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^1H and ^{13}C NMR Assignments for Lanostan-3β-ol Derivatives: Revised Assignments for Lanosterol

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^1H and ^{13}C NMR assignments are presented for 30 oxygenated lanostane derivatives, including lanosterol, dihydrolanosterol, 7-ketolanosterol, agnosterol, 24,25-epoxylanosterol, 8α,9α-epoxylanostan-3β-ol, three 15-oxygenated derivatives of lanost-7-en-3β-ol, lanostane-3β,7α-diol, lanostane-3β,9α-diol and their acetates. These assignments, which were largely determined by a combination of DEPT, one-bond and long-range ^{13}C–^1H chemical shift correlation and lanthanide-induced shift experiments, are not dependent on previously reported assignments, several of which were found to be incorrect. ^1H and ^{13}C acetylation shifts for lanostan-3β-ols were sufficiently invariant among the sterols studied that they were useful for assigning carbons in rings A and B. The acetylation shifts reported for lanostan-3β-ols were extended and partially revised.

KEY WORDS: ^1H and ^{13}C NMR Oxygenated lanostane derivatives Spectral assignment Lanthanide-induced shifts Long-range HETCOR Acetylation shifts

INTRODUCTION

4,4,14α-Trimethylsterols (C_{30} sterols) are widely distributed in nature and play an important role in numerous biological processes. Lanosterol (**1a**) and 24,25-dihydrolanosterol (**2a**) are key intermediates in the biosynthesis of cholesterol. 7-Keto dihydrolanosterol (**4a**), the 24,25-epoxylanosterol epimers (**5a** and **6a**) and many C_{30} sterols oxygenated at C-9, C-15 and C-30 are inhibitors of cholesterol biosynthesis.[1] Several oxygenated 4,4,14α-trimethyl-$\Delta^{7,9(11)}$-sterols isolated from the Chinese medicinal fungus *Ganoderma lucidum* have been shown to exhibit a variety of biological activities.[2]

We recently prepared the 24*R* and 24*S* epimers of 24,25-epoxylanosterol (**5a** and **6a**).[3] In the course of assigning the ^{13}C NMR spectra of these epimers, we noticed that several assignments for lanosterol and dihydrolanosterol were reversed in the seminal papers by Knight[4] and others[5] working in the early 1970s, when few assignment techniques were available. Our further investigation of C_{30} sterols also revealed errors in the assignments for dihydroagnosterol. Several misassignments for these and related C_{30} sterols have gone largely uncorrected[6,7] and have appeared in review papers.[8] Despite the revision[9] of some of these errors and the recognition by others[10] that 2D NMR techniques are generally required to establish sound assignments of triterpenes, many assignments for C_{30} sterols are still based ultimately on the seminal papers. Several incorrect ^1H NMR assignments for C_{30} sterols have also appeared.[1a,b,2,7,11] This situation promotes the proliferation of incorrect assignments in the triterpene literature.

In order to establish reliable ^1H and ^{13}C assignments for C_{30} sterols, we obtained pure samples of a variety of lanostan-3β-ol derivatives and carried out a series of one- and two-dimensional NMR experiments, from which the spectra could be assigned without recourse to comparisons with previously reported assignments of C_{30} sterols. We present here ^1H and ^{13}C NMR assignments for these lanostan-3β-ol derivatives.

RESULTS AND DISCUSSION

The ^{13}C and ^1H NMR assignments for the 30 oxygenated lanostane derivatives shown in Scheme 1 are presented in Tables 1 and 2 and Tables 3 and 4, respectively. ^1H, ^{13}C, DEPT and, in most cases, COSY and ^{13}C–^1H chemical shift correlation (HETCOR) spectra were collected for each of the 30 oxygenated lanostane derivatives. Even with this abundance of data at hand, we could not unambiguously assign many carbons in the δ_C 15–45 region. The method of chemical shift comparisons,[12] often used to establish assignments of sterols and other natural products, was unsuitable here because of the abundant errors in reported ^{13}C assignments of C_{30} sterols. Although reliable assignments exist for numerous C_{27} sterols,[13] chemical shift comparisons with C_{30} sterols were of limited utility because the additional 4α-, 4β- and 14α-methyl groups substantially influence the chemical shifts of carbons up to four bonds away, often in as yet unpredictable ways. The ^{13}C NMR chemical shift differences between 5α-cholest-8-en-3β-ol[14] and dihydrolanosterol (Fig. 1) showed a diversity of γ and δ effects resulting from the addition of the three methyl groups. The magnitude of the *γ-gauche* effects observed for the introduction of C-30 appeared to be generally correlated with the distance from C-30 to the nearest hydrogen atom on the

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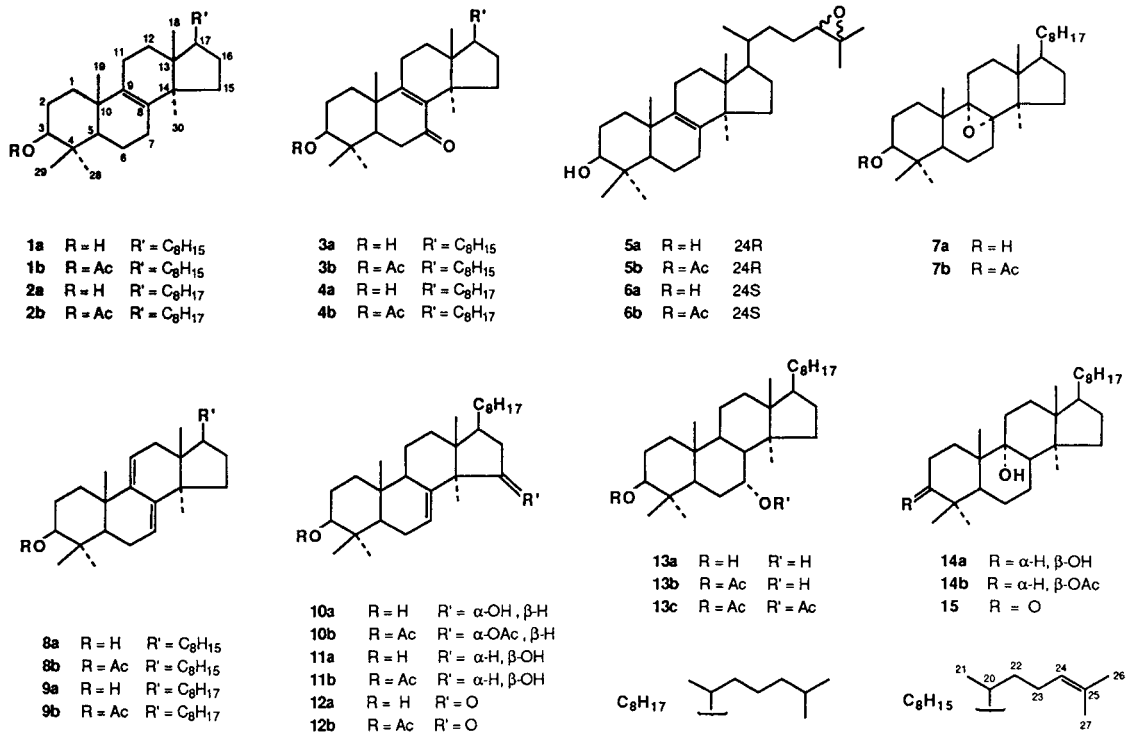

Scheme 1. Structures of C_{30} sterols assigned in this work. Carbons 26–30 are numbered here according to *Chemical Abstracts*. Other numbering systems have been used in some previous work.

Table 1. ^{13}C NMR chemical shifts for lanostan-3β-ol derivatives[a]

