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LANTHANIDE- AND AROMATIC SOLVENT-INDUCED SHIFT EFFECTS ON PROTON RESONANCES IN C-4-METHYLATED STEROIDS AND TETRACYCLIC TRITERPENOIDS

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Lanthanide-induced shifts (LIS) with Eu $(dpm)_3$ and aromatic solvent induced shifts (ASIS) with C_6H_6, C_5H_5N, and C_6F_6 of PMR signals were examined for a series of C-4-methylated steroids and tetracyclic triterpenoids having a hydroxyl, carbonyl or acetoxyl group at position C-3. The magnitude and/or direction of the LIS (or ASIS) of corresponding protons were extensively influenced by the nature of the C-3-functional groups. The possible geometries of Eu $(dpm)_3$-substrate complexes were also discussed on the basis of the LIS data. The above two techniques in the PMR spectroscopy provided the confirmatory evidence for the structural and stereochemical determination of steroids and triterpenoids.

I. Introduction

Proton magnetic resonance (PMR) spectroscopy is conventionally used for the structural and stereochemical analysis of naturally occurring compounds [1]. In steroid and triterpenoid fields, however, the overlap of the proton resonances is often a serious problem, and, consequently, the information which can be obtained from the PMR spectra is severely restricted.

In connection with the problem, Hinckley et al. [2] and Sanders and Williams [3] have suggested the application of tri-(dipivalomethanato) europium Eu $(dpm)_3$, a lanthanide shift reagent. Since the discovery of these workers, many naturally occurring compounds were identified by using Eu $(dpm)_3$ as a shift reagent [4–11]. The use of aromatic solvents such as C_6H_6 [12–14], C_5H_5N [15,16] and C_6F_6 [17,18], instead of normal inert solvent (e.g. $CDCl_3$), has also previously been shown to be of value in

increasing chemical shift differences of specific protons in compounds with a polar functional group. The effectiveness of the above two methods, i.e. lanthanide-induced shift (LIS) and aromatic solvent-induced shift (ASIS), in PMR spectroscopy is now well recognized as useful aids for the signal assignment as well as for the spectral simplification of complex compounds.

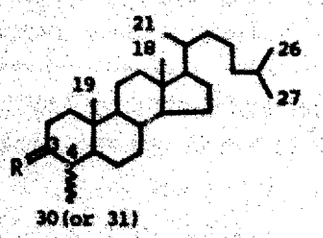
We wish to report here a further extension on the application of LIS and ASIS to the structural and stereochemical determination of biologically important compounds distributed widely in naturally occurring sources. Thus, the aim of this study is to evaluate the LIS and ASIS behaviors of protons in C-4-methylated sterols and tetracyclic triterpene alcohols as well as in their keto and acetate derivatives, since the hydroxyl group is the most frequently found substituent in the fields and since derivatization to such functional groups is a common procedure in characterization and is also informative for physical measurements including PMR spectra; a variety of 4-monomethyl-5α-cholestane, 4,4-dimethyl-5α-cholestane, Δ^8-lanostene, and cycloartane (9β, 19-cyclolanostane) types of compounds were examined here (fig. 1).

II. Experimental

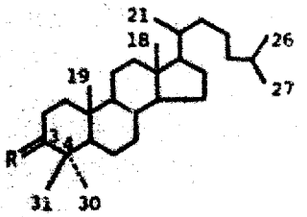
Most of the compounds used in this study were synthesized in our laboratory. Preparations of C-4-methylated steroids (I–V, XI–XV, XXI–XXIV) have been described previously [19]. A mixture of 9β, 19-cyclo-5α-lanost-24-en-3β-ol [VIII; m.p. 112–113°C, $[\alpha]_D$ + 55 (c, 2% in $CHCl_3$); lit. [20] m.p. 112–114°C, $[\alpha]_D$ + 49.2] and 24-methylene-9β, 19-cyclo-5α-lanostan-3β-ol [X; m.p. 119–120°C, $[\alpha]_D$ + 45; lit. [21a] m.p. 121–122°C, $[\alpha]_D$ + 43] was supplied from Riken Vitamin Oil Co., and purified by the preparation of the acetate, followed by silica gel impregnated with 10% (w/v) silver nitrate column chromatography described in detail by Vroman and Cohen [22]. In a similar manner, 5α-lanost-8,24-dien-3β-ol [VI; m.p. 139–140°C, $[\alpha]_D$ + 61] and 5α-lanost-8-en-3β-ol [VII; m.p. 140–141°C, $[\alpha]_D$ + 62] were separated from a mixture of both the alcohols obtained from commercial source (Nakarai Chemicals Ltd.). 9β,19-Cyclo-5α-lanostan-3β-ol [IX; m.p. 101.5–103°C, $[\alpha]_D$ + 49; lit. [21 b] m.p. 99–101°C, $[\alpha]_D$ + 45.5] was obtained by catalytic hydrogenation of VIII over platinum oxide.

