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Moulting Hormones. LIII*
The Synthesis and Biological Activity of Some Ecdysone Analogues

M. Neil Galbraith,[A] *Denis H. S. Horn,*[A,B] *Barry A. Kelly,*[A] *Judith F. Kinnear,*[C] *Marjory-D. Martin,*[D] *E. John Middleton*[A] *and Christopher T. F. Virgona*[E]

[A] Division of Applied Organic Chemistry, CSIRO, P.O. Box 4331, Melbourne, Vic. 3001.
[B] To whom correspondence should be addressed.
[C] Biology Department, Melbourne State College, Carlton, Vic. 3053.
[D] Toorak State College, Malvern, Vic. 3144.
[E] School of Zoology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

Abstract

A number of ecdysone analogues were prepared to study the effect of structural changes on biological activity. It was found that analogues with the 5α-configuration or a 3,5-cyclo structure were inactive, that a 3β-hydroxy group enhances activity but is not essential for activity, and that 3β-substituents decrease activity as follows: OMe (60%), OAc (25%) and OEt (10%). The keto diol (3), keto alcohol (9) and amide (36) were found to be highly toxic to mosquito larvae.

Introduction

Moulting in arthropods is mediated by highly hydroxylated steroid hormones[1] such as 20-hydroxyecdysone (1). However, a number of ecdysteroids occurring in arthropods and plants[1] with fewer hydroxy groups are equally active in the *Calliphora* bioassay[2] for moulting hormones. Surprisingly the synthetic ecdysteroid analogue (3) without hydroxyls at the 2, 20, 22 and 25 positions still retains one-third the activity[3] of 20-hydroxyecdysone. Following this unexpected result it became of interest to determine the essential molecular requirement for moulting hormone activity and, as the simple 5β keto diol (3) is remarkably active in the *Calliphora* bioassay,[2] it was chosen as a control model for structural variation. We now report on the synthesis of a variety of steroidal moulting hormone analogues prepared for structure–biological activity studies. A preliminary account of some of the results so far was reported separately.[4]

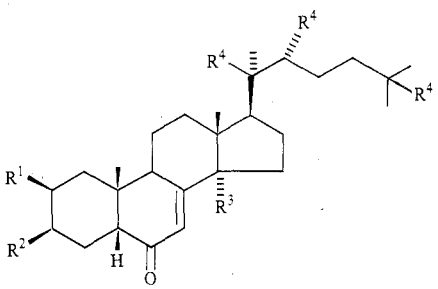
Syntheses and Discussion

As the 3-acetate of 20-hydroxyecdysone (2) is biologically inactive[5] it appeared likely that binding of the 3β-hydroxy group to the active site was important. Also

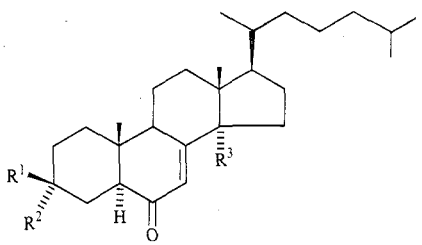
* Part LII, *Aust. J. Chem.*, 1979, **32**, 2017.

[1] Horn, D. H. S., 'The Ecdysones' in 'Naturally Occurring Insecticides' (Eds M. Jacobson and D. G. Crosby) p. 333 (Marcel Dekker: New York 1971).
[2] Thomson, J. A., Imray, F. P., and Horn, D. H. S., *Aust. J. Exp. Biol. Med. Sci.*, 1970, **48**, 321.
[3] Galbraith, M. N., Horn, D. H. S., Middleton, E. J., and Thomson, J. A., *Experientia*, 1973, **29**, 19.
[4] Bergamasco, R., and Horn, D. H. S., 'The Biological Activities of Ecdysteroids and Ecdysteroid Analogues' in 'Developments in Endocrinology' (Ed. J. A. Hoffman) Vol. 7 (Elsevier: Amsterdam 1980).
[5] Robbins, W. E., Kaplanis, J. N., Thompson, M. J., Shortino, T. J., and Joyner, S. C., *Steroids*, 1970, **16**, 105.

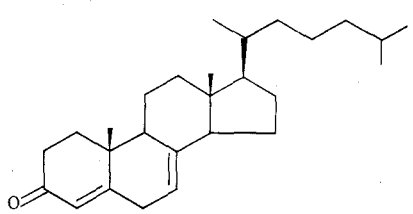
it was considered possible that the inactivity[4] of the 5α keto diol (11) could be due to the 3β-hydroxy group being oriented in space in such a way that binding to the receptor was prevented. From Dreiding models it was evident that there was a near spatial coincidence of the 3α-hydroxy group of the 5α keto diol (14) with a 3β-hydroxy group in the corresponding 5β keto diol (3). Accordingly the 3α-epimer (14) was



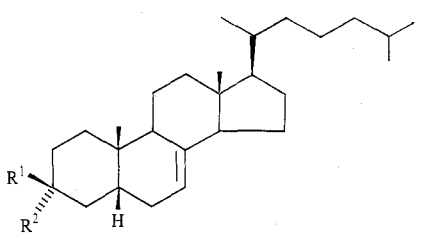
	R^1	R^2	R^3	R^4
(1)	HO	HO	HO	HO
(2)	HO	AcO	HO	HO
(3)	H	HO	HO	H
(4)	H	AcO	HO	H
(5)	H	MeO	HO	H
(6)	H	EtO	H	H
(7)	H	EtO	HO	H
(8)	H	H	H	H
(9)	H	H	HO	H



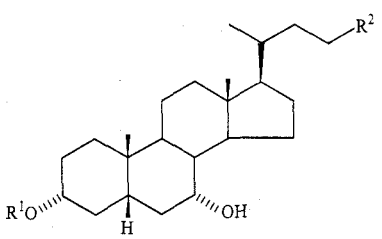
	R^1	R^2	R^3
(10)	HO	H	H
(11)	HO	H	HO
(12)	H	HCOO	H
(13)	H	HCOO	HO
(14)	H	HO	HO
(15)	H	H	H
(16)	H	H	HO



(17)



	R^1	R^2
(18)	O	
(19)	HO	H
(20)	EtO	H
(21)	H	TsO
(22)	H	H



	R^1	R^2
(23)	H	CH_2CHMe_2
(24)	HCO	COOH

synthesized. This was accomplished in the following way. The 5α keto alcohol (10) was converted[6] into the epimeric 3α-formate (12) with diethyl azodicarboxylate and formic acid. Selenium dioxide oxidation afforded the 14α-hydroxy derivative (13), and the required keto diol (14) was isolated after mild hydrolysis.

