H_{32}O_3$ requires: 326·2235; λ(CCl_4) 5·86 broad (ketone and -CO_2H).

Bromination and dehydrobromination of 3-*oxo*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**14**)

A soln of Br_2 (0·46 g) in AcOH (5·7 ml) was added drop-wise to **14** (1·0 g) in the same solvent (50 ml) at 5°, then stirred at room temp for 30 min. The product was worked up in the usual way *via* ether extraction to yield 4β-*bromo*-3-*keto*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**15**) as a pale green gum, $[\alpha]_D$ + 107° (*c*, 0·6); $\lambda(CCl_4)$ 5·76 (bromoketone) and 5·86 μ (CO_2H). It failed to crystallize and was used without further purification.

LiCl (11·5 g) was added to a soln of **15** (1·15 g) in DMF (115 ml) and the mixture heated under reflux in an atmosphere of N_2 for 5 hr. The mixture was then worked up in the usual way to yield 3-*oxo*-12-*methyl*-18-*nor*-17α-*chola*-4,8,11,13-*tetraen*-24-*oic acid* (**21**) as a yellow gum (1·05 g), $[\alpha]_D$ + 199° (*c*, 1·0); high resolution mass spectrum, M^+, *m/e* 366·2191 ($C_{24}H_{30}O_3$ requires: 366·2195; λ 5·86 (CO_2H) and 5·98 μ (conjug. ketone); λ(EtOH) 225 (15,200) and 238 nm (11,800).

3β-*Hydroxy*-12-*methyl*-18-*nor*-5α,7α-*chola*-8,11,13-*trien*-24-*oic acid* (**17**)

A soln of **16** (920 mg) in EtOH (24 ml) was added to anhyd liquid ammonia under an acetone-CO_2 condenser. Li metal (470 mg) was added over 5 min, a further 920 mg added over the next 20 min, and the mixture stirred for a further 40 min. The ammonia was then allowed to evaporate, ether and water added, and the separated alkaline phase acidified with AcOH and then extracted with ether. The washed and dried extract yielded a pale green gum (902 mg) which was crystallized from aqueous MeOH to give 3β-*hydroxy*-12-*methyl*-18-*nor*-5α,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**17**) as needles, m.p. 200–203°, $[\alpha]_D$ + 70° (*c*, 0·42). (Found: C, 77·47; H, 9·10. $C_{24}H_{30}O_3$ requires: C, 77·80; H, 9·25%); $\lambda(CCl_4)$ 5·85 (CO_2H).

Methyl 3-*Oxo*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**19**).

To a soln of **6** (gum, 1·5 g) in acetone (50 ml) was added Jones reagent (8 N CrO_3 in acetone, 1·25 ml) and the mixture allowed to stand at room temp for 30 min, then worked up by dilution with water and extraction with ether. The product was purified by preparative TLC (developed by benzene-ether (3:1)) and the *methyl ester ketone* obtained as a gum, $[\alpha]_D$ + 71° (*c*, 1·5); high resolution mass spectrum, M^+, *m/e* 382·2512 ($C_{25}H_{34}O_3$ requires: 382·2508); $\lambda(CCl_4)$ 5·75 (ester), 5·83 (ketone) and 11·50 μ (Ar-H).

Bromination and dehydrobromination of methyl 3-*oxo*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**19**)

To a soln of **19** (200 mg) in AcOH (10 ml) was added dropwise with stirring and cooling (ice-water) a soln of Br_2 (84 mg) in AcOH (1·1 ml). The Br uptake was complete within 10 min, the mixture was stirred for a further 30 min, then worked up by aqueous dilution and ether extraction. The oily product (220 mg) was purified by TLC to give *methyl* 4β-*bromo*-3-*oxo*-12-*methyl*-18-*nor*-5β,17α-*chola*-8,11,13-*trien*-24-*oate* (**20**) as a gum (195 mg), $[\alpha]_D$ + 107° (*c*, 1·1); $\lambda(CCl_4)$ 5·73 μ (ester and bromoketone) which was rather unstable, decomposing on standing in solution and above 40° in the solid state.

