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Seroflocculating Steroids. VIII.[1] Side Chain Reduction of Deoxycholic, 12α-Hydroxy-3-cholenic and 3α-Hydroxy-11-cholenic Acids[2]

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Methyl deoxycholate, methyl 12α-hydroxy-3-cholenate and methyl 3α-hydroxy-11-cholenate were reduced to the corresponding C-24 hydroxy compounds. The latter were selectively deoxylated at C-24 to give 3α,12α-cholanediol, 3-cholen-12α-ol and 11-cholen-3α-ol, respectively. Some ester and chloro derivatives are active seroflocculants.

Certain derivatives of bile acid esters (*i.e.*, containing unsaturation or chloro or acetoxy substituents) are seroflocculating agents.[4] The reduction of the bile acid side chain to a hydrocarbon moiety comprises one of the structural variations under study. The reduction of a model compound, 3α,24-cholanediol, derivable from the simplest of the bile acids (lithocholic acid), was reported recently;[5] the diol was converted into 3α-cholanol by selective tosylation, and reduction of the 24-monotosylate.

The most sensitive seroflocculants in the bile acid ester group contain two functional groups on the nucleus. In order to prepare reduced side chain analogs of these more sensitive seroflocculants, the reduction sequence has now been applied to methyl deoxycholate, methyl 12α-hydroxy-3-cholenate[6] and methyl 3α-hydroxy-11-cholenate.[7] Lithium aluminum hydride reduction of these esters gave 3α,12α,24-cholanetriol (1), 3-cholen-12α,24-diol (9) and 11-cholen-3α,24-diol (15), respectively.

Under selective tosylation conditions similar to those proved successful on 3α,24-cholanediol, 11-cholen-3α,24-diol (15) gave a low yield[8] of 24-tosyloxy-11-cholen-3α-ol (17), which was easily reduced to 11-cholen-3α-ol (18) with lithium aluminum hydride. Hydrogenation of 18 to 3α-cholanol[5] (rather than 24-cholanol) proved that the tosylation had occurred at C-24. 3α,12α,24-Cholanetriol (1) and 3-cholen-12α,24-diol (9), on the other hand, failed to yield crystalline monotosylates; consequently the crude tosylation products were reduced to give 3α,12α-cholanediol (4) and 3-cholen-12α-ol (11), respectively.

The tosylations of 15 and 1 were not entirely selective, as evidenced by products isolated ultimately in minor amounts from each of these tosylation reactions. Thus, 11-cholene (21) was obtained by chromatography of the lithium reduction products of the crude tosylate fraction from 15. Similarly, 12α-cholanol (3) was obtained from 1. In addition, 3-cholen-12α,24-diol was formed when the tosylate fraction from 1, chromatographically separated on alumina, was dehydrotosylated in lutidine.[9] These products show the presence of 3,24-ditosylates in the tosylate fractions. However, no indication of ditosylation was observed in the tosylation of 9, as might be predicted from the known marked difference in esterification activity between the 3α- and 12α-hydroxy groups in the bile acids.

Under conditions identical to those used in the lithocholate series,[5] 3,11-choladiene (22) was prepared from 11-cholen-3α-yl tosylate (20); 24-chloro-3-cholen-12α-ol (13) and 24-chloro-11-cholen-3α-ol (24) were prepared from crude 24-tosyloxy-3-cholen-12α-ol and 24-tosyloxy-11-cholen-3α-ol (17), respectively, at room temperature; and 3β-chloro-12α-cholanol (7) and 3β-chloro-11-cholene (23) were prepared from the corresponding tosylates at 78°.