Although the 3-acetate of 20-hydroxyecdysone (2) is inactive it is reported that the 3-methyl ether of (2) is active in the *Musca* bioassay.[5] The acetate (4), the methyl ether (5) and the ethyl ether (7) were therefore prepared in order to study the effect of group size on biological activity. The methyl ether (5) was simply prepared from the keto diol (3) with fluoroboric acid and diazomethane[7] while the ethyl ether (7) was obtained indirectly in the following way. The keto diene (17) was catalytically hydrogenated[8,9] affording the mixture of 5α and 5β ketones from which the 5β ketone (18) was tediously separated by chromatography. Reduction of the ketone (18) with K-selectride[10,11] afforded mainly the 3β-alcohol[5,12] (19). Ethylation of the alcohol (19) with diethyl phosphate[13] catalysed by tosyl chloride afforded an unresolved mixture of tosylate and ethyl ether (20) in low yield together with mainly the 3-phosphite ester. Attempts to cause the alcohol (19) to react with bis(acetylacetonato)nickel[14] were also unsuccessful. However, it was found that the ethyl ether (20) could be prepared in 50% yield by treating the alcohol (19) with triethyloxonium fluoroborate[15] as described.[16] Oxidation of the ether (20) with chromium trioxide/pyridine reagent in dichloromethane afforded the corresponding 6-ketone (6) and subsequent reaction with selenium dioxide afforded the required ethyl ether (7).

To further examine the importance of the 3β-hydroxy group the 3-deoxy analogue (9) was prepared. The diol (23) obtained[17] from chenodeoxycholic acid was treated first with tosyl chloride and then with phosphorus oxychloride to give the tosylate (21) which on reduction with lithium aluminium hydride provided the hydrocarbon (22). Oxidation with chromium trioxide/pyridine reagent afforded the 5β ketone (8) which on reaction with selenium dioxide provided the required keto alcohol (9). The corresponding 5α-analogue (16) was also prepared. This was accomplished in the following way. The known[18] acetate (25) was treated with tosyl chloride to give the corresponding tosylate (26) which was reduced with lithium aluminium hydride to the allylic alcohol (27). Oxidation with chromic acid provided the corresponding ketone (15) which with selenium dioxide afforded the required keto alcohol (16).

The 3,5-cyclo analogue (30) was also prepared. This was accomplished in the following way. The 3,5-cyclo alcohol (28) obtained from 7-dehydrocholesterol[19] was

[6] Kinnear, J. F., Martin, M.-D., Horn, D. H. S., Middleton, E. J., Wilkie, J. S., Galbraith, M. N., and Willing, R. I., *Aust. J. Chem.*, 1976, **29**, 1815.
[7] Neeman, M., and Johnson, W. S., *Org. Synth.*, 1973, Coll. Vol. V, 245.
[8] Coombe, M. G., Henbest, H. B., and Jackson, W. R., *J. Chem. Soc. C*, 1967, 2467.
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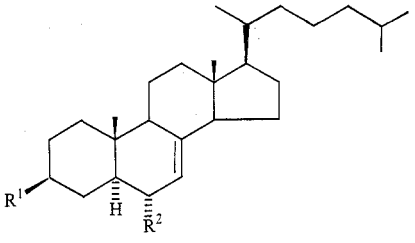
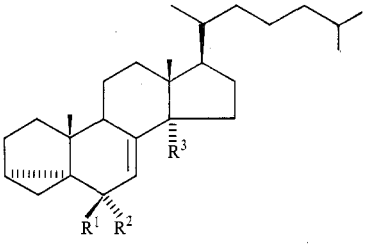
[10] Krishnamurthy, S., *Aldrichimica Acta*, 1974, **7**, 55.
[11] Contreras, R., and Mendoza, L., *Steroids*, 1979, **34**, 121.
[12] Cohen, C. F., Louloudes, S. J., and Thompson, M. J., *Steroids*, 1967, **9**, 591.
[13] Kashman, Y., *J. Org. Chem.*, 1972, **37**, 912.
[14] Yamashita, M., *Synthesis*, 1977, 803.
[15] Meerwein, H., *Org. Synth.*, 1973, Coll. Vol. V, 1080.
[16] Grootveld, H. H., Blomberg, C., and Bickelhaupt, F., *Tetrahedron Lett.*, 1971, 1999.
[17] Bergstrom, S., and Krabisch, L., *Acta Chem. Scand.*, 1957, **11**, 1067.
[18] Galbraith, M. N., Horn, D. H. S., and Middleton, E. J., *Aust. J. Chem.*, 1974, **27**, 1087.
[19] Harvey, W. E., and Bloch, K., *Chem. Ind. (London)*, 1961, 595.

oxidized to the ketone (29)[20] which with selenium dioxide afforded the required keto alcohol (30).

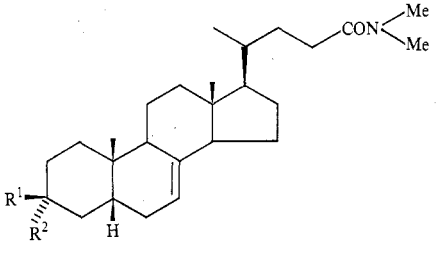
22,25-Dideoxyecdysone shows,[5] besides moulting hormone activity, an inhibitory effect upon insect growth. In addition it is reported that certain azasterols[21] and acyclic dimethyl amides[22] have a similar action. It was therefore of interest to synthesize for testing the ecdysone analogue (36) with a side-chain dimethyl amido group. This was accomplished in the following way. Reaction of chenodeoxycholic acid, in methyl formate, with formic acid at 20° provided mainly the 3α-formate (24) which on reaction, first with thionyl chloride, then with dimethylamine, afforded mainly the dimethyl amide (31) together with a little of the 6-ene isomer. Hydrolysis of the 3α-ester function gave the amide (32) which, on inversion as before,[6] afforded the corresponding 3β-formate (33). The 6-keto and 14α-hydroxy groups were introduced in the usual way to give first the amide (34) and then the amide (35), which afforded, after mild hydrolysis, the required analogue (36).



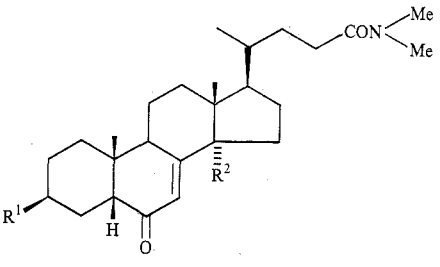
	R^1	R^2
(25)	HO	AcO
(26)	TsO	AcO
(27)	H	HO



	R^1	R^2	R^3
(28)	HO	H	H
(29)	O		H
(30)	O		HO



	R^1	R^2
(31)	H	HCOO
(32)	H	HO
(33)	HCOO	H



	R^1	R^2
(34)	HCOO	H
(35)	HCOO	HO
(36)	HO	HO

[20] Anastasia, M., Scala, A., and Galli, G., *J. Org. Chem.*, 1976, **41**, 1064.
[21] Robbins, W. E., Thompson, M. J., Svoboda, J. A., Shortino, T. J., Cohen, C. F., Dutky, S. R., and Duncan, O. J., *Lipids*, 1975, **10**, 353.
[22] Thompson, M. J., Serban, N. N., Robbins, W. E., Svoboda, J. A., Shortino, T. J., Dutky, S. R., and Cohen, C. F., *Lipids*, 1975, **10**, 615.

