SYNTHESIS OF CHOLESTEROL ANALOGS WITH MODIFIED SIDE-CHAINS.

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Abstract

A number of sterols with modified side-chains have been prepared for tests of their biological availability to insects. The fundamental methods used were the reaction of the chloride of 3β-acetoxy-androst-5-ene-17β-carboxylic acid (I) with appropriate organo-cadmium compounds and the condensation of 3β-acetoxy-pregn-4-en-20-one (IV) with alkylmagnesium bromides.

Bergmann, Rabinovitz and Levinson (1) have shown that certain analogs of cholesterol are capable of replacing this sterol to a certain extent as an essential factor in the development of the larvae of the housefly Musca vicina Macq. However they are not able to sustain pupation, as cholesterol does. Since the sterols tested all had side chains shorter than the isooctyl group of cholesterol, it seemed of interest to test some further analogs carrying more complex, highly branched side-chains. It has been known that more complex natural sterols, e.g. ergosterol and stigmasterol, have the properties of cholesterol, because they are dealkylated (and hydrogenated) to cholesterol (2,3).

A number of such higher sterols have been synthesized, using the reaction of the chloride (I) of 3β-acetoxy-androst-4-ene-17β-carboxylic acid with appropriate cadmium-organic compounds. The ketones (II) so formed were reduced by the Huang-Minlon method to yield the sterols (III).

The groups R' introduced were:

a) 3,4-dimethylpentyl $-CH_2.CH_2CH(CH_3).CH(CH_3)_2$

b) 4,4-dimethylpentyl $-CH_2CH_2.CH_2.C(CH_3)_3$

c) 4-methylpentyl $-CH_2CH_2.CH_2.CH(CH_3)_2$

d) 2,3,4-trimethylpentyl $-CH_2CH(CH_3).CH(CH_3).CH(CH_3)_2$

The synthesis of the appropriate alkyl bromide was carried out by unambiguous methods and is described in the Experimental (4).

I $R.CO.Cl$

II $R.CO.R'$

III $R.CH_2.R'$

IV $R.CO.CH_3$

V $R.C(CH_3)(OH).CH(CH_3).CH_2.CH_2.CH(CH_3)_2$

VI $R.C(CH_3)(OH).CH_2.CH_2.CH(CH_3).CH(CH_3)_2$

VII $R.C(CH_3)=CH.CH(CH_3).CH_2.CH(CH_3)_2$

R= 3β-acetoxy-androst-15-en-17β-yl.

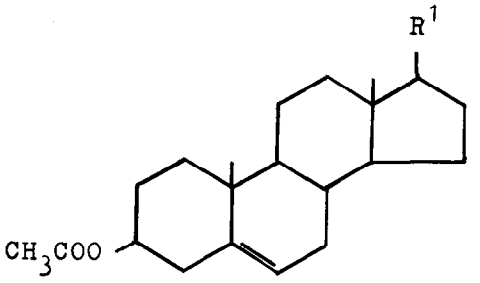
In two additional cases (R' = 2,4-dimethylpentyl, II e, and R' = 5-methyl-2-hexyl, II f), the ketones could be prepared, but not be reduced successfully. Ketone II f gave ill-defined products, and in the case of androsten-15-en-17-yl 2,4-dimethylpentyl ketone (II e) the hydrazone was stable and could not be decomposed even under the most stringent conditions.

Analogous instances are known; thus 24-ketosteroids can be easily reduced to the methylene compounds (5), but their 25-methylhomologs react only under drastic conditions (6).

In a few cases, the synthesis of compounds containing the C-21 methyl group of the natural sterols was attempted. Thus, when the carbinol (V), prepared from IV and 5-methyl-2-hexylmagnesium bromide, was dehydrated, loss of acetic acid occurred simultaneously, and the Δ 3,5,20(22)-triene was isolated. In another instance, the carbinol (VI) from IV and 3,4-dimethylpentylmagnesium bromide, was spontaneously dehydrated to the Δ5,20(22)-diene, but under conditions under which the Δ20(22)- double bond was expected to be hydrogenated selectively, the diene absorbed two molecules of hydrogen to give the saturated compound. No attempts were made to hydrogenate selectively VII, which was obtained only with difficulty from the corresponding tertiary alcohol. The latter was prepared treating IV with 2,4-dimethylpentyl magnesium bromide.

