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CONVERSION OF RUSCOGENIN INTO 1α- AND 1β-HYDROXYCHOLESTEROL DERIVATIVES

STRUCTURE ELUCIDATION BY COMPUTER ASSISTED ANALYSIS OF THEIR LANTHANIDE-INDUCED NMR SHIFTS

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Abstract—The chemistry of ruscogenin (**1**) was studied since its structural features qualify it to serve as a potential starting material for 1-hydroxy vitamin D analogs. Ruscogenin was oxidized to the 1-oxo-derivative **2** which was reduced to a mixture of ruscogenin (**1**) and 1-epiruscogenin (**3**). Both **1** and **3** were converted by Clemmensen reduction to the respective tetrols **12** and **21**, which were further reduced to the triols **15** and **25** and diols **16** and **24** by consecutive treatment with *p*-toluenesulfonyl chloride and LAH. The reduction of triol **15** to diol **20** was achieved by selective benzoylation of positions 1 and 3, and mesylation of position 16 followed by LAH reduction. The utility of the shift reagent Eu(dpm)$_3$ to determine the structures of the products was studied. It was shown that the shifts induced are characteristic of the position and orientation of the OH groups, and can facilitate the elucidation of the structures of hydroxylated steroids.

The importance of the 1α-OH group for vitamin D-like activity was recognized in the early 1970's, when 1α,25-dihydroxy vitamin D$_3$, an active metabolite of vitamin D$_3$, was isolated and characterized.[1-3] Subsequently, it was found that 1α-hydroxy vitamin D$_3$ is very similar in its biological activity to that of the 1α,25-dihydroxy derivative.[4,5] This has been interpreted in terms of fast *in vivo* hydroxylation of the former at the 25 position.[6]

Hydroxylation at the 1α position takes place in the kidney. It may be absent, or decreased, in case of impaired rhenal function. Hence, synthetic 1α-hydroxy-vitamin D is of clinical importance.

All syntheses of 1α-OH vitamin D reported so far introduce the 1α-OH group onto a variety of steroidal starting materials. The novelty of our approach reported in this paper is that it is based on the use of a natural steroid containing a 1-OH group.

Ruscogenin (**1**) contains an OH group at the C-1 position, a 5,6-double bond, and it has all the C atoms of a cholesterol type side chain, although in oxidized form. Epimerization of the 1β-OH group in ruscogenin and conversion of the spiro-ketal moiety by reductive treatment to side chains containing one, two or no OH groups would yield 1α-hydroxycholesterol or its hydroxylated derivatives, which could further be transformed to the 1α-hydroxy vitamin or its analogs by known methods.

The present paper is concerned with a novel synthesis of 1β-hydroxycholesterol, the epimerization of the 1β-OH group in ruscogenin, the transformation of its side chain, and the determination of the structures of the various hydroxysteroids obtained in both the 1α- and 1β-hydroxy series.

Epimerization of ruscogenin at the C-1 position. This can be carried out in two steps: oxidation at the C-1 position in ruscogenin to 3β-hydroxyspirost-5-ene-1-one (**2**) and reduction of the keto group in **2** to a mixture of 1α- and 1β-epimers.

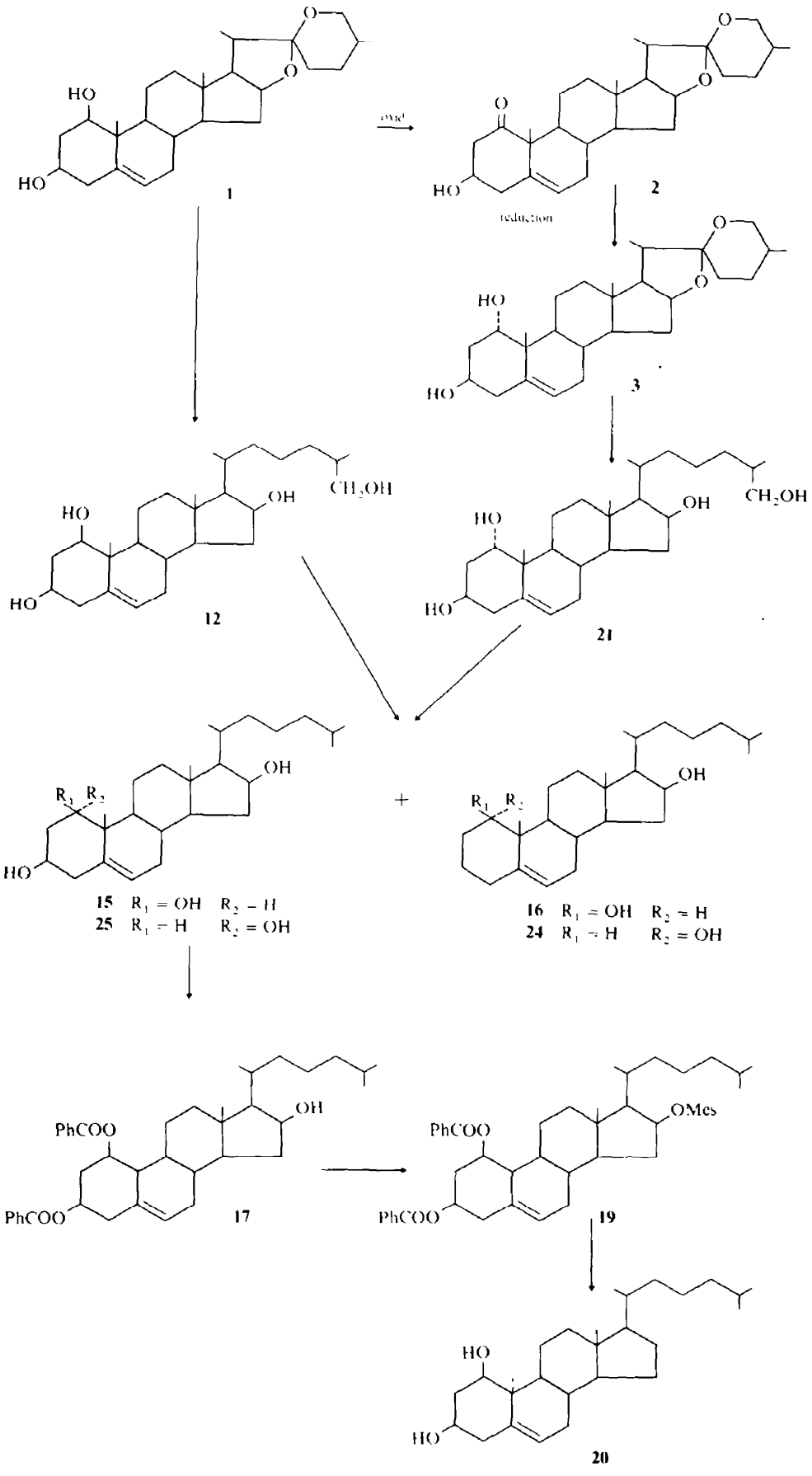
Ruscogenin has previously been oxidized at the C-1 position by Shoppee *et al.*[7] and Kemp[8] both using Jones reagent. Deviation from the precisely defined conditions however might bring about the formation of the 1,3-diketone. Therefore, it is necessary to control exactly the duration and temperature of the reaction and the amount of reagent. We found that the use of pyridinium chlorochromate offers a convenient alternative for this oxidation, giving the desired product (**2**) in a high yield and with no contamination with the 1,3-diketone.