An inspection of the compounds prepared in this work reveals that most of the products that have resisted crystallization efforts are esters (tosylates and acetates) of 12α,24-diols. This behavior probably is related to the erratic melting behavior of the successfully crystallized diols and esters of this group (*e.g.*, compounds 1, 8 and 9 have broad melting ranges and/or variable melting points; 4 and 5 appear to be polymorphic). The property is exhibited to a certain extent by the 12α-hydroxycholanic acid esters.[10]

Infrared spectra of the alcohols reported here (plus those in paper V[6]) contain maxima which are characteristic of the position of the hydroxyl group. Both the 3α- and the 24-hydroxyl groups give rise to a strong band near 3 μ; the 3α-hydroxyl in addition causes a strong band to appear at 9.58–9.68 μ, whereas, with the 24-hydroxyl group this band is shifted to 9.42–9.49 μ. The 12α-hydroxyl group gives rise to a very weak band at 2.77–2.79 μ and a strong band at 9.66–9.68 μ.

(1) Paper VI of this series, *Chemistry & Industry*, 590 (1958); paper VII, THIS JOURNAL, **80**, 2906 (1958).

(2) This investigation was supported in part by grants (C-2249 and C-3407) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

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(4) F. C. Chang, R. T. Blickenstaff, A. Feldstein, J. R. Gray, G. S. McCaleb and D. H. Sprunt, THIS JOURNAL, **79**, 2161 (1957).

(5) R. T. Blickenstaff and F. C. Chang, *ibid.*, **80**, 2726 (1958).

(6) F. C. Chang, A. Feldstein, J. R. Gray, G. S. McCaleb and D. H. Sprunt, *ibid.*, **79**, 2167 (1957).

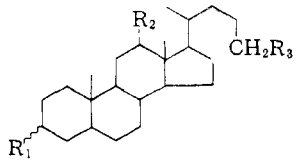
(7) J. Press and T. Reichstein, *Helv. Chim. Acta*, **25**, 878 (1942); L. L. Engle, V. R. Mattox, B. F. McKenzie, W. F. McGuckin and E. C. Kendall, *J. Biol. Chem.*, **162**, 565 (1946).

(8) However, the yield based on 15 actually consumed is 87.5%. The starting diol has a low solubility in pyridine and much of it is recovered unchanged.

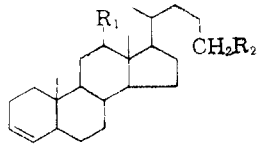
(9) 3-Cholen-12α,24-diol results from the lutidine dehydrotosylation of the triol 3-monotosylate, which most likely was formed from 3α,24-ditosylate by partial hydrolysis (see ref. 5 and paper VI, ref. 1) in the initial alumina separation of the tosylation mixture. The alternative explanation that the 3-monotosylate was directly formed by selective tosylation of the triol appears improbable.

(10) Some examples from the literature are ethyl 3α,12α-diacetoxycholanate (F. C. Chang, *et al.*, THIS JOURNAL, **79**, 2164 (1957)), methyl 12α-benzoyldeoxycholate (B. F. McKenzie, W. F. McGuckin and E. C. Kendall, *J. Biol. Chem.*, **162**, 555 (1946), A. Lardon, P. Grandjean, J. Press, H. Reich and T. Reichstein, *Helv. Chim. Acta*, **25**, 1444 (1942)), methyl 12α-benzoxycholanate (*ibid.*), and methyl 3α-acetoxy-12α-tosyloxycholanate (J. von Euw and T. Reichstein, *ibid.*, **29**, 654 (1946)).

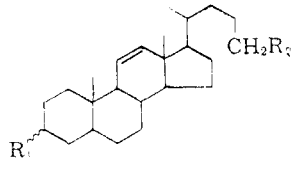
TABLE I



Compd.	R_1	R_2	R_3
1	α-OH	OH	OH
2	α-OAc	OAc	OAc
3	H	OH	H
4	α-OH	OH	H
5	α-OAc	OAc	H
6	α-OTs	OH	H
7	β-Cl	OH	H
8	β-Cl	OAc	H



Compd.	R_1	R_2
9	OH	OH
10	OAc	OAc
11	OH	H
12	OAc	H
13	OH	Cl
14	OAc	Cl
26	H	Br



Compd.	R_1	R_2
15	α-OH	OH
16	α-OAc	OAc
17	α-OH	OTs
18	α-OH	H
19	α-OAc	H
20	α-OTs	H
21	H	H
23	β-Cl	H
24	α-OH	Cl
25	α-OAc	Cl

Screening Results.—Following the scheme used in previous reports[4,5] of seroflocculating activity observed in the screening test, the compounds are classified into three activity groups.