That the stereochemistry in these cases is that of cholesterol, can be concluded from the observation (1) that the natural cholesterol can be prepared by the same sequence of reactions.

The sterol analogs VIII described in this paper and some other analogs have been tested on the hide beetle Dermestes Maculatus Deg. The compounds are inactive (7). Only the previously described 27-norcholesterol which in the less exacting housfly does support growth, but does not permit pupation (1), is active. It is the first synthetic sterol to show direct biological availability in Dermestes (7).



VIII

a: R^1=-CH_2.CH_2.CH_2.CH_2.$CH(CH_3)_2$

b: R^1=-CH_2.CH_2.CH_2.$CH(CH_3)$.$CH(CH_3)_2$

c: R^1=-CH_2.CH_2.CH_2.$C(CH_3)_3$

d: R^1=-CH_2.CH_2.$CH(CH_3)$.$CH(CH_3)$.$CH(CH_3)_2$

e: R^1=-$C(CH_3)(OH)$.$CH(CH_3)$.CH_2.CH_2.$CH(CH_3)_2$

f: R^1=-$C(CH_3)(OH)$.CH_2.$CH(CH_3)$.CH_2.$CH(CH_3)_2$

g: R^1=-$C(CH_3)$=CH.CH_2.$CH(CH_3)$.$CH(CH_3)_2$

Experimental

Ethyl isopropyl ketone was prepared in 90% yield by oxidation of ethyl-isopropylcarbinol (8) with aluminium phenoxide and benzophenone. B.p.114 (lit. (9): 114-115°).

TABLE I

Unsaturated esters

Starting Materials	PRODUCT: Name and formula	b.p. (°/mm)	yield %	Analysis: C, fd.	H, fd.	C, calcd.	H, calcd.
ethyl isopropyl ketone, ethyl bromoacetate	ethyl 3-ethyl-4-methylpentenoate, $C_{10}H_{18}O_2$ a)	90/18	60	70.4	10.6	70.6	10.6
methyl isopropyl ketone, ethyl α-bromopropionate	ethyl 2,3,4-trimethylpentenoate, $C_{10}H_{18}O_2$ b)	83-93/15	55	70.6	10.6	70.6	10.6

Notes: a) $\tilde{\nu}^{liq.}_{max.}$ 1740 cm^{-1} : mainly β,γ-unsaturated esters.

b) $\tilde{\nu}^{liq.}_{max.}$ 1720; 1740 cm^{-1} : mixture of α,β- and β,γ-unsaturated esters

Ethyl 3,4-dimethylpentenoate. To 65 g. of mossy zinc, heated with 0.2 g. of iodine until the iodine vapors filled the whole volume of the flask, a mixture of 86 g. of methyl isopropyl ketone, 167 g. of ethyl bromoacetate and 500 ml. of dry benzene was added with stirring, at a rate which permitted the lively reaction to sustain itself. The heating was continued for 2 hours and the product poured into cold 10% sulfuric acid. The organic layer was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution, dried and concentrated. Ethyl 3-hydroxy-3,4-dimethylpentanoate boiled at 100° (19 mm).

Anal. Calcd. for $C_9H_{18}O_3$: C, 62.1; H, 10.3. Found: C, 62.0; H, 10.4.

A mixture of the crude ester, 400 ml. of pyridine (dried over potassium hydroxide) and 200 ml. of phosphorus oxychloride was kept at room temperature for 12 hours, at 100° for 3 hrs., cooled and treated with 1.5 kg. of ice. The organic layer was separated and the aqueous phase extracted twice with 300 ml. of benzene. The combined organic layers were then washed with 10% hydrochloric acid, until the aqueous layer remained acidic, with 5% sodium hydrogen carbonate solution and saturated sodium chloride solution. The ethyl 3,4-dimethylpentenoate, b.p. 85-87° (20 mm), was according to the infrared spectrum (1720 and 1740 cm^{-1}) a mixture of the α-β - and β,γ - unsaturated esters; yield, 64%.

Anal. Calcd. for $C_9H_{16}O_2$: C, 69.2; H, 10.3. Found: C, 69.0; H, 10.2.

Other unsaturated esters, prepared analogously, are reported in Table I.