	Δ^{8,24}		Δ^{8}		7-Keto Δ^{8,24}		7-Keto Δ^{8}		24*R*,25-Epoxy Δ^{8}		24*S*,25-Epoxy Δ^{8}		8α,9α-Epoxy Δ^{0}	
Atom	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b
C-1	35.55	35.22	35.56	35.22	34.77	34.45	34.77	34.46	35.54	35.23	35.54	35.24	32.82	32.47
C-2	27.81	24.13	27.82	24.13	27.40	23.81	27.41	23.82	27.80	24.14	27.80	24.14	27.09	23.51
C-3	78.96	80.89	78.96	80.88	77.92	79.60	77.94	79.60	78.94	80.89	78.94	80.90	78.44	80.43
C-4	38.85	37.76	38.86	37.74	38.89	37.73	38.90	37.73	38.86	37.78	38.86	37.78	38.47	37.36
C-5	50.35	50.44	50.37	50.44	49.79	49.81	49.79	49.82	50.34	50.45	50.34	50.46	41.70	41.84
C-6	18.22	18.08	18.24	18.09	36.63	36.41	36.63	36.42	18.21	18.09	18.21	18.09	16.44	16.31
C-7	26.46	26.34	26.48	26.34	199.12	198.76	199.12	198.77	26.46	26.35	26.46	26.35	23.43	23.28
C-8	134.35	134.44	134.39	134.45	138.97	139.05	138.99	139.07	134.28	134.39	134.28	134.39	68.19	67.93
C-9	134.35	134.18	134.35	134.16	164.86	164.71	164.86	164.73	134.38	134.24	134.38	134.24	70.70	70.42
C-10	36.97	36.84	36.99	36.84	39.74	39.58	39.74	39.59	36.97	36.86	36.97	36.87	37.83	37.71
C-11	20.97	20.96	20.98	20.96	23.66	23.68	23.67	23.69	20.95	20.96	20.95	20.96	21.44	21.43
C-12	30.94	30.92	30.96	30.92	30.08	30.05	30.09	30.06	30.91	30.91	30.94	30.93	26.81	26.78
C-13	44.43	44.43	44.42	44.39	44.88	44.87	44.86	44.85	44.45	44.46	44.45	44.46	43.55	43.54
C-14	49.76	49.76	49.78	49.74	47.73	47.74	47.72	47.75	49.77	49.78	49.77	49.78	48.78	48.75
C-15	30.82	30.78	30.83	30.77	31.98	31.97	31.99	31.98	30.79	30.77	30.79	30.77	31.85	31.81
C-16	28.18	28.17	28.20	28.18	28.73	28.73	28.75	28.75	28.22	28.22	28.17	28.17	28.44	28.44
C-17	50.35	50.33	50.47	50.44	48.94	48.91	49.04	49.01	50.22	50.24	50.31	50.34	48.25	48.24
C-18	15.71	15.71	15.72	15.71	15.75	15.76	15.75	15.75	15.73	15.73	15.73	15.75	16.22	16.21
C-19	19.11	19.15	19.12	19.14	18.33	18.41	18.33	18.42	19.12	19.16	19.12	19.17	16.99	17.02
C-20	36.24	36.23	36.46	36.46	36.12	36.12	36.38	36.37	36.19	36.20	36.31	36.34	36.23	36.23
C-21	18.61	18.59	18.70	18.68	18.69	18.68	18.78	18.77	18.64	18.65	18.55	18.55	18.94	18.93
C-22	36.33	36.31	36.45	36.42	36.26	36.25	36.38	36.37	32.57	32.57	32.76	32.78	36.30	36.29
C-23	24.89	24.88	24.09	24.09	24.85	24.85	24.06	24.06	25.58	25.60	25.87	25.90	24.02	24.02
C-24	125.22	125.21	39.50	39.48	125.10	125.09	39.46	39.45	64.79	64.77	64.93	64.93	39.43	39.42
C-25	130.91	130.86	27.99	27.97	131.00	130.99	27.99	27.98	58.44	58.41	58.15	58.14	27.94	27.94
C-26	25.72	25.71	22.54	22.52	25.71	25.71	22.52	22.52	24.92	24.92	24.94	24.95	22.50	22.50
C-27	17.62	17.61	22.83	22.81	17.62	17.62	22.82	22.82	18.73	18.73	18.62	18.63	22.79	22.79
C-28	27.93	27.87	27.94	27.87	27.40	27.34	27.41	27.34	27.93	27.89	27.93	27.89	28.23	28.23
C-29	15.40	16.50	15.41	16.51	15.27	16.34	15.27	16.35	15.40	16.52	15.40	16.52	15.03	16.15
C-30	24.23	24.20	24.25	24.22	24.96	24.99	24.98	25.01	24.22	24.21	24.22	24.21	19.90	19.88
Ac-CO		170.98		170.96		170.84		170.84		171.01		171.03		170.75
Ac-CH_3		21.30		21.31		21.23		21.24		21.33		21.34		21.26

[a] Spectra obtained at 75.5 MHz in 0.01–0.1 M $CDCl_3$ solutions.

Table 2. ^{13}C NMR chemical shifts for lanostan-3β-ol derivatives[a]

	$\Delta^{7,9(11),24}$		$\Delta^{7,9(11)}$		15α-OH Δ^7		15β-OH Δ^7		15-Keto Δ^7			7α-OH Δ^0		9α-OH Δ^0		9α-OH 3-keto-Δ^0
Atom	8a	8b	9a	9b	10a	10b	11a	11b	12a	12b	13a	13b	13c	14a	14b	15
C-1	35.69	35.37	35.70	35.37	38.13	37.67	37.64	37.23	38.10	37.73	37.42	37.08	37.10	29.50	29.23	30.66
C-2	27.78	24.23	27.79	24.23	27.36	23.92	27.42	23.91	27.31	23.89	27.53	23.92	23.95	27.34	23.74	34.54
C-3	78.95	80.82	78.94	80.81	79.23	81.03	79.09	80.88	79.14	80.98	78.92	80.92	80.66	78.60	80.64	217.36
C-4	38.68	37.58	38.68	37.58	38.55	37.42	38.61	37.44	38.50	37.36	38.35	37.26	37.22	38.78	37.65	47.54
C-5	49.08	49.21	49.10	49.21	49.93	50.00	50.10	50.20	49.65	49.82	46.37	46.55	47.43	45.14	45.17	46.34
C-6	22.98	22.79	22.98	22.79	22.90	22.76	23.01	22.81	22.78	22.62	31.47	31.35	27.74	21.40	21.31	22.77
C-7	120.13	119.81	120.10	119.77	117.57	117.51	118.09	117.84	120.55	120.36	69.41	69.30	72.02	23.72	23.61	23.42
C-8	142.69	142.78	142.73	142.80	142.73	141.92	142.39	142.41	136.89	137.00	42.48	42.51	41.64	40.51	40.53	40.72
C-9	145.86	145.56	145.87	145.54	47.05	46.77	47.30	47.18	46.98	46.91	41.75	41.64	42.74	77.16	76.94	76.97
C-10	37.34	37.20	37.34	37.19	35.55	35.43	35.60	35.43	35.63	35.50	37.71	37.62	37.38	42.61	42.52	42.41
C-11	116.31	116.58	116.34	116.60	19.76	19.65	19.74	19.72	19.64	19.65	19.45	19.46	19.55	27.95[b]	28.06	27.97[b]
C-12	37.80	37.80	37.82	37.80	32.58	31.97	33.32	33.27	30.57	30.54	31.68	31.65	31.53	29.10	29.10	29.02
C-13	43.73	43.71	43.71	43.67	44.91	44.52	43.21	43.18	43.69	43.69	45.88	45.89	46.03	45.64	45.63	45.67
C-14	50.29	50.30	50.30	50.30	53.70	52.92	56.27	56.23	56.89	56.87	47.98	47.98	47.54	47.42	47.41	47.41
C-15	31.49	31.47	31.50	31.46	74.24	77.06	76.98	76.94	217.43	217.34	33.72	33.75	33.88	33.80	33.81	33.83
C-16	27.88	27.86	27.89	27.88	39.08	36.33	39.54	39.58	41.55	41.55	27.85	27.86	27.74	27.93[b]	27.95[b]	27.94[b]
C-17	50.91	50.89	51.02	51.00	48.48	48.56	51.15	51.10	45.03	45.02	50.41	50.40	50.38	50.45	50.45	50.47
C-18	15.62	15.63	15.63	15.63	16.27	16.28	18.80	18.77	15.94	15.95	14.28	14.30	14.28	14.57	14.57	14.59
C-19	22.74	22.77	22.73	22.77	14.29	14.19	13.67	13.68	14.18	14.21	12.93	12.98	13.13	16.71	16.74	16.75
C-20	36.04	36.03	36.25	36.24	36.04	36.08	36.04	36.02	35.61	35.63	36.26	36.28	36.27	36.01	36.02	36.00
C-21	18.41	18.40	18.50	18.48	18.78	18.68	18.94	18.91	19.09	19.10	18.57	18.58	18.58	18.66	18.66	18.66
C-22	36.27	36.26	36.39	36.37	36.44	36.39	36.37	36.33	36.31	36.31	36.57	36.58	36.55	36.48	36.48	36.47
C-23	24.92	24.92	24.10	24.10	23.96	24.09	23.99	23.98	23.86	23.89	24.05	24.08	24.09	24.03	24.04	24.04
C-24	125.17	125.17	39.50	39.48	39.40	39.37	39.42	39.39	39.27	39.28	39.47	39.47	39.46	39.47	39.47	39.46
C-25	130.98	130.97	27.99	27.98	27.96	27.96	27.96	27.93	27.90	27.91	27.95	27.97	27.97	27.96	27.96[b]	27.96
C-26	25.73	25.72	22.53	22.53	22.52	22.49	22.52	22.50	22.47	22.48	22.51	22.52	22.52	22.51	22.52	22.52
C-27	17.64	17.63	22.82	22.82	22.79	22.81	22.79	22.78	22.75	22.75	22.80	22.82	22.81	22.81	22.81	22.81
C-28	28.12	28.07	28.13	28.06	28.24	28.18	27.94	27.86	28.16	28.10	28.08	28.07	27.94	28.26	28.23	26.29
C-29	15.78	16.92	15.78	16.91	15.52	16.65	15.13	16.26	15.44	16.59	15.80	16.95	16.67	15.40	16.52	21.55
C-30	25.55	25.51	25.57	25.52	16.94	17.89	25.39	25.35	21.26	21.26	19.33	19.37	18.90	18.26	18.27	18.34
Ac-CO		170.98		170.96		171.04		170.94		171.00		170.93	171.02		170.90	
						171.34							170.31			
Ac-CH_3		21.33		21.31		21.33		21.29		21.31		21.32	21.30		21.31	
						21.43							21.85			

[a] Spectra obtained at 75.5 MHz in 0.01–0.1 M $CDCl_3$ solutions.
[b] Assignments within a column may be reversed (see text).

