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# Insect Moulting Hormones: The Synthesis of Ecdysone Analogues from a Bile Acid

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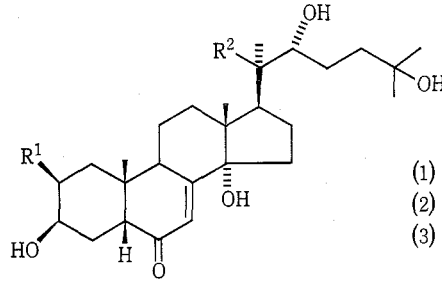
*Abstract*

A new general synthesis of 2-deoxyecdysone analogues starting from the bile acid, chenodeoxycholic acid, has been developed and used to prepare a number of ecdysones for structure–biological activity studies.

## Introduction

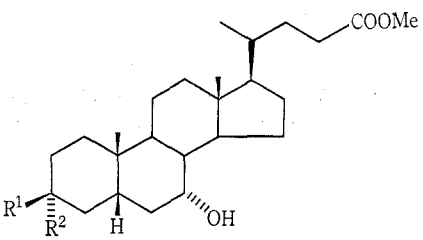
The observation[1] that the naturally occurring 2-deoxyecdysones, deoxycrustecdysone (2-deoxy-$\beta$-ecdysone) (1) and deoxyecdysone (2-deoxy-$\alpha$-ecdysone) (2), are as active as $\beta$-ecdysone (3) in the *Calliphora* bioassay[2] led us to undertake the synthesis of other 2-deoxyecdysone analogues as part of a study to determine the functional groups essential for moulting hormone activity. A major problem has been the synthesis of the required 2-deoxyecdysone analogues. Previously ecdysones have been synthesized from 5$\alpha$-epimers by equilibration with base.[3] In compounds containing a 2$\beta$-hydroxy function the 5$\beta$-epimer is more stable because of the steric interaction between the 2$\beta$-hydroxyl and the C19 methyl group in the 5$\alpha$-form. However, in the case of 2-deoxyecdysone analogues where this steric interaction is absent, the 5$\alpha$-epimer is the more stable and the required 5$\beta$-epimer can be isolated from the equilibration mixture in only small yield by tedious chromatographic procedures.[4] The aim of the present work was to find a better route to these compounds and prepare further analogues for structure–biological activity studies.



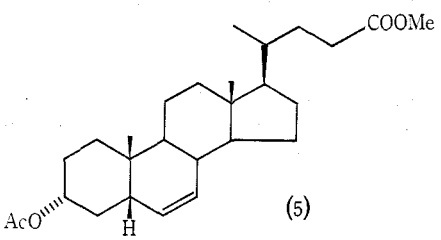

| | $R^1$ | $R^2$ |
|---|---|---|
| (1) | H | HO |
| (2) | H | H |
| (3) | HO | HO |

[1] Chong, Y. K., Galbraith, M. N., and Horn, D. H. S., *Chem. Commun.*, 1970, 1217.
[2] Thomson, J. A., Imray, F. P., and Horn, D. H. S., *Aust. J. Exp. Biol. Med. Sci.*, 1970, **48**, 321.
[3] Horn, D. H. S., 'The Ecdysones' in 'Naturally Occurring Insecticides' p. 333 (Eds M. Jacobson and D. G. Crosby) (Marcel Dekker: New York 1971).
[4] Galbraith, M. N., Horn, D. H. S., and Middleton, E. J., *Aust. J. Chem.*, 1974, **27**, 1087.

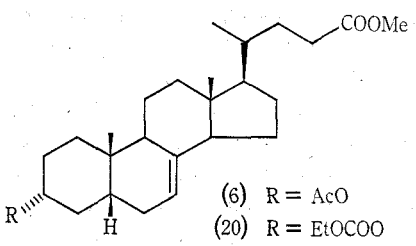
Cholic acids, which already have the 5$\beta$-configuration, were therefore chosen as possible chemical precursors of the required compounds. In addition such compounds are readily available starting materials[5] and many of these compounds possess: (*a*) a 7$\alpha$-hydroxy group which may act as a precursor for the 14$\alpha$-hydroxy-7-en-6-one system; (*b*) a 3-hydroxy group—though of the undesired (3$\alpha$) configuration; (*c*) a partial steroid side chain which permits the synthesis of a variety of side-chain analogues; and (*d*) a 12-hydroxy substituent in the case of cholic acid which enables the preparation of analogues with C-ring substituents. The latter compounds are of interest because the phytoecdysone, ajugalactone, with a C12 keto substituent, is reported[6] to block the biological effect of the phytoecdysone, ponasterone A, in the *Chilo* dipping test.