Group A (with activity comparable to that of the standard compound, ethyl 3β-chloro-11-cholenate):

2 3α,12α,24-Cholanetriol triacetate
5 3α,12α-Cholanediol diacetate
8 3β-Chloro-12α-cholanyl acetate
16 11-Cholen-3α,24-diol diacetate
23 3β-Chloro-11-cholene
25 24-Chloro-11-cholen-3α-yl acetate

Group B (compounds with some activity):

12 3-Cholen-12α-yl acetate
14 24-Chloro-3-cholen-12α-yl acetate
20 11-Cholen-3α-yl tosylate
21 11-Cholene[11]
22 3,11-Choladiene[12]
26 24-Bromo-3-cholene

Group C (inactive compounds):

1 3α,12α,24-Cholanetriol
15 11-Cholen-3α,24-diol[a]
19 11-Cholen-3α-yl acetate
24 24-Chloro-11-cholen-3α-ol

[a] Precipitated from seroflocculant suspension and could not be tested. Since no compound with a free hydroxyl group had shown the least sign of activity in either this or earlier testing, this compound, as well as other hydroxy compounds described in this paper, have been assigned to group C.

Interim Conclusions.—These results substantiate earlier indications that alteration from ω-carboalkoxy to methyl, acetoxymethyl or chloromethyl does not eliminate seroflocculating activity, and that two active groups (chloro, acetoxy, unsaturation) on the steroid nucleus are needed for good activity.

No valid conclusions as to the relative effectiveness of the active groupings, both among the ω-groups which now include bromomethyl, and among the nuclear substituents associated with activity, seem justifiable at present. Thus, in the A group compounds 5, 8 and 23 are ω-methyl analogs of the group A structures in the ω-carbethoxy series,[4] while 16 and 25 are analogs of the structures classified as B in that series. In the B group, compound 22 corresponds to a group A structure in the ω-carbethoxy series; 12, 14 and 21 to group B structures and 20 to a group C structure.

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Experimental[13]

3α,12α,24-Cholanetriol (1).—Lithium aluminum hydride reduction of methyl deoxycholate gave a product which crystallized out of ethyl acetate in the form of needles with a variable m.p. The analytical sample, m. 107–114° (lit. m.p. 106–118°,[14] 123°,[15] 165° and 185°.[16]), $[\alpha]_D$ +58.9° (95% EtOH); λ_{max}^{KBr} 3.08, 9.64 μ.

Anal. Calcd. for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18. Found: C, 76.20; H, 11.19.

The triacetate 2 crystallized out of methanol–water, m.p. 79.7–80.9°, unchanged when mixed with an authentic sample,[17] $[\alpha]_D$ +92.8° (lit.[14] m.p. 79.5–80.5°, $[\alpha]_D$ +93.6°).

Tosylation of 3α,12α,24-Cholanetriol and Reduction of the Tosylate.—A solution of 4.00 g. (21.0 mmoles) of tosyl chloride in 6.7 ml. of anhyd. pyridine was added dropwise over a period of 25 min. to a solution of 5.93 g. (15.7 mmoles) of 1 (which had been dried by distilling benzene from it) in 29.4 ml. of anhyd. pyridine at a bath temp. of 2–5°. The solution stood at 2–5° for 10 min., ice was added, the mixture was acidified with cold, concd. HCl, diluted with ice-water and extracted with ether. The ether solution was washed with aq. 10% $NaHCO_3$, then with water, dried over Na_2SO_4 and evaporated leaving 7.05

(11) Too insoluble to test by regular method. A modified procedure, to be reported elsewhere, was used.

(12) Also too insoluble, but showed some activity even when tested at low concentration. When the modified procedure[11] became available, the sample had deteriorated. Tentatively assigned to this group.