Table 3. ^{1}H NMR chemical shifts for methyl and methine protons of lanostan-3β-ol derivatives[a,b]

		$\Delta^{8,24}$		Δ^{8}		7-Keto $\Delta^{8,24}$		7-Keto Δ^{8}		24*S*,25-Epoxy[d] Δ^{8}		8α,9α-Epoxy Δ^{0}	
Atom	Multiplicity[c]	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**6a**	**6b**	**7a**	**7b**
H-3	dd	3.24	4.50	3.24	4.52	3.28	4.52	3.28	4.52	3.24	4.50	3.21	4.46
H-5	m	1.05	1.15	1.05	1.15	1.64	1.74	[e]	1.71	1.05	1.15	1.64	1.74
H-17	m	1.48	1.48	1.46	1.46	1.44	1.44	[e]	1.41	1.48	1.49	1.33	1.33
H-18	s	0.687	0.687	0.688	0.686	0.652	0.651	0.652	0.650	0.696	0.694	0.766	0.765
H-19	s	0.980	1.003	0.981	1.003	1.167	1.186	1.167	1.185	0.982	1.003	1.118	1.139
H-20	m	1.39	1.39	1.37	1.36	1.38	1.39	[e]	1.35	1.46	1.45	1.36	1.36
H-21	d	0.911	0.911	0.889	0.888	0.924	0.923	0.903	0.902	0.916	0.916	0.875	0.876
H-24	t	5.10	5.10			5.10	5.10			2.69	2.69		
H-25	m			1.52	1.53			[e]	1.52			1.52	1.52
H-26	d or s	1.683	1.685	0.864	0.863	1.684	1.681	0.863	0.863	1.310	1.310	0.859	0.859
H-27	d or s	1.604	1.605	0.869	0.869	1.603	1.602	0.868	0.869	1.268	1.267	0.864	0.864
H-28	s	1.000	0.879	1.000	0.880	0.999	0.885	0.999	0.885	1.001	0.882	0.939	0.821
H-29	s	0.810	0.883	0.810	0.882	0.882	0.954	0.882	0.954	0.811	0.882	0.780	0.852
H-30	s	0.874	0.870	0.875	0.874	0.912	0.911	0.915	0.912	0.877	0.877	0.890	0.890
Ac-CH_3	s		2.05		2.05		2.06		2.06		2.05		2.03

[a] Spectra obtained at 300 MHz in 0.01–0.1 M $CDCl_3$ solutions.
[b] ^{1}H NMR chemical shifts for methylene protons have been omitted because their HETCOR signals were often weak or poorly defined with our present combination of hardware and software.
[c] Coupling constants for methyl and methine protons listed in Tables 3 and 4: H-3, dd, $J = 4.8 \pm 0.6$ Hz, 10.7 ± 0.7 Hz; H-11, br d, 6.4 ± 0.1 Hz; H-15β, dd, 6 Hz, 9 Hz; H-15α, dd, 2 Hz, 7 Hz; H-21, d, $J = 6.3 \pm 0.3$ Hz; H-24, br t, $J = 6.6 \pm 0.6$ Hz; H-26 and H-27 (for compounds with the C_8H_{17} side-chain), d, $J = 6.7 \pm 0.2$ Hz.
[d] The 24*R* isomer **5a** was identical with **6a** to within 0.01 ppm in ^{1}H NMR chemical shifts except for H-17 (δ_H 1.49) and H-20 (δ_H 1.44).
[e] Not measured.

γ-carbon, but this correlation was of limited utility in differentiating closely spaced carbons in the crowded δ_C 15–45 region. Other predictive techniques could usually not be applied because of the presence of the five-membered D ring[9a] or double bonds and other functional groups.[15]

The ^{1}H chemical shifts obtained from the HETCOR spectra were initially of limited value because methods for comparing and predicting ^{1}H chemical shifts of sterols[16] are still in their infancy and because the 300 MHz COSY spectrum was generally uninformative. As our data base of ^{1}H chemical shifts of C_{30} sterols grew, the HETCOR experiments became more useful, especially for differentiating very closely spaced carbons. With the aid of zero-filling and resolution enhancement, ^{13}C signals separated by only 0.02 ppm could be distinguished by their ^{1}H correlations. The value of such fine distinctions was diminished by the limits of reproducibility of ^{13}C chemical shifts. When different samples of the same substance were measured months apart on the same spectrometer, a reproducibility of 0.01 ppm was often obtained for carbons remote from functional groups and in positions insensitive to small conformational changes, such as the C_8H_{17} side chain. In different molecules, the chemical shifts of such carbons frequently matched to within $\leq$0.02 ppm. For other carbons, which appeared to be more affected by small variations in temperature, concentration and trace aromatic or polar impurities, the reproducibility averaged *ca.* 0.02 ppm. Still greater deviations in chemical shift from the values reported here may arise from sample impurities or from spectral measurement in a warm electromagnet and might occasionally lead to interchange of signal assignments.

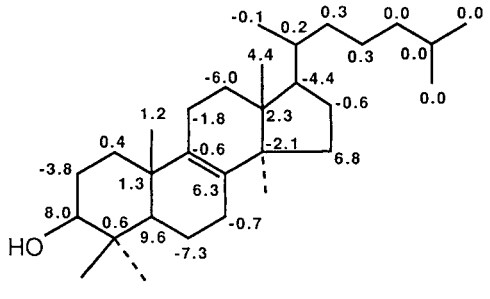

Figure 1. ^{13}C chemical shift changes resulting from the addition of 4α-, 4β- and 14α-methyl groups to 5α-cholest-8-en-3β-ol: δ_C(dihydrolanosterol) − δ_C(5α-cholest-8-en-3β-ol). Data from Ref. 14 for 5α-cholest-8-en-3β-ol have been adjusted to a $CDCl_3$ reference of δ_C 77.0. Distances from C-30 to H-16α, H-7α, H-17α and H-12α were calculated by molecular mechanics to be 3.18, 2.96, 2.78 and 2.61 Å, respectively.

Also useful for establishing ^{13}C assignments was the long-range HETCOR experiment, which gave most of the expected correlations between methyl protons and carbons up to three bonds away and generally much weaker correlations of carbons to methylene or methine protons. This method is especially suitable for C_{30} sterols, which have only six carbons located more than three bonds away from a methyl group. Although resolution in the ^{1}H dimension was severely limited by the few (16–20) increments collected, the set of methyl protons at δ_H 0.85–0.90 could be generally be differentiated from H-18 and H-19. These crude distinctions usually proved adequate to identify the correlated carbon atoms.

Dihydrolanosterol, lanosterol and their acetates

We first rigorously established the ^{13}C NMR assignments of dihydrolanosterol (**2a**). Lanthanide-induced

Table 4. ^{1}H NMR chemical shifts for methyl and methine protons of lanostan-3β-ol derivatives[a,b]

Atom	Multiplicity[c]	$\Delta^{7,9(11),24}$ 8a	8b	$\Delta^{7,9(11)}$ 9a	9b	15α-OH Δ^7 10a	10b	15β-OH Δ^7 11a	11b	15-Keto Δ^7 12a	12b	13a	7α-OH Δ^0 13b	13c	9α-OH Δ^0 14a	14b	9α-OH 3-keto-Δ^0 15
H-3	dd	3.25	4.52	3.25	4.51	3.25	4.51	3.24	4.51	3.23	4.51	3.26	4.53	4.53	3.20	4.47	
H-5	m	1.09	1.19	1.09	1.19	1.12	1.22	1.20	1.30	1.12	1.21	1.42	1.51	1.42	1.54	1.70	2.10
H-7	br s	5.47	5.46	5.46	5.46	5.46	5.14	5.57	5.57	6.50	6.50	4.06	4.06	5.12			
H-8	m											1.58	1.58	1.69	1.86	1.86	1.94
H-9	m					1.99	2.01	2.05	2.07	1.97	1.98	1.54	1.56	1.62			
H-11	br d	5.31	5.32	5.32	5.32												
H-15	ddd					4.24	5.03	4.04	4.04								
H-17	m	1.58	1.58	1.55	1.56	1.56	1.57	1.44	1.45	1.72	1.72	1.57	1.57	1.54	1.51	1.52	1.52
H-18	s	0.563	0.561	0.563	0.562	0.704	0.740	0.966	0.966	0.746	0.744	0.735	0.733	0.736	0.784	0.781	0.807
H-19	s	0.982	1.004	0.983	1.006	0.868	0.879	0.912	0.933	0.879	0.899	0.924	0.949	0.962	1.033	1.052	1.194
H-20	m	1.40	1.40	1.38	1.38	1.31	1.32	1.52	1.52	1.45	1.44	1.35	1.35	1.35	1.39	1.40	1.41
H-21	d	0.906	0.905	0.883	0.884	0.857	0.859	0.897	0.898	0.962	0.962	0.852	0.852	0.850	0.889	0.887	0.894
H-24	t	5.11	5.11														
H-25	m			1.52	1.52	1.52	1.51	1.52	1.52	1.52	1.52	1.52	1.52	1.51	1.51	1.52	1.52
H-26	d or s	1.685	1.685	0.866	0.867	0.862	0.852	0.863	0.863	0.864	0.864	0.861	0.861	0.858	0.862	0.862	0.864
H-27	d or s	1.605	1.606	0.872	0.872	0.867	0.859	0.868	0.868	0.868	0.868	0.867	0.867	0.864	0.867	0.867	0.870
H-28	s	1.008	0.888	1.007	0.889	0.988	0.877	0.990	0.871	0.993	0.875	0.961	0.841	0.754	0.995	0.871	1.086
H-29	s	0.883	0.954	0.883	0.954	0.894	0.957	0.891	0.963	0.890	0.961	0.806	0.878	0.853	0.806	0.876	1.056
H-30	s	0.877	0.872	0.877	0.873	1.030	1.095	0.978	0.978	1.183	1.183	1.077	1.077	0.882	0.923	0.925	0.936
Ac-CH_3	s		2.06		2.06		2.059		2.06		2.05		2.05	2.05		2.04	
							2.058							2.02			