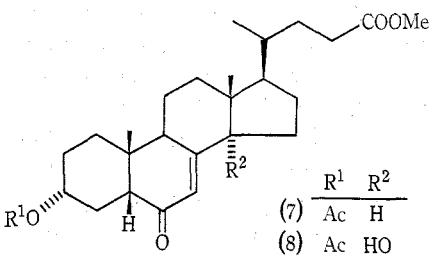

| | $R^1$ | $R^2$ |
|---|---|---|
| (4) | H | AcO |
| (17) | HCOO | H |
| (18) | H | HO |
| (19) | H | EtOCOO |




(5)




(6) R = AcO
(20) R = EtOCOO




| | $R^1$ | $R^2$ |
|---|---|---|
| (7) | Ac | H |
| (8) | Ac | HO |
| (9) | H | HO |

## Results and Discussion

Exploratory experiments were started with the chenodeoxycholic acid ester (4). Dehydration with phosphorus oxychloride in pyridine afforded a product which gave a single spot on t.l.c. but which had a wide melting point. As diaxial elimination may proceed in either direction the product could be formulated as a mixture of 6- and 7-enes, (5) and (6) respectively. This was further confirmed by $^1$H n.m.r. spectroscopy. The 7-ene (6) was isolated by fractional crystallization and on oxidation with chromium trioxide–pyridine complex[7] afforded the corresponding 5$\beta$-7-en-6-one (7), the structure of which was confirmed spectroscopically. Isolation of the 7-ene

[5] Nair, P. P., and Kritchevsky, D., 'The Bile Acids: Chemistry, Physiology and Metabolism' (Plenum Press: New York 1971).
[6] Koreeda, M., Nakanishi, K., and Goto, M., *J. Am. Chem. Soc.*, 1970, **92**, 7512.
[7] Dauben, W. G., Lorber, M., and Fullerton, D. S., *J. Org. Chem.*, 1969, **34**, 3587.

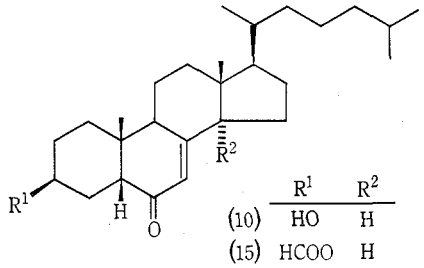
(6) from the mixture was not necessary, however, because the 6-ene is resistant to oxidation and is readily separated from the 5β-7-en-6-one (7) by chromatography. Finally the 14α-hydroxy group was introduced in the usual way by treating the ketone (7) with selenium dioxide.[3] Hydrolysis of the product (8) with potassium hydrogen carbonate in aqueous methanol afforded the free equatorial 3α-alcohol (9) without epimerization at C 5 as was evident from the shape (width at half-height) of the C 3 proton signal in its n.m.r. spectrum. However, attempts to hydrolyse the side-chain ester under mild conditions preparatory to the elaboration of the normal steroid side chain were not very satisfactory and it was evident that extension of the side chain would be better accomplished prior to the introduction of the 6-keto function.

As the configuration of the 3-hydroxyl in the naturally occurring ecdysones is β and the biological activity of the 3α-hydroxy analogues is lower,[8] attention was next given to methods of epimerizing the 3α-hydroxy group of the bile acid derivatives. For this purpose the reaction[9] of the equatorial 3α-hydroxyl with diethyl azodicarboxylate, triphenylphosphine and an organic acid appeared to have a number of advantages. Only equatorial alcohols react under mild conditions and epimerization and protection of the alcoholic group is accomplished in one step. It was found that the reaction worked well and the only problem encountered with its use was where the ester obtained had the same chromatographic mobility as the triphenylphosphine oxide formed in the reaction. In this instance the problem was overcome by choosing another sequence of synthetical steps. Formic acid is the acid of choice since the axial formate ester formed is sufficiently reactive to be hydrolysed under mild conditions (potassium hydrogen carbonate in aqueous methanol). These conditions do not bring about concomitant epimerization at C 5 of the 2-deoxyecdysone analogue being synthesized.

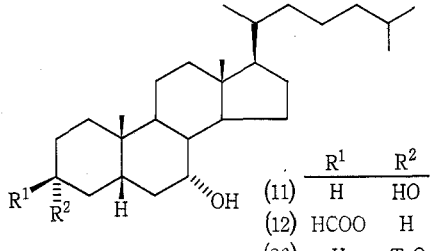
The above reactions were then used to synthesize the known[4] 2-deoxyecdysone analogue (10) as follows: 5β-cholestane-3α,7α-diol (11), prepared from chenodeoxycholic acid by anodic coupling,[10] was treated with the epimerizing reagent to afford the hydroxy formate (12) in 60–70% yield. The orientation of the ester group was confirmed as axial from the shape in its n.m.r. spectrum of the peak due to the C 3 proton. Dehydration of the hydroxy formate (12) afforded a mixture of the 6-ene (13) and 7-ene (14) which was oxidized directly to the required ester (15) in a yield of 20–25% from the hydroxy formate (12). Hydrolysis of this ester afforded the ecdysone analogue (10) in nearly quantitative yield. This new route to this compound is far superior to that used previously. A similar reaction procedure was used to synthesize the analogue (16), which was required to determine the effect on biological activity of shortening the steroid side chain. Initial attempts to prepare (16) via the intermediate (17), available from methyl chenodeoxycholate (18), were abandoned when it was found that the corresponding 3β-formate ester (17) could not be easily separated from the triphenylphosphine oxide produced. Instead another route via the intermediate (19) was used. Dehydration of the ester (19) with phosphorus oxychloride and crystallization of the product afforded the 7-ene (20), which on reaction with methylmagnesium bromide and hydrolysis was converted into the

[8] Galbraith, M. N., Horn, D. H. S., Middleton, E. J., and Hackney, R. J., *Aust. J. Chem.*, 1969, **22**, 1059.
[9] Bose, A. K., Lal, B., Hoffman, W. A., III, and Manhas, M. S., *Tetrahedron Lett.*, 1973, 1619.
[10] Bergström, S., and Krabisch, L., *Acta Chem. Scand.*, 1957, **11**, 1067.

