carded. Later fractions contained the 18-oxo-derivative XXVI (2.25 g.) already described above, whilst further elution afforded the desired 19-oxocortisol 3,20-bis-ethylene ketal 21-acetate (XXVIII, R = Ac, R′ = H) (8.01 g.) as needles from ethyl acetate, m.p. 283–301°. The analytical sample had m.p. 294–302°, $[\alpha]D$ +30° (*c* 0.9), ϵ at 207 mμ 2,100; γ_{max}^{KBr} 3600(s), 1750(s) and 1240(s); $\gamma_{max}^{CHCl_3}$ 3650(m), 1740(s) and 1240(s) cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{38}O_9$: C, 64.01; H, 7.56; O, 28.43. Found: C, 64.14; H, 7.71; O, 28.21.

The above 19-oxo-21-acetate (XXVIII, R = Ac, R′ = H) (500 mg.) in methanol (100 ml.) and benzene (25 ml.) was refluxed with aqueous sodium hydroxide (20 ml., 5%) for 15 min., then diluted with water (50 ml.) and extracted with methylene dichloride (450 ml.). The product, crystallized from ethyl acetate, gave 19-oxocortisol 3,20-bisethylene ketal (XXVIII, R = R′ = H) (420 mg.), m.p. 311–328°. The analytical sample had m.p. 309–331°, ϵ at 208 mμ 2,200, γ_{max}^{KBr} 3600(s) and 1660(w) cm.$^{-1}$. The compound was too insoluble for determination of $[\alpha]D$.

Anal. Calcd. for $C_{25}H_{36}O_8$. C, 64.63; H, 7.81; O, 27.55. Found: C, 64.51; H, 7.74; O, 27.55.

19-Oxocortisol 3,20-bisethylene ketal 21-acetate on treatment with pyridine–acetic anhydride on the steam-bath for 15 min. gave, after crystallization from ethyl acetate–hexane, the 19,21-diacetate (XXVIII, R = R′ = Ac) as needles, m.p. 236–245°, $[\alpha]D$ −23° (*c* 1.0) ϵ at 208 mμ 2,700; γ_{max}^{KBr} 3550(m), 1745(s) and 1250(s) cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{40}O_{10}$: C, 63.52; H, 7.35; O, 29.16. Found: C, 63.43; H, 7.55; O, 29.07.

19-Oxocortisol (XXIII) and Related Compounds.—19-Oxocortisol 3,20-bisethylene ketal (XXVIII, R = R′ = H) (see above) (1.63 g.) was taken up in dioxane (80 ml.) and water (20 ml.) and treated with water (20 ml.) and aqueous hydrochloric acid (40 ml., 2 *N*) at room temperature for 12 hr. The solution was concentrated *in vacuo* and the solid product filtered off. Chromatography over alumina in methylene dichloride containing increasing amounts of methanol gave first 19-oxocortisol 20-monoethylene ketal (XXX) (700 mg.). Crystallized from ethyl acetate–methanol this had m.p. 205–209°, λ_{max} 246 mμ (ϵ 13,000) (the max. disappeared on addition of a trace of alkali); γ_{max}^{KBr} 3500(s), 1665(s) and 1615(m) cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{32}O_7$: C, 65.69; H, 7.67; O, 26.64. Found: C, 65.78; H, 7.74; O, 26.69.

Further elution afforded 19-oxocortisol (XXIII) as needles (180 mg.) from ethyl acetate–methanol or ethyl acetate–hexane, m.p. 208–241°, λ_{max} 246 mμ (ϵ 13,000), disappearing on addition of trace of alkali and reappearing (at 241 mμ) with the original intensity on the addition of more alkali; γ_{max}^{KBr} 3500(s), 1705(s), 1655(s) and 1610(m) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50; O, 25.50. Found: C, 66.70; H, 7.60; O, 25.67.