LiCl (3 g) was added to a soln of **20** (320 mg) in DMF (37 ml) and the mixture heated under reflux in an atmosphere of N_2 for 5 hr, concentrated under reduced pressure, diluted with water and worked up *via* ether extraction. The mixture was treated with diazomethane in ether soln and separated by preparative TLC. The fastest running zone gave a gum (29 mg) considered to be (see NMR spectrum; Table 4) *methyl* 3-*oxo*-12-*methyl*-18-*nor*-5β,17α-*chola*-1,8,11,13-*tetraen*-24-*oate* (**22**); $\lambda(CCl_4)$ 5·57 (ester) and 5·93 μ (conjug. ketone). The second-fastest running zone yielded *methyl* 3-*oxo*-12-*methyl*-18-*nor*-17α-*chola*-4,8,11,13-*tetraen*-24-*oate* (**21**) as a gum (98 mg); $\lambda(CCl_4)$ 5·75 (ester) and 5·98 μ (conjug. ketone).

Lithium and ammonia reduction of methyl 3-*oxo*-12-*methyl*-18-*nor*-17α-*chola*-4,8,11,13-*tetraen*-24-*oate* (**21**)

A soln of **21** (60 mg) in EtOH (1·5 ml) was added to anhyd ammonia, cooled by an external CO_2 acetone bath. Li metal (31 mg) was added over 2–3 min (soln turned blue), a further quantity (62 mg) added over a further 20 min, then the mixture stirred for a further 20 min. Ether was then added, and the mixture allowed to stand at room temp until ammonia had evaporated, and the product isolated by dilution with water and ether extraction. Evaporation of the ether yielded the product mixture as a green gum, the NMR spectrum of which showed the absence of olefinic and carbomethoxyl protons, but the presence of an aromatic proton (δ 6·92 br) and consequently was considered to contain 12-*methyl*-18-*nor*-5α,17α-*chola*-8,11,13-*trien*-3,24-*diol* (**23**).

(a) The crude diol (730 mg) dissolved in acetone (36 ml) was treated with Jones reagent (1·6 ml) at 5° with stirring. Working up in the usual way yielded an acid fraction (495 mg), isolated by NaOHaq extraction and re-acidification and considered to be (see NMR spectrum, Table 3) 3-*oxo*-12-*methyl*-18-*nor*-5α,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**24**). It was isolated as a pale green gum, λ (CCl_4) 5·83 (ketone and carbonyl).

(b) The crude diol was acetylated with pyridine and Ac_2O in the usual way and the product (red gum, 70 mg) purified by TLC. The presence of at least 4 products was indicated; the major fraction (40 mg, second-fastest running) was isolated as an almost colourless gum considered to be 3β,24-*diacetoxy*-12-*methyl*-18-*nor*-5α,17α-*chola*-8,11,13-*triene*, λ 5·77 (esters); NMR spectrum: δ 0·60 d. (*J* 6·5) (C-12 Me), 1·13 (C-19 Me), 2·03 (acetate, 6 protons), 2·25 (C-18 Me), 4·10 t (*J* 6) (CH_2OAc), 4·73 br (3α-H) and 6·88 (H-11).

3β-*Acetoxy*-12-*methyl*-18-*nor*-5α,17α-*chola*-8,11,13-*trien*-24-*oic acid* (**18**)

(a) A soln of **17** in pyridine-Ac_2O (1:2) was set aside overnight at room temp and worked up in the usual way. The gummy product was then heated at 90° with pyridine-water (1:1) for 3 hr and isolated *via* ether extraction to yield **18** as a gum, $\lambda(CCl_4)$ 5·74 (acetate), 5·85 (CO_2H) and 8·10 μ (acetate).

(b) The crude **24** (490 mg, pale green gum) was dissolved in MeOH (25 ml), cooled to *ca* 3° and $NaBH_4$ (490 mg) added portionwise with stirring over 15 min. The mixture was stirred for a further 30 min, diluted with water, acidified with AcOH, concentrated under reduced pressure and extracted with ether. The washed and dried extract was acetylated by treatment with pyridine and Ac_2O overnight at room temp. The product, isolated in

the usual way, was dissolved in a mixture of pyridine (10 ml) and water (10 ml), heated at 100° for 2 hr (to decompose mixed anhydride), then the solvents removed under reduced pressure to give a residual gum, dried by addition of benzene and distillation of the azeotrope. This gave the same *acetoxy acid* (**18**) as a pale yellow gum, with same IR and NMR spectrum as obtained in (a).

