6,7-Unsaturated 5β-Steroids as Intermediates for Labelling with Isotopic Hydrogen

By **David N. Kirk*** and **David R. A. Leonard,** Medical Research Council Steroid Reference Collection, Chemistry Department, Westfield College, London NW3 7ST

5β-Pregn-6-ene-3α,20α-diol (I) (Δ^6-pregnanediol) and 3α-hydroxy-5β-androst-6-en-17-one (II) (Δ^6-aetiocholanolone) have been prepared and reduced to give their parent saturated compounds. Deuteriation experiments with model Δ^6-5β-cholestanes show that catalytic hydrogenation is not stereospecific.

THE 6,7-unsaturated derivatives of 5β-pregnane-3α,20α-diol (pregnanediol) and 3α-hydroxy-5β-androstan-17-one (aetiocholanolone) were envisaged as convenient precursors of the [6,7-$^3H_2$]-compounds. The unsaturated materials were expected to be stable to long-term storage, and to be capable of being tritiated as the need arose to provide material of high specific activity in a single step, with the labels in chemically stable positions.

The key steps used in the synthesis of Δ^6-5β-steroids were: (i) hydroboration–oxidation of a 3,3-ethylenedioxy-Δ^5-derivative (III); this is stereoselective in favour of the 6β-hydroxy-5β-isomer (IV) because of steric hindrance on the α-face from the axial 3α-oxygen substituent;[1] (ii) regiospecific dehydration of the 6β-hydroxy-5β-compounds (IV) with phosphoryl chloride–pyridine, with loss of the only available *anti*periplanar proton (7α) to give the Δ^6-bond.[2] The other steps in the syntheses, which are unexceptional, are indicated in the Scheme.

The Δ^6-compounds were characterised from their n.m.r. spectra, and by hydrogenation to give products identical with pregnanediol and aetiocholanolone, respectively. Hydrogenation over palladium–charcoal catalysts was slow and not always complete; Adams platinum was a satisfactory alternative. Tris(triphenylphosphine)rhodium chloride[3] could also be used (see later).

Stereochemistry of Hydrogenation at the Δ^6*-Bond.*—In order to examine the stereochemistry of hydrogenation of the olefinic bond, 5β-cholest-6-en-3-one and 5β-cholest-6-en-3α-ol were prepared as model compounds from cholest-4-en-3-one. Catalytic deuteriation, either of the 6-en-3-one in the presence of the soluble tris-(triphenylphosphine)rhodium chloride, or of the 6-en-3α-ol over Adams platinum, gave materials containing essentially two deuterium atoms per molecule (mass spectra). Reduction with deuterium over Pd–C was slow and incomplete.

Direct n.m.r. study. Examination of the deuteriated 5β-cholestan-3α-ol by n.m.r. (at 100 MHz) with added shift reagent Eu(dpm)$_3$[4] gave inconclusive evidence, suggesting that the reduction was non-stereospecific but favoured the [6β,7β-$^2H_2$]isomer. The spectrum showed three distinct groups of signals shifted to lower field than the C-19 protons. Since the europium-induced shifts are roughly proportional to the inverse cube of the distance from the proton to the europium atom com-

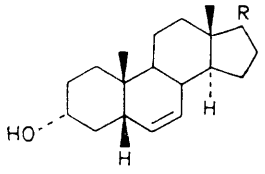
[1] (*a*) A. S. Clegg, W. A. Denny, Sir Ewart R. H. Jones, V. Kumar, G. D. Meakins, and V. E. M. Thomas, *J.C.S. Perkin I*, 1972, 492; (*b*) D. N. Kirk and M. P. Hartshorn, 'Steroid Reaction Mechanisms,' Elsevier, Amsterdam, 1968, p. 70.

[2] Ref. 1*b*, p. 103.

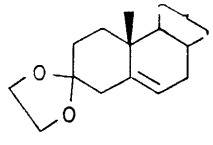
[3] J. F. Young, J. A. Osborn, F. H. Jardine, and G. Wilkinson, *Chem. Comm.*, 1965, 131; C. Djerassi and J. Gutzwiller, *J. Amer. Chem. Soc.*, 1966, **88**, 4537.

[4] Specialist Periodical Reports: Terpenoids and Steroids, The Chemical Society, vol. 2, pp. 237—239.