[a–c] See footnotes in Table 3.

shifts (LIS) were measured for **2a** using Yb(fod)$_3$ in $CDCl_3$. The observed LIS were compared with LIS calculated from the McConnell–Robertson equation [17] using atomic coordinates obtained from molecular mechanics calculations. The correspondence between the observed and calculated LIS (Table 5) was sufficiently close that, in conjunction with the multiplicity information, nearly all carbons could be unambiguously assigned. The side-chain carbons C-22–C-27 were assigned based on the similarity of their chemical shifts with those in the side chain of cholesterol. Only the assignments of C-12 and C-15 to signals at δ_C 30.83 and 30.96 could not be established by LIS, multiplicity or chemical shift comparisons. Although the ^{1}H NMR chemical shifts determined from the one-bond HETCOR spectrum were of little diagnostic value at this point, a long-range HETCOR experiment optimized for 10 Hz couplings (Table 6) established the assignments of the analogous signals of acetate **2b** by their correlations with the H-18 and H-30 resonances. Because the acetylation shifts for C-12 and C-15 are negligible, these assignments also apply to the free sterol **2a**. The assignments presented here for dihydrolanosterol agree with those given by Beierbeck *et al.*[9a] except for the interchange of C-6 and C-19. In addition to this interchange, our assignments differ from those given by Knight[4] for C-7, C-12, C-15 and C-16. Our assignments agree fully with those in a report[9c] appearing after submission of this work.

The ^{13}C assignments for **2b** are most easily derived from the average acetylation shifts given in Table 7. These shifts, which result from acetylation of the 3β-hydroxy group, have been tabulated.[4b,8a] Our reassignments of C-6 and C-19 led to an interchange of the previously reported acetylation shifts for these carbons.

Table 5. Observed and calculated relative lanthanide-induced shifts for dihydrolanosterol (2a)[a,b]

Atom	Observed LIS	Calculated LIS	Atom	Observed LIS	Calculated LIS
C-1	189	184	C-20	13	14
C-2	467	471	C-21	11	12
C-3	1000	1000	C-22	9	10
C-4	438	449	C-23	8	8
C-5	208	206	C-24	6	6
C-6	119	113	C-25	5	5
C-7	71	68	C-26	4	5
C-8	62	62	C-27	4	5
C-9	80	77	C-28	300	296
C-10	156	155	C-29	330	328
C-11	49	49	C-30	26	25
C-12	28	26	H-18	32	32
C-13	28	28	H-19	113	115
C-14	36	35	H-21	11	13
C-15	27	28	H-26	4	6
C-16	18	18	H-27	4	5
C-17	18	18	H-28	238	241
C-18	32	31	H-29	286	282
C-19	125	125	H-30	22	23

[a] The LIS values are normalized to the LIS of C-3.
[b] Agreement factor (*R*), 1.24%; Yb—O distance, 2.65 Å; Yb—O—C-3 angle, 129°; Yb—O—C-3—C-4 angle, −141°; magnetic axis deviation from Yb—O bond, 3.3°.

Table 6. Long-range HETCOR signals for dihydrolanosterol acetate (2b)

Carbon atom	Correlated ^{1}H chemical shift(s)[a]	Assignment
C-1	1.00 (s), 1.76	H-19, H-1
C-2	1.69	H-2
C-3	0.88	H-28 and H-29
C-4	0.88	H-28 and H-29
C-5	0.88	H-28 and H-29
C-6	1.15	H-5
C-7	1.63	H-6
C-8	0.88 (w)	H-30
C-9	1.00	H-19
C-10	0.99, 1.15	H-19, H-5
C-11	1.99 (w)	H-11
C-12	0.69	H-18
C-13	0.69, 0.88	H-18, H-30
C-14	0.69, 0.86	H-18, H-30
C-15	0.88	H-30
C-16		
C-17	0.68, 1.67	H-18, H-12
C-18	0.69	
C-19	1.00 (s), 1.16 (w), 1.31	H-19, H-5, H-1
C-20	0.90	H-21
C-21	0.89	H-21
C-22	0.90	H-21
C-23		
C-24	0.87	H-26 and H-27
C-25	0.88 (s)	H-26 and H-27
C-26	0.86	H-26 and H-27
C-27	0.86	H-26 and H-27
C-28	0.88	H-28 and H-29
C-29	0.88 (s)	H-28 and H-29
C-30	0.87 (s)	H-30

[a] s, strong intensity; w, weak intensity.

Aside from discrepancies in the sign of the acetylation shifts for C-4 and C-10, our other acetylation shifts are comparable with those of Knight.[4b,8a] Acetylation shifts for the *cis* methyl group (4β-CH_3) were similar to the effect of acetylation of the 15α-hydroxy group on 14α-CH_3 signals and very different from the 3β-acetylation shifts for the *trans* methyl group (4α-CH_3). The methyl carbons (C-29, C-30) *cis* to the 3β- and 15α-hydroxy groups both showed *ca.* 1 ppm downfield acetylation shifts, and the corresponding ^{1}H signals were shifted 0.07 ppm downfield. In contrast, the shift for the *trans* methyl carbon (C-28) was negligible, and a large (0.12 ppm) upfield 3β-acetylation shift was observed for the *trans* methyl protons (H-28).

Although comparison of 3β-acetylation shifts between C_{27} and C_{30} sterols indicates a substantial influence from the C-4 methyl groups, the acetylation shifts of the compounds studied here proved to be remarkably constant, partly because no additional functional groups were introduced to ring A. Based on the constancy of the acetylation shifts, the chemical shifts of an acetate could be accurately estimated by adding the average acetylation shifts in Table 7 to the chemical shifts of the corresponding free sterol. Deviations were usually <0.05 ppm for sp^3 carbons and <0.3 ppm for sp^2 carbons. Consequently, assignment of the acetate from the free sterol or vice versa was trivial except for carbons of identical multiplicity differing in chemical

Table 7. ^{1}H and ^{13}C NMR acetylation shifts for lanostan-3β-ol derivatives[a]

