Hydroxy-steroids. Part 20.[1] Distinction between 19-Norergosta-5,7,9-trien-3β-ol (Dihydroneoergosterol) and its 3α-Epimer

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Two methods have been used for epimerising the 3-hydroxy-group of 19-norergosta-5,7,9-trien-3β-ol. Although the 3β- and 3α-alcohols show a close resemblance in many of their properties it is possible to distinguish between them by examining the ^{19}F n.m.r. spectra of their α-methoxy-α-trifluoromethylphenylacetates and the ^{1}H n.m.r. spectra of their complexes with shift reagents. Purification of the alcohols is most reliably achieved by crystallising their 3,5-dinitrobenzoates. Measurement of optical rotation is the best method for the quantitative analysis of mixtures of the epimers.

CONFORMATIONAL mobility in ring A of steroids having an aromatic ring B was first demonstrated by i.r. measurements, which showed that both neorgosterol (19-norergosta-5,7,9,22-tetraen-3β-ol (1; $R^1 = H$, $R^2 = C_9H_{17}$) and epineoergosterol (the 3α-epimer) (2; $R^1 = H$, $R^2 = C_9H_{17}$) have an equatorial hydroxy-group; this was attributed to their preferences for different half-chair conformations.[2] The consideration that these forms should be 'about equally free of destabilising interactions' led Levine and Ghosh[3] to question the

[1] Part 19, A. D. Boul, R. Macrae, and G. D. Meakins, *J. Chem. Soc. Perkin I*, 1974, 1138.

[2] D. H. R. Barton, R. C. Cookson, W. Klyne, and C. W. Shoppee, *Chem. and Ind.*, 1954, 21.

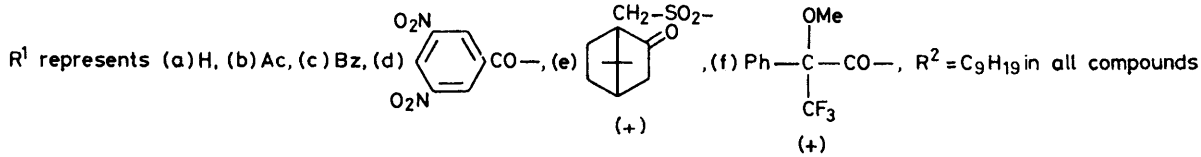
[3] S. G. Levine and A. C. Ghosh, *Tetrahedron Letters*, 1969, 39.

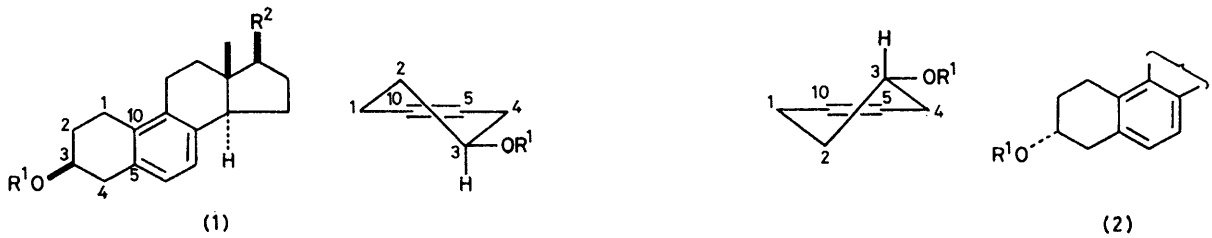
early report [4] that neoergosterol is converted completely into epineoergosterol by treatment with sodium in boiling pentyl alcohol. After developing a sequence (3β-OH ⟶ 3β-O·SO_2Me ⟶ 3α-O·CHO ⟶ 3α-OH) for preparing epineoergosterol, these authors found that (i) the 3-epimers had almost identical i.r. and ^{1}H n.m.r. spectra, and (ii) mixtures of the alcohols did not exhibit m.p. depression and could not be separated chromatographically. Assessment of purity was based on rotations; from neoergosterol ($[\alpha]_D$ −6.0°) the new method stereospecificity of the inversion procedure. Since several of the derivatives to be studied were expected to have crucial ^{1}H n.m.r. signals in the region τ 4—5 it was decided to work with 22,23-dihydro-compounds based on dihydroneorgesterol (1; R^1 = H, R^2 = C_9H_{19}) so as to avoid interference from the neoergosterol olefinic signal at τ 4.75. (This change of substrate does not appear to influence the problems under investigation.)