Little is known about the steric course of the reduction of 1-keto groups in a steroid.[9] The results found in the literature for the reduction of cholestan-1-one along with those for 1-oxo-diosgenin (**2**) are listed in Table 1. From this Table it is apparent that there is a predominant formation of the 1α-hydroxy epimer in all cases.

The percentage of the 1α-epimer decreases with the increase in the bulkiness of the reducing agent, (cf entries 1 to 2 and 3 to 5) and with the increase of the size of the 3β-substituent (cf 3 to 4). The results are consistent with the principle of product development control assuming that non-bonded interactions between the 11α (equatorial) H and the 1β-equatorial OH group would cause the 1β-epimer to be thermodynamically unfavoured. Consequently, 1-epiruscogenin (**3**) was best obtained by sodium borohydride reduction of **2**. Separation of the epimers **1** and **3** was accomplished by chromatography on silica gel.

Transformation of the side chain. The conversion of the spirostan side chain of diosgenin (**4**) to a cholesterol one has previously been accomplished by Marker using a tedious multistep route in low yield.[12]

We set out to develop the spirostane side chain degradation using first diosgenin as a model due to reasons of availability. Following Marker, the Clemmensen reduction of **4** gave the triol cholest-5-ene-3β, 16β-26-triol (**5**). For the conversion of **5** to cholesterol it is necessary to protect selectively the 3-OH group. Since this is impossible to achieve by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15 R1 = OH R2 = H
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OH
16 R1 = OH R2 = H
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PhCOO
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OH
17
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19
HO
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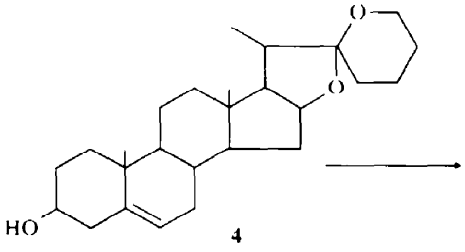
starting from **5**, and since during the Clemmensen reduction of diosgenin acetate, the acetate undergoes hydrolysis, milder modifications of the Clemmensen reduction[13] were tried but gave no satisfactory results. Consequently we decided to take advantage of the fact that the 16β position in **5** is the most sterically hindered one. Benzoylation of **5** gave cholest-5-ene-3β,26-dibenzoyloxy-16β-ol (**6**). Mesylation of **6** gave **7** which upon reduction by LAH afforded 26-hydroxy-cholesterol (**8**). Tosylation of **8** using an equimolar amount of *p*-toluenesulfonyl chloride gave **9** which was reduced with LAH to cholesterol (**10**). It is worthy of note that tosylation of the triol **5** using one equivalent of tosylchloride followed by LAH reduction gave 16-hydroxy-cholesterol (**11**).



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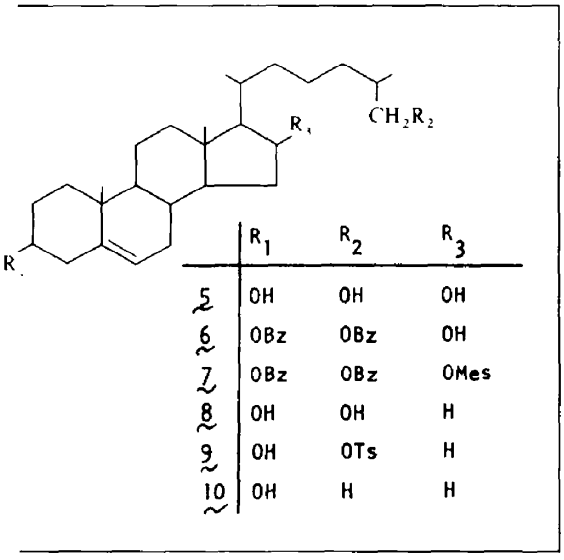