Atom	$\Delta^{8,24}$ 1b − 1a	Δ^{8} 2b − 2a	7-Keto $\Delta^{8,24}$ 3b − 3a	7-Keto Δ^{8} 4b − 4a	24*S*,25-Epoxy Δ^{8} 6b − 6a	8α,9α-Epoxy Δ^{0} 7b − 7a	$\Delta^{7,9(11),24}$ 8b − 8a	$\Delta^{7,9(11)}$ 9b − 9a	15α-OH Δ^{7} 10b − 10a	15β-OH Δ^{7} 11b − 11a	15-Keto Δ^{7} 12b − 12a	7α-OH Δ^{0} 13b − 13a	9α-OH Δ^{0} 14b − 14a	Mean (±range)
C-1	−0.33	−0.34	−0.32	−0.31	−0.31	−0.34	−0.32	−0.34	−0.46	−0.41	−0.37	−0.34	−0.27	−0.34 (0.12)
C-2	−3.68	−3.69	−3.59	−3.59	−3.66	−3.58	−3.55	−3.57	−3.45	−3.51	−3.42	−3.61	−3.60	−3.58 (0.16)
C-3	1.93	1.92	1.67	1.67	1.95	2.00	1.87	1.87	1.80	1.80	1.84	2.00	2.04	1.87 (0.20)
C-4	−1.09	−1.12	−1.17	−1.17	−1.08	−1.12	−1.10	−1.11	−1.13	−1.17	−1.13	−1.10	−1.13	−1.12 (0.05)
C-5	0.10	0.07	0.02	0.03	0.11	0.14	0.13	0.11	0.07	0.09	0.17	0.17	0.02	0.10 (0.08)
C-6	−0.14	−0.15	−0.21	−0.21	−0.12	−0.13	−0.19	−0.19	−0.15	−0.21	−0.16	−0.13	−0.09	−0.16 (0.07)
C-7	−0.12	−0.14	−0.36	−0.35	−0.11	−0.15	−0.33	−0.33	−0.06[c]	−0.25	−0.20	−0.11	−0.11	−0.20 (0.15)
C-8	0.09	0.06	0.08	0.08	0.11	−0.25	0.09	0.07	[b]	0.03	0.12	0.04	0.02	0.04 (0.30)
C-9	−0.17	−0.19	−0.15	−0.13	−0.14	−0.28	−0.29	−0.33	−0.28[c]	−0.13	−0.08	−0.11	−0.21	−0.19 (0.14)
C-10	−0.13	−0.15	−0.15	−0.15	−0.11	−0.12	−0.14	−0.15	−0.13	−0.16	−0.13	−0.09	−0.09	−0.13 (0.04)
C-19	0.03	0.02	0.09	0.09	0.04	0.02	0.04	0.04	−0.10[c]	0.01	0.03	0.05	0.05	0.03 (0.13)
C-28	−0.06	−0.07	−0.06	−0.06	−0.04	0.00	−0.06	−0.07	−0.06	−0.08	−0.07	−0.01	−0.01	−0.05 (0.05)
C-29	1.10	1.10	1.07	1.08	1.12	1.11	1.14	1.14	1.13	1.13	1.15	1.14	1.14	1.12 (0.04)
H-3	1.26	1.28	1.24	1.24	1.26	1.25	1.27	1.26	1.26	1.27	1.28	1.27	1.27	1.26 (0.02)
H-5	0.10	0.10	0.10	[d]	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.16	0.10 (0.06)
H-19	0.023	0.022	0.019	0.018	0.021	0.021	0.022	0.023	0.011	0.021	0.020	0.025	0.019	0.020 (0.009)
H-28	−0.119	−0.120	−0.114	−0.114	−0.119	−0.118	−0.120	−0.118	−0.111	−0.119	−0.118	−0.120	−0.124	−0.118 (0.007)
H-29	0.071	0.070	0.072	0.072	0.071	0.072	0.071	0.071	0.063	0.072	0.071	0.072	0.070	0.071 (0.008)

[a] δ(acetate) − δ(free sterol).
[b] Omitted because the acetylation shift of 0.81 for C-8 is primarily attributable to acetylation at C-15.
[c] 3-Acetylation shift influenced by acetylation at C-15.
[d] Not measured.

shift by <0.2 ppm (sp^3 carbons) or <0.5 ppm (sp^2 carbons). Although formally circular reasoning lurks in the practice of using average acetylation shifts to make assignments that are in turn used to calculate those same average acetylation shifts, we point out that the 3β-acetylation shifts have been previously well-established and that we have confirmed most assignments by other techniques. Based on acetylation shifts, the assignments for dihydrolanosterol acetate follow directly from those of the free sterol **2a** except for two closely spaced pairs of peaks. C-8 and C-9 were assigned by their correlation to H-30 and H-19 in the long-range HETCOR spectrum, and C-2 and C-23 were distinguished by the correlation of C-23 to signals at δ_H 1.35 and 1.15. These chemical shifts are characteristic for the 23-protons of a C_8H_{17} sterol side chain.

Lanosterol and its acetate **1b** were assigned by comparison with the ^{13}C assignments for the side-chain carbons of desmosterol[18] and the ring carbons of dihydrolanosterol. Except for C-17 and side-chain carbons, the carbon signals of **1a** and **1b** fell within 0.04 ppm of the corresponding values for **2a** and **2b**. The C-5 and C-17 resonances, which could not be resolved in **1a**, were assigned in **1b** based on their correlated ^{1}H NMR chemical shifts (δ_H 1.15 for H-5 and δ_H 1.48 for H-17) compared with typical values for H-5 and H-17 in Tables 3 and 4. This technique of assigning ^{13}C signals by comparing their correlated ^{1}H chemical shifts extracted from HETCOR spectra becomes increasingly useful as predictive methods and data bases are developed for ^{1}H chemical shifts of sterols. Our assignments for lanosterol revise those given by Knight[4] for C-6, C-7, C-12, C-15, C-16, C-19, C-26 and C-27.

Other C_{30} sterols

The ^{13}C NMR spectra of the epimeric 24,25-epoxy sterols **5a** and **6a** and their acetates **5b** and **6b** were assigned based on their epoxide-induced shifts[19] referenced to the parent olefins **1a** and **1b**. Closely neighboring peaks were distinguished by HETCOR (C-21 and C-27) or long-range HETCOR spectra (C-8 and C-9). C-5 and C-17 were assigned from the spectrum of the 1:1 mixture of 24*R* and 24*S* epimers, showing a pair of peaks 0.10 ppm apart (C-17) adjacent to a single peak of double intensity (C-5). ^{13}C NMR spectra of mixtures of epimers **5b** and **6b** showed that epimeric pairs of peaks differed by $\geq$0.02 ppm only for side-chain carbons and for C-12, C-16 and C-17.[3]

The ^{13}C NMR spectrum of dihydroagnosterol (**9a**) was assigned largely by comparison of observed and calculated LIS, as described for dihydrolanosterol. The vinyl carbon assignments were confirmed by HETCOR and COSY (C-7 and C-11) or long-range HETCOR spectra of the acetate derivative (C-8 and C-9). Application of the average acetylation shifts from Table 7 gave the assignments for **9b**. The assignments for agnosterol (**8a**) and its acetate **8b** followed directly from the assignments for **9a**, **9b** and lanosterol. The assignments for dihydroagnosterol differ from those given by Knight[4b] in the interchange of C-2 and C-28, C-15 and C-16, C-20 and C-22, and C-26 and C-27.

Assignment of the remaining C_{30} sterols proved considerably more formidable because changes in functional group or double bond position often led to chemical shift changes of >1 ppm for carbons up to five bonds away. In the case of the 8α,9α-epoxide **7a**, reasonable (albeit weak) LIS values were measured for its *tert*-butyldimethylsilyl ether derivative, but no acceptable agreement could be obtained between observed and calculated values. LIS analysis of other sterols with two functional groups was often precluded because of limited amounts of material. The following alternative strategy was used for these C_{30} sterols: (i) multiplicities were determined; (ii) carbons bearing functional groups (C=O, C—OH, C=C) were assigned; (iii) the side-chain carbons were assigned by comparison with lanosterol or dihydrolanosterol; (iv) a rough estimate (often $\pm$3 ppm) of chemical shifts of the remaining carbons was obtained by the methods of chemical shift comparisons and substituent effects;[13] (v) for each set of multiplicities, the remaining unassigned carbons were differentiated based on ^{13}C acetylation shifts (C-1, C-2, C-3, C-4, C-6, C-7, C-8, C-9, C-10, C-28, C-29), ^{1}H acetylation shifts (C-3, C-5, C-19, C-28, C-29), long-range HETCOR experiments (C-1, C-3, C-4, C-5, C-8, C-9, C-10, C-12, C-13, C-14, C-15), HETCOR/COSY experiments (carbons adjacent to a functional group), ^{1}H multiplicity (C-21), ^{1}H chemical shift comparisons and by process of elimination.

For example, after application of steps i–iii above, half of the carbons of 7-ketodihydrolanosterol acetate (**4b**) could be assigned from very rough chemical shift estimates ($\pm$3 ppm for carbons more than two bonds distant from C-7) obtained by applying the Blunt and Stothers substituent effects[13] for the 7-oxo group to the chemical shifts of dihydrolanosterol acetate. Several pairs of the remaining closely spaced carbon signals (C-4, C-10; C-5, C-17; C-19, C-21) were differentiated by both acetylation shifts and long-range HETCOR spectra. C-6 and C-22 were distinguished by their correlated ^{1}H NMR chemical shifts (δ_H 2.4 and 1.2, 1.0, respectively). Correlation of C-12 to H-18 in the long-range HETCOR spectrum distinguished it from C-15 and C-16. C-15 was identified by the significant downfield shift of at least one of its protons (δ_H 2.1, 1.7) relative to dihydrolanosterol acetate ($\delta_{H\text{-}15}$ 1.2, 1.6). Similar downfield shifts have been observed in C_{27} sterols for H-7β of $\Delta^{8(14)}$-15-keto sterols and of H-15α of $\Delta^{8(14)}$-7-keto sterols.[20] Of the three methylene peaks in the δ_C 23.7–24.1 region, C-23 was identified by its characteristic ^{1}H chemical shifts, C-2 by the correlation of its protons with H-3α in the COSY spectrum and C-11 by process of elimination. Only C-13 and C-14 could not be distinguished except by the method of chemical shift comparisons. Assignments for the free sterol **4a** followed readily from the acetylation shifts in Table 5. The assignments for the Δ^{24} analogs **3a** and **3b** were also trivial because the ^{13}C chemical shifts differed from those of **4a** and **4b** by $\leq$0.03 ppm except for C-17 and in the side chain, where they were identical within 0.13 ppm with those of **1a** and **1b**.