In the first approach, summarised in the Scheme, dihydroneoergosterol (1a) was epimerised by the method

SCHEME

The epimeric 3-hydroxy-19-norergosta-5,7,9-trienes and their derivatives





(1) (2)

Rotations were measured using solutions in $CHCl_3$ (*c* 0.5—1.0). The i.r. bands ($CHCl_3$ solutions, positions in cm^{-1}) are the two strongest absorptions of each compound associated with C(3)–O stretching. N.m.r. spectra were examined using solutions in $CDCl_3$; the positions of the 3-H signals are τ values, and those of the ^{19}F signals are p.p.m. downfield from external CF_3·CO_2H

M.p. (°C)	$[\alpha]_D$	I.r.	3-H	^{19}F	Epimeric pairs i, ii, iii	M.p. (°C)	$[\alpha]_D$	I.r.	3-H	^{19}F
146—147	−2°	1 050, 1 029	5.89		(1a) [a] ⟶ (2a) [b]	165—166	+49°	1 088, 1 045	5.90	
120—121	+2	1 040, 1 032	4.88		(1b) [a] iv ⟶ (2b) [b] iv	120—122	+46	1 208, 1 015	4.90	
121—122	−8	1 120, 1 029	4.57		(1c) vii ⟵ v ⟶ (2c) v vii	124—126	+50	1 117, 1 026	4.55	
220—222	−4	1 175, 1 041	4.51		(1d) [c] iii ⟵ v ⟶ (2d) v iii	165—167	+46	1 172, 1 042	4.47	
115—117	+17	1 171, 1 055	4.82		(1e) v ⟶ (2e) v	120—122	+48	1 171, 1 053	4.80	
108—109	+19	1 171, 1 022	4.57	3.75	(1f) v ⟶ (2f)	98—100	+67	1 171, 10 22	4.54	3.95

Reagents: i, Me·SO_2Cl–C_5H_5N; ii, NEt_4^+ HCO_2^-–Me_2CO; iii, $NaHCO_3$; iv, Ac_2O–C_5H_5N; v, Appropriate acid chloride–C_5H_5N; vi, EtO_2C·N=N·CO_2Et–PPh_3–Ph·CO_2H; vii, KOH–MeOH.

* The product of this reaction had m.p. 126—128 °C, $[\alpha]_D$ +49°.

[a] Ref. 7: (1a), m.p. 146—148 °C, $[\alpha]_D$ −3°; (1b), m.p. 120—121 °C, $[\alpha]_D$ +1°. [b] Ref. 4: (2a), m.p. 167 °C; (2b), m.p. 83 °C. [c] G. A. D. Haslewood and E. Roe, *J. Chem. Soc.*, 1935, 465: (1d), m.p. 216—218 °C.

gave epineoergosterol with $[\alpha]_D$ +50.7°, and the various literature products ($[\alpha]_D$ values no higher than +27°) were concluded to be mixtures of epimers.

Although the inversion sequence is expected to be stereospecific this feature is not rigorously established. It is conceivable that in the displacement of the 3β-methylsulphonyloxy-group there is some S_Ni contribution or participation by the neighbouring aromatic ring, and the presence in the final product of a certain amount of the 3β-alcohol, however formed, would not have been revealed by the previous study.[3] The main objects of the present investigation were, therefore, to find other criteria for distinguishing between such similar epimeric alcohols, and to use these in assessing the used with neoergosterol, and corresponding series of derivatives were prepared from the original alcohol and the product. Compounds (1a—f) and (2a—f) were obtained in crystalline form and their properties were examined; repeated recrystallisation of the samples had little effect on the properties, and at no stage did t.l.c. reveal evidence of inhomogeneity in any of the samples. The i.r. spectra of solutions of epimeric pairs are similar but not identical; although there are distinct differences in the bands associated with the C(3)–O stretching modes of the alcohols (1a) and (2a) these differences are not sufficient to exclude the possibility that the 3α-alcohol

[4] A. Windaus and M. Deppe, *Chem. Ber.*, 1937, **70**, 76.

(2a) contains a small amount (say, up to 5%) of the starting material (1a). Even greater similarity was found in the ^1H n.m.r. spectra of epimeric pairs, including those [(1e) and (2e), (1f) and (2f)] in which it had been hoped that esterification with a chiral acid would produce appreciable differences in the steroidal 3-H signals. The ^{19}F n.m.r. signals of the methoxytrifluoromethylphenylacetates, (1f) and (2f), provided the first clear spectrometric distinction between the series. These signals are sufficiently well separated that contamination of the 3α-ester (2f) by *ca.* 2% or more of the 3β-epimer (1f) would have been detected.