Carboxylation or acetylation of alcohols was carried out by standard methods, and the products were checked by appropriate physical constants and gas chromatography: compounds XVI and XVII were prepared following the procedure of Wigfield et al. [23], using dimethyl sulfoxide-dicyclohexyl carbodiimide; compounds XVIII–XX were prepared by the addition of chromium trioxide in C_5H_5N to the corresponding alcohols in the same solvent; compounds XXV–XXIX were obtained by the addition of a solution of acetic anhydride-C_5H_5N to the corresponding alcohols.

All PMR spectra were obtained on a Hitachi R-22 spectrometer (90 MHz) operating in the field sweep mode, at ambient probe temperature of 34°C. Substrate concentrations were 0.06 to 0.12 M depending upon the sample quantity available: $CDCl_3$ was



[1]



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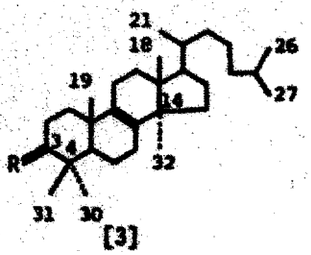

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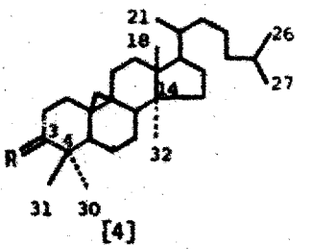

[4]

	3β-ol ($R<^{OH}_{H}$)	3-one (R = O)	3β-ylacetate ($R<^{OCOCH_3}_{H}$)
4-Monomethyl steroids [1]			
4α-Methyl-5α-cholestan-	I	XI	
4β-Methyl-5α-cholestan-	II	XII	
4α-Methyl-5α-cholest-7-en-			XXI
4,4-Dimethyl steroids [2]			
4,4-Dimethyl-5α-cholestan-	III	XIII	XXII
4,4-Dimethylcholest-5-en-	IV	XIV	XXIII
4,4-Dimethyl-5α-cholest-7-en-	V	XV	XXIV
Δ^8-Lanostene triterpenes [3]			
5α-Lanost-8,24-dien-	VI	XVI	XXV
5α-Lanost-8-en-	VII	XVII	XXVI
Cycloartane trierpenes [4]			
9β,19-Cyclo-5α-lanost-24-en-	VIII	XVIII	XXVII
9β,19-Cyclo-5α-lanostan-	IX	XIX	XXVIII
24-Methylene-9β,19-cyclo-5α-lanostan-	X	XX	XXIX

Fig. 1. Basic skeletons and the numbering systems of methyl groups for compounds examined.

used as standard solvent; C_5H_6, C_5H_5N, and C_6F_6 (for solubility reason of substrates in C_6F_6, ca. 5% $CDCl_3$ contain) were used as induced shift solvents. Eu $(dpm)_3$ was purchased from Wako Pure Chemicals Industries Ltd., and used as such: exactly weighed amounts (2–4 mg) of Eu $(dpm)_3$ were added in increasing amounts to $CDCl_3$

(0.4 ml) solutions of weighed quantities of substrates (9.2–20.6 mg) in the PMR sample tube, and spectra recorded after each addition; usually five or six such additions of the shift reagent were made for each compound. In all cases, tetramethylsilane (TMS) was employed as internal reference standard and chemical shifts were denoted on δ (ppm) unit relative to internal TMS. Error of the measurements was estimated to be less than ± 0.02 ppm for the chemical shifts.