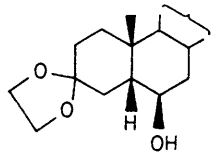
plexed with the 3α-OH group,[4] measurements of O · · · H distances on a Dreiding model allowed the three groups of signals to be assigned tentatively to the following sets



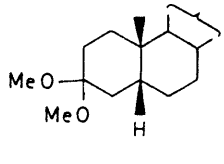
(I) R = CHOH(α)Me
(II) R = :O



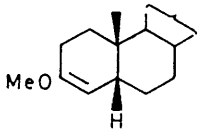
(III)



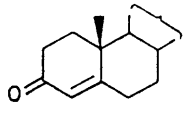
(IV)



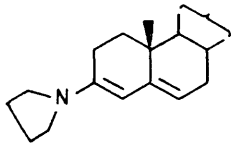
(V)



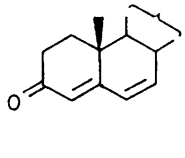
(VI)



(VII)

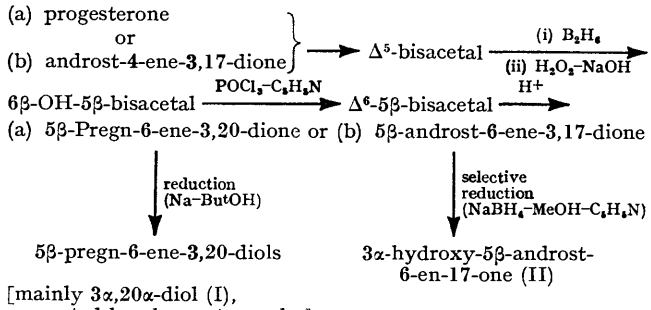


(VIII)



(IX)

of protons: (i) at lowest field (five protons), 2α, 2β, 3β, 4α, and 4β; (ii) at higher field, multiplets totalling four protons: 1α, 1β, 5β, and 9α; (iii) two adjacent bands, which comprised together rather less than four protons. The component at lower field was tentatively assigned to the 6α- and 7α-protons; its fine structure was poorly resolved, but was dominated by a doublet (*W* 5 Hz). [The spectrum of unlabelled 5β-cholestan-3α-ol showed this signal as a quartet (*W* 8 Hz), but was virtually identical elsewhere.] Unfortunately the integral of this signal in the spectrum of the deuteriated sample, though less than that from the unlabelled material, could not be measured with sufficient reliability to give a good estimate of deuterium at the 6α- and 7α-positions. The 6β- and 7β-proton signals could not be separated from a fourth region of the spectrum totalling about 9—10 protons, which included those at C-19; however, the integral over this region was *ca.* 1—1·5 protons less for the labelled than for the unlabelled material, suggesting



SCHEME Preparation of 6,7-unsaturated-5β-steroids

that the deuterium atoms occupied predominantly β-positions.

Indirect method. An alternative approach was therefore explored. This depended upon a stepwise conversion of the [6,7-$^2H_2$]-5β-3-one into the 4,6-dien-3-one. A reaction sequence having some novel features was employed, after preliminary exploration using unlabelled 5β-cholestan-3-one. Reaction with 2,2-dimethoxypropane gave the 3,3-dimethoxy-derivative (V),[5] which eliminated methanol in refluxing naphthalene to give mainly 3-methoxy-5β-cholest-3-ene (VI), in accord with the known preference for 3,4- rather than 2,3-unsaturation in the 5β-series.[6] Dehydrogenation of the 3-enol ether with dichlorodicyanobenzoquinone (DDQ) [7] gave cholest-4-en-3-one (VII), which was purified by chromatography. The derived 3,5-dienamine (VIII) [8] was dehydrogenated, again by DDQ, to give the 4,6-dien-3-one (IX).

Analysis of the n.m.r. and mass spectra of the 4,6-dienone (IX) showed rather more than half of the material to have a deuterium atom at C-7. Since DDQ dehydrogenates 3,5-dienolic derivatives by stereospecific abstraction of 7α-H as hydride ion,[7] and would be expected to show similar selectivity in the 3,5-dienamine, the modest preponderance of 6β,7β-deuteriation is confirmed. Another sample of 4,6-dienone, obtained *via* deuteriation of 5β-cholest-6-en-3-one with the soluble rhodium catalyst, gave evidence from its spectra of a slight preponderance (*ca.* 60%) of 6α,7α-deuteriation.