With five of the pairs in the Scheme, mixtures of epimers had melting points *between* those of the components, and only the 3,5-dinitrobenzoates, (1d) and (2d) (mixed m.p. 154—161 °C), behaved normally. (The reluctance of 3,5-dinitrobenzoates to form mixed crystals has long been recognised in the traditional use of these derivatives for separating sterol mixtures.) Crystallisation was effective in purifying samples of the esters (1d) and (2d) to which 10% of the other isomer had been added; hydrolysis of the esters gave the parent alcohols (1a) and (2a) with unchanged characteristics, thus establishing the purity of the 3α-alcohol (2a) produced in the epimerisation. The more direct method of inversion using diethyl azodicarboxylate [5] gave the 3α-benzoate (2c) stereoselectively, and this is a more efficient route to compounds of the 3α-series.

A different approach, involving n.m.r. shift reagents, is shown in the Table. (The standard techniques [6] used in obtaining and processing the data are described briefly at the head of the Table.) Here, the intention was to differentiate between the signals of specific protons (say the 3-H) in the diastereoisomeric alcohols (1a) and (2a) by studying the spectra of their complexes. Since chiral reagents are known to induce a difference between the chemical shifts of enantiomeric protons and achiral reagents to increase the difference between those of diastereoisomeric protons, a reagent of each type was employed.

The most complete analysis emerged with the system 3β-alcohol (1a) plus Eu(facam)$_3$ * where the resonance of each ring A proton was identified as a separate signal. Elsewhere difficulties of assignment arose from the overlapping and crossing over of some signals caused by varying rates of shift (with respect to concentration). With both reagents the epimeric alcohols gave different values for the bound shifts (ν_b) of their 3-H signals. As expected from this result mixtures of the alcohols in a solution of either reagent give distinguishable 3-H signals; however, these are so broad and closely overlapped that their use for estimating 3β : 3α ratios leads to inaccurate values. The sharper 4-H signals of the Eu(facam)$_3$ complexes are much more suitable for quantitative work, and with solutions of known composition the epimer ratios found by integration (at ρ values of *ca.* 0.26) were accurate to ±5%.

The present work establishes the stereochemical homogeneity of the 3α-alcohol (2a) having the constants reported in the Scheme and, almost certainly, of the epineoergosterol described by Levine and Ghosh.[3] There is a close correspondence between the $[\alpha]_D$ values of the

The effect of shift reagents on the ^1H n.m.r. signals of the epimeric alcohols (1a) and (2a)

S = steroid, L = shift reagent, and ρ = [L]/[S]. For each system portions of S were added to a solution of L in $CDCl_3$ to give 10 solutions in the range ρ = 0.1—0.5; a selection of the results obtained by examining these solutions at 90 MHz is shown below. The descriptions low and high refer to the field strength at which the signals of a CH_2 group occur; the positions (in Hz downfield from $SiMe_4$) of a signal in the presence and absence of L are denoted by ν_{sh} and ν_o respectively, and $(\nu_{sh}-\nu_o) = \Delta\nu$. Extrapolation to ρ = 0 of ν_{sh} *versus* ρ plots (which were linear up to ρ = *ca.* 0.30) gave the ν_0 values. Plots of [S] *versus* $1/\Delta\nu$ were also linear; the gradients of these plots are L.ν_b, where ν_b represents the bound shifts (in Hz) of particular protons

3β-Alcohol (1a) in 0.13M-Eu(fod)$_3$ *

		1-H		2-H		3-H	4-H	
ρ		Low	High	Low	High		Low	High
0.10						584	417	372
0.15						673	474	415
0.19	ν_{sh}	401	348			777	524	463
0.23		426	364	462		839	568	491
0.26		453	384	517		910	613	526
	ν_b	990 ‡	590 ‡			2 130	1 475	1 320

3α-Alcohol (2a) in 0.10M-Eu(fod)$_3$

0.11						562	394	355
0.14						623	437	383
0.17	ν_{sh}					679	477	409
0.24						788	555	460
0.27						832	586	481
	ν_b					1 870	1 100	1 050

Average ν_o values (both alcohols)

247 ‡ 240 238 ‡ 238 ‡ 364 274 250

3β-Alcohol (1a) in 0.10M-Eu(facam)$_3$ †

		1-H		2-H		3-H	4-H	
ρ		Low	High	Low	High		Low	High
0.11		310	292			575	403	364
0.17						684	470	418
0.22	ν_{sh}		333	409	377	773	530	463
0.28		382	350	451	410	836	573	500
0.34		410	362	483	451	881	605	544
	ν_b	670 ‡	580	860 ‡	780 ‡	2 120	1 250	1 190

3α-Alcohol (2a) in 0.13M-Eu(facam)$_3$

0.14						607	418	377
0.19			312			684	472	415
0.24	ν_{sh}		326			747	513	444
0.29			341			801	549	470
0.33			351			835	570	489
	ν_b		410			1 770	1 000	820

* Tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III). † Tris(trifluoroacetyl-*d*-camphorato)europium-(III). ‡ Values less precise than the others in the same set.