III. Results and Discussion

A. LIS effects

Upon the addition of a lanthanide shift reagent, Eu $(dpm)_3$, to normal $CDCl_3$ solutions, almost all of methyl protons in compounds examined suffered paramagnetic shifts [4] due to complex formation between Eu^{3+} in the reagent and oxygen lone pairs in the substrates. The magnitude of the LIS was enhanced successively by increasing in the concentration of Eu $(dpm)_3$ added, and the first-order spectra were obtained without serious line broadening. The assignment of each methyl signal in normal $CDCl_3$ solvent spectra was performed using criteria discussed in the previous works [1, 24, 25], and confirmed in this study, on the basis of the LIS data (discussed below). The signal assignments in the presence of Eu $(dpm)_3$ were based on integration, careful inspections of each signal as increments of the reagent were added, and the measurement of approximate spatial distance between protons under consideration and a C-3-functional group.

Plots of the chemical shifts vs. the molar ratio of Eu $(dpm)_3$ added to substrates were found to be linear within the molar ratio up to about 1. Fig. 2 shows sample plots of methyl protons in compounds VII, XVII and XXVI under the same concentration of the substrates. As can be seen in fig. 2, the straight lines were accurately extrapolated to zero concentration of Eu $(dpm)_3$ to provide valuable informations about ambiguous chemical shifts. In fact, δ_{CDCl_3} values of compounds examined can be ascertained more easily from the plotting data than the substituent effects on the chemical shifts [1, 24, 25]. This method is therefore useful for confirming obscured and unidentified chemical shifts in the normal PMR spectra of steroids and triterpenoids.

From the plotting data, ΔEu values [26], namely, paramagnetic induced shifts, for each methyl proton were then determined by the equation defined as $\delta_{n=1}^{Eu(dpm)_3} - \delta_{CDCl_3}$, where $\delta_{n=1}^{Eu(dpm)_3}$ is the chemical shift obtained by extrapolating to the point where the molar ratio of Eu $(dpm)_3$ to substrate is 1. Because of the great dependence of the ΔEu values on substrate concentration [27,28], the observed ΔEu values for the fastest moving signal within the same molecule were further normalized to a value of 100. Tables 1–3 show the normalized ΔEu values obtained here, together with chemical shift data; negative value represents an upfield shift. As expected, the normalized ΔEu values were surely almost independent of substrate concentrations and essentially identical for corresponding protons in compounds possessing the same