(13) Microanalyses by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Melting points were taken on an electrical micro-hot-stage and are uncorrected. Optical rotations were determined in 2% chloroform solutions, except where noted, at about 25°, using a Keston polarimeter attachment (Standard Polarimeter Co., 6 Banta Place, Hackensack, N. J.) to a Beckman DU spectrophotometer, with accuracy estimated to be better than ±2°. The infrared spectra were recorded on a Baird spectrophotometer equipped with NaCl optics. Common C–H stretching and bending bands are not reported; all other medium and strong bands are. The alumina for chromatography was a Fisher Scientific Company product A-540.

(14) G. B. Spero, *et al.*, THIS JOURNAL, **70**, 1907 (1948).

(15) L. Ruzicka and M. W. Goldberg, *Monatsh.*, **82**, 437 (1951).

(16) K. Matsumoto, *J. Biochem. (Japan)*, **42**, 207 (1955).

(17) We wish to take this opportunity to thank Dr. G. B. Spero of the Upjohn Co. for furnishing us with samples of 3α,12α,24-cholanetriol and its triacetate.

g. of viscous liquid.[18] This was extracted with three portions of boiling ether (100, 50 and 30 ml.) leaving 1.24 g. of undissolved solid (unreacted 1).

The ether solution was added dropwise to a stirred suspension of 1.57 g. of $LiAlH_4$ in 50 ml. of anhyd. ether. After refluxing for 5.5 hr. the reaction mixture was worked up in the usual fashion. An ether solution of the crude product was chromatographed on 132 g. of Al_2O_3.

The first fraction, 262 mg., eluted with ether, was crystallized from methanol–water, and from acetone–water to give **12α-cholanol** (**3**), needles, m.p. 100.9–103.3°, $[\alpha]_D$ +41.0° (2.2%); λ_{max}^{KBr} 2.78. 9.67 μ.

Anal. Calcd. for $C_{24}H_{42}O$: C, 83.17; H, 12.22. Found: C, 83.19; H, 12.01.

After a very small intermediate fraction, the second major fraction, 2.448 g., was eluted with 5% ethanol in ether. It crystallized from ether, from ethyl acetate and from acetone; ethyl acetate gave prisms, m.p. 171.2–172.2°, while acetone gave needles which begin melting at 70°, resolidify at 109–120°, and finally melt at 168.0–169.5°; **3α,12α-cholanediol** (**4**), $[\alpha]_D$ +46.6°; λ_{max}^{KBr} 2.99, 9.15, 9.38, 9.58, 9.86 μ.

Anal. Calcd. for $C_{24}H_{42}O_2$: C, 79.49; H, 11.68. Found: C, 79.17; H, 11.33.

The **diacetate 5** was prepared with acetic anhydride and acetic acid in refluxing pyridine. It crystallizes out of methanol to give prisms which change appearance without melting at 112–120°, finally m.p. 146.7–148.0°, $[\alpha]_D$ +104.0° (1.9%); λ_{max}^{KBr} 5.77, 8.05, 9.72, 10.32, 10.51, 11.30 μ.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.10; H, 9.99.

The final fraction, 958 mg., eluted with 10% ethanol in ether, was recovered **3α,12α,24-cholanetriol** (**1**).

3α-Tosyloxy-12α-cholanol (**6**) was prepared by room temperature tosylation of 4, and after several recrystallizations from acetone–ligroin[19] gave needles, m.p. 141.2–143.0°, $[\alpha]_D$ +37.3° (1.5%); λ_{max}^{KBr} 2.77, 8.49, 9.11, 10.71, 11.46, 11.77, 12.25, 14.94 μ.

Anal. Calcd. for $C_{31}H_{48}O_4S$: C, 72.05; H, 9.36; S, 6.20. Found: C, 71.77; H, 9.14; S, 6.06.