* See footnote to Table.

[5] A. K. Bose, B. Lal, W. A. Hoffman, and M-S. Manhas, *Tetrahedron Letters*, 1973, **18**, 1619.

[6] G. A. Webb, ' Annual Reports on N.m.r. Spectroscopy,' Vol. 6A, Academic Press, 1975; ' Nuclear Magnetic Resonance Shift Reagents,' ed. R. E. Sievers, Academic Press, London, 1973.

3β- and 3α-alcohols in the two series (see earlier) and a large difference between those of each 3β/3α pair; hence, once reference data are available, optical rotation measurement is the most accurate and convenient method for analysing mixtures of such similar diastereoisomeric alcohols.

EXPERIMENTAL

General directions were as described in *J. Chem. Soc.(C)*, 1968, 2674 except that routine ^1H n.m.r. spectra were recorded at 100 MHz. Petrol refers to light petroleum, b.p. 60—80 °C. The constants and main spectrometric properties of compounds are shown in the Scheme and are repeated here only when required by the context.

Epimerisation of 19-*Norergosta*-5,7,9-*trien*-3β-*ol* (*Dihydroneoergosterol*) (1a).—(*a*) *Three-stage sequence* (*see Scheme*). Reactants, solvents, and apparatus required in the first stage were carefully dried, and atmospheric moisture was excluded during this stage.

A solution of 19-norergosta-5,7,9-trien-3β-yl methanesulphonate (following paper; 6 g) in Me_2CO (240 ml) was contained in a dry box under N_2. $NEt_4^+HCO_2^-$ (10 g) was weighed in the box and added to the solution, which was then removed from the box, boiled under reflux for 12 h, and evaporated. The residue was partitioned between H_2O and C_6H_6, and the organic layer was washed twice with water, and evaporated. Saturated aqueous $NaHCO_3$ (22 ml) and MeOH (420 ml) were added, and the mixture was boiled under reflux for 1 h. After evaporation the residue was extracted with Et_2O to give material which was adsorbed on to neutral Al_2O_3 (200 g). Elution with petrol gave olefinic material (2.9 g). Et_2O eluted material which crystallised from MeOH to give 19-norergosta-5,7,9-trien-3α-ol (dihydroepineoergosterol) (2a) (1.62 g) (Found: C, 84.8; H, 11.2. Calc. for $C_{27}H_{42}O$: C, 84.75; H, 11.1%).

(*b*) *Two-stage sequence* (*see Scheme*). Diethyl diazodicarboxylate (309 mg) was added to a solution of the 3β-alcohol [7] (1a) (300 mg), PPh_3 (450 mg), and $PhCO_2H$ (195 mg) in dry tetrahydrofuran (6 ml). The solution was kept in a stoppered flask at 20 °C for 22 h, and then applied to a 1-m SiO_2 p.l.c. plate which was developed with petrol–Me_2CO. The material in the band of highest R_F was collected and applied to a second plate which was developed with petrol. The material of lowest R_F was collected and crystallised from Et_2O to give 19-*norergosta*-5,7,9-*trien*-3α-*yl benzoate* (2c) (181 mg) (Found: C, 83.9; H, 9.4. $C_{34}H_{46}O_2$ requires C, 83.9; H, 9.5%). Hydrolysis of this material (140 mg) as described later gave the 3α-alcohol (2a) (94 mg), m.p. 164—165 °C, $[\alpha]_D$ +48° (*c* 0.8).

Esters of the Epimeric Alcohols (1a) *and* (2a).—*Compounds* (1b) *and* (2b). Treatment of the 3β-alcohol (1a) (200 mg) with Ac_2O (2 ml)–C_5H_5N (2 ml) at 90 °C for 1 h gave the 3β-acetate (1b) (195 mg, from EtOH). The 3α-alcohol (2a) (200 mg) similarly gave the 3α-acetate (2b) (191 mg, from Et_2O).