The assignments of **7b**, **10a**, **11a**, **12a**, **13a**, **14b** and **15** were carried out in similar fashion by performing steps i–v. The model compounds used in step iv and the assignment criteria used in step v are given in Table 8. Once these assignments were complete, the ^{13}C spectra

Table 8. Criteria for establishing ^{13}C assignments for 7b, 11a, 10a, 12a, 13a, 14b and 15[a–d]

8α,9α-Epoxylanostan-3β-ol, acetate (7b)
Model for rings A and D: dihydrolanosterol acetate (**3b**)
C-13; C-14: epoxidation-induced shifts[19]
C-18, C-19: LR HETCOR (H-18⇔C-12, C-13, C-14; H-19⇔C-1, C-9, C-10)
C-8; C-9: LR HETCOR (H-30; H-19)
C-5; C-17: C-5 and H-5 acetylation shifts
C-1; C-12; C-15; C-16: LR HETCOR (H-19; H-18; H-30; —)
C-2; C-7: HETCOR (δ_H 1.8, 1.5; 2.0, 1.8) and COSY (H-2⇔H-3)
C-7; C-11: acetylation shift for C-7

Lanost-7-ene-3β,15β-diol (11a)
Model for rings A and B: dihydroagnosterol (**9a**)
C-18; C-19: LR HETCOR (H-18⇔C-12, C-13, C-14; H-19⇔C-1, C-9 C-10)
C-5; C-9; C-17: HETCOR (δ_H 1.20; 2.05; 1.44)
C-16; C-24: HETCOR (δ_H 2.5, 1.4; 1.1)
C-2; C-6; C-23: HETCOR (δ_H 1.7; 2.0; 1.1)

Lanost-7-ene-3β,15α-diol (10a)
Model for rings A, B and C: lanost-7-ene-3β,15β-diol (**11a**)
C-18; C-30: HETCOR (δ_H 0.704; 1.030)
C-5; C-9; C-17: HETCOR (δ_H 1.12; 1.99; 1.56)

3β-Hydroxylanost-7-en-15-one (12a)
Model for rings A and B: lanost-7-ene-3β,15α-diol (**10a**)
C-18; C-19; C-29: ^1H and ^{13}C acetylation shifts
C-5; C-9; C-17: HETCOR (δ_H 1.12; 1.97; 1.72)

Lanostane-3β-7α-diol (13a)
Model for rings A and D: 3β-hydroxylanost-8-en-7-one (**4a**)
C-18; C-19: H-19 acetylation shift
C-13; C-14: ^{13}C acetylation shifts for 7α-acetoxy group of **13c**
C-5; C-8; C-9; C-17: LR HETCOR (H-28; H-30; H-19; H-21) and acetylation shifts
C-6; C-12; C-15: LR HETCOR (—; H-18; H-30)
C-2; C-16: HETCOR (δ_H 1.6. 1.5; 1.9, 1.3) and COSY (H-2⇔H-3)

Lanostane-3β,9α-diol, 3-acetate (14b)
Model for rings A and D: dihydrolanosterol acetate (**2b**)
C-18; C-19; C-29: ^1H and ^{13}C acetylation shifts
C-13; C-14: chemical shift comparisons with **2b**
C-5; C-8; C-17: LR HETCOR (H-19; H-30; H-21)
C-1; C-12; C-15: LR HETCOR (H-19; H-18; H-30)
C-11; C-16; large methanol-induced shift for C-11
C-2; C-7: HETCOR (δ_H 1.7, 1.6; 1.5, 1.3) and COSY (H-2⇔H-3)
C-6; C-7: chemical shift comparisons with **15**

9a-Hydroxylanostan-3-one (15)
Model for rings B, C and D: lanostane-3β,9α-diol (**14a**)
C-28; C-29: 3-Ketonization shifts calculated from Ref. 28
C-4; C-10; C-14: LR HETCOR (—; H-19; H-18; H-30)
C-5; C-8: LR HETCOR (H-28; H-29; H-30)
C-6; C-7: LR HETCOR (C-9⇔H-7α, H-7β), HETCOR (1.6, 1.5; 1.5, 1.4) and COSY (H-5⇔H-6)

[a] The notations C-8; C-9: LR HETCOR (H-30; H-19) signifies that C-8 and C-9 were distinguished by their correlations to H-30 and H-19, respectively, in the long-range HETCOR spectrum. The notation C-16; C-24: HETCOR (δ_H 2.5, 1.4; 1.1) means that H-16 signals were observed at δ_H 2.5 and 1.4 and that one signal was observed for H-24 at δ_H 1.1 (in this case, because the protons were nearly isochronous). The symbol ⇔ indicates a correlation in the COSY or long-range HETCOR spectrum.
[b] In each case, C-21 was identified by its correlation to a ^1H doublet. C-4 and C-10 were differentiated by their acetylation shifts (except for **15**).
[c] Most assignments were also based on additional information, such as acetylation shifts and ^1H NMR chemical shift comparisons.
[d] Model refers to the sterol used for chemical shift comparisons.

of the corresponding acetates were easily assigned from those of the free sterol or vice versa, except for a few very closely spaced pairs of carbons that were differentiated by HETCOR experiments. Diacetates **10b** and **13c** were assigned similarly; signals for **13c** were differentiated by long-range HETCOR (C-4, C-10 and C-8, C-9) and HETCOR/COSY (C-8, C-9).

Assigning C-11, C-16 and C-25 of the 9-hydroxy derivatives **14a**, **14b** and **15** presented a special problem. These signals lie in a 0.03 ppm interval in **14a** and **15**, and the C-11 and C-16 protons are nearly isochronous (δ_H 1.3, 1.9). Also, the original resolution of the ^{13}C signals was difficult to reproduce consistently in later experiments. When a later DEPT spectrum of **14b**

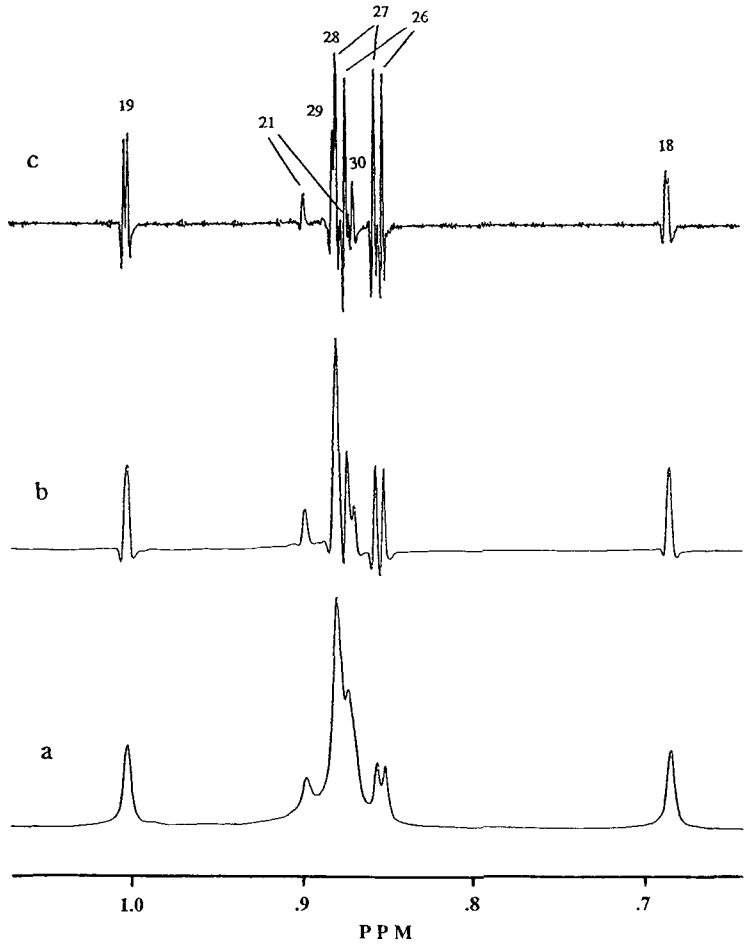

Figure 2. ^{1}H NMR spectrum of dihydrolanosterol: (a) no resolution enhancement; (b) moderate resolution enhancement (Gaussian multiplication, LB −1.7, GB 0.37); (c) strong resolution enhancement (Gaussian multiplication, LB −2.3, GB 0.65). Digital resolution 0.0005 ppm.

showed that the peaks for C-16 and C-25 (0.01 ppm apart) had reversed positions relative to an earlier spectrum, attempts were abandoned to differentiate such closely spaced signals as C-11, C-16 and C-25 of **14a** and **15**. In **14b**, C-11 and C-16 (0.11 ppm apart) were distinguished by methanol-induced shifts (MIS), which were measured analogously to lanthanide-induced shifts in $CDCl_3$ solutions containing 1–3% CD_3OD. MIS experiments on **13a** and **14b** indicated that carbons remote from any oxygen atom have negligible MIS. Consequently, C-11 of **14b** was assigned to the signal with the large MIS and C-16 to the signal with the small MIS.