Ethyl 3,4-dimethylpentanoate. A solution of 36 g. of ethyl 3,4-dimethylpentenoate in 200 ml. of anhydrous ethanol was hydrogenated (2 atm. pressure) in the presence of 0.1 g. of palladium charcoal. Filtration and distillation gave 36 g. of ethyl 3,4-dimethylpentanoate, b.p. 81° (27 mm).

Anal. Calcd. for $C_9H_{18}O_2$: C, 68.4; H, 11.4. Found: C,68.5; H, 11.5.

Analogously were prepared in practically quantitative yield:

Ethyl 3-ethyl-4-methylpentanoate, b.p. 83° (20 mm).

Anal. Calcd. for $C_{10}H_{20}O_2$: C, 70.1; H, 11.7. Found: C,69.9; H, 11.6.

Ethyl 2,3,4-trimethylpentanoate, b.p. 93° (20 mm).

Anal. Calcd. for $C_{10}H_{20}O_2$: C, 70.1; H, 11.7. Found: C, 69.8; H, 11.6.

2,4-Dimethylpentanoic acid. The synthesis was carried out essentially according to Bentley and Burrows (10) by condensation of diethyl methylmalonate (115 g.) and isobutyl bromide (69 g.). The solution of the crude condensation product was refluxed for 36 hrs. with 80 g. of potassium hydroxide, and after addition of 75 ml. of water (which dissolved the potassium salt that had precipitated), 280 ml. of the solvent was distilled off in a vacuum of 30 mm. The solution was then neutralized with 18% hydrochloric acid and precipitated with a saturated solution of calcium chloride. The calcium salt was filtered, washed with 50 ml. of cold water and treated with 18% hydrochloric acid. The brown oil that separated was drawn off and the aqueous layer extracted three times with 100 ml. of ether. The ethereal extracts were added to the brown oil, dried and evaporated. The residue which consisted of isobutylmethyl malonic acid and some 2,4-dimethylpentanoic acid, was heated at 200° until the evolution of carbon dioxide ceased, and distilled. B.p. 115-116° (28 mm); yield, 26 g. (40%, calculated on isobutyl bromide). This method gives better yields than the isolation and subsequent decarboxylation of the substituted malonic acid.

Anal. Calcd. for $C_7H_{14}O_2$: C, 64.6; H, 10.8. Found: C,64.6; H, 10.8.

For the synthesis of the corresponding primary alcohols from the saturated esters, lithium aluminum hydride in ether was used: for 0.4 mole of the ester in 150 ml. of ether, 9 g. of the reducing agent in 150 ml. of ether. The addition was carried out at 0° and the mixture then refluxed for 2 hrs and worked up as usual. The primary alcohols so obtained retained small quantities of water tenaciously, which did not interfere in the subsequent step of conversion into the primary bromides. In order to obtain the pure alcohols, azeotropic distillation with benzene was necessary.

Thus was obtained 3,4-dimethylpentan-1-ol, b.p. 152°, in 80% yield, and 2,3,4-trimethylpentan-1-ol, b.p. 110-112° (20 mm), in 73% yield.

2,4-Dimethylpentan-1-ol, which had been described before (11,12), was prepared from 2,4-dimethylpentanoic acid in a manner similar to that proposed by Dirscherl and Nahm (13), b.p. 156°; yield 79%.

Anal. Calcd. for $C_7H_{16}O$: C, 72.4; H, 13.8. Found: C, 71.9; H, 13.8.

5-Methylhexan-2-ol was prepared from 27.6 g. of magnesium, 151 g. of isoamyl bromide and 39.6 g. of acetaldehyde in 450 ml. of ether at -50°. B.p. 145° (lit.(14): 150-155°); yield, 97 g. (75%).

Isohexyl alcohol was obtained from isobutylmagnesium bromide and ethylene oxide (15).

For the preparation of the corresponding bromides, 21.5 g. of phosphorus tribromide was added, with stirring, to 0.25 mole of the alcohol at -8°; the temperature was not allowed to rise above 3°. As the alcohols contained small quantities of water, the reaction tended to be lively in the first stage. The stirring was continued until the (internal) temperature reached 20° and the mixture kept for 12 hrs. and distilled at a temperature not exceeding 100° (suitable vacuum). Towards the end of the first distillation, the sirupy residue tends to decompose and to spoil the vacuum. The distillate was washed three times with 15 ml. of cold conc. sulfuric acid, cooled at 0° and neutralized with solid potassium carbonate, filtered and distilled again.