	R_1	R_2	R_3
5	OH	OH	OH
6	OBz	OBz	OH
7	OBz	OBz	OMes
8	OH	OH	H
9	OH	OTs	H
10	OH	H	H

By analogy to the reaction with diosgenin (**4**) Clemmensen reduction of ruscogenin (**1**) afforded the tetrol **12** which was characterized as tetraacetate and tetrabenzoate. Attempts to follow the course developed for diosgenin in this case failed since it was not found possible to protect selectivity the OH groups at positions 1 and 3 in order to remove those at 16 and 26. Attempts to benzoylate **12** selectively led to mixtures of the 3,26-dibenzoate and 1,3,16,26-tetrabenzoate. Consequently, **12** was reacted with one equivalent of *p*-toluenesulfonylchloride to give the 26-tosylate (**13**) and 3,26-ditosylate (**14**). Reductions of these tosylates with LAH gave 1β, 3β, 16β-triol (**15**) and 1β, 16β-diol (**16**) respectively. Benzoylation of triol **15** with controlled amount of benzoyl chloride gave 1,3-dibenzoate (**17**) as the main product with some 1,3,16-tribenzoate (**18**). Mesylation of **17** gave **19** which was reduced to 1β-hydroxycholesterol (**20**).[20]

The side chain degradation of 1-epiruscogenin (**3**) followed the same course as that of ruscogenin. Tetrol **21** was tosylated to a mixture of **22** and **23** which was reduced without separation to a mixture of diol **24** and triol **25** that could easily be separated by chromatography. All 1α-hydroxy derivatives were characterized also as the corresponding acetates.

Structure determination of products. NMR spectroscopy is a helpful diagnostic tool for the determination of the position of a 26-OH group on a cholesterol side chain. All 26-hydroxysteroids prepared in this work exhibit a characteristic doublet of two protons at around 3.50 ppm. In the respective acetates or benzoates this doublet is shifted to 3.90 ppm. Consequently, it was easy to confirm spectroscopically the removal of the 26-OH group from compounds **8**, **12**, and **21**. In contrast the protons α to oxygen at positions 1, 3 and 16 appear as ill defined broad signals often overlapping each other and therefore difficult to identify conclusively by NMR spectroscopy. Consequently there was some uncertainty as regards the structure of **20** which could have either the structure shown or that of the 3β, 16β-dihydroxy isomer.

In order to establish the structure of **20** it was decided to use a lanthanide shift reagent.[14] We chose tris-(dipivalomethanato)-europium [Eu(dpm)$_3$] as the shift reagent for the study of our system. This lanthanide shift reagent was shown by Demarco *et al.*[15] to coordinate at the 2-OH group of 5-androstane-2β-ol and the 3-OH group of friedlan-3β-ol.

To solutions of compounds **1**, **3**, **4**, **16**, **24**, **15** and **25** were added portions of a solution of Eu(dpm)$_3$ and the spectra were determined after the addition of each portion. The chemical shifts, $\Delta\delta$, obtained were plotted against the molar ratio of the shift reagent to substrate which never exceeded 0.36. The straight lines obtained in these titrations indicate the formation of 1:1 complexes between substrates and the lanthanide shift reagent.[16] The slopes of the straight lines obtained from the plots of Me-19, H-1, H-3, H-6 and Me-18 are summarized in Table 2.

Examination of this Table reveals that the slope which has the highest diagnostic value for 1-hydroxysteroids is that of Me-19. The value obtained for the shift of this signal is the highest for a 1β-hydroxysteroid like ruscogenin (**1**) (Table 2 entry 1) in which the OH group is equatorially oriented (Fig. 1a). In contrast the corresponding value is much lower in the 1α-hydroxy epimer (Table 2, entry 2) (Fig. 1b) in which the 1-OH group and the 19-Me are *trans* related. In the absence of 1-OH group (Fig. 1c) the shift is reduced to half the value of that of the 1α-epimer (diosgenin, Table 2 entry 3). These values are reconfirmed by the other 1α- and 1β-hydroxysteroids examined as shown by entries 4–7 in Table 1, although some differences are apparent.

Table 1. Reductions of 1-ketosteroids

No.	Substrate	Reagent	Products	
1.	Cholestane-1-one	$NaBH_4$	100%α[10]	
2	Cholestane-1-one	$LiAlH_4$	52%α	28%β[11]
3	3β-hydroxyspirost-5-ene-1-one (2)	$NaBH_4$	70%α	30%β
4	3β-acetoxyspirost-5-ene-3-one (2a)	$NaBH_4$	66%α	34%β
5	3β-hydroxyspirost-5-ene-3-one (2)	LTBH*	60%α	40%β

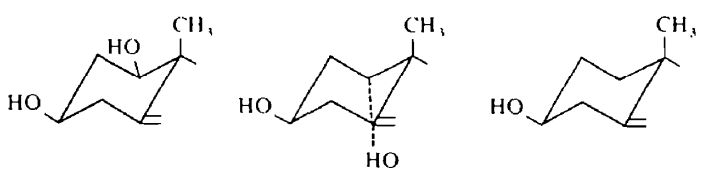
* Lithium aluminum tri-t-butoxyhydride

Table 2. The magnitudes of the slopes derived from the plots of the paramagnetic shifts against the lanthanide/substrate molar ratio, obtained for hydroxysteroids

		SLOPE				
No.	Compound	Me-19	Me-18	H-1	H-3	H-6
1	1	2.15	0.41	4.58	6.02	1.21
2	3	1.41	0.34	3.91	5.10	1.12
3	4	0.77	0.16	-	4.24	0.48
4	16	3.85	2.12	6.00	-	
5	24	1.59	0.96	5.00	-	
6	15	2.73				1.22
7	25	1.50		3.08	4.47	1.00
8	20	2.27				

The shifts obtained for 1, 16-dihydroxysteroids **16** and **24** are considerably larger than those obtained for 3-hydroxysteroids. This is consistent with the assumption that the OH group with the highest ability for complexation is that in the 3 position. When the 3-OH group, that competes most strongly for complexation, is removed, relatively more transition metal is complexed with the 1-position resulting in high paramagnetic shifts of the neighboring protons (see values for Me-19 and H-1).