For the purpose of these analyses, the n.m.r. spectra of the dienone samples were examined in the presence of Eu(dpm)$_3$, which separated the olefinic proton signals by shifting them to lower field (shifts were in the order 4-H > 6-H > 7-H, depending upon the distance of the protons from the C-3 oxygen atom). The 6-H and 7-H signals are superimposed as a sharp singlet in the ordinary

[5] 'Organic Reactions in Steroid Chemistry,' ed. J. Fried and J. A. Edwards, Van Nostrand-Reinhold, New York, 1972, vol. 1, p. 388.
[6] E. P. Oliveto, C. Gerold, and E. B. Hershberg, *J. Amer. Chem. Soc.*, 1954, **76**, 6113; ref. 1*b*, p. 161.
[7] S. K. Pradhan and H. J. Ringold, *J. Org. Chem.*, 1964, **29**, 601; ref. 1*b*, p. 189.
[8] J. L. Johnson, M. E. Herr, J. C. Babcock, A. E. Fonken, J. E. Stafford, and F. W. Heyl, *J. Amer. Chem. Soc.*, 1956, **78**, 430.

n.m.r. spectrum, but they separate into an AB quartet when the added lanthanide complex renders these protons non-equivalent.

EXPERIMENTAL

I.r. spectra were measured for Nujol mulls. N.m.r. spectra were obtained for solutions in $CDCl_3$ at 100 MHz. Alumina was Spence grade H. 'Deactivated alumina' refers to grade H deactivated with 5% of aqueous 10% acetic acid.

3,3;20,20-*Bisethylenedioxy*-5β-*pregnan*-6β-*ol*.— 3,3;20,20-Bisethylenedioxypregn-5-ene (6·28 g) in anhydrous tetrahydrofuran (30 ml) was treated at 0° with an excess of diborane gas in a stream of nitrogen. After a further 2 h at room temperature, the solution was treated with crushed ice, followed by 3M-NaOH (50 ml). Hydrogen peroxide (30%; 30 ml) was then added slowly. The mixture was then left to stir overnight. The product, extracted *via* ethyl acetate, showed the presence of three minor components (t.l.c.) as well as the desired 6β-hydroxy-5β-derivative: the main part was dehydrated without further purification. A pure sample of the 6β-*hydroxy-bisacetal* was obtained by crystallising a small portion from acetone–hexane: m.p. 187—190°; $\nu_{max.}$ 3360, 1120, 1070, and 1050 cm^{-1} (Found: C, 71·6; H, 9·8. $C_{25}H_{40}O_5$ requires C, 71·4; H, 9·6%).

5β-*Pregn*-6-*ene*-3,20-*dione*.—The crude 6β-hydroxy-bisacetal (5·87 g) in anhydrous pyridine (50 ml) was treated dropwise at 0° with phosphoryl chloride (5 ml), and left at room temperature overnight. The mixture was then poured into aqueous 70% acetic acid (150 ml), and the solution was warmed on a steam-bath for 2 h. Most of the solvents were then removed under reduced pressure, and the residual oil, dissolved in benzene–ethyl acetate, was washed until neutral, dried, and evaporated, to give a gum (2·03 g) which showed six spots on t.l.c. Chromatography on deactivated alumina allowed removal of by-products of low polarity, before the desired 5β-*pregn*-6-*ene*-3,20-*dione* (740 mg) was eluted with ether–benzene (1 : 9). The dione crystallised from acetone–hexane: m.p. 152—154°; $\nu_{max.}$ 3010, 1662 (*cis*-olefin), 1717, and 1704 cm^{-1}; τ 9·29 (18-H_3; calc.[9] 9·30), 9·01 (19-H_3; calc. 9·00); 7·84 (21-H_3), and 4·40 (s, *W* 3 Hz, 6,7-H_2) (Found: C, 79·8; H, 9·3. $C_{21}H_{30}O_2$ requires C, 80·2; H, 9·6%).