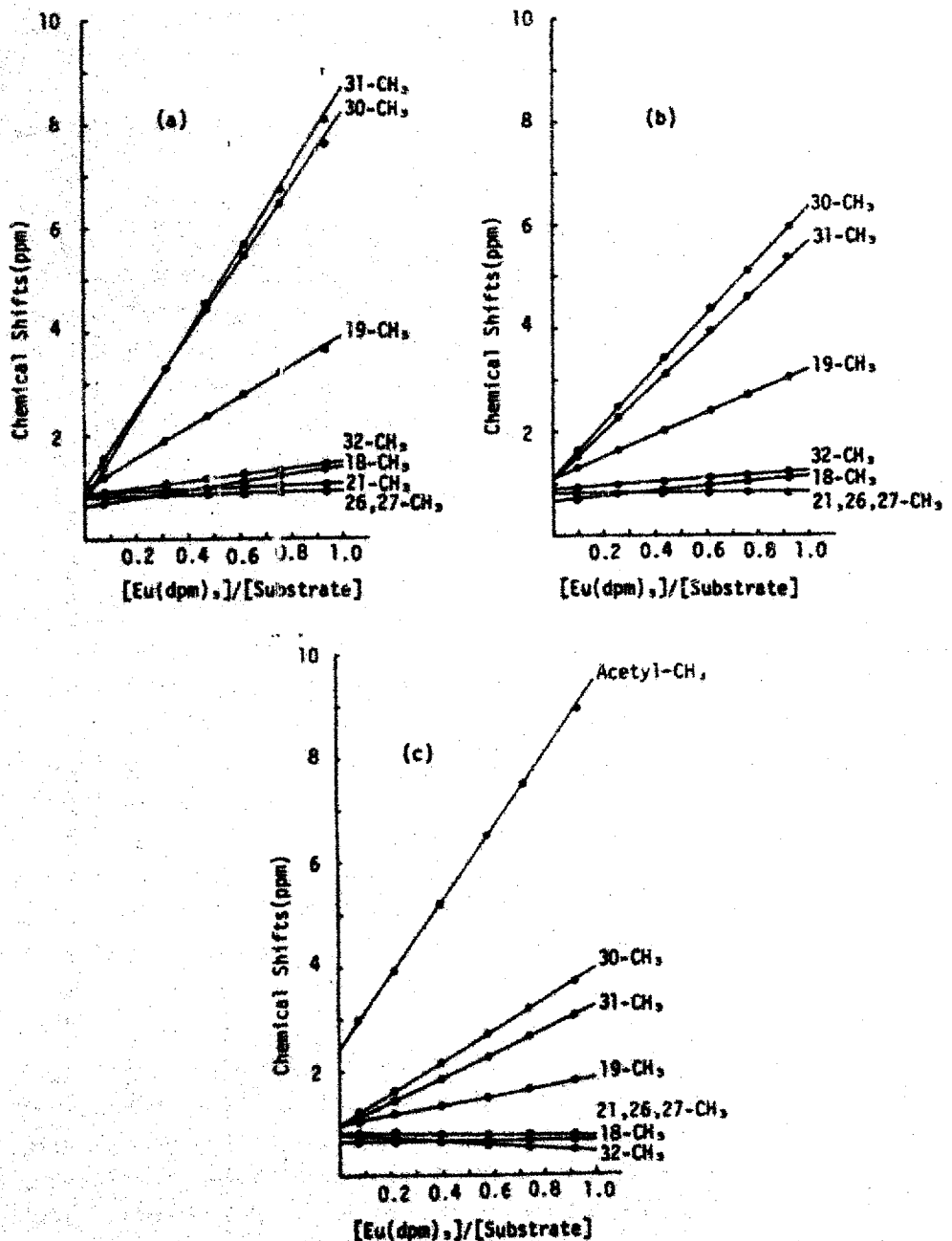

Fig. 2. Plots of the chemical shifts vs. the molar ratio of $Eu(dpm)_3$ to substrates of methyl protons for 5α-lanost-8-en-3β-ol (VII, a), 5α-lanost-8-en-3-one (XVII, b), and 5α-lanost-8-en-3β-ylacetate (XXVI, c). (Concentrations of the substrates are shown in table 1–3).

C-3-functional group. All the compounds are therefore believed to form adduct with $Eu(dpm)_3$ of the same type.

The complexiting ability of substrates with $Eu(dpm)_3$, i.e. the slopes of linear plots for corresponding protons, decreased in the order, alcohols > ketones > acetates (see fig. 2). This is the expected order based on the dissociation constant of these

Table 1

Ptoton chemical shifts (δ, ppm) and LIS data on sterols and triterpene alcohols examined[a]

Compounds	Conc. of substrate ($\cdot 10^{-5}$ M)		30-CH_3[b]	31-CH_3[b]	19-CH_3[b]	18-CH_3[b]	32-CH_3[b]	21-CH_3[c]	26,27-di-CH_3
I	4.29	δ_{CDCl_3}	0.94[c]		0.82	0.65		0.90	0.86
			(100)		(41)	(8)		(3)	(1)
II	4.30	δ_{CDCl_3}		0.87[c]	0.83	0.64		0.88	0.86
				(100)	(39)	(8)		(3)	(0)
III	4.28	δ_{CDCl_3}	0.95	0.79	0.85	0.64		d	0.86
			(95)	(100)	(37)	(5)		(2)	(0)
IV	4.95	δ_{CDCl_3}	1.13	1.05	1.05	0.65		0.91	0.85
			(95)	(100)	(38)	(9)		(3)	(1)
V	4.25	δ_{CDCl_3}	0.98	0.85	0.88	0.52		0.91	0.85
			(90)	(100)	(35)	(9)		(3)	(1)
VI	2.86	δ_{CDCl_3}	0.98	0.80	1.00	0.70	0.87	0.91	1.63
			(91)	(100)	(37)	(10)	(7)	(3)	(1)
VII	4.39	δ_{CDCl_3}	0.98	0.82	0.98	0.69	0.89	d	0.86
			(92)	(100)	(36)	(10)	(8)	(3)	(1)
VIII	4.46	δ_{CDCl_3}	0.95	0.81		0.95	0.89	d	1.64
			(94)	(100)		(10)	(9)	(3)	(1)
IX	4.47	δ_{CDCl_3}	0.96	0.80		0.96	0.89	d	0.86
			(94)	(100)		(10)	(10)	(3)	(1)
X	4.30	δ_{CDCl_3}	0.97	0.81		0.97	0.91	d	1.05
			(94)	(100)		(10)	(10)	(3)	(1)

[a]Proton chemical shifts were obtained on a Hitachi R-22 spectrometer (90 MHz) using internal TMS (δ = 0 ppm); values in parentheses show normalized ΔEu values (see text for definition); negative normalized ΔEu value represents an upfield shift of a proton by the addition of $Eu(dpm)_3$.