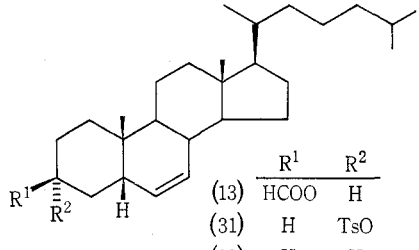
diol (21), the structure of which was consistent with its n.m.r. spectrum. The corresponding 3$\beta$-formate ester (22), prepared as before,[9] was oxidized to the ketone (23) the structure of which was confirmed spectroscopically. Further reaction with selenium dioxide afforded the ester (24) which on mild hydrolysis was converted into the ecdysone analogue (16).



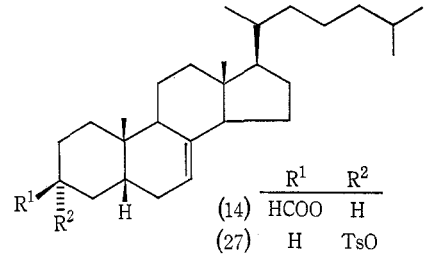
| | $R^1$ | $R^2$ |
|---|---|---|
| (10) | HO | H |
| (15) | HCOO | H |
| (25) | H | HO |
| (29) | H | H |
| (30) | HO | HO |



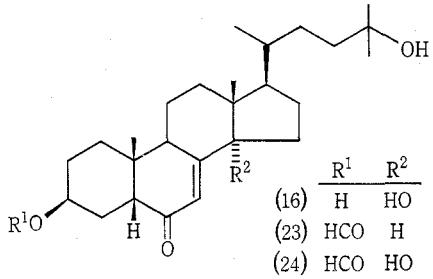
| | $R^1$ | $R^2$ |
|---|---|---|
| (11) | H | HO |
| (12) | HCOO | H |
| (26) | H | TsO |



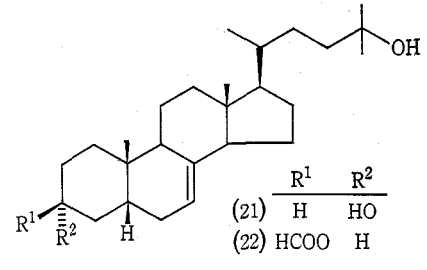
| | $R^1$ | $R^2$ |
|---|---|---|
| (13) | HCOO | H |
| (31) | H | TsO |
| (32) | H | H |



| | $R^1$ | $R^2$ |
|---|---|---|
| (14) | HCOO | H |
| (27) | H | TsO |
| (28) | H | H |



| | $R^1$ | $R^2$ |
|---|---|---|
| (16) | H | HO |
| (23) | HCO | H |
| (24) | HCO | HO |



| | $R^1$ | $R^2$ |
|---|---|---|
| (21) | H | HO |
| (22) | HCOO | H |

The synthetic method was also used to synthesize 14$\alpha$-hydroxy-5$\beta$-cholest-7-en-6-one (25) required for the study of the contribution of the 3$\beta$-hydroxy group of ecdysones to their biological activity. Thus 5$\beta$-cholestane-3$\alpha$,7$\alpha$-diol (11) was converted into the 3$\alpha$-tosyl derivative (26), which was dehydrated to a mixture of unsaturated 3$\alpha$-*p*-toluenesulphonates, from which the 7-ene isomer (27) could be obtained by fractional crystallization. Reduction of the crude mixture of unsaturated tosylates afforded a mixture of hydrocarbons containing about 60% of the 7-ene (28). Further oxidation of this mixture afforded the pure ketone (29) which on hydroxylation with selenium dioxide provided the required ecdysone analogue (25).

## Biological Results

The ecdysone analogues (16) and (25) were examined in the moulting hormone bioassay with *Calliphora stygia*.[2] The triol (16) proved to be completely inactive at a concentration of 0·05% whereas β-ecdysone (3) gave a 60% response at 0·001% and the keto diol (30) a similar response at 0·003%. Thus it appears that the full steroid side chain may be required for biological activity. Robbins *et al.*[11] have also reported that a 27-norecdysone analogue was inactive in the bioassay with the housefly *Musca domestica*. However, *M. domestica* is more sensitive to side-chain variations than *C. stygia*. For example podecdysone A (24-ethyl β-ecdysone), which is as active as β-ecdysone in the bioassay with *C. stygia*,[12] does not elicit a response in *M. domestica*.

The ketone (25) proved to be remarkably active in the *Calliphora* bioassay (1/7th that of α-ecdysone) in spite of the fact that it has only one hydroxy group whereas α-ecdysone has five. The ketone (25) has a little less than half the activity of the keto diol (30) and thus the 3β-hydroxy group makes only a modest contribution to moulting hormone activity. The keto alcohol (25) has also about the same activity as 14-deoxy-α-ecdysone.[13] Thus it appears, from these and earlier[14,15] observations, that the C 2, C 3, C 22 and C 25 hydroxy groups make only small contributions to the overall activity of α-ecdysone whereas the 14α-hydroxy group makes a relatively large one.

## Experimental

Microanalyses were performed by the Australian Microanalytical Service, Melbourne. N.m.r. spectra were measured on Varian HA-100 or T-60 spectrometers and chemical shifts are relative to tetramethylsilane (δ 0·00). Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. In the case of spectra measured with perdeuteropyridine solutions chemical shift values were obtained with benzene as a lock signal. I.r. spectra were measured as KBr discs, and u.v. spectra as ethanol solutions. Mass spectra were recorded on a Finnigan 3300 mass spectrometer using chemical ionization (methane reagent gas) and the direct inlet probe at a probe heater temperature of 150–200° (c.i.), or on an Hitachi Perkin–Elmer RMU-6D mass spectrometer with probe temperature 100–200° and ionizing voltage of 70 eV (e.i.). The peak intensity is given as a percentage of the base peak.