Biological Results

All the ecdysone analogues with the 5α-configuration and the 3,5-cyclo compound (30) were inactive in the *Calliphora* bioassay[2] for moulting hormone activity. Even the analogue (14) with a favourably oriented 3α-hydroxy group was inactive. Thus analogues with the more planar A/B-ring structure cannot be accommodated at the moulting hormone receptor. However, it is possible, if binding to the receptor takes place to the α-face of the steriod molecule, that activity may be obtained by attaching additional groups to the α-face of the A ring of 5α-ecdysone analogues so that the binding in this region of the molecule with the receptor can take place.

The methyl ether (5) had 60%, the acetate (4) 40% and the ethyl ether only 10% of the activity of the free alcohol (3). Thus it would appear that there is decreasing activity with decreasing polarity of the 3-substituent of the alcohol (3). Also it is possible that with increasing size of the substituent there is increasing interference with the hormone–receptor interaction.

Binding of the 3β-hydroxyl to the receptor is not strong or essential since the 3-deoxy analogue (9) had nearly half (45%) of the activity of the keto diol (3). It is quite remarkable that this relatively non-polar compound has such high activity. A possible explanation is that, because of its lower water solubility, non-specific binding to the receptor compensates for specific interactions of the various hydroxy groups of the natural hormone.

The keto diol (3) and the keto alcohol (9) were toxic to the mosquito *Aedes aegypti*, completely inhibiting its development at concentrations of 0·01 and 0·1 ppm respectively. None of the 5α-analogues showed activity at concentrations up to 1 ppm. The dimethyl amide (36) was also toxic to the mosquito but only to the same order as the keto diol (3). However, the amide (36), unlike the keto diol (3), was inactive in the *Calliphora* bioassay.[2] Thus it is likely that the amide has a different mechanism of action and it is possible, because of its close similarity to ecdysone precursors, that it interferes with ecdysone synthesis.[23]

Experimental

Microanalyses were performed by the Australian Microanalytical Service, Melbourne. ^{1}H n.m.r. spectra were measured on Varian HA-100 or T-60 spectrometers and ^{13}C n.m.r. on a Varian CFT-20 spectrometer. Chemical shifts are relative to tetramethylsilane (δ 0·00). Chemical shift values were obtained, unless stated otherwise, with deuterochloroform as solvent. I.r. spectra were measured, unless stated otherwise, as KBr discs, and u.v. spectra as ethanol solutions.

Plates for thin-layer chromatography were prepared with Kieselgel HF 254 (Merck, Darmstadt) (layer thickness 0·2 mm) and were not activated. The spots were visualized with u.v. light or by spraying with vanillin/sulfuric acid reagent and heating at 120° for 2–4 min. The silica gel used for column chromatography was Mallinckrodt SilicAR CC-7, 100–200 mesh, activated at 120° overnight. Columns were prepared and eluted by gravity as described.[24] Columns for high-pressure liquid chromatography were prepared and used as described.[25] Melting points were determined on a Kofler hot stage and are uncorrected. Mass spectra (EI) were determined with a Hitachi Perkin–Elmer RMU-6D mass spectrometer with probe temperature 100–200° and ionizing voltage of 70 eV, or with a Finnigan 3300 instrument, having a direct inlet, methane and helium being used to obtain

[23] Marks, E. P., Robbins, W. E., and Thompson, M. J., *Lipids*, 1978, **13**, 263.
[24] Horn, D. H. S., Middleton, E. J., Thomson, J. A., and Wilkie, J. S., *J. Insect. Physiol.*, 1974, **20**, 2433.
[25] Kinnear, J. F., Martin, M.-D., Faux, A. F., Horn, D. H. S., and Wilkie, J. S., *Aust. J. Chem.*, 1979, **32**, 2017.

chemical ionization (CI) and charge exchange (CE) spectra (equivalent to electron impact spectra) respectively.

Calliphora bioassays were carried out as described.[2] The mosquito bioassay was carried out in the following way. Samples for testing were dissolved in a minimum volume of acetone and the solutions vigorously stirred into distilled water to make up the concentration required. The prepared solutions were tested for toxicity on *Aedes aegypti* larvae. Eggs were hatched early on the morning of the test. First instar larvae were transferred to the solutions, fed with 'Tetramin' fish food and maintained at 26°. Each analogue was tested in triplicate at 1·0, 0·1 and 0·01 ppm. Dead larvae were counted after 5, 10 and 15 days.

3β-Methoxy-14α-hydroxy-5β-cholest-7-en-6-one (5)

A solution of fluoroboric acid was prepared by the addition of concentrated fluoroboric acid (0·13 ml) to anhydrous diethyl ether (19 ml) at 0°, after which the solution was made up to 25 ml with dichloromethane.

A solution of the keto diol (3) (50 mg) in dichloromethane (10 ml) and the fluoroboric acid solution (400 μl) was cooled to 0°. A 2·5% (w/v) solution of diazomethane in dichloromethane (15 ml) was added slowly with stirring; and the mixture stirred for 0·5 h. The solution was then washed with saturated sodium hydrogen carbonate and water, dried and evaporated. The residue (55 mg) was chromatographed on silica gel (10% water, 10 g) in chloroform. Appropriate fractions were combined and recrystallized from aqueous methanol to give 3β-methoxy-14α-hydroxy-5β-cholest-7-en-6-one (5) (12·8 mg) as plates, m.p. 142–147° (phase change to needles at 126°). *m/e* (CI) 431 (M+1, 38%), 415 (M−15, 25), 413 (M+1−18, 46), 99 (100).

6-Oxo-5α-cholest-7-en-3α-yl Formate (12)

3β-Hydroxy-5α-cholest-7-en-6-one (10) (205 mg) and triphenylphosphine (276 mg) under argon were dissolved in anhydrous tetrahydrofuran (5·5 ml) containing formic acid (40 μl) in a sealed flask and a solution of diethyl azodicarboxylate (187 mg) in dry tetrahydrofuran (1 ml) added dropwise by syringe to the stirred solution over 15 min. After 6 h the solvent was evaporated, and the yellow residue chromatographed on a column of silica gel (5% water, 25 g). Elution with dichloromethane afforded triphenylphosphine (13 mg), and elution with chloroform afforded the formate (181 mg), which was crystallized from light petroleum and then aqueous methanol to give *6-oxo-5α-cholest-7-en-3α-yl formate* (12) as needles, m.p. 177–179° (Found: C, 78·3; H, 10·4. $C_{28}H_{44}O_3$ requires C, 78·5; H 10·4%). λ_{max} 244 nm (ε 15200). ν_{max} 1735, 1670 cm^{-1}. δ 0·61 (s, H 18), 0·86 (s, H 19), 0·87 (d, *J* 6 Hz, H 26/27), 0·94 (d, *J* 6 Hz, H 21), 5·30 (m, $W_{h/2}$ 7·5 Hz, H 3), 5·72 (m, $W_{h/2}$ 6 Hz, H 7), 8·03 (s, HCO). *m/e* (EI) 428 (M, 100%), 413 (M−CH_3, 23), 383 (M+1−HCO_2H, 35), 382 (M−HCO_2H, 57), 367 (M−HCO_2H−CH_3, 32), 315 (M−C_8H_{17}, 17), 289 (M−C_8H_{17}−C_2H_2, 40), 269 (M−C_8H_{17}−HCO_2H, 15), 243 (M−C_8H_{17}−HCO_2H−C_2H_2, 25).