The characteristics of the bromides prepared are summarized in Table II.

3β-Acetoxy-androst-5-en-17-yl 4-methylpentyl ketone (IIc). The chloride (I) (18) (3 g.) of 3β-acetoxyetiocholenic acid (1,19) was added to the cadmium derivative of 4.5 g. of isohexyl bromide (20) and the ether replaced by benzene. The usual work-up gave a semi-solid material which was dissolved in benzene and freed from water by azeotropic distillation with benzene. The solution was then concentrated to a volume of 10 ml. and chromatographed on alumina, a mixture of benzene-petroleum ether serving as eluent: the initial ratio of the two solvents was 1:6, and the benzene content was gradually increased. The ketone moved as a narrow brown band. It was dissolved in 200 ml. of hot methanol; upon cooling and reheating, some drops of a dark-yellow oil remained, from which the methanolic solution was decanted. This was repeated until no more oily material separated. Finally cooling of the methanolic solution gave colorless crystals, m.p. 135-138° (lit.(20): 136-140°), $[\alpha]_D^{24}$ + 11°; yield, 1.75 g. (58%).

The analogous ketones prepared in this study are described in Table III.

21-Norcholesteryl acetate (VIIIa). A suspension of 2.9 g. of IIc in 25 ml. of anhydrous diethylene glycol was heated for 30 mins. with 7.5 ml. of hydrazine hydrate. After cooling, 2.3 g. of powdered potassium hydroxide was added and the heating continued for 90 mins. until the internal temperature had reached 190° . The heating was then continued for 10 hrs.; in the beginning, some water was still formed which was evaporated by removing the condenser for a short time. The product was poured into 150 ml. of cold water and filtered after neutralization with 10% hydrochloric acid. The dried precipitate was acetylated by keeping it for 12 hr. with 15 ml. of pyridine and 15 ml. of acetic anhydride, and the solution poured into a mixture of 50 g. of ice and 10 ml. of conc. hydrochloric acid. The solid product was then dissolved in benzene and chromatographed on alumina. Thus 1.5 g. (53%) of m.p. 84°, $[\alpha]_D^{24}$ -33° was obtained.

Anal. Calcd. for $C_{28}H_{46}O$: C, 81.1; H, 11.1. Found: C,81.0; H, 11.1.

The analogs of this compound are reported in Table IV.

TABLE II

BROMIDES

Bromides	yield (%)	B.p. (°/mm)	Analysis C, fd.	H, fd.	C, calcd.	H, calcd.
2,4-Dimethyl-1-pentyl[a)]	55	69/30	46.7	8.6	46.9	8.4
3,4-Dimethyl-1-pentyl	65	66-67/18	47.4	8.0	46.9	8.4
5-Methyl-2-hexyl	69	58-58/18	47.1	8.7	46.9	8.4
2,3,4-Trimethyl-1-pentyl	49	84/18	50.2	9.2	49.8	8.8
4,4-Dimethyl-1-pentyl[b)]	20	70-71/35				
Isohexyl		143-145/750				

Notes: a) ref. 16 b) ref. 17.

TABLE III

3ß-Acetoxy-androst-5-en-17-yl alkyl ketones (II)

R =	Recryst. from	M.p.(°)	Yield (%)	$[\alpha]_D^{24}$	Analysis			
					C,fd.	H,fd.	C,calcd.	H,calcd.
3,4-dimethylpentyl (IIa) ($C_{29}H_{46}O_3$)	EtOH	96-97	51	+3.1	78.3	10.7	78.7	10.4
4,4-dimethylpentyl (IIb) ($C_{29}H_{46}O_3$)	MeOH	143-144	40	+4.2	78.7	10.5	78.7	10.4
2,3,4-trimethylpentyl (IId) ($C_{30}H_{48}O_3$)	MeOH	138-140	60	+15.6	79.1	10.5	79.0	10.5
2,4-dimethylpentyl (IIe) ($C_{29}H_{46}O_3$)	MeOH	93-94	56	+3.0	78.5	10.2	78.7	10.4
5-methyl-2-hexyl (IIf) ($C_{29}H_{46}O_3$)	MeOH	83-85	36	+2.5	78.5	10.3	78.7	10.4