Plates for thin-layer chromatography were prepared with Kieselgel HF254 (Merck, Darmstadt) (layer thickness 0·20 mm) and were not activated. The spots were visualized with u.v. light or by spraying with vanillin–sulphuric acid reagent and heating at 120° for 5 min. The silica gel used for column chromatography was Mallinckrodt SilicAR Cc-7, 100–200 mesh, activated at 120° overnight. Alumina for chromatography was Woelm neutral grade.

Evaporation of solvents was carried out below 40°. Melting points were determined on a Kofler hot stage and are uncorrected.

*Methyl 3α-Acetoxy-7α-hydroxy-5β-cholan-24-oate (4)*

Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (6·7 g) was acetylated with a mixture of pyridine (30 ml) and acetic anhydride (15 ml) at 20°. When the proportion of monoacetate in the reaction mixture had reached a maximum (7 h) as determined by t.l.c., methanol (5 ml) was added and the mixture evaporated to dryness. Chromatography of the residue on silica gel afforded methyl

[11] Robbins, W. E., Kaplanis, J. N., Thompson, M. J., Shortino, T. J., and Joyner, S. C., *Steroids*, 1970, **16**, 105.
[12] Galbraith, M. N., Horn, D. H. S., Middleton, E. J., and Hackney, R. J., *Chem. Commun.*, 1969, 402.
[13] Hocks, P., Jager, A., Kerb, U., Wiechert, R., Furlenmeier, A., Fürst, A., Langemann, A., and Waldvogel, G., *Angew. Chem., Int. Ed. Engl.*, 1966, **5**, 673.
[14] Galbraith, M. N., Horn, D. H. S., Middleton, E. J., and Thomson, J. A., *Experientia*, 1973, **29**, 19.
[15] Galbraith, M. N., Horn, D. H. S., and Thomson, J. A., *Experientia*, 1975, **31**, 873.

3α-acetoxy-7α-hydroxy-5β-cholan-24-oate (4) as needles from cyclohexane, m.p. 68–76°. Mass spectrum (c.i.): *m/e* 477 (M+29, 4%), 449 (M+1, 2), 431 (M+1−$H_2O$, 5), 389 (M+1−HOAc, 7), 371 (M+1−HOAc−$H_2O$, 100). N.m.r. spectrum ($CDCl_3$): δ 0·67 (s, C18–$H_3$), 0·92 (s, C19–$H_3$), 2·03 (s, $CH_3COO$), 3·68 (s, $COOCH_3$), 3·68 (m, $W_{h/2}$ 8 Hz, C7–H equatorial), 4·67 (m, $W_{h/2}$ 20 Hz, C3–H axial).

*Methyl 3α-Acetoxy-5β-chol-7-en-24-oate (6)*

To the monoacetate (4) (5·25 g) in dry pyridine (50 ml) was added phosphorus oxychloride (5 ml) and the reaction mixture allowed to stand overnight. It was then poured onto ice (200 g) and the mixture extracted twice with chloroform (100 ml). Each volume of chloroform was washed in turn with water (100 ml) containing enough dilute hydrochloric acid to make it just acid and then finally water (100 ml). The combined chloroform layers were evaporated to dryness to provide a crystalline solid (4·6 g) with a wide melting point. Fractional crystallization from ether afforded the purified *methyl 3α-acetoxy-5β-chol-7-en-24-oate* (6), m.p. 124–130° (Found: C, 75·3; H, 9·9. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). N.m.r. spectrum ($CDCl_3$): δ 0·57 (s, C18–$H_3$), 0·89 (s, C19–$H_3$), 2·03 (s, $OCOCH_3$), 3·71 (s, $COOCH_3$), 4·70 (m, C3–H), 5·2 (C7–H). The presence of a minor peak at 0·70 (C18–$H_3$) indicated the presence of about 5% of the isomeric 6-ene (5).

*Methyl 3α-Acetoxy-6-oxo-5β-chol-7-en-24-oate (7)*

Chromium trioxide (501 mg) (in later oxidations the chromium trioxide–pyridine complex[7] in dichloromethane was used instead and gave better yields) in acetic acid (24 ml) was added to a solution of the above 7-ene (6) (2·0 g) in acetic acid (35 ml) and the mixture stirred for 2 days at room temperature. Water (100 ml) was added and the tarry suspension extracted twice with carbon tetrachloride. Each extract was washed in turn with 0·1 M sodium carbonate, and water. The combined carbon tetrachloride extracts were evaporated to a resin (2·2 g) which was chromatographed on silica gel. The u.v.-absorbing fractions were combined (300 mg) and recrystallized from methanol to afford *methyl 3α-acetoxy-6-oxo-5β-chol-7-en-24-oate* (7) as needles (222 mg), m.p. 153–154° (Found: C, 73·1; H, 9·1. $C_{27}H_{40}O_5$ requires C, 72·9; H, 9·1%). $\lambda_{max}$ 245 nm (ε 14800). N.m.r. spectrum ($CDCl_3$): δ 0·61 (s, C18–$H_3$), 0·95 (s, C19–$H_3$), 2·00 (s, $OCOCH_3$), 3·68 (s, $COOCH_3$), 4·75 (m, $W_{h/2}$ 30 Hz, 3β-H axial), 5·7 (m, C7–H).