3β-Chloro-12α-cholanol (**7**) was prepared from the tosylate 6 by reaction with pyridinium chloride in pyridine at 78°. The crude product, an oil, solidified when seeded with 3β-chloro-11-cholene (23); it was washed with dil. HCl, aq. $NaHCO_3$ and water, then crystallized from methanol as a dense, white solid, m.p. 117.0–119.5°, $[\alpha]_D$ +13.2° (1.2%); λ_{max}^{KBr} 2.78, 7.82, 9.66, 10.52, 14.03 μ.

Anal. Calcd. for $C_{24}H_{41}OCl$: C, 75.65; H, 10.85. Found: C, 75.56; H, 10.58.

The **acetate 8**, prepared by the action of acetic acid, acetic anhydride and toluenesulfonic acid at room temp.,[20] crystallized from acetone–water (2:1) as fine needles, m.p. 122.5–136.0°, $[\alpha]_D$ +69.8°; λ_{max}^{KBr} 5.79, 8.04, 9.73, 10.13, 14.04 μ.

Anal. Calcd. for $C_{26}H_{43}O_2Cl$: C, 73.81; H, 10.24; Cl, 8.38. Found: C, 73.81; H, 10.20; Cl, 8.23.

3-Cholen-12α,24-diol (**9**), prepared by $LiAlH_4$ reduction of methyl 12α-hydroxy-3-cholenate,[6] crystallized out of ethanol or 2-propanol–water in the form of laths with a variable m.p. The analytical sample, recrystallized from 2-propanol–water, had m.p. 56–62°, $[\alpha]_D$ +26.9°; λ_{max}^{KBr} 2.99, 9.47, 9.67, 15.1 μ.

Anal. Calcd. for $C_{24}H_{40}O_2$: C, 79.94; H, 11.18. Found: C, 79.99; H, 11.20.

This compound was also obtained by dehydrotosylation of **3α,12α,24-cholanetriol 3-monotosylate** (see ref. 9). Tosylation of 1 (as described previously) gave a mixture which after chromatography on alumina yielded a tosylate fraction in about 10% yield, when eluted by ether–ethanol (19:1); and the remainder as recovered 1 eluted by ether–ethanol (3:1). The non-crystalline tosylate fraction was heated in refluxing collidine; the product was worked up in the usual fashion and chromatographed on alumina. The major fraction, eluted by ether–ethanol (19:1) and crystallized from ethanol–water (5:1), showed m.p. 56–66°, not reduced on admixture with an authentic sample; infrared spectrum identical with that of the compound prepared from methyl 12α-hydroxy-3-cholenate.

The **diacetate 10** was prepared from 9 by the acetic acid, acetic anhydride, pyridine method and by the acetic acid, acetic anhydride, toluenesulfonic acid method, and purified by chromatography on alumina, but could not be induced to crystallize.

3-Cholen-12α-ol (**11**).—Tosylation of 3-cholen-12α,24-diol (9) at 11–14° under the conditions described previously for the tosylation of 3-cholen-24-ol,[5] and chromatography of a portion of the crude product on Florisil gave a clear oil which would not crystallize. Reduction of this oil with $LiAlH_4$ gave a mixture of 9 and 11, which was separated easily by chromatography on alumina. 3-Cholen-12α-ol, eluted with benzene, crystallized from methanol–water (20:1) in the form of waxy crystals, m.p. 34–60°, and after vacuum drying m.p. 68.0–69.5°, $[\alpha]_D$ +31.5°; λ_{max}^{KBr} 2.79, 9.68, 15.16 μ.

Anal. Calcd. for $C_{24}H_{40}O$: C, 83.65; H, 11.70. Found: C, 83.57; H, 11.72.

The **acetate 12** prepared similarly to 8, crystallized from methanol in the form of irregularly edged laths, m.p. 98.2–102.4°, $[\alpha]_D$ +70.3°; λ_{max}^{KBr} 5.78, 8.05, 9.73, 15.06, 15.16 μ.

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.93; H, 10.99.