6-Oxo-5α-cholest-7-ene-3α,14α-diol 3-Formate (13)

6-Oxo-5α-cholest-7-en-3α-yl formate (12) (127 mg) and selenium dioxide (250 mg) under argon were heated in dioxan (10 ml) at 90° for 1·5 h, whereupon t.l.c. indicated reaction was complete. The mixture was diluted with ethyl acetate (50 ml) and filtered through a plug of Celite, which was washed with ethyl acetate (50 ml). The filtrate was washed with water, 5% potassium cyanide solution, water, and dried over sodium sulfate. The crude product (130 mg) was crystallized from light petroleum and then methanol/water to give *6-oxo-5α-cholest-7-ene-3α,14α-diol 3-formate* (13) as needles, m.p. 208–228° (dec.) (Found: C, 75·7; H, 10·2. $C_{28}H_{44}O_4$ requires C, 75·6; H, 10·0%). λ_{max} 241 nm (ε 12400). ν_{max} 3450, 1705, 1670 cm^{-1}. δ 0·70 (s, H 18), 0·86 (s, H 19), 0·87 (d, *J* 6 Hz, H 26/27), 0·88 (d, *J* 6 Hz, H 21), 5·31 (m, $W_{h/2}$ 7·5 Hz, H 3), 5·89 (d, $J_{7,9}$ 2·8 Hz, H 7), 8·03 (s, HCO). *m/e* (EI) 444 (M, 30%), 426 (M−H_2O, 68), 416 (M−CO, 8), 411 (M−H_2O−CH_3, 24), 398 (M−HCO_2H, 100), 383 (M−HCO_2H−CH_3, 11), 370 (M−HCO_2H−CO, 46), 365 (M−HCO_2H−H_2O−CH_3, 14), 355 (M−HCO_2H−CO−CH_3, 22), 313 (M−H_2O−C_8H_{17}, 43).

3α,14α-Dihydroxy-5α-cholest-7-en-6-one (14)

The formate (13) (88 mg) and potassium hydrogen carbonate (80 mg) were stirred together under argon in water (0·9 ml), methanol (12 ml) and tetrahydrofuran (6 ml) at room temperature for 16 h.

The mixture was diluted with water, the organic solvents were evaporated under reduced pressure, and the products extracted into ethyl acetate. Crystallization of the ethyl acetate residue (84 mg) from acetone/light petroleum and then methanol/water afforded *3α,14α-dihydroxy-5α-cholest-7-en-6-one* (14) as needles, m.p. 210–233° (dec.) (Found: C, 77·8; H, 10·7. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·7%). λ_{max} 242 nm (ε 12000). ν_{max} 3500, 3400, 1660 cm^{-1}. δ 0·69 (s, H 18), 0·80 (s, H 19), 0·86 (d, *J* 6 Hz, H 26/27), 0·91 (d, *J* 6 Hz, H 21), 4·14 (m, $W_{h/2}$ 7·5 Hz, H 3), 5·82 (m, $W_{h/2}$ 5 Hz, H 7). *m/e* (EI) 416 (M, 34%), 398 (M−H_2O, 89), 388 (M−CO, 10), 383 (M−H_2O−CH_3, 20), 370 (M−CO−H_2O, 16), 360 (M−$2H_2O$, 20), 355 (M−H_2O−CO−CH_3, 13), 285 (M−H_2O−C_8H_{17} 100), 270 (M−H_2O−C_8H_{17}−CH_3, 13), 267 (M−$2H_2O$−C_8H_{17}, 7).

Cholesta-4,7-dien-3-one (*17*)

An Oppenauer oxidation of 7-dehydrocholesterol (15 g) was carried out essentially as described[12] affording cholesta-4,7-dien-3-one (17) (7·36 g, 49%) as yellow prisms, m.p. 89–90° (lit.[20] 87–89°). δ 0·61 (s, H 18), 0·88 (d, *J* 6 Hz, H 26/27), 0·94 (d, *J* 5 Hz, H 21), 1·18 (s, H 19), 5·18 (m, $W_{h/2}$ 5 Hz, H 7), 5·78 (d, *J* 2 Hz, H 4). ν_{max} 1690, 1630, 1470 cm^{-1}. The mother liquors from the crystallization were combined and chromatographed on silica gel by using cyclohexane with a gradient to chloroform. The fractions giving a u.v.-absorbing spot on t.l.c. were combined affording a further quantity of ketone (17) (5 g, total yield 82%).

5β-Cholest-7-en-3-one (*18*)

Piperidine (125 μl) was added to a mixture of cholesta-4,7-dien-3-one (17) (5 g) in ethyl acetate (100 ml) and palladium on calcium carbonate (Merck, 10%, 2·5 g). The mixture was shaken under an atmosphere of hydrogen for 2 h at room temperature and then filtered through Celite. The filtrate on evaporation afforded a waxy solid (5·3 g) which was purified by chromatography on silica gel (Woelmn, 5% water, 300 g) from cyclohexane with a gradient to chloroform. The early fractions, shown by g.l.c. to contain mainly one compound, were combined and crystallized from acetone affording 5β-cholest-7-en-3-one (18) as plates, m.p. 88–90° (lit.[9] 87–88°) (Found: C, 84·2; H, 11·3. Calc. for $C_{27}H_{44}O$: C, 84·3; H, 11·5%). δ 0·56 (s, H 18), 0·87 (d, *J* 7 Hz, H 26/27), 0·94 (d, *J* 6 Hz, H 21), 0·96 (s, H 19), 5·10 (m, $W_{h/2}$ 6 Hz, H 7). ν_{max} 1725 cm^{-1}. *m/e* (CI) 385 (M+1, 100%), 384 (M, 25), 369 (M+1−CH_3, 10).

5β-Cholest-7-en-3β-ol (*19*)

5β-Cholest-7-en-3-one (18) (1·5 g) in tetrahydrofuran (20 ml) was reduced with a solution of potassium tri-s-butyl borohydride (0·5 M, 9·32 ml) in tetrahydrofuran for 40 min at −78° and then for 2 h at 0°. The mixture was then slowly poured into water (100 ml) and extracted with ether. The extract after washing with dilute hydrochloric acid, water, and drying over sodium sulfate, was evaporated to an oil (1·9 g) which on crystallization first from methanol, then acetonitrile/ether, afforded 5β-cholest-7-en-3β-ol (19) (0·8 g) as irregular crystals, m.p. 104–106° (lit.[12] 104–106°). δ 0·55 (s, H 18), 0·87 (d, *J* 6 Hz, H 26/27), 0·90 (s, H 19), 0·93 (d, *J* 5 Hz, H 21), 4·0 (m, $W_{h/2}$ 8 Hz, H 3), 5·06 (m, $W_{h/2}$ 10 Hz, H 7). ν_{max} 3500 cm^{-1}.