*Methyl 3α-Acetoxy-14α-hydroxy-6-oxo-5β-chol-7-en-24-oate (8)*

The ester (7) (200 mg) in dioxan (20 ml) and finely divided selenium dioxide (400 mg) were stirred overnight at 60°. The reaction mixture was filtered through Celite and evaporated to small volume. Chloroform (20 ml) was added and washed with 5% aqueous potassium cyanide (5 ml) then with water. Evaporation of the solution afforded a residue (222 mg) which was chromatographed on silica gel. The main fractions (114 mg) after recrystallization from methanol afforded *methyl 3α-acetoxy-14α-hydroxy-6-oxo-5β-chol-7-en-24-oate* (8), m.p. 228–230° (Found: C, 70·1; H, 8·8. $C_{27}H_{40}O_6$ requires C, 70·4; H, 8·9%). N.m.r. spectrum ($CDCl_3$): δ 0·68 (s, C18–$H_3$), 0·93 (s, C19–$H_3$), 2·00 (s, $OCOCH_3$), 3·68 (s, $COOCH_3$), 4·66 (m, $W_{h/2}$ 32 Hz, C3–H axial), 5·90 (m, C7–H). $\lambda_{max}$ 242 nm (ε 13000).

*5β-Cholestane-3β,7α-diol 3-Formate (12)*

5β-Cholestane-3α,7α-diol (11) (250 mg) and triphenylphosphine (330 mg) were dissolved in dry tetrahydrofuran (7·5 ml) containing formic acid (58 mg). To this solution was added diethyl azodicarboxylate (220 mg) dissolved in dry tetrahydrofuran (1·25 ml) and the mixture allowed to stand at room temperature for 2 h. It was then evaporated to dryness, the residue stirred with cyclohexane–chloroform (1 : 1) and the solution filtered from the crystalline diethyl hydrazodicarboxylate. The solution was chromatographed on silica gel in the same solvent and afforded *5β-cholestane-3β,7α-diol 3-formate* (12) as a waxy low-melting solid (Found: C, 77·7; H, 11·2. $C_{28}H_{48}O_3$ requires C, 77·7; H, 11·2%). N.m.r. spectrum ($CDCl_3$): δ 0·68 (s, C18–$H_3$), 0·87 (d, *J* 6 Hz, C26/27–$H_6$), 0·95 (s, C19–$H_3$), 3·90 (m, $W_{h/2}$ 4 Hz, C7–H equatorial), 5·2 (m, $W_{h/2}$ 4 Hz, C3–H equatorial), 8·15 (s, HCOO).

*Dehydration of 5β-Cholestane-3β,7α-diol 3-Formate (12)*

The formate (12) (383 mg) was dissolved in dry pyridine (3·8 ml) and phosphorus oxychloride (380 μl) added. After standing for 2 h the mixture was poured into water and worked up as before. The product was chromatographed on silica gel from chloroform–cyclohexane (40 : 60) and afforded an oil which consisted mainly of the 7-ene (14). N.m.r. spectrum ($CDCl_3$): δ 0·55 (s, C18–$H_3$), 0·88 (d, *J* 6 Hz, C26/27–$H_6$), 0·90 (s, C19–$H_3$), 5·20 (m, C3/7–$H_2$), 8·10 (s, HCOO); minor peaks 0·68 (s, C18–$H_3$) and 5·5 (m, C6/7–$H_2$) indicated the presence of about 25% of the isomeric 6-ene (13).

*6-Oxo-5β-cholest-7-en-3β-yl Formate (15)*

The mixture of 6- and 7-enes (13) and (14) (360 mg) in dry methylene chloride (20 ml) was oxidized with chromium trioxide–pyridine complex[7] (1·9 g) at room temperature. After 2 h the mixture was poured into water (40 ml) and extracted with ether (40 ml). The extract was washed with dilute hydrochloric acid, water, dilute sodium hydrogen carbonate, water and finally dried over sodium sulphate and evaporated. The residue (330 mg) was chromatographed on silica gel with cyclohexane–ether (85 : 15) as eluting solvent. The combined ketone fractions (79 mg) afforded *6-oxo-5β-cholest-7-en-3β-yl formate* (15) as needles from methanol, m.p. 113–117° (Found: C, 78·8; H, 10·4. $C_{28}H_{44}O_3$ requires C, 78·5; H, 10·4%). N.m.r. spectrum ($CDCl_3$): δ 0·60 (s, C18–$H_3$), 0·87 (d, *J* 6 Hz, C26/27–$H_6$), 1·02 (s, C19–$H_3$), 5·2 (m, $W_{h/2}$ 4 Hz, C3–H equatorial), 5·7 (m, C7–H), 8·1 (s, HCOO). $\lambda_{max}$ 245 nm (ε 13400).

*5β-Cholest-6-en-3β-yl Formate (13)*

The early non-u.v.-absorbing fraction from the chromatography of the ketone (15) above was crystallized and afforded purified 5β-cholest-6-en-3β-yl formate (13) as needles from methanol, m.p. 87–97°. Mass spectrum (c.i.): *m/e* 415 (M+1, 12%), 400 (M+1−$CH_3$, 10), 370 (M+1−HCOOH, 100). N.m.r. spectrum ($CDCl_3$): δ 0·70 (s, C18–$H_3$), 0·87 (d, *J* 6 Hz, C26/27–$H_6$), 0·92 (s, C19–$H_3$), 5·20 (m, C3–H), 5·50 (m, C6/7–$H_2$), 8·1 (s, HCOO).