[b]singlet.

[c]Doublet (*J*, 6.3–7.2 Hz).

[d]Not determined due to signal overlapping.

adducts within the range of the substrate concentrations examined [3]. We also found that the addition of Eu $(dpm)_3$ has little or no effect upon the proton resonances in C-3β-methyl ether derivatives [29], which are often used in structural identification of sterols by their excellent gas chromatographic and mass spectrometric properties [30].

1. LIS of sterols and triterpene alcohols

As shown in table 1, the addition of Eu $(dpm)_3$ to $CDCl_3$ solutions of sterols and triterpene alcohols (I–X) produced the downfield shifts of all of the substrate methyl protons. The magnitude of the normalized ΔEu values decreased in the order, 31–(100) > 30–(90–95) > 19–(35–41) > 18–(5–10) ≅ 32–(7–10) > 21–(2–3) > 26,27–(0–1) methyls, in accord with the prediction of the crude distance treatment [1]: the shift of a proton close to the co-ordinating site of Eu^{3+} is usually larger than a proton further remoted.

With those compounds, the most interesting finding concerned the LIS behaviors of methyls geminally attached at C-4. Thus, the *axial* 31-methyl signal was moved more rapidly than the *equatorial* 30-counterpart, when increasing amounts of Eu $(dpm)_3$ were added, and the plots of the two methyls crossed each other (see fig. 2a). This crossover indicates that the relative position of the *axial* and *equatorial* methyl signals in the spectra is influenced with the concentration of the shift reagent added: the 31-methyl occurs at a higher field than the geminal 30-methyl in the low concentration of Eu $(dpm)_3$, but the reverse occurs in the high concentration of the reagent. The observation was justified by a careful comparison of the spectra of an isomeric pair of 4α-methyl-5α-cholestan-3β-ol (I) and 4β-methyl-5α-cholestan-3β-ol (II). Particular attention should therefore be paid in the assignments of the two siganls from the spectra alone without the plotting data. The addition of Eu $(dpm)_3$ also brought about the separation of the doublets of C-17 side chain methyls, i.e. 21-methyl and 26,27-dimethyl, in C-24 saturated compounds (I-V, VII, IX), and revealed the presence of the 21-methyl doublet whose signal was often invisible in the normal PMR spectra.

According to Hinckley's consideration [1], LIS is ascribed to be mainly of the pseudo-contact shift [31] which is expressed as $K(3\cos^2\theta_i - 1)/R_i^3$, where K is a constant for a given complex, R_i is the distance between Eu^{3+} and H_i proton and θ_i is the corresponding internuclear angle between Eu-H_i axis and Eu-O axis. As K is the same for different protons within the same molecule, the pseudo-contact shift is essentially a function of the distance R and the angular factor θ. If one assumes that in Eu $(dpm)_3$-alcohol complexes examined here, the Eu^{3+} is located 3 Å [32, 33] from the oxygen with the Eu-O-C(3) angle of 130° [34, 35], the spatial distance of the Eu^{3+} from 31-methyl group will be closer than that from 30-methyl group, thereby the former will be deshielded more strongly than the latter.

2. LIS of keto derivatives

In the case of C-3-keto derivatives (XI–XX, table 2), the decreasing order of the normalized ΔEu values was as follows: 30–(100) > 31–(65–88) > 19–(32–48) > 18–(4–8) ≅ 32–(4–8) > 21–(1–4) ≧ 26,27–(−1–1) methyls. This order in the

Table 2

Proton chemical shifts (δ, ppm) and LIS data on Keto derivatives examined[a].