The fact that there is also some complexation involving the hindered 16β-position is indicated by the values of the shifts obtained for the 18-Me groups (cf entries 4 and 5 to 1 3) and the split observed in the signal corresponding to the 25 and 27-Me groups. This split indicates that in the complex derived from the 16β-hydroxysteroid there is magnetic non equivalence of these Me groups presumably due to inhibition of free rotation of the steroid side chain. Furthermore, it appears that this split can be used as a diagnostic tool



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for the identification of a 16β-OH group. The shifts of other hydrogens in ring A are of lesser significance. The vinylic proton at position 6 is not influenced by the orientation of the 1-OH group but it seems to depend on the number of OH groups in ring A.

Due to the small quantities of compound **20** available for NMR studies, titration of the solution of **20** with $Eu(dpm)_3$ gave very large shifts in the spectra. Consequently it was not possible to assign the peaks in these spectra with a reasonable degree of certainty to the various protons. In order to overcome this difficulty it was necessary to turn to the use of computer simulation of the titration curves for variously substituted hydroxysteroids.

This method enabled us to identify the correct peaks of Me-19 (out of several possibilities in every spectrum) since only one set of induced shifts was found to fit the corresponding calculated equilibrium constants and intrinsic shifts (*vide infra*). From the shifts thus obtained it was possible to arrive at a value of 2.27 for the slope of the Me-19. See Table 2 entry 8. This slope is nearly identical with that obtained for ruscogenin, therefore, it provides evidence for the structure of **20** as a 1β,3β-dihydroxysteroid.

Computer analysis of the titration curves. For the computer simulation we assumed that during the course of titration there exist simultaneous equilibria between the complexes formed (denoted SR) at positions 1 and 3 with a single molecule of $Eu(dpm)_3$ at each site, and substrate (S) and reagent (R) which can be described by the eqn set (1).

$$\begin{aligned} SR(3) &\overset{K_3}{\rightleftharpoons} S + R \\ SR(1) &\overset{K_1}{\rightleftharpoons} S + R \\ SR(1)R(3) &\overset{K_3}{\rightleftharpoons} SR(1) + R \\ SR(1)R(3) &\overset{K_1}{\rightleftharpoons} SR(3) + R \end{aligned} \qquad (1)$$

Coordination sites 1 and 3 were characterized by their dissociation constants K_1 and K_3 respectively assuming that the second lanthanide molecule does not alter the dissociation constant for the first one. Thus, the observed lanthanide induced shift of Me-19 is the sum of two contributions given by eqn (2), where

$$\delta = \frac{\Delta 1}{St}([SR(1)] + [SR(1)R(3)]) + \frac{\Delta 3}{St}([SR(3)] + [SR(1)R(3)]) \qquad (2)$$

Δ_1 and Δ_3 are the intrinsic shifts of SR(1) and SR(3) complex species, in which the lanthanide is bound at positions 1 and 3 respectively (the subscript t denotes total concentration and the square brackets equilibrium concentrations).

The analysis of the experimental results (a set of observed shifts and total concentrations) was carried out as follows: Solution of the following compounds at constant concentrations was titrated by $Eu(dpm)_3$: diosgenin (**4**), cholest-5-ene-1β, 16β-diol (**16**) and cholest-5-ene-1α, 16β diol (**24**). All these compounds have a single coordination site on ring A. (Position 16 is considerably more hindered for complex formation than positions 1 and 3). The observed chemical shifts of the 19-Me groups in each compound were analysed utilizing a computer program for the calculation of the concentrations of all the species in an equilibrium system as described by Reed *et al.*[21] A dissociation constant K_1 or K_3 was chosen and the corresponding equilibrium concentrations were calculated by the iterative process. These values are used in eqn (2) to determine the intrinsic shifts $\Delta_{1\alpha}$ and $\Delta_{1\beta}$ respectively for every point in the titration curve. The average values for the intrinsic shifts (Δ) are computed and the induced chemical shift values of Me-19 are recalculated and compared with the observed δ values to compute the standard deviation.[17] The cycle is repeated varying the values of K_1 or K_3 over a wide range until a minimum in the standard deviation is obtained.[18] The results ($K_{1\alpha}$, $K_{1\beta}$, $K_{3\beta}$, $\Delta_{1\alpha}$, $\Delta_{1\beta}$ and $\Delta_{3\beta}$) are summarized in Table 3 entries 1, 2 and 3.

This set of parameters corresponding to these minima are considered to be the best ones describing the data for a single hydroxylic coordination site of positions 1α, 1β or 3β and $Eu(dpm)_3$ in this system. Since the compound of interest, **20**, has two sites of coordination in ring A we proceeded by using an identical analysis of the observed results for two similar compounds, ruscogenin (**1**) and 1-epiruscogenin (**3**). We assumed that the intrinsic shifts $\Delta_{1\alpha}$, $\Delta_{1\beta}$ and $\Delta_{3\beta}$ will not change with the variation of the side chain, while the values of the dissociation constants, k's, might vary from compound to compound. We used the previously obtained Δ_i values, have chosen K_3 values and varied the corresponding values of K_1. The set of four parameters which give the minimum in the standard deviation (σ) for these two compounds are also listed in Table 2 (entries 4 and 5). Thus, all four parameters necessary for the analysis of the titration

Table 3. Equilibrium constants and intrinsic chemical shifts for complexes of $Eu(dpm)_3$ with various hydroxy steroids

No.	Compound	$K_{1\alpha}10^{-3}$	$K_{1\beta}10^{-3}$	$K_{3\beta}10^{-3}$	$\Delta_{1\alpha}$ ppm	$\Delta_{1\beta}$ ppm	$\Delta_{3\beta}$ ppm
1	**4**			2.0±1.0			0.75±0.08
2	**16**		3.0±1.0			3.4±0.14	
3	**24**	2.0±1.0			1.4±0.1		
4	**1**		2.2±0.7	3.0±1.0		3.4	0.75
5	**3**	1.0±1.0		2.0±0.2	1.4		0.75
6	**20**		7.0±0.2	1.0±0.1		3.4	0.75

curve of compound **20** were available. Using the values of the intrinsic shifts $\Delta_{1\beta}$ and $\Delta_{3\beta}$, choosing the value of K_3 and varying in a wide range the value of K_1 its titration curve could be reconstructed. An excellent fit of 5% in the value of δ with a sharp minimum was obtained for the set of four parameters listed in Table 3, entry 6. These parameters give the best description of the coordination of compound **20** with Eu(dpm)$_3$. These data reconfirm the structure of **20**.