The mother liquors were chromatographed on silica gel and afforded a further amount of alcohol (19) (0·44 g, total yield of 80%).

5β-Cholest-7-en-3β-yl Ethyl Ether (*20*)

Triethyloxonium fluoroborate (1·7 g), prepared[15] by treating boron trifluoride etherate with epichlorohydrin, was added to a stirred solution of 5β-cholest-7-en-3β-ol (19) (1·2 g) in dichloromethane (15 ml) and ethyldiisopropylamine (2·3 ml), at 0°. After 4 h the reaction mixture was poured into water and extracted with ether. The ether extract was successively washed with saturated sodium hydrogen carbonate solution, water, hydrochloric acid (1 M), water, dried over sodium sulfate and evaporated. The residue (1·2 g), which t.l.c. showed to still contain some starting alcohol (19), was chromatographed on silica gel from cyclohexane with a gradient to chloroform. Crystallization of early fractions (600 mg, 50%) afforded *5β-cholest-7-en-3β-yl ethyl ether* (20) as rectangular blocks, m.p. 62–64° (Found: C, 83·9; H, 12·0. $C_{29}H_{50}O$ requires C, 84·0; H, 12·2%). δ 0·54 (s, H 18), 0·87 (d, *J* 6 Hz, H 26/27), 0·88 (s, H 19), 0·93 (d, *J* 5 Hz, H 21), 3·40 (q, *J* 7 Hz, OCH_2), 3·53 (m, $W_{h/2}$ 8 Hz, H 3), 5·07 (m, $W_{h/2}$ 10 Hz, H 7). *m/e* (CI) 415 (M+1, 19%), 414 (M, 38), 399 (M+1−CH_4,

23), 369 (M+1−EtOH, 100). ν_{max} 2970, 2890 cm^{-1}. Further elution afforded starting material (600 mg, 50%).

3β-Ethoxy-5β-cholest-7-en-6-one (6)

A solution of 5β-cholest-7-en-3β-yl ethyl ether (20) (600 mg) in dichloromethane (50 ml) was oxidized with Collins reagent[26] (CrO_3,2py, 5·0 g) for 3 h at room temperature. The reaction mixture was poured into a saturated solution of sodium hydrogen carbonate (200 ml) and extracted with ether. The ether extract was washed successively with saturated sodium hydrogen carbonate solution, water, hydrochloric acid (1 M), water, and dried over sodium sulfate and evaporated to dryness. The residual yellow oil (700 mg) was chromatographed on alumina (10% water, 100 g) from cyclohexane with a gradient to ether.

The u.v.-absorbing fractions were combined affording 3β-ethoxy-5β-cholest-7-en-6-one (6) as a yellow oil (225 mg, 38%) which could not be crystallized. λ_{max} 244 (ε 12000). *m/e* (CI) 429 (M+1, 100%), 428 (M, 30), 413 (M+1−CH_4, 22), 400 (10), 383 (M+1−EtOH, 85). ν_{max} (CS_2) 2980, 2890, 1680 cm^{-1}.

3β-Ethoxy-14α-hydroxy-5β-cholest-7-en-6-one (7)

3β-Ethoxy-5β-cholest-7-en-6-one (6) (95 mg) and freshly sublimed selenium dioxide (250 mg) were heated in dioxan (5 ml) at 65° for 4 h. The reaction mixture was then poured into water (50 ml) and extracted with ethyl acetate. The extract was washed successively with potassium cyanide solution (5%), water, and dried over sodium sulfate. The residue (91 mg) was chromatographed on alumina (8% water, 17 g) from cyclohexane with a gradient to ether. Appropriate fractions (56 mg, 60%) were crystallized from methanol/water affording *3β-ethoxy-14α-hydroxy-5β-cholest-7-en-6-one* (7) as long rectangular prisms, m.p. 169–171° (Found: C, 78·1; H, 10·6. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%). λ_{max} 244 (ε 11000). δ 0·64 (s, H 18), 0·87 (d, *J* 7 Hz, H 26/27), 0·93 (d, *J* 5 Hz, H 21), 0·95 (s, H 19), 3·38 (q, *J* 7 Hz, OCH_2), 3·46 (m, H 3), 5·81 (d, *J* 7 Hz, H 7). *m/e* (CI) 445 (M+1, 100%), 444 (M, 13), 427 (M+1−H_2O), 416 (M−CO, 9), 399 (M−OEt, 65). ν_{max} (CS_2) 3500, 2985, 2895, 1680 cm^{-1}.

5β-Cholest-7-en-3α-yl p-*Toluenesulfonate (21)*

5β-Cholestane-3α,7α-diol (23) (410 mg) and *p*-toluenesulfonyl chloride (410 mg) were dissolved in dry pyridine (5 ml) and the mixture was allowed to react for 18 h at room temperature. Phosphorus oxychloride (400 μl) was then added, the mixture stirred for 4 h, poured into ice-water and extracted with ether. The extract was washed successively with cold hydrochloric acid (1 M), sodium hydrogen carbonate (1 M), saturated brine and then dried over sodium sulfate. Evaporation of the solvent afforded a clear resin (544 mg) shown by n.m.r. spectroscopy to be a 7:3 mixture of cholest-7- and -6-enes. δ 5·13 (m, $W_{h/2}$ 11 Hz, H 7) and 5·48 (m, $W_{h/2}$ 3 Hz, H 6) respectively.

Crystallization of the mixed esters from acetone/methanol gave a poor yield of *5β-cholest-7-en-3α-yl* p-*toluenesulfonate* (21) as needles, 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%). δ 0·56 (s, H 18), 0·83 (s, H 19), 0·87 (d, *J* 6 Hz, H 26/27), 0·91 (d, *J* 6 Hz, H 21), 1·23 (s, CH_3Ar), 4·44 (m, $W_{h/2}$ 18 Hz, H 3), 5·03 (m, $W_{h/2}$ 12 Hz, H 7), 7·29, 7·76 (A_2B_2 centres, H_4Ar). *m/e* (EI) 368 (M−$C_7H_7SO_3H$, 100%), 353 (M−$C_7H_7SO_3H$−CH_3, 35), 339 (metastable, calc. for 368 → 353 = 338·6).

5β-Cholest-7-en-6-one (8)

The mixed 6- and 7-ene sulfonates above (477 mg) were dissolved in dry ether (20 ml), lithium aluminium hydride (430 mg), was added, and the mixture refluxed under argon for 0·5 h. Isolation of the products in the usual way afforded an oily mixture of 5β-cholest-7- and -6-enes, in ratio of 3:2 as estimated from the n.m.r. spectrum, δ 5·13 ($W_{h/2}$ 11 Hz, H 7) and 5·47 ($W_{h/2}$ 7 Hz, H 6), respectively. Chromatography on silica gel (10% silver nitrate) failed to separate the mixture, which could not be induced to crystallize.