In a related experiment 19-oxocortisol 3,20-bisethylene ketal (XXVIII), R = R = H) (4.0 g.) in dioxane (200 ml.), methanol (70 ml.) and aqueous hydrochloric acid (90 ml., 1 *N*) was warmed briefly to effect dissolution and then kept at room temperature for 24 hr. Chromatography of the product over alumina (120 g.) gave, as major crystalline product, 19-oxocortisol 19-methyl ether (XXIX, R = H, R′ = Me). Crystallized from ethyl acetate this formed needles (600 mg.), m.p. 220–241, $[\alpha]D$ +73° (*c* 1.0), λ_{max} 244 mμ (ϵ 15,600), unchanged on addition of alkali; γ_{max}^{KBr} 3550(s), 1660(s) and 1615(m) cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74; O, 24.59; OMe, 7.95. Found: C, 67.73; H, 7.66; O, 24.99; OMe, 9.44.

19-Oxocortisol 3,20-bisethylene ketal 21-acetate (XXVIII, R = Ac, R′ = H) (3.96 g.) in tetrahydrofuran (800 ml.) was refluxed with lithium aluminum hydride (4.5 g.) for 3 hr. Crystallization of the product from aqueous methanol furnished 19-hydroxycortisol 3,20-bisethylene ketal (2.34 g.). The analytical sample, crystallized from ethyl acetate–methanol, had m.p. 248–251°, γ_{max}^{KBr} 3600(s), 3500(s), 3400(s), 3250(s) and 1670(w) cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{38}O_8$: C, 64.36; H, 8.21; O, 27.44. Found: C, 64.01; H, 8.01; O, 27.58.

18,21-Anhydro-17α-hydroxyaldosterone (XXVII).—18-Oxocortisol 3,20-bisethylene ketal 21-acetate (XXVI) (300 mg.) in dioxane (18 ml.) containing water (42 ml.) and concd. sulfuric acid (3.6 ml.) was heated under nitrogen on the steam bath for 105 min. Addition of water and extraction into methylene dichloride gave, on crystallization from ethyl acetate, 18,21-anhydro-17α-hydroxyaldosterone (XXVII) as needles (95 mg.), m.p. 201–210°. The analytical sample had m.p. 203–214°, $[\alpha]D$ +200° (*c* 1.0), λ_{max} 239 mμ (ϵ 16,500); γ_{max}^{KBr} 3550(s), 1730(s), 1670(s), and 1620(m); $\gamma_{max}^{CHCl_3}$ 3600(m), 1730(s), 1665(s) and 1620(m) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31; O, 22.32. Found: C, 70.18; H, 7.35; O, 22.46.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO., KALAMAZOO, MICH.]

Nuclear Magnetic Resonance Studies on Some Hydrocarbon Side Chains of Steroids

BY GEORGE SLOMP AND FORREST A. MACKELLAR

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Nuclear magnetic resonance spectra of some common steroids with hydrocarbon side chains have been studied. The methyl regions have been factored and assigned. The results are applied to the identification of an unknown methylated steroid.

Chemists often perform exploratory reactions on sterols because they offer a convenient source of rigid molecules with known stereochemistry. However, the structural analysis of the products by proton magnetic resonance spectroscopy is quite difficult. The methyl–hydrogen region of the spectrum is so cluttered with absorptions from the side chains that those from the angular methyls[1] or from other methyl substitutents[2] have been difficult to identify. A study of the methyl absorptions of some of the common sterols, which is reported herein, has clarified this region of the spectrum to the point where useful structural data can be obtained from it.

The absorption frequencies of the angular methyls in the steroid molecule vary[1] depending on the nature and position of other substituents nearby and the anisotropy corrections form the basis for many structural assignments. Hydrocarbon chains at the 17β-position caused the 18-methyl absorption frequency for most steroids to be about 2 c.p.s.[3] lower than those for the corresponding 20-keto analogs. There was no contribution from the 22,23-double bond.

(1) J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).

(2) G. Slomp and B. R. McGarvey, *ibid.*, **81**, 2200 (1959).