^{1}H NMR assignments

^{1}H NMR assignments (Tables 3 and 4) were usually made directly from the HETCOR spectrum. This process was complicated by the congestion in the δ_H 0.85–0.90 region, which in many cases contained signals for three methyl singlets (H-28, H-29 and H-30) and three methyl doublets (H-21, H-26 and H-27). As shown in Fig. 2, the signals in this crowded region could be resolved at 300 MHz only with strong resolution enhancement. Nevertheless, when HETCOR spectra were acquired using a small spectral window in the ^{1}H dimension (δ_H 0.80–0.97) with ample increments and digital resolution, ^{1}H signals separated by only 0.002 ppm could be differentiated (Fig. 3). No attempt was made to distinguish peaks less than 0.002 ppm apart, as they might be interchanged by small variations in concentration, temperature or trace impurities.

CONCLUSION

A variety of one- and two-dimensional NMR techniques have been used in this work to obtain reliable ^{1}H and ^{13}C assignments for a representative selection of lanostan-3β-ol derivatives. Numerous ^{13}C assignments from the early 1970s for lanosterol, dihydrolanosterol and agnosterol were shown to be incorrect. More important, several assignment techniques have been refined for C_{30} sterols. The utility of ^{1}H and ^{13}C acetylation shifts has been extended by new estimates of the magnitude and precision of these shifts. An improved implementation of the LIS technique, giving good agreement between observed and calculated LIS, has proved particularly effective in establishing ^{13}C assignments. The long-range HETCOR experiment has been carried out under conditions permitting a 20-mg sample

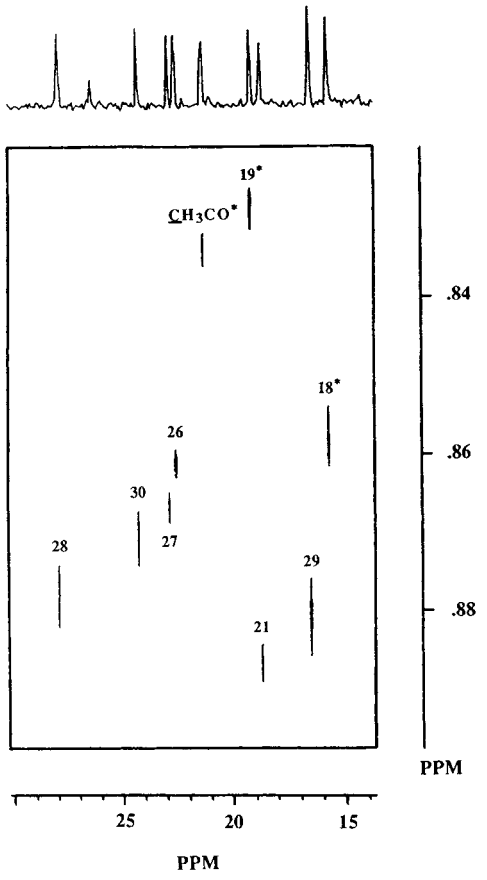

Figure 3. HETCOR spectrum of dihydrolanosterol acquired using a small spectral window in the ^1H dimension in order to resolve methyl protons. Parameters: δ_H 0.80–0.97 spectral window in f_1, 100 increments, zero-filled in t_1 to 256 points, 0.25 Hz exponential line-broadening in t_1, absolute value mode. Cross-peaks marked with an asterisk are folded in the f_1 dimension

of a C_{30} sterol to give useful data in 2 h. Finally, a modest database of reliable ^1H and ^{13}C chemical shifts has been established for C_{30} sterols. This database should be useful for developing and testing predictive methods and for assigning spectra of new triterpenes by chemical shift comparisons.

EXPERIMENTAL

Lanosterol (**1a**), dihydrolanosterol (**2a**) and agnosterol (**8a**) were isolated from a commercial sample of lanosterol (Mann Research Laboratories, New York, USA) by recrystallization from methanol followed by preparative reversed-phase HPLC on a Dynamax 60A column, 8 μm, 250 × 21.4 mm i.d. (Rainin Instrument, Woburn, MA, USA) with methanol as eluent. Treatment of the free sterols with acetic anhydride–pyridine (1:1) at room temperature for 12 h gave acetates **1b** (m.p. 128–129.5 °C; lit.[21] m.p. 127–128 °C), **2b** (m.p. 118.5–120 °C; lit.[21] m.p. 119–120 °C) and **8b** (m.p. 172.5–174.5°C; lit.[22] m.p. 174–175 °C). The epimeric 24,25-epoxylanosterols **5a** and **6a** were prepared[23] from lanosterol and separated by HPLC of their acetate derivatives.[3] The 7-keto derivatives (**3a** and **4a**) were isolated by saponifying lanolin (USP) (E. J. Fougera, Melville, NY, USA) and subjecting the crude material to a combination of normal-phase HPLC on a Spherisorb silica column with ethyl acetate–hexane (5:95) as eluent and reversed-phase HPLC on a Spherisorb ODS-II column, with water–methanol (6:94) as eluent. Both ketosterols showed a UV λ_{max} at 253 nm, had the expected molecular ions at m/z 440 (**3a**) and 442 (**4a**) in their mass spectra and were assayed for purity by capillary gas chromatography on an 11-m DB5 fused-silica column (**3a**, single peak, >99% pure and **4a**, >97% pure). Acetylation gave **3b** and **4b** (m.p. 147–148.5 °C; lit.[24] m.p. 149–151 °C). The structure of acetate **4b** derived from lanolin was confirmed by chemical synthesis of **4b** (m.p. 150.5–152 °C) by treatment of dihydrolanosterol acetate with acetic acid containing sulfuric acid and hydrogen peroxide[24] followed by purification of the major product by reversed-phase HPLC on a Dynamax column (8 μm, 250 × 10 mm i.d.). Treatment of dihydrolanosterol acetate with *m*-chloroperbenzoic acid followed by purification by reversed-phase HPLC on a Dynamax column (8 μm, 250 × 10 mm i.d.) gave the acetate of 8α,9α-epoxylanostan-3β-ol[25] (**7b**, m.p. 140.5–141.5 °C; lit.[25] m.p. 140–142 °C). Dihydroagnosterol acetate (**9b**, m.p. 166.5–168 °C; lit.[25] m.p. 166.5–167 °C) was prepared from **7b** as described previously.[25] Saponification of **5b**, **6b**, **7b** and **9b** gave the corresponding free sterols. Compounds **10–15** were prepared either as described previously or by acetylation of the known free sterol.[1c,d,11] Tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium [Yb(fod)$_3$] was obtained from Aldrich Chemical (Milwaukee, WI, USA) and used as received.

NMR spectra of C_{30} sterols were acquired in $CDCl_3$ solution (6–50 mg ml^{-1}) at ambient temperature (22 °C) on an IBM AF300 spectrometer equipped with an ASPECT 3000 computer and a 5-mm ^1H–^{13}C dual probe. ^1H NMR spectra (300.1 MHZ) were referenced to tetramethylsilane and ^{13}C NMR spectra (75.5 MHz) were referenced to $CDCl_3$ at δ_C 77.0. ^{13}C NMR spectra were measured with WALTZ decoupling using a 20000-Hz spectral window, 0.82-s acquisition time, 0.9-s recycle time and 0.008-ppm digital resolution. ^1H NMR spectra were obtained using a 5000-Hz spectral window, 3.3-s acquisition time, 3.4-s recycle time and 0.001-ppm digital resolution. Resolution enhancement was done by Gaussian multiplication as exemplified in Fig. 2. Standard Bruker microprograms were used for DEPT and 2D experiments. ^{13}C multiplicities were determined from DEPT experiments. COSY spectra were acquired using a 45° read pulse (*ca.* 200 increments of 8–16 scans each), transformed with squared sine bell multiplication in both dimensions and viewed in the absolute value mode. HETCOR spectra optimized for 125 Hz couplings were acquired using a DEPT pulse sequence[26] (XHDEPTD; typical f_2 parameters: δ_C 5–77 spectral window, 2–4K data points, sine bell multiplication). An observed ^1H chemical shift accuracy of *ca.* 0.02 ppm over a range of δ_H of 0.6–2.6 in the f_1 dimension was achieved by collecting *ca.* 50 increments of 16–64 scans with a 1.5-s recycle time, zero-filling to 256 points and applying a 3-5-Hz exponential line broadening in t_1. A smaller ^1H range and/or additional increments were occasionally used for better resolution. For long-range

HETCOR spectra, an INEPT pulse sequence[27] (XHCORRD) was used with decoupling in the f_1 dimension and optimization for 10 Hz couplings (Δ_1 = 50 ms, Δ_2 = 30 ms). With 20-mg samples, more than half of the possible correlations between methyl protons and carbons up to three bonds away were visible after 2 h (16 increments, *ca.* 300 scans, 1.5-s recycle time, zero-filling to 64 points in t_1, 8-Hz line broadening in t_1). An observed chemical shift accuracy of *ca.* ±0.05 ppm (δ_H 0.6–2.6 range) in f_1 permitted several of the ^{1}H methyl signals to be differentiated.