5β-*Pregn*-6-*ene*-3α,20α-*diol* (I).—The 6-ene-3,20-dione (211 mg) in anhydrous t-butyl alcohol was heated under reflux with sodium (1·9 g) with stirring for 1 h. A little methanol was then added to destroy unchanged sodium, the solution was cooled and acidified, the butanol was evaporated off, and the products were extracted with chloroform, to give a mixture of diols (210 mg), containing 55% of the desired 3α,20α-diol (g.l.c.). The isomers were separated by chromatography on alumina (20 g). Elution with chloroform gave a mixture (74 mg) comprising mainly the 3α,20β- and 3β,20α-diols, identified by catalytic hydrogenation and comparison (g.l.c.) of the products with the corresponding saturated 5β-pregnanediols. Elution with ether–chloroform (1 : 9) and ethyl acetate–chloroform (1 : 9) gave the crude 6-*ene*-3α,20α-*diol* (116 mg), which crystallised from acetone–hexane to give pure diol (69 mg), m.p. 193—195°; $\nu_{max.}$ 3340sh, 3270, 3010, 1650, 1077, 1017 and 745 cm^{-1}; τ 9·29 (18-H_3; calc.[9] 9·30), 9·12 (19-H_3; calc. 9·00), and 4·52 (s, 6,7-H_2) (Found: C, 79·1; H, 10·4. $C_{21}H_{34}O_2$ requires C, 79·2; H, 10·8%).

5β-*Pregnane*-3α,20α-*diol*.—The unsaturated 3α,20α-diol was hydrogenated in ethanol over 5% Pd–C, giving 5β-pregnane-3α,20α-diol, m.p. and mixed m.p. 230—233°, identical (i.r. and g.l.c.) with an authentic sample.

5β-*Androst*-6-*ene*-3,17-*dione*.— 3,3:17,17-Bisethylenedioxyandrost-5-ene (5·7 g) was treated with diborane followed by alkaline hydrogen peroxide, essentially as described for the pregnane analogue. The product (6·1 g) was a gum, which was used without purification. The gum (4·36 g) in pyridine (25 ml) was added dropwise during 2 h to a stirred solution of phosphoryl chloride (5 ml) in pyridine (45 ml) at 50°. After further heating on a steam-bath for 1 h the solution was added to 1 : 1 benzene–ethyl acetate (100 ml), treated with ice, and then washed neutral. Removal of the organic solvents gave of a gum (2·65 g), which was treated on a steam-bath for 1 h with aqueous 70% acetic acid, and the product was isolated by use of benzene, giving a gum (2·13 g). Chromatography on alumina (200 g) allowed minor amounts of by-products to be eluted by light petroleum, followed by benzene, and ether–benzene (1 : 4). Elution with ether–benzene (1 : 1) then gave 5β-*androst*-6-*ene*-3,17-*dione* (844 mg), which crystallised from hexane as plates (750 mg), m.p. 104·5—106°; $\nu_{max.}$ 3020 (vinylic C–H), 1742, 1717, 1220, 1170, 1098, 1057, 1010, and 738 cm^{-1}; τ 9·06 (18-H_3; calc.[9] 9·05), 9·01 (19-H_3; calc. 9·03), and 4·43 (s, 6,7-H_2) (Found: C, 79·8; H, 8·9. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·15%). Later fractions, eluted with 1 : 1 ether–benzene (319 mg), were mixtures of the 6-ene-3,17-dione and androst-4-ene-3,17-dione.

Selective Reduction of 5β-*Androst*-6-*ene*-3,17-*dione*.—The unsaturated dione (286 mg, 1 mmol) in methanol (80 ml) was stirred and treated at room temperature with sodium borohydride (15 mg, 0·31 mmol) in a mixture of pyridine (10 ml), methanol (10 ml), and aqueous 2·5N-sodium hydroxide (0·3 ml). After 10 min stirring the solution was acidified with N-hydrochloric acid and extracted with ethyl acetate–chloroform (1 : 1), which afforded a mixture of products (299 mg). Chromatography on deactivated alumina (60 g) gave unchanged dione as the first fraction (42 mg, 15%), eluted by ether–benzene (1 : 19). Elution with ether–benzene (1 : 9) gave 3β-*hydroxy*-5β-*androst*-6-*en*-17-*one* (41 mg, 14%), needles (from hexane), m.p. 160—162°; $\nu_{max.}$ 3490, 3015, 1728, and 1650 cm^{-1}; τ 1·07 (18-H_3; calc.[9] 1·08), 1·07 (19-H_3; calc. 1·03), 6·25 (q, *W* 10·5 Hz, 3α-H), and 4·40 (s, 6,7-H_2) (Found: C, 78·8; H, 9·5. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%). The structure of this product was confirmed by catalytic hydrogenation in ethanol, over 5% Pd–C, to give 3β-hydroxy-5β-androstan-17-one, m.p. 156—157° (lit.,[10] 152°) [3-acetate, m.p. 149—154° (lit.,[11] 157—159°)].