*3β-Hydroxy-5β-cholest-7-en-6-one (10)*

The ester (15) (8·4 mg) and potassium hydrogen carbonate (10 mg) were placed in a flask closed with a serum cap and the flask flushed out with argon. Tetrahydrofuran (800 μl), methanol (1·6 ml) and water (100 μl) were injected, and the mixture was stirred at room temperature (about 20°). When the hydrolysis was complete (2–3 h) as shown by thin-layer chromatography, water (1 ml) was added and the mixture extracted with chloroform. The chloroform extract was evaporated to afford an oil (6·8 mg) which showed the same behaviour on thin-layer chromatography and gave the same n.m.r. spectrum as a sample of 3β-hydroxy-5β-cholest-7-en-6-one (10) prepared previously.[4] The free alcohol readily undergoes autoxidation and is best stored at −20° under an inert gas.

*Methyl 3α-Ethoxycarbonyloxy-7α-hydroxy-5β-cholate (19)*

Ethyl chloroformate (9 ml) was added over a period of 10 min to methyl chenodeoxycholate (18) (5·5 g) dissolved in dry pyridine (14 ml). After stirring for 1 h the suspension was poured into ice water (100 g) and the mixture extracted twice with ether (100 ml). The extracts were washed with water and dried over sodium sulphate. The residue (6·5 g) was chromatographed on silica gel (100 g) in chloroform–cyclohexane (1 : 1) and the main fractions crystallized from aqueous ethanol afforded *methyl 3α-ethoxycarbonyloxy-7α-hydroxy-5β-cholate* (19) as needles, m.p. 100–105° (Found: C, 70·3; H, 9·8. $C_{28}H_{46}O_6$ requires C, 70·3; H, 9·7%). N.m.r. spectrum ($CDCl_3$): δ 0·67 (s, C18–$H_3$), 0·93 (s, C19–$H_3$), 1·30 (t, *J* 7 Hz, $CH_3CH_2OCOO$), 3·68 (s, $COOCH_3$), 4·17 (q, *J* 7 Hz, $CH_3CH_2OCOO$).

*6-Oxo-24-nor-5β-cholest-7-ene-3β,25-diol 3-Formate (23)*

(A) *Dehydration of ester (19).*—Phosphorus oxychloride (3·6 ml) was added with stirring to the crystalline ester (19) (3·6 g) dissolved in dry pyridine (36 ml). The temperature of the reaction

mixture was kept below 30° by cooling the reaction flask in ice. After 2 h the reaction was worked up as before. The crude product (3·5 g) was crystallized from ethanol to afford a mixture containing mainly *methyl 3α-ethoxycarbonyloxy-5β-chol-7-en-24-oate* (20), m.p. 130–133° (Found: C, 72·8; H, 9·5. $C_{28}H_{44}O_5$ requires C, 73·0; H, 9·6%). N.m.r. spectrum ($CDCl_3$): $\delta$ 0·55 (s, C18–$H_3$), 0·88 (s, C19–$H_3$), 1·32 (t, $J$ 7 Hz, $CH_3CH_2OCOO$), 3·70 (s, $COOCH_3$), 4·2 (q, $J$ 7 Hz, $CH_3CH_2OCOO$), 5·15 (m, C3/7–$H_2$); a minor methyl peak at 0·70 (s, C18–$H_3$) indicated the presence of the 6-ene isomer (about 20%).

(B) *Grignard reaction.*—The substantially pure 7-ene (20) above (1·55 g) was dissolved in ether (150 ml) and methylmagnesium iodide [prepared from magnesium (10 g) and methyl iodide (20 ml) in dry ether (280 ml)] added with stirring at room temperature over 30 min. After 1 h the reaction mixture was worked up in the usual way, and the products (1·15 g) were chromatographed on silica gel. The main fractions were recrystallized from ethyl acetate to afford substantially pure *24-nor-5β-cholest-7-ene-3α,25-diol* (21), m.p. 164–166° (Found: C, 80·4; H, 11·4. $C_{26}H_{44}O_2$ requires C, 80·4; H, 11·4%). N.m.r. spectrum ($CDCl_3$): $\delta$ 0·55 (s, C18–$H_3$), 0·87 (s, C19–$H_3$), 1·22 (s, C26/27–$H_6$), 5·1 (m, C3/C7–$H_2$). A minor peak at $\delta$ 0·70 indicated the presence of about 10% of the corresponding 6-ene isomer.

(C) *Epimerization of diol (21).*—The diol (672 mg) and triphenylphosphine (880 mg) were dissolved in tetrahydrofuran (20 ml) containing formic acid (154 mg). Diethyl azodicarboxylate (585 mg) dissolved in dry tetrahydrofuran (3·35 ml) was added and the mixture stirred at room temperature overnight. The reaction was worked up in the usual way and the product chromatographed on silica gel to afford a non-crystalline foam (560 mg) consisting mainly of the 7-ene (22). N.m.r. spectrum ($CDCl_3$): $\delta$ 0·54 (s, C18–$H_3$), 0·88 (s, C19–$H_3$), 1·21 (s, C26/27–$H_6$), 5·2 (m, C7–H), 8·1 (s, HCOO). Minor peaks at $\delta$ 0·70 and 5·50 indicated the presence of about 25% of the 6-ene isomer.