[Note : a) The hydrazone, m.p. 74.5° ; $[\alpha]_D^{24}$ -22.8° , could not be decomposed even under drastic conditions. (Calcd. for $C_{29}H_{48}N_2O_2$: C,76.2; H,10.5; N,6.1. Found: C,76.3; H,10.5; N,5.6)]

TABLE IV

3β-Acetoxy-17-alkyl-androst-5-enes (VIII)

R =	Prepared from	Recryst. from	M.p.(°)	yield (%)	$[\alpha]_D^{24}$	Analysis			
						C, fd.	H, fd.	C, calcd.	H, calcd.
-dimethylhexyl IIb) ($C_{29}H_{48}O_2$)	IIa	EtOH	94–95	61	-36.4	81.2	11.6	81.3	11.2
-dimethylhexyl IIc) ($C_{29}H_{48}O_2$)	IIb	MeOH	130–131	57	-31.5	81.8	11.6	81.3	11.2
,5-trimethylhexyl IId) ($C_{30}H_{50}O_2$)	IIf	EtOH	82–83	20[a)]	-63	81.2	11.4	81.5	11.3

e : a) A nitrogen-containing by-product, m.p.216° (from ethanol) was not further investigated.

3ß-Acetoxy-20-hydroxy-22-methylcholest-5-ene (VIIIe). A solution of 3.6 g. of IV in 50 ml. of dry benzene was added to the Grignard solution, prepared - as described above - from 1.8 g. of magnesium in 20 ml. of ether and 10.5 g. of 5-methyl-2-hexyl bromide in 50 ml. of the same solvent. The reaction mixture was refluxed for 60 mins., the ether was distilled off and replaced by benzene, and the mixture refluxed for 4 hr. and decomposed with 100 ml. of a saturated solution of ammonium chloride. The product was then distilled with steam until all the benzene and the unreacted bromide were removed, and taken up again in 100 ml. of benzene. The oil, remaining after removal of the benzene in vacuo, was kept for 12 hr. with 30 ml. of pyridine and 30 ml. of acetic anhydride, and the volatile constituents were distilled off in vacuo. In order to remove the last traces of acetic anhydride, the product was taken up alternatively in methanol and benzene and recovered by evacuation. Finally, it was chromatographed on alumina from a mixture of chloroform and benzene (1:9) and eluted with an 1:7 mixture of the same solvents. After a yellow oil, the desired product was isolated; it was recrystallized from isopropyl alcohol. M.p. 180-182°; $[\alpha]_D^{24}$ - 3° ; ν_{max}^{KBr} 3450 (OH); 1730 (acetate carbonyl) cm^{-1}

yield, 1.7 g. (37%).

Anal. Calcd. for $C_{30}H_{50}O_3$: C,78.6; H,10.9. Found : C,78.2; H,10.4.

Under the conditions, under which the dehydration of this alcohol succeeded, it also caused loss of acetic acid, and 22-methylcholesta-3,5,20(22)-triene was obtained, e.g. with pyridine and phosphorus oxychloride. The hydrocarbon was isolated as a white powder of m.p. 50° (unsharp), which gave the correct analysis after recrystallization from methanol.

Anal. Calcd. for $C_{28}H_{44}$: C,88.4; H,11.6. Found: C,88.0; H,11.7.

3ß-Acetoxy-20-hydroxy-23-methylcholest-5-ene (VIIIf). The Grignard compound from 7.1 g. of 2,4-dimethyl-1-pentyl bromide in 50 ml. of ether gave with 3.6 g. of IV in 50 ml. of benzene 1.8 g. (41%) of the above tertiary alcohol, from methanol colorless crystals of m.p. 188-190° ; $[\alpha]_D^{20}$ -8.5; ν_{max}^{KBr} 3450 (OH), 1730 (acetate carbonyl) cm^{-1}.

Anal. Calcd. for $C_{30}H_{50}O_3$: C,78.6; H,10.9. Found : C,78.5; H,10.7.