Compounds (1c) *and* (2c). A solution of the 3β-alcohol (1a) (200 mg) in BzCl (0.15 ml)–C_5H_5N (2 ml) was kept at 20 °C for 2 d. Work-up gave 19-*norergosta*-5,7,9-*trien*-3β-*yl benzoate* (1c) (210 mg, from petrol) (Found: C, 83.6; H, 9.5. $C_{34}H_{46}O_2$ requires C, 83.9; H, 9.5%). The 3α-alcohol (2a) (200 mg) similarly gave the 3α-benzoate (2c) (212 mg, from Et_2O).

Separate solutions of these esters (150 mg) in MeOH (10 ml) containing KOH (200 mg) were boiled under reflux for 1 h. Work-up gave the alcohols (1a) and (2a) in 90% yield.

Compounds (1d) *and* (2d). A solution of the 3β-alcohol (1a) (500 mg) and 3,5-dinitrobenzoyl chloride (450 mg) in C_5H_5N (6 ml) was kept at 40 °C for 1 d. Work-up gave the 3β-ester (1d) (678 mg, from EtOAc) (Found: C, 70.6; H, 7.7. Calc. for $C_{34}H_{44}N_2O_6$: C, 70.8; H, 7.7%). The 3α-alcohol (2a) (500 mg) similarly gave 19-*norergosta*-5,7,9-*trien*-3α-*yl* 3,5-*dinitrobenzoate* (2d) (672 mg, from EtOAc) (Found: C, 70.5; H, 7.7%).

Separate solutions of these esters (200 mg) in MeOH (30 ml)–saturated $NaHCO_3$ aq. (1 ml) were boiled under reflux for 3 h. Work-up gave the alcohols (1a) and (2a) in 85% yield.

Two crystallisations from EtOAc of a mixture of the ester (1d) (180 mg) and the ester (2d) (20 mg) gave the ester (1d) (105 mg), m.p. 219—221 °C, $[\alpha]_D$ −3 °C (*c* 0.6). A mixture of the ester (2d) (180 mg) and the ester (1d) (20 mg) similarly gave the ester (2d) (98 mg), m.p. 166—167 °C, $[\alpha]_D$ +47° (*c* 0.7).

Compounds (1e) *and* (2e). A solution of the 3β-alcohol (1a) (125 mg) and (+)-camphor-10-sulphonyl chloride [8] (100 mg) in C_5H_5N (2 ml) was kept at 20 °C for 2 d. Work-up gave 19-*norergosta*-5,7,9-*trien*-3β-*yl* (+)-*camphor*-10-*sulphonate* (1e) (175 mg, from petrol) (Found: C, 75.4; H, 7.8. $C_{37}H_{46}O_4S$ requires C, 75.7; H, 7.9%). The 3α-alcohol (2a) (125 mg) similarly gave 19-*norergosta*-5,7,9-*trien*-3α-*yl* (+)-*camphor*-10-*sulphonate* (2e) (171 mg, from petrol) (Found: C, 75.5; H, 7.7%).

Compounds (1f) *and* (2f). Following the published method,[9] (±)-α-methoxytrifluoromethylphenylacetic acid was treated with (+)-α-methylbenzylamine, $[\alpha]_D$ +37.5° (liquid), and the less soluble salt was crystallised three times from EtOH to give the α-methylbenzylammonium salt, m.p. 193—195 °C, $[\alpha]_D$ +62° (*c* 0.8) (lit.,[9] m.p. 195—198 °C, $[\alpha]_D$ +59.1°). The acid was liberated, dried, and treated with $SOCl_2$ to give (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride, b.p. 56—58 °C/1 mmHg (lit.,[9] 54—56 °C/1 mmHg), $[\alpha]_D$ +94° (*c* 0.8), $\nu_{max.}$ (liquid film) 1 790 cm^{-1}.

A solution of the 3β-alcohol (1a) (200 mg) and the foregoing chloride (170 mg) in CCl_4 (1 ml)–C_5H_5N (1 ml) was kept at 20 °C for 2 d. Work-up gave 19-*norergosta*-5,7,9-*trien*-3β-*yl* (+)-α-*methoxy*-α-*trifluoromethylphenylacetate* (1f) (245 mg, from petrol) (Found: C, 73.9; H, 8.3. $C_{37}H_{49}F_3O_3$ requires C, 74.2; H, 8.2%). The 3α-alcohol (2a) (200 mg) similarly gave 19-*norergosta*-5,8,9-*trien*-3α-*yl* (+)-α-*methoxy*-α-*trifluoromethylphenylacetate* (2f) (251 mg, from petrol) (Found: C, 74.0; H, 8.2%).

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[8] P. D. Bartlett and L. H. Knox, *Org. Synth.*, 1965, **45**, 14.

[9] J. A. Dale, D. L. Dull, and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.