EXPERIMENTAL

Preparation of ruscogenin (**1**). Ruscogenin (**1**) was prepared from a commercially available (Inverni and Della Beffa, Milano, Italy) 1:1 mixture with neoruscogenin (spirost-5,25(27)-diene-1β,3β-diol) by selective reduction of the 25 27 double bond by catalytic transfer hydrogenation as follows: 1 g of Pd/C 5%, 80 ml EtOH, and 2 ml cyclohexene were refluxed for 1 hr. A soln of 4 g of a 1:1 mixture of ruscogenin and neoruscogenin in 20 ml EtOH was added and refluxed for 16 hrs. Filtration and evaporation gave 3.58 g of white crystals (recovery 90%). GC analysis of the silylated product (on a 1% XE 60 on Gas Chrom Q 100 120 mesh column at 230°) showed only the peak corresponding to ruscogenin.

Spirost-5-ene-1-oxo-3β-ol (**2**). 0.107 g of **1** and 0.080 g pyridinium chlorochromate were dissolved in 2 ml CH_2Cl_2 and the mixture was allowed to stand at room temp for 2½ hr. Water was added, the soln was concentrated and the resulting ppt was filtered off. Crystallization from MeOH gave **2** (0.100 g, 95%) m.p. 200°; IR ($CHCl_3$): ν 1705, 921, 901 cm^{-1}; NMR ($CDCl_3$): δ 0.80 3 H s, 1.30 3 H s, 1.60 3 H s, 3.36–3.38 2 H m, 3.87 1 H m, 4.40 1 H m, 5.53 14 H m.

3β-Acetoxyspirost-5-ene-1-one (**2a**). 0.22 g of **2** was acetylated to yield 0.021 g (87.5%) of **2a** m.p. 199°; IR ($CHCl_3$): 1750, 1655 cm^{-1}; NMR ($CDCl_3$): δ 0.80 3 H s, 1.28 3 H s, 1.60 3 H s, 2.00 3 H s, 4.80 1 H m, 5.68 1 H m; MW. Calc. 470. Found (MS) *m/e* = 470 (2%), *m/e* = 410 (35%), *m/e* = 408 (58%) *m/e* = 296 (100%).

Reductions of spirost-5-ene-1-oxo-3β-ol derivatives

a. *Reduction of* **2** *by sodium borohydride.* Sodium borohydride was added in a large excess (0.100 g) to a soln of 0.100 g of **2** in 15 ml MeOH. The soln was allowed to stand at room temp for 2 hrs and then neutralized carefully with dil HCl. Most of the solvent was taken out by evaporation. Water was added and the product was filtered and dried. Recrystallization from MeOH gave 0.090 g of white solid, a mixture of the epimers **3**, 70% and **1**, 30%. The two epimers were separated by dry column chromatography over silica gel. Elution by a mixture of EtOAc (70%) petroleum ether 80–100° (30%), gave **1** followed by the slightly more polar **2**, m.p. 190–191°; IR ($CHCl_3$): 3500, 1440, 1370 cm^{-1}. NMR ($CDCl_3$): δ 0.79 3 H s, 1.04 3 H s, 3.40 2 H m, 3.82 1 H t, 3.95 1 H bm, 4.38 1 H m, 5.59 1 H m. MW calc. 430. Found (MS) *m/e* = 430 (10%), *m/e* = 412 (10%), *m/e* 316 (100%); (Found: C, 75.68; H, 9.75; Calc. for $C_{27}H_{42}O_4$: C, 75.37; H, 9.92%).

b *Reduction of* **2** *by lithium aluminum tri-t-butoxyhydride.* To a cooled soln of 0.054 g (0.125 mM) **2** was added with stirring a soln of lithium aluminum tri-t-butoxyhydride (0.005 mol) in THF. The mixture was kept 1 hr at this temp and another hr at room temp, and was decomposed with 5% AcOH, extracted with ether, dried over Na_2SO_4 and evaporated to give 0.052 g white solid, a mixture of the epimers **1** and **3**. The epimers were separated by chromatography over silica plates to give **1** (40%) and **3** (60%).

c. *Reduction of* **2a** *by lithium aluminum tri-t-butoxyhydride.* 117.5 mg (0.25 mM) of **2a** were reduced as described above to give 96 mg of white crystals, a mixture of **1**, 34% and **3**, 66% which was separated as above.

1α,3β-Diacetoxyspirost-5-ene (**3a**). 0.508 g of **3** were acetylated to yield 0.442 g (72%) of **3a** white crystals, m.p 149–151°; IR ($CHCl_3$): 1700–1720, 1440, 1360 cm^{-1}; NMR ($CDCl_3$): δ 0.77 3 H s, 1.09 3 H s, 1.60 3 H s, 2.00 3 H s, 2.02 3 H s, 3.42 2 H m, 4.42 1 H bm, 4.87 1 H bm, 5.03 1 H t, 5.55 1 H m. MW calc. 514. Found (MS) *m/e* = 514 (3%), *m/e* = 454 (5%), *m/e* = 394 (100%), *m/e* = 325 (63%); (Found: C, 72.10; H, 8.98; Calc. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01%).