Further elution with ether–benzene (1 : 4) gave a mixture (28 mg; three spots on t.l.c.), probably containing the 3β- and 3α-hydroxy-17-ones and a little 17β-hydroxy-5β-androst-6-en-3-one; $\nu_{max.}$ 1730 [C(17)O] and 1712 cm^{-1} [C(3)O].

Ether–benzene (3 : 7) and (2 : 3) desorbed the required 3α-*hydroxy*-5β-*androst*-6-*en*-17-*one* (II) (154 mg, 53%) which crystallised from hexane–acetone as prisms, m.p. 172—174°, τ 9·11 (18-H_3; calc.[9] 9·08), 9·08 (19-H_3; calc. 9·075),

[9] N. S. Bhacca and D. H. Williams, 'Applications of N.M.R. Spectroscopy in Organic Chemistry,' Holden-Day, San Francisco, 1964, pp. 19—24.

[10] L. F. Fieser and M. Fieser, 'Steroids,' Reinhold, New York, 1959, p. 505.

[11] T. Reichstein and A. Lardon, *Helv. Chim. Acta*, 1941, **24**, 955.

6·39 (m, *W* 27 Hz, 3β-H), and 4·44 (s, 6,7-H_2) (Found: C, 79·2; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%). A sample (50 mg) was hydrogenated over 5% Pd–C, to give 3α-hydroxy-5β-androstan-17-one, m.p. 148—151°, identified by comparison (g.l.c., t.l.c., i.r.) with an authentic sample, m.p. 150—153°.

Elution with ethyl acetate–chloroform (3 : 7 and 1 : 1) gave 5β-*androst*-6-*ene*-3α,17β-*diol* (20 mg, 7%), needles (from acetone–hexane), m.p. 197—200°; $\nu_{max.}$ 3325, 3020, 1066, 1050, 1039, and 743 cm^{-1} (Found: C, 78·2; H, 10·5. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%).

The diol (11 mg) was hydrogenated over 5% Pd–C, to give 5β-*androstane*-3α,17β-*diol*, m.p. 233—234°, identified by comparison (g.l.c., t.l.c., i.r.) with an authentic sample, m.p. 237—238°.

5β-*Cholest*-6-*en*-3-*one*.—Hydroboronation and oxidation of 3,3-ethylenedioxycholest-5-ene, as described for the pregnane analogue, gave the crude 6β-hydroxy-acetal. The product was characterised by hydrolysing a small sample (aqueous 70% acetic acid), followed by acetylation. Chromatography of the resulting mixture on alumina gave 6β-acetoxy-5β-cholestan-3-one (60%), m.p. 109—112°, (lit.,[12] 113—115°); $\nu_{max.}$ 1740, 1714, 1248, and 1023 cm^{-1}, and 6α-acetoxy-5α-cholestan-3-one (19%), m.p. 132—134° (lit.,[13] 133—143°); $\nu_{max.}$ 1740, 1712, 1240, and 1022 cm^{-1}.

The main part of the crude 6β-hydroxy-compound (9 g) was treated with phosphoryl chloride (10 ml) and pyridine (140 ml) and subsequently with aqueous 70% acetic acid (300 ml), as in the synthesis of 5β-androst-6-ene-3,17-dione. The resulting gum (4·84 g) was chromatographed on deactivated alumina (160 g). Light petroleum eluted a hydrocarbon fraction (0·84 g), then light petroleum–benzene mixtures, and benzene, eluted 5β-cholest-6-en-3-one (3·1 g), m.p. 108·5—109° (from ethanol) (lit.,[14] 109—110°); $\nu_{max.}$ 3013, 1722, 1675, 734, and 703 cm^{-1}.

5β-*Cholest*-6-*en*-3α-*ol*.—5β-Cholest-6-en-3-one (190 mg) was reduced in tetrahydrofuran (8 ml) with lithium aluminium hydride (200 mg). The product (186 mg) crystallised from acetone to give the 3α-ol, m.p. 85—90°; $\nu_{max.}$ 3310, 3002, 1636, 1066, 1010, and 742 cm^{-1}.