The mixed olefins (287 mg) and Collins reagent (2·09 g) were dissolved in dry dichloromethane (25 ml) under argon and the mixture was stirred vigorously for 2 h. Isolation of the products in the

[26] Dauben, W. G., Lorber, M., and Fullerton, D. S., *J. Org. Chem.*, 1969, **34**, 3587.

usual way with ether afforded a brown resin (260 mg) which was chromatographed on silica gel (5% water, 50 g). Gradient elution from cyclohexane to dichloromethane afforded *5β-cholest-7-en-6-one* (8) (36 mg), which crystallized from methanol as needles, m.p. 120–121° (Found: C, 84·5; H, 11·4. $C_{27}H_{44}O$ requires C, 84·3; H, 11·5%). λ_{max} 246 nm (ε 14600). δ 0·54 (s, H 18), 0·86 (d, *J* 6 Hz, H 26/27), 0·88 (s, H 19), 0·91 (d, *J* 6 Hz, H 21), 5·66 (m, $W_{h/2}$ 5 Hz, H 7). *m/e* (EI) 384 (M, 100%), 369 (M−CH_3, 32), 355 (metastable, calc. for 384 → 369 = 354·63), 341 (M−CO−CH_3, 4), 303 (metastable, calc. for 384 → 341 = 302·8), 271 (M−C_8H_{17}, 5).

14α-Hydroxy-5β-cholest-7-en-6-one (*9*)

5β-Cholest-7-en-6-one (8) (40 mg) and freshly sublimed selenium dioxide (160 mg) in dry dioxan (2 ml) under argon were heated at 90° for 3 h. The product was extracted into ethyl acetate (100 ml) and the solution washed with water and dried over sodium sulfate. Evaporation of the solvent afforded a partly crystalline yellow resin (47 mg) which was chromatographed on silica gel (5% water, 20 g). Elution with dichloromethane gave *14α-hydroxy-5β-cholest-7-en-6-one* (9), which crystallized from methanol as needles, m.p. 123–125°, analysing as the hemihydrate (Found: C, 79·5; H, 11·0. $(C_{27}H_{44}O_2)_2,H_2O$ requires C, 79·2; H, 11·1%). λ_{max} 242 nm (ε 12100). δ 0·64 (s, H 18), 0·88 (d, *J* 6 Hz, H 26/27), 0·89 (s, H 19), 0·92 (d, *J* 6 Hz, H 21), 3·11 (m, $W_{h/2}$ 22 Hz, H 9), 5·80 (d, $J_{7,9}$ 2·6 Hz, H 7). *m/e* (EI) 400 (M, 31%), 382 (M−H_2O, 25), 372 (M−CO, 100), 367 (M−H_2O−CH_3, 9), 358 (M+1−CO−CH_3, 8), 357 (M−CO−CH_3, 16), 354 (M−H_2O−CO, 4), 339 (M−H_2O−CO−CH_3, 3), 269 (M−H_2O−C_8H_{17}, 24).

*5α-Cholest-7-ene-3β,6α-diol 3-*p*-Toluenesulfonate 6-Acetate* (*26*)

p-Toluensulfonyl chloride (1·0 g) was added to 5α-cholest-7-ene-3β,6α-diol 6-acetate (25) (747 mg) dissolved in dry pyridine (10 ml), the flask flushed with argon, and the mixture allowed to react for 18 h at room temperature. Isolation of the product in the usual way with ether afforded *5α-cholest-7-ene-3β,6α-diol 3-p-toluenesulfonate 6-acetate* (26) which was crystallized from light petroleum at −20° as thick needles (680 mg), m.p. 128–131° (Found: C, 72·0; H, 9·2; S, 5·3. $C_{36}H_{54}O_5S$ requires C, 72·2; H, 9·1; S, 5·4%). δ 0·51 (s, H 18), 0·86 (s, H 19), 0·86 (d, *J* 6 Hz, H 26/27), 0·90 (d, *J* 7 Hz, H 21), 1·99 (s, CH_3CO), 2·43 (s, CH_3Ar), 4·40 (m, $W_{h/2}$ 24 Hz, H 3), 4·98 (m, $W_{h/2}$ 15 Hz, H 6), 5·03 (m, $W_{h/2}$ 5 Hz, H 7), 7·31, 7·79 (A_2B_2 centres, H_4Ar). *m/e* (EI) 598 (M, 0·1%), 538 (M−CH_3CO_2H, 7), 523 (M−CH_2CO_2H−CH_3, 0·05), 426 (M−$C_7H_7SO_3H$, 0·2), 366 (M−$C_7H_7SO_3H$−CH_3CO_2H, 65), 337 (metastable, calc. for 538 → 426 = 337·3), 253 (M−$C_7H_7SO_3H$−CH_3CO_2H−C_8H_{17}, 100), 175 (metastable, calc. for 366 → 253 = 174·9).

5α-Cholest-7-en-6α-ol (*27*)

The *p*-toluenesulfonate (26) (615 mg) was dissolved in dry ether (50 ml), lithium aluminium hydride (0·5 g) added in portions over 10 min, and the mixture refluxed gently for 2 h. The excess hydride was destroyed with saturated sodium sulfate solution and the product, isolated in the usual way, was chromatographed on silica gel (5% water). Gradient elution from dichloromethane to chloroform/ethanol (95:5) afforded a fraction (245 mg), which, after crystallization from ethyl acetate and then aqueous ethanol, gave *5α-cholest-7-en-6α-ol* (27) as needles, m.p. 126–128° (Found: C, 84·0; H, 12·0. $C_{27}H_{46}O$ requires C, 83·9; H, 12·0%). δ 0·55 (s, H 18), 0·87 (d, *J* 6 Hz, H 26/27), 0·90 (s, H 19), 0·92 (d, *J* Hz, H 21), 3·73 (m, $W_{h/2}$ 15 Hz, H 6), 5·16 (m, $W_{h/2}$ 6 Hz, H 7). *m/e* (EI) 386 (M, 7%), 368 (M−H_2O, 100), 353 (M−H_2O−CH_3, 33), 339 (metastable ion, calc. for 368 → 353 = 338·6), 255 (M−H_2O−C_8H_{17}, 86).

5α-Cholest-7-en-6-one (*15*)

A solution of Collins reagent[26] (650 mg) in dichloromethane (10 ml) was added to a solution of the alcohol (27) (168 mg) in dichloromethane (5 ml), the flask stoppered and shaken vigorously for 15 min. The crude product was extracted with ether and the ether residue chromatographed on a column of silica gel (5% water). Gradient elution from cyclohexane to dichloromethane afforded *5α-cholest-7-en-6-one* (15) (110 mg), which crystallized from methanol as prisms, m.p. 117–120° (Found: C, 84·6; H, 11·6. $C_{27}H_{44}O$ requires C, 84·3; H, 11·5%). λ_{max} 246 nm (ε 13200). δ 0·59 (s, H 18), 0·86 (s, H 19), 0·88 (d, *J* 6 Hz, H 26/27), 0·94 (d, *J* 6 Hz, H 21), 5·69 (dd, $J_{7,9}$ 2·8 Hz, $J_{7,14}$ 2·0 Hz, H 7). *m/e* (EI) 384 (M, 100%), 369 (M−CH_3, 36), 356 (M−CO, 1), 355 (metastable ion, calc. for 384 → 369 = 354·6), 341 (M−CH_3−CO, 4), 271 (M−C_8H_{17}, 48), 256 (M−C_8H_{17}−CH_3, 4).