(D) *Oxidation of the 7-ene (22).*—The formate (585 mg) was oxidized with chromium trioxide–pyridine complex as described above. The product, a brown oil (552 mg), was chromatographed on silica gel and the fractions with a u.v. absorption at 246 nm combined (80 mg). Crystallization of the material from methanol afforded 6-oxo-24-nor-5β-cholest-7-ene-3β,25-diol 3-formate (23) as needles, double m.p. 74–90°, remelted 120–136°. Mass spectrum (c.i.): $m/e$ 459 (M+29, 10%), 431 (M+1, 100), 413 (M+1−$H_2O$, 37), 403 (M+1−CO, 25), 385 (M+1−HCOOH, 56). $\lambda_{max}$ 246 nm ($\varepsilon$ 11200). N.m.r. spectrum ($CDCl_3$): $\delta$ 0·58 (s, C18–$H_3$), 1·03 (s, C19–$H_3$), 1·23 (s, C26/27–$H_6$), 5·20 (m, $W_{h/2}$ 9 Hz, C3–H equatorial), 5·85 (m, C7–H), 8·1 (s, HCOO).

*6-Oxo-24-nor-5β-cholest-7-ene-3β,14α,25-triol 3-Formate (24)*

The ketone (23) (47 mg) and selenium dioxide (120 mg) in dry dioxan (5 ml) were heated to 60–70° under argon for 2·5 h and the product worked up as before. Chromatography of the crude product on silica gel afforded the pure *6-oxo-24-nor-5β-cholest-7-en-3β,14α,25-triol 3-formate* (24) as needles from methanol–water, m.p. 199–201° (Found: C, 72·1; H, 9·6. $C_{27}H_{42}O_5$ requires C, 72·6; H, 9·5%). Mass spectrum (c.i.): $m/e$ 475 (M+29, 10%), 447 (M+1, 20), 429 (M+1−$H_2O$, 100), 411 (M+1−2$H_2O$, 43), 401 (M+1−HCOOH, 32). $\lambda_{max}$ 243 nm ($\varepsilon$ 12600). N.m.r. spectrum ($CDCl_3$): $\delta$ 0·68 (s, C18–$H_3$), 1·00 (s, C19–$H_3$), 1·22 (s, C26/27–$H_6$), 5·2 (m, $W_{h/2}$ 10 Hz, 3α-H equatorial), 5·85 (m, C7–H), 8·1 (s, HCOO).

*3β,14α,25-Trihydroxy-24-nor-5β-cholest-7-en-6-one (16)*

Hydrolysis of the formate (24) (10·8 mg) was accomplished as before by means of aqueous methanolic potassium hydrogen carbonate. The product was crystallized to afford pure 3β,14α,25-trihydroxy-24-nor-5β-cholest-7-en-6-one, m.p. 228–230°. Mass spectrum (c.i.): $m/e$ 447 (M+29, 15%), 419 (M+1, 30), 401 (M+1−$H_2O$, 50), 383 (M+1−2×$H_2O$, 43), 365 (M+1−3×$H_2O$, 15). N.m.r. spectrum ($CDCl_3$–5% $CD_3OD$): $\delta$ 0·68 (s, C18–$H_3$), 0·98 (s, C19–$H_3$), 1·22 (s, C26/27–$H_6$), 4·0 (m, $W_{h/2}$ 9 Hz, C3–H equatorial), 5·8 (m, C7–H). $\lambda_{max}$ 243 nm ($\varepsilon$ 12200).

*5β-Cholest-7-en-3α-yl* p-*Toluenesulphonate (27)*

5β-Cholestane-3α,7α-diol (410 mg) and *p*-toluenesulphonyl chloride (410 mg) were dissolved in dry pyridine (5 ml) in a stoppered flask and stored overnight at room temperature, when t.l.c. indicated

a single product with $R_F(CH_2Cl_2)$ 0·38, corresponding to the diol monoester (26). To the reaction mixture was then added phosphorus oxychloride (400 μl) and after a further 4 h the mixture was poured into water. Isolation of the product with ether in the usual way afforded a resin (434 mg) whose composition was analysed by n.m.r. spectrometry. Two distinct olefinic proton resonances were observed, one at δ ($CDCl_3$) 5·03 (m, $W_{h/2}$ 10 Hz, C7–H in 7-ene) and the other at δ 5·47 (m, $W_{h/2}$ 3 Hz, C6/7–$H_2$ in 6-ene), and integration indicated the mixture contained about 70% of the desired 7-ene. Fractional crystallization of a portion of the crude product from light petroleum–ether and then acetone–methanol afforded pure *5β-cholest-7-en-3α-yl* p-*toluenesulphonate* (27), m.p. 139–140° (Found: C, 75·6; H, 9·4; S, 6·2. $C_{34}H_{52}O_3S$ requires C, 75·5; H, 9·7; S, 5·9%). N.m.r. spectrum ($CDCl_3$): δ 0·53 (s, C18–$H_3$), 0·84 (s, C19–$H_3$), 0·86 (d, J 6 Hz, C26/27–$H_6$), 0·91 (d, J 6 Hz, C21–$H_3$), 2·52 (s, tosyl $CH_3$), 4·46 (m, $W_{h/2}$ 28 Hz, C3–H), 5·03 (m, $W_{h/2}$ 10 Hz, C7–H) and 7·29, 7·76 (AA′BB′ multiplet, Ar–$H_4$).