(3) All absorption frequencies are recorded in c.p.s. at 60 megacycles (unless otherwise noted) to obviate the need for analyzing unknown multiplets.

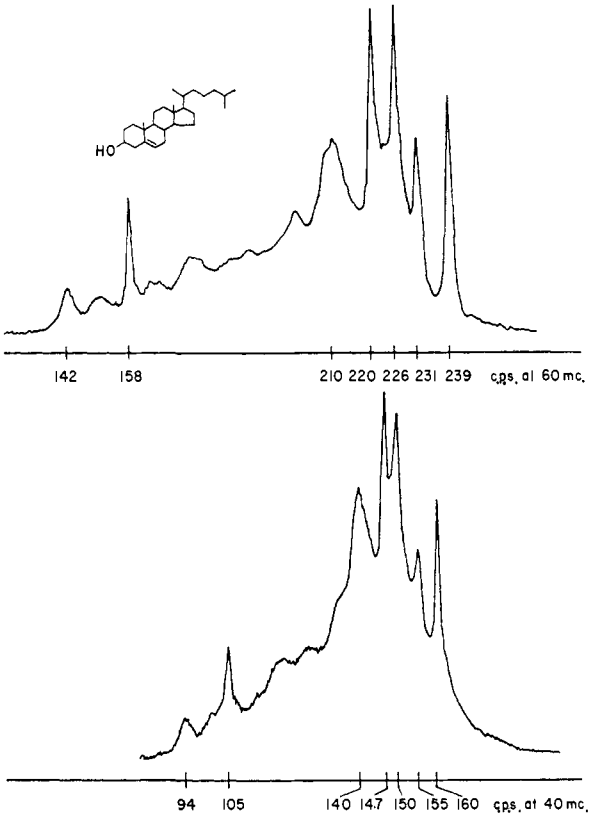
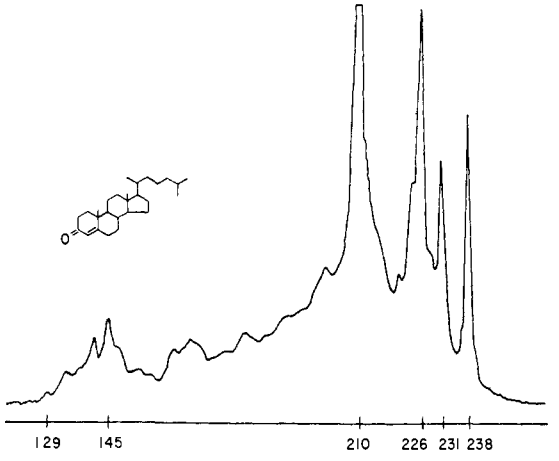


Fig. 1.—Aliphatic portion of the spectrum of cholesterol observed at 60 mc. and at 40 mc. Chain methylenes at about 210; 19-hydrogens at 220; 21-, 26-, 27-hydrogens at about 226, 231; 18-hydrogens at 239; OH at 158 c.p.s.



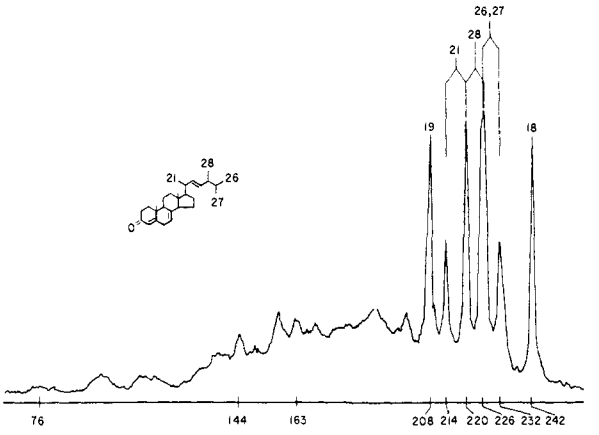
C.p.s. at 60 mc.