14α-Hydroxy-5α-cholest-7-en-6-one (16)

Freshly sublimed selenium dioxide (250 mg), the ketone (15) (110 mg) and anhydrous dioxan (10 ml) were stirred under argon at about 85° for 1 h. The flask was cooled, ethyl acetate (50 ml) added and the solution filtered through a plug of Celite. The filtrate was washed with 5% potassium cyanide solution, then water, and dried over sodium sulfate. The crude product obtained by evaporation of the solvent was crystallized from light petroleum to give *14α-hydroxy-5α-cholest-7-en-6-one* (16) as needles (96 mg), m.p. 199–207° (dec.) (Found: C, 80·6; H, 11·0. $C_{27}H_{44}O_2$ requires C, 80·9; H, 11·1%). λ_{max} 242 nm (ε 12100). δ 0·66 (s, H 18), 0·82 (s, H 19), 0·87 (d, *J* 6 Hz, H 26/27), 0·92 (d, *J* 6 Hz, H 21), 5·86 (d, $J_{7,9}$ 2·8 Hz, H 7). *m/e* (EI) 400 (M, 31%), 385 (M−CH_3, 5), 382 (M−H_2O, 100), 372 (M−CO, 20), 367 (M−H_2O−CH_3, 37), 357 (M−CO−CH_3, 12), 354 (M−H_2O−CO, 4), 353 (metastable ion, calc. for 382 → 367 = 352·6), 269 (M−H_2O−C_8H_{17}, 83), 259 (M−CO−C_8H_{17}, 4).

3,5-Cyclocholest-7-en-6-one (29)

Cholesta-5,7-dien-3β-yl *p*-toluenesulfonate (1·38 g) was converted into 3,5-cyclocholest-7-en-6β-ol (28) (0·25 g) by treatment with potassium hydrogen carbonate in acetone.[19] The cyclo alcohol (28) (252 mg) was then oxidized with Collins reagent[26] (1·0 g) in dichloromethane (25 ml) for 15 min, and the product extracted with ether. The ether residue was dissolved in chloroform and filtered through a plug of alumina (15% water) to eliminate chromium residues. Evaporation of the solvent gave a brown resin (197 mg), which was chromatographed on silica gel (5% water, 30 g). Gradient elution from cyclohexane/dichloromethane (3:1) to dichloromethane afforded 3,5-cyclocholest-7-en-6-one[19] (29) (116 mg). δ 0·64 (s, H 18), 0·87 (d, *J* 6 Hz, H 26/27), 0·94 (d, *J* 6 Hz, H 21), 1·06 (s, H 19), 5·84 (m, $W_{h/2}$ 7 Hz, H 7).

14α-Hydroxy-3,5-cyclocholest-7-en-6-one (30)

3,5-Cyclocholest-7-en-6-one (29) (116 mg), selenium dioxide (250 mg) and dioxan (10 ml) were stirred under argon at 80° for 1·5 h. The crude product was extracted with ethyl acetate and the residue chromatographed on silica gel (25 g). The major peak fractions were rechromatographed on alumina (3% water, 50 g). Gradient elution from cyclohexane to ethyl acetate afforded *14α-hydroxy-3,5-cyclocholest-7-en-6-one* (30) (35 mg), which crystallized from aqueous methanol as needles, m.p. 181–185° (Found: C, 81·4; H, 10·7. $C_{27}H_{42}O_2$ requires C, 81·4; H, 10·6%). λ_{max} 243 nm (ε 10700). δ 0·68 (s, H 18), 0·88 (d, *J* 7 Hz, H 26/27), 0·92 (d, *J* 7 Hz, H 21), 1·04 (s, H 19), 5·75 (m, H 7). *m/e* (EI) 398 (M, 29%), 380 (M−H_2O, 20), 370 (M−CO, 100), 365 (M−H_2O−CH_3, 14), 355 (M−CO−CH_3, 14), 352 (M−H_2O−CO, 2), 341 (metastable ion, calc. for 370 → 355 = 340·6), 337 (M−H_2O−CO−CH_3, 2), 285 (M−C_8H_{17}, 2).

3α-Formyloxy-7α-hydroxy-5β-cholic Acid (24)

Formic acid (20 ml) was added to a solution of chenodeoxycholic acid (2 g) in methyl formate (20 ml). After the mixture had been stirred at room temperature (20°) for 2 h, methanol (20 ml) was added and stirring continued for 10 min. Saturated brine (100 ml) was added and the mixture extracted with ether (100 ml). The ether residue (2·03 g) consisted of an equimolar mixture of chenodeoxycholic acid and its 3α-formate (24) together with only a trace of 3α,7α-diformate. The mixture was separated by chromatography on silica gel by using cyclohexane/ethyl acetate (70:30) with a gradient to ethyl acetate affording 3α-formyloxy-7α-hydroxy-5β-cholic acid (24) (919 mg, 46%) as a low melting solid. δ 0·67 (s, H 18), 0·92 (s, H 19), 0·95 (d, *J* 5 Hz, H 21), 3·86 (m, $W_{h/2}$ 4 Hz, H 7), 4·72 (m, $W_{h/2}$ 13 Hz, H 3), 8·05 (s, OCOH). ν_{max} 3430, 1735, 1720 cm^{-1}. *m/e* (EI) 420 (M, 15%), 402 (M−H_2O, 38), 357 (M−H_2O−HCOO, 100).

24-Oxo-25-aza-5β-cholest-7-en-3α-yl Formate (31)

Redistilled thionyl chloride (1·02 g, 8·56 mmol) was added dropwise to a stirred solution of chenodeoxycholic acid 3-formate (24) (900 mg, 2·14 mmol) in dry toluene (10 ml) and dry pyridine (0·5 ml, 6·4 mmol) at below 15°. The excess of thionyl chloride was removed under vacuum and a solution of dimethylamine (0·74 ml, 11 mmol) in dry ether (5 ml) added with stirring. After 10 min the mixture was poured into water (100 ml) and extracted with ether. The ether residue (864 mg) was crystallized from hexane as irregular prisms affording *24-oxo-25-aza-5β-cholest-7-en-3α-yl formate* (31), m.p.

133–137° (Found: C, 75·3; H, 10·2; N, 3·1. $C_{27}H_{43}NO_3$ requires C, 75·5; H, 10·1; N, 3·3%). δ 0·56 (s, H 18), 0·88 (s, H 19), 0·96 (d, J 5 Hz, H 21), 2·93 (s, $CONCH_3$), 3·0 (s, $CONCH_3$), 3·60 (m, $W_{h/2}$ 12 Hz, H 3), 5·10 (m, H 7), 8·03 (s, OCOH). ν_{max} (CCl_4) 1730, 1670 cm^{-1}. *m/e* (EI) 429 (M, 22%), 414 (M − CH_3, 2), 384 (M − OCOH, 13), 369 (M − OCOH − CH_3, 26), 87 ($(CH_3)_2NCOCH_3$).