24-Chloro-3-cholen-12α-ol (**13**).—A portion of the crude tosylate of 9 was treated with pyridinium chloride in N,N-dimethylformamide under conditions used to prepare 24-chloro-3-cholene.[5] Chromatography of the product on alumina gave a chlorine-containing fraction eluted with benzene, and a chlorine-free fraction eluted with 10% ethanol in ether (presumably recovered 9). The first fraction crystallized from methanol–water, but rapidly liquefied on standing. Chromatography of this material on silicic acid and elution with ligroin–ether (1:1) gave an oil which would not crystallize; it was characterized as the acetate.

The **acetate 14**, prepared similarly to 8, crystallized from methanol as needles, m.p. 140.0–142.2°, $[\alpha]_D$ +62.9°; λ_{max}^{KBr} 5.81, 8.04, 9.75, 13.20, 15.16, 15.51 μ.

Anal. Calcd. for $C_{26}H_{41}O_2Cl$: C, 74.18; H, 9.82; Cl, 8.42. Found: C, 73.99; H, 9.96; Cl, 8.02.

11-Cholen-3α,24-diol (**15**) was prepared by $LiAlH_4$ reduction of methyl 3α-hydroxy-11-cholenate (obtained from the acid[7]) and crystallized from methanol as plates, m.p. 192.0–193.5°, $[\alpha]_D$ +34.1° (0.8%) (lit.[21] m.p. 193–194°, $[\alpha]_D$ +42°); λ_{max}^{KBr} 3.01, 9.5, 9.9, 13.8 μ.

Anal. Calcd. for $C_{24}H_{40}O_2$: C, 79.94; H, 11.18. Found: C, 79.38; H, 10.84.

The **diacetate 16** crystallized as needles from methanol, m.p. 65.0–68.2°, $[\alpha]_D$ +39.9° (1%) (lit.[21] m.p. 67–69°, $[\alpha]_D$ +48°); λ_{max}^{KBr} 5.75, 5.81, 8.0, 9.60, 13.78 μ.

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.75; H, 10.06.

24-Tosyloxy-11-cholen-3α-ol (**17**) was prepared by a low temperature reaction with 13.50 g. of 15, similar to the tosylation of 1 except that a larger volume of pyridine was used; in spite of this not all of the 15 was in solution. At the end of the reaction period the supernatant was decanted from the insol. portion and diluted with ice and water. The crude product separated as a solid, was filtered, washed with aq. 5% HCl and water, and vacuum dried over P_2O_5: 8.59 g. This was extracted with benzene and the resulting solution chromatographed on 230 g. of Florisil. The material originally insol. in benzene was extracted with ether and the ether solution also placed on the column. There remained 3.83 g. of ether-insol. material, m.p. 189–193°.

The first fraction, eluted by benzene–ether (1:1) and by ether, 2.66 g., comprised the tosylate 17, prisms out of benzene–ligroin, m.p. 109.9–112.5°, $[\alpha]_D$ +26.3°; λ_{max}^{KBr} 2.94, 8.42, 8.52, 9.68, 10.45, 10.62, 12.09, 12.32, 15.07 μ.

Anal. Calcd. for $C_{31}H_{46}O_4S$: C, 72.33; H, 9.01; S, 6.23. Found: C, 72.26; H, 8.98; S, 6.26.

(18) In a similar run the crude product at this point was chromatographed on Florisil. A tosylate fraction was separated as an oil whose infrared spectrum resembled those of 24-tosylates, but efforts to crystallize the product were unsuccessful.

(19) The "ligroin" used in these experiments was Skellysolve B (Skelly Oil Co.), b.p. 63–70°, purified by sulfuric acid treatment and distillation.

(20) R. B. Turner, THIS JOURNAL, **74**, 4220 (1952); Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952).

(21) P. Bladon, H. B. Henbest and G. W. Wood, *J. Chem. Soc.*, 2737 (1952).

The second fraction, 2.05 g., m.p. 188–192°, eluted with 10% ethanol in ether, is recovered 15. The original pyridine-insol. residue was washed and dried: 5.49 g.; this plus the ether-insol. portion plus fraction two represent a recovery of 11.37 g. of 15.