Fig. 2.—Aliphatic portion of the spectrum of cholestenone. Chain methylenes and 19-hydrogens at about 210; 21-, 26- and 27-hydrogens at about 226, 231; 18-hydrogens at 238; allylic hydrogens at 129–145 cps.

Experimental

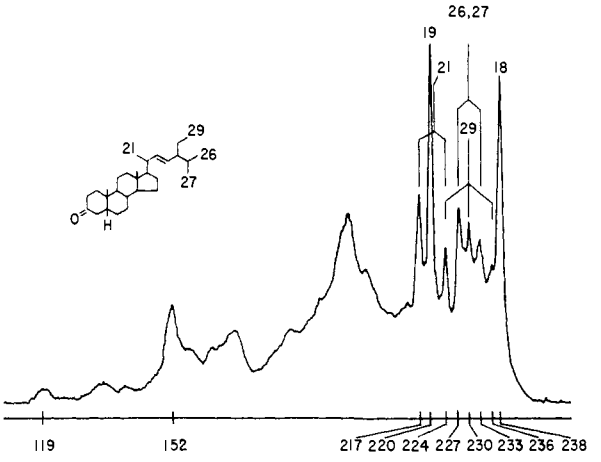
The spectra were observed with a Varian 4300-2 spectrometer operating at 60 mc. on solutions (*ca.* 0.3 ml., *ca.* 0.15 molar) of the steroids in deuteriochloroform. The spectra were calibrated against internal tetramethylsilane as 282 c.p.s.[4] (which makes external water 0 c.p.s.) by the audiofrequency sideband technique.[5]

(4) To convert to c.p.s. at 60 mc. relative to benzene add 102 c.p.s.



C.p.s. at 60 mc.

Fig. 3.—Aliphatic portion of the spectrum of 4,7,22-ergostatrien-3-one.



C.p.s. at 60 mc.

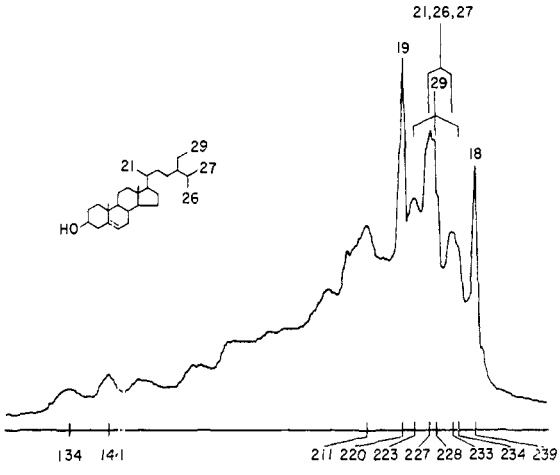
Fig. 4.—Aliphatic portion of the spectrum of 5β-stigmast-22-en-3-one.

Discussion

Cholestane Side Chain.—The aliphatic portion of the spectrum of cholesterol is shown in Fig. 1. The lines at approximately 226 and 231 c.p.s., which retain the same spacing at a lower applied field, are attributed to the three nearly superimposed sets of doublets arising from the three similar methyls on the cholesterol side chain. The freely-wobbling methylenes of the side chain are observed at about 210 c.p.s. In cholestenone, Fig. 2, the 19-methyl absorption is shifted out of the way and the side chain methyl absorption lines can be seen more clearly. The two intense lines are probably from the isopropyl hydrogens. The absorptions of the 21-hydrogens may be the shoulders at 224, 229 c.p.s., since this is the frequency at which they were observed in desmosterol (24-dehydrocholesterol). It is also to be noted that the pattern observed in the region of 129–145 c.p.s. is a typical fingerprint for the allylic hydrogens associated with the 3-keto-Δ^4-system.