3α-Hydroxy-25-aza-5β-cholest-7-en-24-one (32)

Potassium carbonate (800 mg) in water (4 ml) was added to a stirred solution of the amido formate (31) (840 mg) dissolved in methanol (36 ml). After 1 h, the mixture was poured into brine and extracted with ether. The ether residue (667 mg) afforded *3α-hydroxy-25-aza-5β-cholest-7-en-24-one* (32) as rectangular prisms from methanol, m.p. 199–203° (Found: C, 77·5; H, 10·8; N, 3·3. $C_{26}H_{43}NO_2$ requires C, 77·7; H, 10·8; N, 3·5%). δ 0·56 (s, H 18), 0·87 (s, H 19), 0·97 (d, J 6 Hz, H 21), 2·94 (s, $CONCH_3$), 3·01 (s, $CONCH_3$), 3·60 (m, $W_{h/2}$ 12 Hz, H 3), 5·10 (m, H 7). ν_{max} 3310, 2960, 2890, 1630 cm^{-1}. *m/e* (EI) 401 (M, 43%), 386 (M − CH_3, 2), 383 (M − H_2O, 9), 368 (M − H_2O − CH_3, 24), 87 ($(CH_3)_2NCOCH_3$).

24-Oxo-25-aza-5β-cholest-7-en-3β-yl Formate (33)

Diethyl azodicarboxylate (540 mg, 3·1 mmol) in dry tetrahydrofuran (5 ml) was added with stirring to a mixture of ketone (32) (520 mg, 1·55 mmol), triphenylphosphine (1·22 g, 4·65 mmol), formic acid (143 mg, 3·1 mmol) and tetrahydrofuran (15 ml). After being stirred overnight at room temperature, the mixture was evaporated to dryness. The residue was stirred with cyclohexane/chloroform (70:30), filtered from diethyl hydrazodicarboxylate and evaporated. The residue was chromatographed on silica gel by using cyclohexane/chloroform (70:30) with a gradient to chloroform. The main fractions (527 mg, 87%) afforded *24-oxo-25-aza-5β-cholest-7-en-3β-yl formate* (33) as irregular prisms from hexane, m.p. 153–159° (Found: C, 75·2; H, 9·9; N, 3·0. $C_{27}H_{43}NO_3$ requires C, 75·4; H, 10·1; N, 3·2%). δ 0·57 (s, H 18), 0·92 (s, H 19), 0·95 (d, J 6 Hz, H 21), 2·86 (s, $CONCH_3$), 2·98 (s, $CONCH_3$), 5·12 (m, $W_{h/2}$ 6 Hz, H 3), 5·12 (m, H 7), 7·97 (s, OCOH). ν_{max} 1730, 1660, 1200 cm^{-1}. *m/e* (EI) 429 (M, 27%), 384 (M − HCOO, 73), 369 (M − HCOO − CH_3, 55), 87 ($(CH_3)_2NCOCH_3$, 100).

6,24-Dioxo-25-aza-5β-cholest-7-en-3β-yl Formate (34)

The formate (33) (500 mg, 1·17 mmol) in dry dichloromethane (15 ml) was oxidized with Collins reagent[26] (3·6 g, 14 mmol) at 20° for 16 h. The mixture was poured into water and extracted with ether. The ether residue (396 mg) was chromatographed on silica gel from cyclohexane with a gradient to chloroform. The u.v.-absorbing fractions (125 mg, 25%) afforded *6,24-dioxo-25-aza-5β-cholest-7-en-3β-yl formate* (34) as a foam. δ 0·62 (s, H 18), 0·96 (d, J 6 Hz, H 21), 1·01 (s, H 19), 2·94 (s, $CONCH_3$), 3·0 (s, $CONCH_3$), 5·20 (m, $W_{h/2}$ 6 Hz, H 3), 5·86 (m, H 7), 8·04 (s, OCOH). ν_{max} 1730, 1670, 1650, 1200 cm^{-1}. *m/e* (CI) 444 (M+1, 65%), 443 (M, 10), 415 (M+1−OCH, 13), 398 (M+1−HCOOH, 40), 87 ($(CH_3)_2NCOCH_3$, 100).

6,24-Dioxo-25-aza-5β-cholest-7-ene-3β,14α-diol 3-Formate (35)

Selenium dioxide (250 mg) was added to the keto amide (34) (120 mg) dissolved in dry dioxan (10 ml) and heated at 80°. After 2 h the mixture was poured into saturated brine and extracted with ethyl acetate. The residue was chromatographed on silica gel by using cyclohexane/chloroform (70:30) with a gradient to chloroform. Early fractions afforded unchanged keto amide (34) (30 mg, 25%), while later fractions (42 mg, 35%) afforded *6,24-dioxo-25-aza-5β-cholest-7-ene-3β,14α-diol 3-formate* (35) as prisms from acetone/light petroleum, m.p. 229–238° (dec.) (Found: C, 70·3; H, 9·2; N, 3·0. $C_{27}H_{41}NO_5$ requires C, 70·5; H, 9·0; N, 3·0%). δ 0·66 (s, H 18), 0·94 (d, J 6 Hz, H 21), 0·97 (s, H 19), 2·88 (s, $CONCH_3$), 2·94 (s, $CONCH_3$), 5·18 (m, $W_{h/2}$ 5 Hz, H 3), 5·82 (m, H 7). ν_{max} 1730, 1680, 1620, 1200 cm^{-1}. *m/e* (EI) 459 (M, 6%), 441 (M − H_2O, 6), 429 (M − CO, 15), 414 (M − HCOO, 2), 87 ($(CH_3)_2NCOCH_3$, 100).

3β,14α-Dihydroxy-25-aza-5β-cholest-7-ene-6,24-dione (36)

Potassium hydrogen carbonate (38 mg) in water (0·6 ml) was added at 20° with stirring to the formate (35) in tetrahydrofuran (2 ml). After 3 h the mixture was evaporated to dryness and extracted

with chloroform. The residue (32 mg, 85%) afforded *3β,14α-dihydroxy-25-aza-5β-cholest-7-ene-6,24-dione* (36) as fine plates from ethanol, m.p. 275–280° (dec.) (Found: C, 72·6; H, 9·6; N, 3·2. $C_{26}H_{41}NO_4$ requires C, 72·3; H, 9·6; N, 3·2%). δ 0·67 (s, H 18), 0·97 (d, J 5 Hz, H 21), 0·99 (s, H 19), 2·95 (s, $CONCH_3$), 3·07 (s, $CONCH_3$), 4·04 (m, $W_{h/2}$ 6 Hz, H 3), 5·84 (m, H 7). ν_{max} 3440, 3280, 1660, 1600 cm^{-1}. *m/e* (EI) 431 (M, 8%), 413 (M−H_2O, 55), 403 (M−CO, 40), 398 (M−H_2O−CH_3, 38), 87 ($(CH_3)_2NCOCH_3$, 60). λ_{max} 243 nm (ε 12300).

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