3β-Acetoxy-12-methyl-18,24-bisnor-5α,17α-chola-8,11,13,22-tetraene (**25**)

To a soln of **18** (gum, 605 mg) in AcOH (50 ml) was added lead tetraacetate (1·8 g, in 10% AcOH), and the mixture heated to *ca* 50°, then taken to dryness below 40° under reduced pressure, with last traces of solvent removed by keeping under continuous vacuum at room temp for 18 hr. The dry solid was dissolved in dry benzene (12 ml) under N_2, pyridine (46·5 mg) and cupric acetate (64·5 mg) added, and the mixture heated under reflux for 2 hr, filtered, and the filtrate taken to dryness under reduced pressure. The residual green gum was dissolved in ether, washed successively with water, 5% NaOHaq, water, dried and evaporated. The neutral fraction (372 mg) was purified by TLC to yield as major product (262 mg) the *acetoxy tetraene* (**25**) as a gum, $[\alpha]_D + 41°$ (*c*, 1·77); high resolution mass spectrum, M^+, *m/e* 366·2558 ($C_{25}H_{34}O_2$ requires: 366·2558); λ(CCl_4) 5·75 (acetate), 6·12 and 10·97 μ (methylene). The olefin is rather unstable. A TLC examination after exposure to the atmosphere indicated the presence of several impurities.

3β-Hydroxy-12-methyl-18,23,24-trisnor-5α,17α-chola-8,11,13-trien-22-al (**26**)

A soln of osmium tetroxide (13·8 mg) in ether (10 ml) was added to a soln of **25** (200 mg) in ether (20 ml). The soln turned black after 5 min whereupon 30% H_2O_2 soln (0·34 ml) was added and the mixture stirred at room temp for 18 hr. It was then washed with water, 5% NaOHaq, and water, and the dried ether soln stirred with LAH (200 mg) at room temp for 1 hr. Work up in the usual way yielded the triol fraction (140 mg) as a white gum, which was dissolved in EtOH (90 ml), mixed with a soln of sodium metaperiodate (145 mg) in water (7 ml), and kept in the dark for 20 hr. The product was isolated by aqueous dilution and ether extraction to give the *aldehyde* (**26**) as a gum, $[\alpha]_D + 112·5°$ (*c*, 1·4); high resolution mass spectrum, M^+, *m/e* 326·2246 ($C_{22}H_{30}O_2$ requires: 326·2235); λ(CCl_4) 2·74 and 2·89 (OH) and 5·79 (CHO).

Treatment of the aldehyde (25 mg) in a minimum volume of EtOH with a slight excess of Brady's reagent (0·75 ml) and slight warming gave a ppt, which was collected, dissolved in benzene and filtered through a small column of alumina. A yellow-brown band was eluted by ether-benzene (1:9) and crystallized twice from EtOH to give the 2,4-dinitrophenylhydrazone derivative as dark yellow prisms, m.p. 172–174°, $[\alpha]_D +$ 25° (*c*, 1·1); high resolution mass spectrum, M^+, *m/e* 504·2394 ($C_{28}H_{32}N_4O_5$ requires: 504·2373.) NMR spectrum: δ 0·98d. (C-21 Me), 1·13s. (C-19 Me), 2·28s. (C-18 Me), 6·95s. (H-11), 7·62d. (*J* 4·5, H-22), 7·92d. (*J* 9·5, H-6'), 8·33dd. (*J* 9·5 and 3, H-5'), 9·13d. (*J* 3, H-3') and 11·03s. (NH).

X-Ray diffraction

All X-ray data were taken on a Picker full-circle diffractometer with Ni-filtered Cu radiation (λ = 1·54178 A). The crystal used was a prism of dimensions 0·1 × 0·3 × 0·1 mm. mounted on the long (b) axis. Cell dimensions were obtained from 2θ and φ values for selected reflections. Intensities were measured by the stationary-crystal stationary-counter method using a 4·3° take-off angle. Background was obtained from a plot against 2θ and was found to be independent of other angles. All calculations were done on a teletype terminal interfaced to a time-shared CDC 3800 computer.[21] The least-squares program of Gantzel, Sparks, and Trueblood was used, minimizing $\Sigma w(F_{obs}-F_{cal})^2$ where the weight, w, is determined from the estimated standard deviation in F_{obs}. Atomic scattering factors for neutral atoms are from the "International Tables for X-ray Crystallography."[22]

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