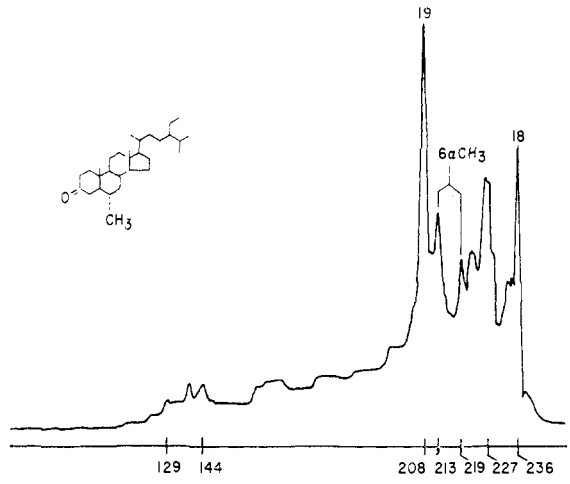
Ergostane Side Chain.—The aliphatic portion of the spectrum of 4,7,22-ergostatrien-3-one is shown

(5) J. T. Arnold and M. E. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).



C.p.s. at 60 mc.

Fig. 5.—Aliphatic portion of the spectrum of β-sitosterol.



C.p.s. at 60 mc.

Fig. 6.—Aliphatic portion of the spectrum of 6α-methyl-4-sitosten-3-one.

in Fig. 3. This compound was chosen because the absorptions from the angular methyls were sufficiently spread by the effects of the ring substituents to uncover the absorptions due to the side chain methyls. The spectrum therefore shows 19-hydrogen at 208 c.p.s., and 18-hydrogen at 242 as expected from the contributions[1] of the ketone and the three double bonds. The assignment of the four remaining lines arising from the side chain methyls was made as follows: the isopropyl doublet (226,232 c.p.s.) remains the same as in cholesterol, the 21-hydrogens (214, 220 cps.) are unshielded by the anisotropy of the Δ^{22} and the 28-hydrogen doublet (220, 226 c.p.s.) is also unshielded and is superimposed on two of the above lines as is apparent from their areas and shapes. As expected, this doublet becomes a triplet in the stigmastane side chain.

Stigmastene Side Chain.—The aliphatic portion of the spectrum of 5β-stigmast-22-en-3-one is shown in Fig. 4. The absorptions of the angular methyl groups are found at 220 and 238 c.p.s. The isopropyl doublet (227, 233 c.p.s.) remains unchanged from the previous example. The 29-hydrogens have the same shielding as those at 26 and 27 but are now observed as an irregular triplet[6] at 224, 230, 236 c.p.s. The 21-hydrogen doublet is at 217, 224 c.p.s. There is nothing underneath the 19-methyl absorption. This was shown from the spectrum of stigmastadienone where the 19-methyl absorption was lowered to 210 c.p.s. The above assignments were consistent also with the 40-mc. spectrum obtained for this material.

Sitostane Side Chain.—The aliphatic portion of the spectrum of sitosterol is shown in Fig. 5. The absorptions of the angular methyl groups are found at 220 and 239 c.p.s. The isopropyl doublet remains unchanged from the previous samples, at 227 and 233 c.p.s., and is approximately superimposed on the doublet from the 21-hydrogens. The 29-hydrogens are observed as an irregular triplet[6] at 223, 228, 234±.

6α-Methyl-4-sitosten-3-one.—Application of the method is illustrated by the identification of this dehydration product of a 5α-hydroxy-6β-methyl steroid from the nuclear magnetic resonance spectrum (see Fig. 6). From the foregoing information the absorptions due to the sitosterol side chain were readily identified. The angular methyl absorptions were identified as the two intense lines at 208 and 236 c.p.s. The remaining doublet at 213, 219 c.p.s. must be from the 6-methyl group and is consistent[2] only with an isomerization to the more stable 6α-configuration (*i.e.*, the 6β-methyl was expected[2] at 203, 210 c.p.s.). The absorption pattern at 129–144 c.p.s. is in agreement with the 3-keto-Δ^4-structure.

(6) B. R. McGarvey and G. Slomp, Jr., *J. Chem. Phys.*, **30**, 1586 (1959).