11-Cholen-3α-ol (18) was prepared by $LiAlH_4$ reduction of 17, and crystallized from methanol as needles, m.p. 135.3–136.3°, $[\alpha]D$ +31.3° (1.77%); λ_{max}^{KBr} 3.05, 9.68, 13.81 μ.

Anal. Calcd. for $C_{24}H_{40}O$: C, 83.65; H, 11.70. Found: C, 83.71; H, 12.01.

The **acetate 19** crystallized from methanol as needles, m.p. 86.5–88.0°, $[\alpha]D$ +45.9°; λ_{max}^{KBr} 5.77, 8.01, 9.75, 13.83 μ.

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.99; H, 11.02.

The **tosylate 20** crystallized from acetone as plates, m.p. 141.5–143.5°, $[\alpha]D$ +31.7°; λ_{max}^{KBr} 8.53, 10.71, 10.83, 14.97 μ. Although repeated recrystallizations did not raise the m.p., analyses were erratic.

Anal. Calcd. for $C_{31}H_{46}O_3S$: C, 74.65; H, 9.30; S, 6.43. Found: C, 75.16, 74.80, 74.10; H, 9.10, 8.71, 9.08; S, 6.31, 6.72, 6.30.

Hydrogenation of 11-cholen-3α-ol in ethanol over Adams catalyst gave 3α-cholanol, m.p. 135.5–138.0°, mixture with authentic 3α-cholanol[5] m.p. 139.5–143.5°, mixture with 24-cholanol m.p. 110–130°, infrared spectrum (KBr) identical with that of known 3α-cholanol.

11-Cholene (21).—In a separate preparation of 11-cholen-3α-ol from crude tosylate 17, the total reduction product was chromatographed on alumina. The first fraction, eluted with ligroin, crystallized from methanol–benzene (4:1) as plates, m.p. 77.9–80.5°, $[\alpha]D$ +36.6°, λ_{max}^{KBr} 13.84 μ.

Anal. Calcd. for $C_{24}H_{40}$: C, 87.73; H, 12.27. Found: C, 87.79; H, 12.27.

3,11-Choladiene (22) was prepared by dehydrotosylation of 11-cholen-3α-yl tosylate (20) in refluxing 2,6-lutidine. The crude product crystallized when seeded with 3-cholene;[5] when recrystallized from methanol–benzene (2:1) it was obtained in the form of platelets, m.p. 53.0–56.0°, $[\alpha]D$ +37.2° (1.5%); λ_{max}^{KBr} 13.56, 13.82 μ.

Anal. Calcd. for $C_{24}H_{38}$: C, 88.27; H, 11.73. Found: C, 88.01; H, 11.81.

3β-Chloro-11-cholene (23) was prepared from the tosylate 20 and pyridinium chloride in pyridine at 78°. The crude product crystallized from methanol, m.p. 110–115°; this fraction was chromatographed on alumina and the portion eluted by ligroin crystallized from acetone in the form of platelets, m.p. 112.5–116.5°, $[\alpha]D$ +16.8° (1.66%); λ_{max}^{KBr} 7.82, 13.85, 14.06 μ.

Anal. Calcd. for $C_{24}H_{39}Cl$: C, 79.38; H, 10.82; Cl, 9.96. Found: C, 79.12; H, 11.04; Cl, 9.69.

24-Chloro-11-cholen-3α-ol (24), prepared similarly to 13 from the tosylate 17, crystallized from methanol–water (10:1), m.p. 103.5–104.8°, $[\alpha]D$ +32.3°; λ_{max}^{KBr} 3.01, 9.66, 13.79, 15.4 μ.

Anal. Calcd. for $C_{24}H_{39}OCl$: C, 76.05; H, 10.37; Cl, 9.36. Found: C, 75.98; H, 10.34; Cl, 9.53.