Cholest-5-en-3β,16β, 26-triol (**5**). Diosgenin **4** (20 g) was subjected to Clemmensen reduction according to the procedure described in detail for the reduction of ruscogenin. This reduction gave **5** (20%) m.p. 178°; IR (KBr): 3350 cm^{-1}. NMR ($CDCl_3$): δ 3.46 3 H m, 4.36 1 H m, 5.33 1 H m, MW calc. 418. Found (MS) *m/e* = 418 (13%); *m/e* = 400 (100%) *m/e* = 382 (30%) *m/e* = 271 (97%). These physical constants are in good agreement with those described in the literature.[19]

3β,26-Dibenzoyloxycholest-5-en-16β-ol (**6**). 11.1 g (0.0027 mol) of **5** were benzoylated using 80 ml pyridine and 12 ml benzoyl chloride. After the usual workup crystallization gave 12.8 g of a product mixture which was separated by dry column chromatography over silica gel. Elution by a mixture of EtOAc (15%) petroleum ether 80–100° (**85**%) gave 5.9 g of **6**, m.p. 150°; IR ($CHCl_3$): 3500 cm^{-1}. NMR ($CDCl_3$): δ 4.25 2 H d, 4.37 1 H m, 4.90 1 H m, 5.50 1 H m, 7.20–7.45 6 H m, 7.76–8.16 4 H m. MS Found: *m/e* = 502 (40%), *m/e* = 486 (30%) *m/e* = 471 (20%) *m/e* = 380 (100%); $[\alpha]_D^{25} = -7.1°$ ($CHCl_3$). (Found: C, 79.33; H, 8.58; Calc. for $C_{41}H_{54}O_5$C, 78.59; H, 8.78%).

3β,26-Dibenzoyloxycholest-5-ene-16β-ol mesylate (**7**). A soln of methanesulfonyl chloride (2 ml) in 10 ml pyridine was added to a soln of **6** (1.03 g; 1.6 mM) in 10 ml dry pyridine at 0°. The mixture was kept at 0° overnight. The red soln was poured into ice water and the resulting mixture extracted with ether. The ether extract was washed successively with 2N cold HCl, 2% $NaHCO_3$ aq, water, and dried over $MgSO_4$. Evaporation gave 1.626 g of **7**. NMR ($CDCl_3$): δ 2.96, 3 H s, 4.23 2 H d, 4.90 1 H m, 5.20 1 H m, 5.46 1 H m, 7.63–7.40 6 H m, 8.0–8.2 4 H m.

Cholest-5-ene-3β,26-diol (**8**). **7** (1.63 g) was dissolved in 80 ml dry ether and reduced by LAH as described in the preparation of **15** and **16**, to give 0.653 g (100%) of **8**, m.p. 170°. NMR ($CDCl_3$): δ 3.50 3 H m, 5.33 1 H m, MW calc. 402. Found (MS) *m/e* = 402 (35%), *m/e* = 384 (100%), *m/e* = 369 (18%); $[\alpha]_D^{22} = -34.8$ ($CHCl_3$). (Found: C, 80.00; H, 11.43; Calc. for $C_{27}H_{46}O_2$: C, 80.10; H, 11.44%)

Cholest-5-ene-3β-ol (**10**). (0.158 g, 0.39 mM) was subjected to tosylation using equimolar amount of *p*-toluenesulfonyl chloride, and to LAH reduction according to the procedure described for **15**, to give white crystals of **10** which were identical in all respects with a commercial sample of cholesterol.

Cholest-5-ene-1β,3β,16β,26-tetrol (**12**). A soln of **1** (5.00 g, 0.016 mol) in 500 ml 95% EtOH was treated with 160 g amalgamated Zn and the mixture was heated to boiling. Then 160 ml HCl was added slowly over a period of 2½ hr to the boiling mixture, which was refluxed for an additional ½ hr. The soln was poured into water and the resulting mixture extracted with ether. The ether extract was successively washed with $NaHCO_3$ aq, water, dried over $MgSO_4$, and evaporated. The residue was crystallized from EtOAc to give compact white crystals of **12** (1.52 g, 33%), m.p. 200°, MS: *m/e* = 416 (M-H_2O, 60%), *m/e* = 414 (90%), *m/e* = 398 (30%). (Found: C, 74.74; H, 10.32; Calc. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.64%).

1β,3β,16β,26-Tetracetoxycholest-5-ene (**12a**). **12** (1.00 g, 2.3 mM) was acetylated with Ac_2O pyridine. Crystallization from pentane gave white crystals of **12a** (0.52 g, 0.87 mM, 38%) m.p. 105–106°, IR ($CHCl_3$): 1700 cm^{-1}. NMR ($CDCl_3$): δ 0.90 3 H s, 1.17 3 H s, 2.00 12 H s, 3.90 2 H d, 4.70 2 H m, 5.20 1 H m, 5.60 1 H m. MS: *m/e* = 542 (M-CH_3COOH, 1%), *m/e* = 482 (M-2CH_3COOH, 100%). *m/e* = 422 (M 3CH_3COOH, 60%). (Found: C, 69.14; H, 9.10; Calc. for $C_{35}H_{54}O_8$: C, 69.74; H, 9.03%).

1β,3β,16β,26-Tetrabenzoyloxycholest-5-ene (**12b**). **12** (1.00 g, 2.3 mM) was benzoylated with benzoyl chloride in pyridine to yield white crystals of **12b**, (0.40 g, 0.66 mM 29%). m.p. 83°, IR ($CHCl_3$): 1710, 1280 cm^{-1}. NMR ($CDCl_3$): δ 3.85 2 H d, 5.00 2 H m, 5.45 1 H m, 5.65 1 H m, 7.40 12 H bm, 7.93 8 H

bm. M.W. Calc. 850. Found (MS): *m/e* = 850 (< 1 %), *m/e* = 728 (M–PhCOOH, 1.5%), *m/e* = 606 (M–2PhCOOH, 90%), *m/e* = 484 (M–3PhCOOH, 100%); $[\alpha]_D^{25} = +20.5°$ ($CHCl_3$) (Found: C, 77.01; H, 7.47; Calc. for $C_{55}H_{62}O_8$: C, 77.65; H, 7.29%).