Compounds	Conc. of substrate ($\cdot 10^{-5}$ M)		30-CH_3[b]	31-CH_3[b]	19-CH_3[b]	18-CH_3[b]	32-CH_3[b]	21-CH_3[c]	26,27-di-CH_3[c]
XI	2.66	δ_{CDCl_3}	0.97[c]		1.04	0.67		0.89	0.86
			(100)		(32)	(7)		(1)	(1)
XII	2.69	δ_{CDCl_3}		1.11[c]	1.05	0.67		0.89	0.85
				(100)	(52)	(11)		(1)	(0)
XIII	4.37	δ_{CDCl_3}	1.04	1.04	1.04	0.66		0.89	0.86
			(100)	(79)	(36)	(6)		(3)	(0)
XIV	4.39	δ_{CDCl_3}	1.21	1.21	0.85	0.68		0.91	0.86
			(100)	(73)	(48)	(7)		(4)	(0)
XV	4.68	δ_{CDCl_3}	1.12	1.07	1.07	0.56		0.92	0.86
			(100)	(76)	(32)	(8)		(3)	(0)
XVI	2.79	δ_{CDCl_3}	1.08	1.06	1.11	0.73	0.89	0.91	1.64
			(100)	(85)	(42)	(4)	(4)	(1)	(0)
XVII	4.41	δ_{CDCl_3}	1.09	1.05	1.11	0.72	0.89	d	0.86
			(100)	(88)	(42)	(8)	(7)	(1)	(0)
XVIII	4.55	δ_{CDCl_3}	1.10	1.05		1.00	0.90	d	1.66
			(100)	(67)		(7)	(8)	(1)	(−1)
XIX	2.27	δ_{CDCl_3}	1.09	1.04		0.99	0.90	d	0.86
			(100)	(66)		(8)	(8)	(2)	(0)
XX	4.29	δ_{CDCl_3}	1.10	1.05		0.99	0.91	0.92	1.03
			(100)	(65)		(7)	(7)	(2)	(0)

[a–d] See in table 1.

ketones is similar to that found in their parent hydroxyl compounds mentioned above, but with one significant difference. Thus, contrary to the measurements with the presence of a C-3β-hydroxyl group, the fact that the 30-methyl signal in the presence of a C-3-carbonyl group is much more subject to the LIS with increasing addition of Eu·(dpm)$_3$ than the geminal 31-counterpart was verified by comparing the spectrum of 4α-methyl-5α-cholestan-3-one (XI) with that of 4β-methyl isomer (XII) (fig. 2b). Assuming that in Eu (dpm)$_3$-ketone complexed state, the Eu-O distance is 3 Å at an angle of 20° to the carbonyl-bond axis towards protons at C-2 [10,36], then the above decreasing shift order of each methyl proton can reasonably be interpreted.

It is of importance to note that the magnitude of the normalized ΔEu values for the 31-methyl proton in the ketones is obviously dependent on the nature of skeletal structure: cycloartane (XVIII–XX), 4,4-dimethyl-5α-cholestane (XII–XV) and Δ^8-lanostene (XVI, XVII) skeletones are characterized by the values of 65–67, 73–79 and 85–88, respectively. The observed differences are probably associated with both the angular dependence and the fact that a carbonyl group is bifunctional, and serve as a useful tool for evaluating each skeleton of steroids and triterpenoids.

3. LIS of acetate derivatives

In our LIS study, C-3β-acetate derivatives (XXI–XXIX, table 3) provided many informations about the structures of steroids and triterpenoids. The decreasing order of the normalized ΔEu values in those compounds was as follows: acetyl- (100) > 30– (39–45) > 31– (28–34) > 19– (10–12) > 32– (−3 ~ −6) > 21– (−1 ~ −3) ≅ 26,27– (−1 ~ −3) ≧ 18– (0–1) methyls. Acetyl methyl proton attached to the coordinating site of Eu^{3+} undergoes the greatest downfield shifts. A signal suffering a larger LIS within the geminal C-4-methyl groups is believed to be the 30-methyl proton (see fig. 2c), in analogy with results obtained by Shingu et al. [7] for some pentacyclic triterpene acetates related to β-amyrin series. The assignment of the geminal C-4-methyls in the C-3β-acetate derivatives is therefore the same as that made in the corresponding keto derivatives mentioned above. Comparing the differences in the chemical shifts between the 30- and 31-methyl groups, it can be seen that they become somewhat larger in the acetates than in the ketones by increasing Eu(dpm)$_3$ (see fig. 2b and c). It is also noted that the induced shift value of 18-methyl proton is negligibly small.