The **acetate 25** crystallized from acetone as transparent plates, m.p. 162.0–163.5°, $[\alpha]D$ +47.5°; λ_{max}^{KBr} 5.78, 5.80, 7.93, 8.02, 9.74, 13.83, 14.00 μ.

Anal. Calcd. for $C_{26}H_{41}O_2Cl$: C, 74.18; H, 9.82; Cl, 8.42. Found: C, 74.41; H, 9.81; Cl, 8.11.

24-Bromo-3-cholene (26).—To a solution of 3-cholene-24-yl tosylate[5] (1 g.) in 10 ml. of N,N-dimethylformamide was added 1.2 g. of pyridinium bromide (Metro Industries). The mixture was shaken and left at room temperature for 88 hours. The long needles which had formed and additional solid resulting from precipitation on addition of ice and water, after thorough washing with water, weighed 809 mg. (97%), m.p. 86.5–90°. Crystallization from acetone–water, then from acetone–methanol gave long rods, m.p. 88.5–91.0°, $[\alpha]D$ +17.5°; λ_{max}^{KBr} 14.70, 15.07, 15.58 μ.

Anal. Calcd. for $C_{24}H_{39}Br$: C, 70.72; H, 9.65; Br, 19.61. Found: C, 70.49; H, 9.85; Br, 19.30.

Further recrystallizations, although yielding products of sharper melting point, gave poorer analyses.

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[CONTRIBUTION FROM NATIONAL BUREAU OF STANDARDS, CHEMISTRY DIVISION]

Branched-chain Higher Sugars. II. A Diethylidene-octose[1]

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2,4-*O*-Ethylidene-D-erythrose (I) in calcium hydroxide solution undergoes aldol condensation to a branched-chain octose derivative, which is proved to be 1,3:5,7-di-*O*-ethylidene-3-*C*-formyl-D-*glycero*-D-*talo*-heptitol-3(1),6-pyranose. The 3-*C*-formyl-heptitol obtained on acid hydrolysis is converted by acid to a novel 3(1),1-anhydro-3(1),6-pyranose and converted by lead tetraacetate oxidation to D-*manno*-3-heptulose. Compound I is shown to crystallize as a dimer with the structure bis-(2,4-*O*-ethylidene-D-erythrose)-1,1′:1′,3-cyclic acetal.

Schaffer and Isbell[2] have shown that 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose[3] in calcium hydroxide solution undergoes aldol condensation to the branched-chain decose, 9-aldo-4-*C*-formyl-1,2:8,9-di-*O*-isopropylidene-L-*xylo*-L-*ido*-nono-1,4:9,6-difurano-4(1),7-α-pyranose. As aldol formation is not a characteristic reaction of tetroses or larger sugars, its occurrence with the pentose derivative was attributed to an inhibiting effect (of the substitution) on the enolizing and ring-forming properties of the pentose, with consequent enhancement of conditions for the condensation. In the present paper, a tetrose derivative, substituted (a) at carbon atom 2 to limit enolization to carbon atoms 1 and 2 only, and (b) at carbon atom 4 to preclude intramolecular hemiacetal formation, is shown to undergo aldol condensation in fulfillment of the prediction that suitably substituted sugars generally would undergo the reaction.[2] A proof of structure and configuration of the newly formed aldol product is also presented.

Discussion

The precursor to the aldol condensation used in this study, 2,4-*O*-ethylidene-D-erythrose (I), has been prepared in several laboratories,[4–6] but here-

(1) This work was conducted as part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Division of Research, Atomic Energy Commission, Dr. H. S. Isbell, project leader.

(2) (a) R. Schaffer and H. S. Isbell, THIS JOURNAL, **80**, 756 (1958); (b) R. Schaffer and H. S. Isbell, *ibid.*, **81**, 2178 (1959).

(3) K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941).

(4) E. J. Bourne, G. T. Bruce and L. F. Wiggins, *J. Chem. Soc.*, 2708 (1951).

(5) H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **51**, 307 (1953).

(6) A. C. Neish, *Can. J. Chem.*, **32**, 334 (1954).