Cholest-5-ene-1β,3β,16β-triol (**15**) *and cholest-5-ene-1β-diol* (**16a**). A soln of *p*-toluenesulfonyl chloride 0.457 g (0.0022 mol) in 3 ml pyridine was added over a period of 1 hr to a soln of 0.868 g (0.002 mol) of **12** in 15 ml dry pyridine which was cooled to −10° while maintaining the temp between −6 and −10°. The mixture was kept at room temp (23°) overnight. The soln was poured into ice water and the resulting mixture extracted with ether. The ether extract was washed successively with cold 5% HCl, 2% $NaHCO_3$ aq. water, and dried over $MgSO_4$. Evaporation gave 1.050 g of white foam as a mixture of **13** and **14**. 5.6 g of this mixture were dissolved in dry ether and was added dropwise to a mixture of excess LAH in 100 ml dry ether. The mixture was refluxed for 16 hr, cooled and decomposed first by the addition of a few drops of EtOAc, followed by 6N HCl. The aqueous layer was separated and washed with ether. The etheral extracts were combined and washed successively with 10% $NaHCO_3$ aq, water, dried over Na_2SO_4 and evaporated to give 3.4 g white crystals, a mixture of **15** and **16**. The products were separated by dry column chromatography over silica gel. Elution by a mixture of EtOAc (65%) petroleum ether 80–100° (35%) gave first 0.46 g diol (**16**). NMR ($CDCl_3$) δ 0.80 3 H s, 0.90 6 H s, 0.96 3 H s, 1.00 3 H s, 3.50 1 H m, 4.30 1 H m, 5.50 1 H m. MW Calc. 402. Found (MS) *m/e* = 402 (8%), *m/e* = 384 (80%), *m/e* = 271 (80%). Further elution gave 1.43 g triol (**15**) m.p. 180°, IR ($CHCl_3$): 3500–3600, 1440, 1370 cm^{-1}; NMR ($CDCl_3$): δ 0.80 3 H s, 0.90 6 H s, 1.00 3 H s, 3.43 2 Hm, 4.26 1 H m, 5.53 1 H m. MW Calc. 418. Found (MS) *m/e* = 418 (2%), *m/e* = 400 (100%), *m/e* 382 (39%). $[\alpha]_D^{25} = -41°$ ($CHCl_3$). (Found: C, 77.23; H, 11.66; Calc. for $C_{27}O_3H_{46}$: C, 77.46, H, 11.07%).

1β,16β-Diacetoxycholest-5-ene (**16a**). (**16**) (0.395 g, 0.98 mM) was acetylated to yield 0.367 g of **16a** (0.75 mM, 76%). m.p. 177–179°. NMR ($CDCl_3$): δ 0.81 3 H s, 0.90 6 H s, 1.00 3 H s, 2.01 6 H s, 4.90–5.40 3 H m; MS. *m/e* = 426 (M − CH_3COOH, 40%), *m/e* = 366 (M − $2CH_3COOH$, 60%), *m/e* = 253 (100%). $[\alpha]_D^{25} = +8.1$ ($CHCl_3$, C = 0.105).

1β,3β,16β-Triacetoxycholest-5-ene (**15a**). (**15**) (0.408 g, 0.99 mM) was acetylated to yield 0.346 g **15a** (0.65 mM, 64%) white crystals m.p. 165°; IR ($CHCl_3$): 1740–1715 cm^{-1}; NMR ($CDCl_3$): δ 0.77 3 H s, 0.87 6 H s, 1.12 3 H s, 1.99 9 H s, 4.3–4.8 2 H m, 4.92–5.38 1 H m, 5.56 1 H m; MS: *m/e* = 424 (*M* − $2CH_3COOH$, 58%), *m/e* = 364 (M − $3CH_3COOH$, 48%), *m/e* = 251 (100%); $[\alpha]_D^{25} = +23.7°$ ($CHCl_3$). (Found: C 70.65; H, 9.65; Calc for $C_{33}H_{52}O_6$: C, 70.43; H, 9.67%).

1β,3β,16β-Tribenzoyloxycholest-5-ene (**18**). (**15**) (0.31 g, 0.74 mM) was benzoylated to yield a mixture which was separated over a dry silica column. Elution with 10% EtOAc in petroleum ether 80–100° gave **18** m.p. 213–215°, 0.185 g, IR ($CHCl_3$). 1700–1730 cm^{-1}; NMR ($CDCl_3$): δ 4.76–5.16 2 H m, 5.37 1 H m, 5.66 1 H m, 7.47 9 H bm and 8.00 6 H bm; MS: *m/e* 608 (M − PhCOOH, 1%), *m/e* = 486 (M–2PhCOOH, 100%), *m/e* 364 (M − 3PhCOOH, 80%), $[\alpha]_D^{25} = +17.3°$ ($CHCl_3$). (Found: C, 77.00; H, 7.70; Calc. for $C_{48}H_{58}O_6.H_2O$: C, 77.01, H, 8.02%). Further elution gave 0.158 g **17**.