A by-product was isolated, having m.p. 83-86° and being probably 3ß-acetoxy-23-methylcholesta-5,20(22)-diene; the quantity was too small for further purification.

3β-Acetoxy-24-methylcholesta-5,20(22)-diene (VIIIg). When in the same manner 3.6 g. of pregnenolone acetate reacted with the magnesium derivative from 7.1 g. of 3,4-dimethyl-1-pentyl bromide, and the product was worked up with dilute hydrochloric acid, reacetylated and chromatographed, the dehydration product (3.0 g.; 69%) of the expected tertiary alcohol was isolated. From isopropyl alcohol, it crystallized in colorless plates of m.p. 137°; $[\alpha]_D^{24}$ -44.9°. $\tilde{\nu}_{max}^{KBr}$ 1730 (acetate carbonyl) cm^{-1}.

Anal. Calcd. for $C_{30}H_{48}O_2$: C, 81.8; H, 10.9. Found: C, 81.5; H, 11.2.

24-Methylcholestanyl acetate. Hydrogenation of the foregoing compound proved more complicated than expected. Only in two cases could absorption of hydrogen be observed, viz. with 10% palladium-charcoal in absolute ethanol (not ethyl acetate) and with platinum oxide in a mixture of 18 ml. dioxane and 12 ml. of glacial acetic acid (for 100 mg. of the compound, 50 mg. of catalyst). However, in these cases 2 mole of hydrogen were absorbed without any break in the rate of reaction. The product which crystallized from ethanol in large, colorless plates, melted at 133-134°.

Anal. Calcd. for $C_{30}H_{52}O$: C, 81.1; H, 11.7. Found: C, 80.8; H, 12.0.

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REFERENCES

1. Bergmann, E.D., Rabinovitz, M., and Levinson, Z.H., J. AM. CHEM. SOC., 81, 1239 (1959).

2. See, e.g. Dadd, R.H., J. INSECT PHYSIOL., 5, 161 (1960).

3. Bergmann, E.D., and Levinson, Z.H., NATURE, 182, 723 (1958).

4. In several cases, the introduction of R added at least one asymmetric centre to the molecule. However, only one sterol was isolated in each case, the other (or others) were perhaps not separable. As the biological tests with the new sterols were negative, no attempt was made to repeat the syntheses with the optically active alkyl bromides.

5. Barton, D.H.R., and Laws, G.G., J. CHEM. SOC., 52 (1954).

6. Woodward, R.B., Patchett, A.A. Barton, D.H.R., Ives, D.A.J., and Belly, R.B., J. CHEM. SOC., 1131 (1957).

7. Bergmann, E.D., ISRAEL J. CHEM., 1, 91 (1963).

8. Whitmore, F.C., and Johnston, F., J. AM. CHEM. SOC., 60, 2265 (1938).

9. Faworsky, A., and Szyborsky, D., J. PRAKT. CHEM., [2]88, 674 (1913).

10. Bentley, H.W., and Burrows, M.W., J. CHEM. SOC., 67, 510 (1895).

11. Graves, C.D., IND. ENG. CHEM.,23, 1381 (1931).

12. Chu, T.T., and Marvel, C.S., J. AM. CHEM. SOC., 53, (1931).

13. Dirscherl, W., and Nahm, H., BER., 76, 709 (1943).

14. Clark, D.J., and Bloch, K., J. BIOL. CHEM., 234, 2578,2583 (1959).

15. Huston, R.C., and Agett, A.H., J. ORG. CHEM., 6, 123 (1941).

16. The compound has already been mentioned by Shonle, H.A., Waldo, J.W., Keltch, A.K., and Coles, H.W., J. AM. CHEM. SOC., 58, 585 (1936).

17. Whitmore, F.C., and Homeyer, A.H., J. AM. CHEM. SOC., 55, 4555 (1933); cf. Thomas, J., and Marlow, W., J. MED.CHEM., 7, 75 (1964).

18. Steiger, M., and Reichstein, T.. HELV. CHIM. ACTA, 20 1164 (1937).

19. King, L., J. AM. CHEM.SOC., 66, 1612 (1944).

20. Kurath, P., and Capezzuto, M., J. AM. CHEM. SOC., 78, 3527 (1956). See also Shimizu, K., J. OF BIOCHEM., 56, 201 (1964).