LIS were determined by adding 1–32 μl of a *ca.* 0.12 mM solution of Yb(fod)$_3$ in $CDCl_3$ to a solution of 25–28 mg of sterol in 0.4 ml of $CDCl_3$. ^{1}H and ^{13}C NMR spectra were collected at 22 °C for five molar ratios (0.005–0.2) of Yb(fod)$_3$ to sterol. Induced shifts were measured to 0.001 ppm precision. The relative LIS values were obtained by linear regression calculations (fixing the intercept at the origin) on the plot of induced shifts *vs.* apparent lanthanide shift reagent (LSR) concentration, followed by normalization to the LIS of C-3. Extrapolated to a 1:1 molar ratio of LSR to substrate, the LIS for C-3 of **2a** was 117 ppm. The apparent LSR concentrations, calculated for each LSR concentration from weighted ratios of all non-zero ^{1}H and ^{13}C induced shifts relative to those of a 0.016 molar ratio data set, were similar to the LSR concentrations measured volumetrically. No corrections were made for contact shifts. Atomic coordinates for LIS computations were obtained from molecular mechanics calculations using PC Model (Serena Software, Bloomington, IN, USA) on an IBM-compatible computer (Tandy 4000). The sterol side chain was considered to exist only in its extended conformation. Average positions were calculated for each group of methyl hydrogen atoms. For each set of coordinates and each set of observed LIS values, the position of the coordinating Yb atom was adjusted to give a minimum value of the agreement factor R using the simplified McConnell–Robertson equation.[17] The position of Yb was optimized to a location *ca.* 2.6 Å from O-3. A further set of minimizations, which alternately optimized the position of Yb and the location of the principal magnetic axis, reduced R for dihydrolanosterol from 2.6 to 1.2%. A single site model was used. The minimizations were performed with a Microsoft QuickBASIC program on a Macintosh II computer.

Sets of ^{1}H and ^{13}C NMR assignments were manipulated on a microcomputer spreadsheet (Microsoft Excel, Macintosh version). Macros were used to print the chemical shifts and chemical shift differences in the form of a C_{30} sterol and to rapidly sort the chemical shifts by multiplicity and highlight closely spaced pairs of chemical shifts.

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REFERENCES

1. (a) Y. Sonodo and Y. Sato, *Chem. Pharm. Bull.* **31**, 1698 (1983); (b) Y. Sonodo, Y. Sekigawa and Y. Sato, *Chem. Pharm. Bull.* **36**, 966 (1988); (c) G. J. Schroepfer, Jr, E. J. Parish, M. Tsuda and A. A. Kandutsch, *Chem. Phys. Lipids* **47**, 187 (1988); (d) G. J. Schroepfer, Jr, E. J., Parish and A. A. Kandutsch, *Biochem. Int.* **2**, 257 (1981); (e) G. F. Gibbons, C. R. Pullinger, H. W. Chen, W. K. Cavenee and A. A. Kandutsch, *J. Biol. Chem.* **255**, 395 (1980).
2. L.-J. Lin, M.-S. Shiao and S.-F. Yeh, *J. Nat. Prod.* **51**, 918 (1988).
3. G. T. Emmons, W. K. Wilson and G. J. Schroepfer, Jr, *J. Lipid Res.* **30**, 133 (1989).
4. (a) S. A. Knight, *Tetrahedron Lett.* 83 (1973); (b) S. A. Knight, *Org. Magn. Reson.* **6**, 603 (1974).
5. G. Lukacs, F. Khuong-Huu, C. R. Bennett, B. L. Buckwalter and E. Wenkert, *Tetrahedron Lett.* 3515 (1972).
6. (a) T. Itoh, Y. Kikuchi, N. Shimizu, T. Tamura and T. Matsumoto, *Phytochemistry* **20**, 1929 (1981); (b) M. de Bernardi, G. Mellerio, G. Vidari, P. Vita-Finzi, G. Fronza, M. Kocor and J. St. Pyrek, *J. Nat. Prod.* **44**, 351 (1981); (c) I. Muhammad and C. M. Hasan, *Indian J. Chem., Sect. B* **26**, 722 (1987); (d) P. Majumder, A. Pal and S. Lahiri, *Indian J. Chem. Sect. B* **26**, 297 (1987).
7. (a) A. B. Harref and J.-P. Lavergne, *Bull. Soc. Chim. Fr.* 965 (1985); (b) J. de Pascual Theresa, J. G. Urones, I. S. Marcos, P. Basabe, M. J. Sexmero Cuadrado and R. Fernandez Moro, *Phytochemistry* **26**, 1767 (1987); (c) J.-F. Cheng, J. Kobayashi, H. Nakamura, Y. Ohizumi, Y. Hirata and T. Sasaki, *J. Chem. Soc., Perkin Trans. 1* 2403 (1988).
8. (a) F. W. Wehrli and T. Nishida, *Fortschr. Chem. Org. Naturst.* **36**, (1979); (b) T. Akihisa and T. Matsumoto, *Yukagaku* **36**, 301 (1987).
9. (a) H. Beierbeck, J. K. Saunders and J. W. ApSimon, *Can. J. Chem.* **55**, 2813 (1977); (b) Y. Takaishi, Y. Murakomi, T. Ohashi, K. Nakano, K. Murakami and T. Tomimatsu, *Phytochemistry* **26**, 2341 (1987); (c) J. Martynow and Z. Paryzek, *Magn. Reson. Chem.* **27**, 258 (1989).
10. (a) T. Kikuchi, S. Kanomi, Y. Murai, S. Kadota, K. Tsubono and Z. Ogita, *Chem. Pharm. Bull.* **34**, 4018 (1986); (b) W. Kamisako, C. Honda, K. Suwa and K. Isoi, *Magn. Reson. Chem.* **25**, 683 (1987); (c) S. Seo, A. Uomori, Y. Yoshimura, K. Takeda, H. Seto, Y. Ebizuka, H. Noguchi and U. Sankawa, *J. Chem. Soc., Perkin Trans. 1* 2407 (1988); (d) M. Arisawa, A. Fujita, T. Hayashi, M. Shimizu, N. Morita, T. Kikuchi, S. Kadota and Y. Tezuka, *J. Nat. Prod.* **51**, 54 (1988); (e) M. Tori, R. Matsuda, M. Sono and Y. Asakawa, *Magn. Reson. Chem.* **26**, 581 (1988).
11. E. J. Parish and G. J. Schroepfer, Jr, *J. Lipid Res.* **22**, 859 (1981).
12. (a) F. W. Wehrli, A. P. Marchand and S. Wehrli, *Interpretation of Carbon-13 NMR Spectra*, 2nd ed. Wiley, New York (1988); (b) J. K. Whitesell and M. A. Minton, *Stereochemical Analysis of Alicyclic Compounds by C-13 NMR Spectroscopy*. Chapman and Hall, New York (1987).
13. J. W. Blunt and J. B. Stothers, *Org. Magn. Reson.* **9**, 439 (1977).
14. M. Tsuda and G. J. Schroepfer, Jr, *J. Org. Chem.* **44**, 1290 (1979).

15. (a) J. Bastard, J. M. Bernassau, M. Bertranne and E. R. Maia, *Magn. Reson. Chem.* **26**, 992 (1988); (b) D. H. Smith and P. C. Jurs, *J. Am. Chem. Soc.* **100**, 3316 (1978).
16. (a) H.-J. Schneider, U. Buchheit, N. Becker, G. Schmidt and U. Siehl, *J. Am. Chem. Soc.* **107**, 7027 (1985); (b) W. R. Croasmun and R. M. K. Carlson, in *Two Dimensional NMR Spectroscopy*. VCH, New York (1987).
17. T. Wenzel, *NMR Shift Reagents*. CRC Press, Boca Raton, FL (1987).
18. P. Joseph-Nathan, G. Mejia and D. Abramo-Bruno, *J. Am. Chem. Soc.* **101**, 1289 (1979).
19. H.-J. Schneider and P. K. Agrawal, *Magn. Reson. Chem.* **24**, 718 (1986).
20. H.-S. Kim, W. K. Wilson, D. H. Needleman, F. D. Pinkerton, D. K. Wilson, F. A. Quiocho and G. J. Schroepfer, Jr, *J. Lipid Res.* **30**, 247 (1989).
21. J. D. Johnston, F. Gautschi and K. Bloch, *J. Biol. Chem.* **224**, 185 (1957).
22. L. Ruzicka, R. Denss and O. Jeger, *Helv. Chim. Acta* **29**, 204 (1946).
23. S. R. Panini, R. C. Sexton, A. K. Gupta, E. J. Parish, S. Chitrakorn and H. Rudney, *J. Lipid Res.* **27**, 1190 (1986).
24. J. T. Pinhey, B. J. Ralph, J. J. H. Simes and M. Wootton, *Aust. J. Chem.* **23**, 2141 (1970).
25. I. G. Guest and B. A. Marples, *J. Chem. Soc. C* 1468 (1971).
26. T. T. Nakashima, B. K. John and R. E. D. McClung, *J. Magn. Reson.* **59**, 124 (1984).
27. J. A. Wilde and P. H. Bolton, *J. Magn. Reson.* **59**, 343 (1984).
28. B. L. Buckwalter, I. R. Burfitt, A. A. Nagel, E. Wenkert and F. Näf, *Helv. Chim. Acta* **58**, 1567 (1975).