1β,3β-Dibenzoyloxycholest-5-ene-16β-ol (**17**). To **15** (0.60 g 1.4 mM) dissolved in 30 ml dry pyridine 0.8 ml (0.968 g) of benzoyl chloride was added dropwise, and the mixture was kept at room temp (25°) for 50 min. After the addition of a few drops of MeOH, the soln was poured into water and extracted with ether. The ether extract was washed successively with 2N HCl water, 2% $NaHCO_3$ aq. water and was dried over $MgSO_4$. Evaporation gave 0.619 g (1.0 mM) (71%) of **17** m.p. 172–174°; IR ($CHCl_3$): 3610, 1700 cm^{-1}; NMR ($CDCl_3$): δ 3.50 1 H m, 4.38 1 H m, 5.42 1 H m, 5.60 1 H m, 7.47 6 H bm and 8.0 4 H bm; MS: *m/e* = 504 (M − PhCOOH, 3%), *m/e* = 400 (20%), *m/e* = 382 (M–2PhCOOH, 100%); $[\alpha]_D^{25} = +11.1$ ($CHCl_3$). (Found: C, 78.37; H, 8.67. Calc. for $C_{41}H_{54}O_4$: C, 78.60 H, 8.63%).

Cholest-5-ene-1β,3β-diol (**20**). A soln of 0.6 ml methanesulfonyl chloride in 3 ml pyridine was added to a soln of 0.510 g (0.8 mM) of **17** in 3 ml dry pyridine at 0°. The mixture was kept at 0° overnight. The red soln was poured into ice water and the resulting mixture extracted with ether. The ether extract was washed successively with 2N cold HCl, 2% $NaHCO_3$ aq. water, and dried over $MgSO_4$. Evaporation gave 0.544 g of **19**. NMR ($CDCl_3$): 3.05 3 H s, 4.35–5.45 3 H bm, 5.70 1 H m, 7.52 6 H bm, 7.954 H m. 0.544 g of **19** was dissolved in 10 ml of dry ether and reduced by LAH as described in the preparation of **15** and **16** to give 0.346 g of crude **20**. The product was purified by dry column chromatography over silica gel (elution by a mixture of EtOAc (15%) in petroleum ether 80–100°). NMR ($CDCl_3$): δ 0.82 3 H s, 0.88 6 H s, 1.02 3 H s, 4.16–4.67 2 H m, 5.45 1 H m, MW calc. 402. Found (MS). *m/e* = 402 (45%), *m/e* = 384 (M − H_2O, 52%), *m/e* = 369 (28%), *m/e* = 271 (65%).

1β,3β-Diacetoxycholest-5-ene (**20a**). **20** (18 mg) was acetylated to yield 7 mg of **20a** as white crystals m.p. 110° Lit.[20] m.p. 96–98° MW calc. 486. Found (MS): *m/e* = 486 (1%), *m/e* = 426 (M − CH_3COOH, 42%), *m/e* = 366 (M − $2CH_3COOH$, 34%), *m/e* = 253 (100%). NMR ($CDCl_3$): δ 0.82 3 H s, 0.90 6 H s, 1.00 3 H s, 2.00 6 H s, 5.0–5.4 3 H m.

Cholest-5-ene-1α,3β,16β,26-tetrol (**21**). **3** (5 g, 0.016 mol) was subjected to Clemmensen reduction, as described for **1**, to give 1.5 g of **21** (0.035 mol, 30%). MS: *m/e* = 416 (M − H_2O, 25%), *m/e* = 398 (M − $2H_2O$, 18%) *m/e* = 370 (M − $3H_2O$, 35%), *m/e* = 287 (100%). (Found: C, 74.41; H, 10.37. Calc. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67%).

1α,3β,16β,26-Tetraacetoxycholest-5-ene (**21a**). **21** (6.9 g) was acetylated to yield 8.3 g (87%) of **21a**. MS: *m/e* = 540 (2%), *m/e* = 480 (100%), *m/e* = 420 (70%).

Cholest-5-ene-1α,3β,16β-triol (**25**) *and cholest-5-ene-1α,16β-diol* (**24**). **21** (4.340 g, 0.01 mol) was converted to **24** and **25** in analogy to the preparation of **15** and **16**. The products were separated by dry column chromatography over silica gel. Elution by a mixture of EtOAc (65%) petroleum ether 80–100° (35%) gave first 1.00 g diol (**24**) m.p. 95°; NMR ($CDCl_3$): δ 0.80 3 H s, 0.88 6 H s, 0.98 3 H s, 3.70 1 H m, 4.30 1 H m, 5.50 1 H m; MW, Calc. 402. Found (MS): *m/e* = 402 (30%), *m/e* = 384 (22%), *m/e* = 270 (54%), *m/e* = 271 (60%), *m/e* = 159 (100%). Further elution gave 1.09 g triol (**25**) m.p. 126–128°, IR ($CHCl_3$) 3500 cm^{-1}; NMR ($CDCl_3$). δ 0.80 3 H s, 0.86 6 H s, 1.00 3 H s, 3.63–4.50 3 H bm, 5.53 1 H m, MW. Calc. 418 Found (MS) *m/e* = 418 (19%), *m/e* = 400 (100%), *m/e* = 386 (19%), *m/e* = 384 (23%), *m/e* = 383 (36%). $[\alpha]_D^{25} = -41.8$ ($CHCl_3$). (Found C, 77.15; H, 11.03; Calc. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07%).

1α,3β,16β-Triacetoxycholest-5-ene (**25a**). **25** (0.730 g, 1.8 mM) was acetylated to yield 0.996 g of **25a** (1.8 mM). NMR ($CDCl_3$): δ 2.00 9 H s, 4.7–5.4 3 H m, 5.55 1 H m. MS: *m/e* = 424 (M − $2CH_3COOH$, 100%), *m/e* = 380 (10%), *m/e* = 364 (M − $3CH_3COOH$, 6%).

1α,16β-Diacetoxycholest-5-ene (**24a**). **24** (0.699 g, 0.17 mM) was acetylated to yield 0.704 g (0.14 mM) 80% of **24a**, m.p. 101–103°. NMR ($CDCl_3$): δ 2.00 6 H s, 4.90 1 H m, 5.16 1 H m, 5.33 1 H m. MW calc. 486. Found (MS) *m/e* = 486 (2%) *m/e* = 426 (M − CH_3COOH, 65%), *m/e* = 366 (M − $2CH_3COOH$, 100%).

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