*5β-Cholest-7-en-6-one* (*29*)

The crude mixture of *p*-toluenesulphonates (31) and (27) (477 mg) was reduced with lithium aluminium hydride (432 mg) in ether (20 ml) during 2 h at reflux under argon. The crude hydrocarbon product, isolated as an oil, was found by n.m.r. to be a mixture of 5β-cholest-6- and -7-enes (32) and (28) in the same ratio as in the crude toluenesulphonates before reduction. An attempt to prepare pure 5β-cholest-7-ene (28) by reduction of purified toluenesulphonate (27) also gave an oil, but apparently pure 5β-cholest-7-ene (28) from its n.m.r. spectrum ($CDCl_3$): δ 0·54 (s, C18–$H_3$), 0·85 (s, C19–$H_3$), 0·88 (d, C26/27–$H_6$), 5·10 (m, $W_{h/2}$ 10 Hz, C7–H).

The mixture of 6- and 7-enes (32) and (28) was dissolved in dry dichloromethane (25 ml) and chromium trioxide–pyridine complex (2·09 g) added. After 2 h shaking, t.l.c. indicated no further change in the product composition, and the mixture was poured into saturated sodium hydrogen carbonate solution and the products were extracted into ether. The organic layer was separated, washed with sodium hydrogen carbonate solution and water, dried over sodium sulphate and evaporated to yield a brown gum (260 mg). The gum was chromatographed on a column of silica gel (5% water, 50 g), when elution with cyclohexane afforded 5β-cholest-6-ene (32) as an oil (73 mg), which could not be induced to crystallize. N.m.r. spectrum ($CDCl_3$): δ 0·69 (s, C18–$H_3$), 0·88 (d, J 6 Hz, C26/27–$H_6$), 0·93 (s, C19–$H_3$), 5·47 (m, $W_{h/2}$ 4 Hz, C6/7–$H_2$). Further elution with cyclohexane–dichloromethane (1 : 1) afforded the conjugated ketone (50 mg), which was recrystallized from methanol to give *5β-cholest-7-en-6-one* (29) as thick needles, m.p. 120–121° (Found: C, 84·4; H, 11·4. $C_{27}H_{44}O$ requires C, 84·3; H, 11·5%). $\lambda_{max}$ 246 nm (ε 14600). N.m.r. spectrum ($CDCl_3$): δ 0·54 (s, C18–$H_3$), 0·88 (s, C19–$H_3$), 0·88 (d, J 6 Hz, C26/27–$H_6$), 5·66 (m, $W_{h/2}$ 4 Hz, C7–H). Significant peaks in the mass spectrum (e.i.) were observed at *m/e* 384 ($M^+$, 100%), 369 (M − $CH_3$, 32), 355 (M − H − CO, 3), 341 (M − $CH_3$ − CO, 4), 329 (M − $C_4H_7$, 8), with metastable ions apparent at about *m/e* 354 (calc. for M to M − $CH_3$: 354·6) and at *m/e* 303 (calc. for M to M − $CH_3$ − CO: 302·8).

*14α-Hydroxy-5β-cholest-7-en-6-one* (*25*)

5β-Cholest-7-en-6-one (29) (40 mg) and resublimed selenium dioxide (84 mg) were dissolved in dry dioxan (4 ml) under argon in a sealed flask and the mixture was heated to 90° for 4 h whereupon t.l.c. indicated the reaction was complete. The cooled mixture was filtered through a pad of Celite to remove the red precipitate of selenium and the filter bed washed well with ethyl acetate. The combined filtrate and washings were washed with water, 5% potassium cyanide solution, and water, dried over sodium sulphate and evaporated to yield a yellow resin (46 mg). The crude product was chromatographed on a column of silica gel (5% water, 20 g) when elution with chloroform afforded the keto alcohol (25) as a resin (26 mg) that crystallized only with difficulty. The material was rechromatographed on alumina (3% water, 15 g) and the product (22 mg) eluted with cyclohexane–ethyl acetate (9 : 1). Recrystallization from light petroleum–ether and then methanol afforded *14α-hydroxy-5β-cholest-7-en-6-one* (25) as needles, m.p. 123–125°, analysing as the hemihydrate (Found: C, 79·5; H, 11·0. $C_{27}H_{44}O_2,\frac{1}{2}H_2O$ requires C, 79·2; H, 11·1%). $\lambda_{max}$ 243 nm (ε 12400). N.m.r. spectrum ($CDCl_3$): δ 0·64 (s, C18–$H_3$), 0·88 (d, J 5 Hz, C26/27–$H_6$), 0·89 (s, C19–$H_3$), 3·09 (m, $W_{h/2}$ 11 Hz, C9–H), 5·80 (d, J 2·6 H7, C7–H). Irradiation at δ 3·09 caused the doublet

observed at $\delta$ 5·80 to collapse to a singlet, and irradiation at 5·80 reduced the multiplet at 3·09 to a quartet. Significant ions in the mass spectrum were observed at *m/e* 400 ($M^+$, 31%), 382 ($M-H_2O$, 25), 372 ($M-CO$, 100), 367 ($M-H_2O-CH_3$, 9) 357 ($M-CO-CH_3$, 16), 354 ($M-CO-H_2O$, 4), 339 ($M-CO-H_2O-CH_3$, 3), 268 ($M-H_2O-C_8H_{16}$ side chain, 24).

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