6,7-*Dideuterio*-5β-*cholestan*-3-*one*.—(a) *With a soluble catalyst*. 5β-Cholest-6-en-3-one (245 mg) and tris(triphenylphosphine)rhodium chloride (200 mg) in propan-2-ol (10 ml) and benzene (10 ml) containing aqueous 3% hydrogen peroxide (0·02 ml) was shaken in an atmosphere of deuterium for 40 h; reaction was then complete. The solvents were removed, and the residue was chromatographed on alumina (20 g). Elution with chloroform–benzene (1 : 1) gave 6,7-dideuterio-5β-cholestan-3-one, identical (t.l.c., g.l.c.) with the unlabelled material, *m/e* 388 (M^+).

(b) 5β-Cholest-6-en-3α-ol (91 mg) in benzene (2 ml) was reduced with deuterium over Adams platinum (19 mg). Removal of the catalyst and solvent, and crystallisation from aqueous acetone, gave 5β-cholestan-3α-ol as needles, m.p. 107—109°, identical with an authentic sample. The alcohol was then oxidised by Jones reagent in acetone to give 6,7-dideuterio-5β-cholestan-3-one, *m/e* 388 (M^+).

Deuterium-labelled Cholesta-4,6-*dien*-3-*one* (IX).—The following reaction sequence was followed with 6,7-dideuterio-5β-cholestan-3-one, after a preliminary run with the unlabelled ketone.

5β-Cholestan-3-one (198 mg) in 2,2-dimethoxypropane (1·0 ml), methanol (0·4 ml), and toluene-*p*-sulphonic acid (5 mg) was heated under reflux for 3·5 h. The cooled solution was taken up in chloroform (20 ml) containing a few drops of pyridine, and washed with aqueous sodium hydrogen carbonate and brine. Removal of solvents left the crude 3,3-dimethoxy-derivative (V) (221 mg) as an oil, $\nu_{max.}$ 1101 and 1058 cm^{-1}.

The crude dimethoxy-compound (200 mg) and naphthalene (2·0 g) were heated at 190° for 45 min. The naphthalene was then removed by sublimation under reduced pressure, leaving a gum (200 mg) consisting essentially of the 3-methoxy-Δ^3-compound (VI), $\nu_{max.}$ 1669, 1210, 1172, 810, and 783 cm^{-1}. A small proportion of the 5β-cholestan-3-one was also present (t.l.c.).

A solution of the crude 3-methoxy-5β-cholest-3-ene (125 mg, 0·31 mmol) in acetone (2 ml) was treated with DDQ (74 mg, 0·325 mmol) in aqueous 95% acetone (2 ml). The mixture was stirred overnight and then added to an excess of aqueous sodium hydrogen carbonate. Extraction with chloroform, and chromatography on alumina (20 g), gave 5β-cholestan-3-one (12 mg) and cholest-4-en-3-one (VII) (84 mg), characterised by comparison (t.l.c., i.r., u.v., and n.m.r.) with an authentic sample.

A solution of cholest-4-en-3-one (90 mg) in methanol (0·5 ml) was heated to boiling, treated with pyrrolidine (0·1 ml), and immediately set aside to cool. The dienamine (VIII), which separated as a yellow solid (63 mg) ($\nu_{max.}$ 1640 and 1610 cm^{-1}) was treated in aqueous 95% acetone (2 ml) with a solution of DDQ (48 mg) in aqueous 95% acetone (2 ml). The mixture was stirred overnight, then added to aqueous 95% ethanol (30 ml) and heated under reflux for 1 h. The solvents were removed under reduced pressure, and the product was isolated by use of benzene and chromatographed on alumina (20 g). Chloroform eluted cholesta-4,6-dien-3-one (51 mg), identified by comparison with an authentic sample. The mass spectra of samples prepared from 6,7-dideuterio-5β-cholestan-3-one showed the principle parent ion at *m/e* 383 ($^2H_1$ species); τ 4·50 (4-H) and 3·99 (s, 6,7-H_2) [the latter signal was smaller in the spectra of the deuteriated samples, and appeared as a quartet when the signals were shifted to lower field by added $Eu(dpm)_3$ (see text)].

[3/571 *Received*, 19*th March*, 1973]

[12] D. N. Jones, J. R. Lewis, C. W. Shoppee, and G. H. R. Summers, *J. Chem. Soc.*, 1955, 2876.

[13] C. W. Shoppee, R. Lack, and B. McLean, *J. Chem. Soc.*, 1964, 4996.

[14] A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, 1959, **81**, 6330.