It might be difficult to assess the presence or absence of the 32-methyl group in tetracyclic triterpenoids using only the normal PMR spectra. However, the methyl group could easily and unequivocally be characterized by measuring the Eu(dpm)$_3$-induced shift spectra of the C-3β-acetate derivatives, because it shifted to the higher field and showed negative ΔEu values. Minor but consistent negative ΔEu values was also detected for the doublets of the 21-methyl and 26,27-di-methyl, in accord with the previous findings of Kishi et al. [37] in cholesteryl acetate. The abnormal shift of proton resonances to upfield is attributable to a particularly geometrical disposition of those methyl groups against Eu^{3+}, and mean that the sign of the angular dependence term, $(3\cos^2\theta - 1)$, in the pseudo-contact shift equation is negative [4]; θ has a value

Table 3

Proton chemical shifts (δ, ppm) and LIS data on acetate derivatives examined[a].

Compounds	Conc. of substrate ($\cdot 10^{-5}$ M)		30-CH_3[b]	31-CH_3[b]	19-CH_3[b]	18-CH_3[b]	32-CH_3[b]	21-CH_3[c]	26,27-di-CH_3[c]	Acetyl-CH_3[e]
XXI	2.58	δ_{CDCl_3}	0.86[c]		0.83	0.54		0.90	0.86	2.01
			(43)		(12)	(0)		(−2)	(−2)	(100)
XXII	4.19	δ_{CDCl_3}	0.86	0.88	0.86	0.85		d	0.87	2.03
			(42)	(31)	(12)	(0)		(−2)	(−2)	(100)
XXIII	4.47	δ_{CDCl_3}	1.02	1.13	1.08	0.68		0.91	0.86	2.01
			(39)	(34)	(12)	(0)		(−1)	(−1)	(100)
XXIV	4.50	δ_{CDCl_3}	0.88	0.97	0.91	0.54		0.92	0.86	2.03
			(39)	(30)	(10)	(0)		(−2)	(−2)	(100)
XXV	4.34	δ_{CDCl_3}	0.86	0.86	1.00	0.70	0.86	0.91	1.64	2.02
			(40)	(31)	(11)	(0)	(−6)	(−3)	(−2)	(100)
XXVI	4.38	δ_{CDCl_3}	0.87	0.87	1.00	0.69	0.87	0.88	0.85	2.01
			(41)	(32)	(11)	(0)	(−6)	(−2)	(−1)	(100)
XXVII	4.36	δ_{CDCl_3}	0.85	0.88		0.98	0.88	d	1.66	2.01
			(42)	(28)		(1)	(−3)	(−3)	(−3)	(100)
XXVIII	2.16	δ_{CDCl_3}	0.85	0.89		0.97	0.89	d	0.86	2.02
			(45)	(34)		(1)	(−3)	(−1)	(−1)	(100)
XXIX	2.88	δ_{CDCl_3}	0.85	0.89		0.98	0.89	d	1.02	2.03
			(44)	(34)		(1)	(−3)	(−1)	(−1)	(100)

[a–d] See in table 1.
[e] Singlet.

of 54.7–125.3°.

On the basis of the above facts, it seemed resonable to presume that in $Eu(dpm)_3$-acetate complexes, the Eu^{3+} is located approximately beneath the ring system of the substrate molecule with the Eu–O distance of 3 Å, though the exact Eu–O=C angle is not apparent now. This presumption may be supported by the fact that C-3β-acetoxyl group in steroidal acetates exists predominantly in a *cis*-conformation with the *axial* C-3α-methine proton eclipsed by the C=O group [38,39].

B. ASIS Effects

Tables 4–6 show the ASIS (Δ values = $\delta_{\text{aromatic solvent}} - \delta_{CDCl_3}$) observed for protons in compounds examined; negative Δ value represents an upfield shift. The proton assignments in aromatic solvents, i.e. C_6H_6, C_5H_5N, C_6F_6, were based almost exclusively on the comparison of the spectra of those compounds which differ from one another in the presence or absence of certain methyl groups and of an unsaturated bond in the skeletons. It is apparent from the data shown in tables 4–6 that the ASIS are obviously detected on the solute protons, particularly on protons situated in the vicinity of a C-3-functional group where co-ordination with solvent molecule occurs [16, 17, 40], and that all compounds possessing the same C-3-functional group usually give similar Δ values for corresponding protons. Although the shift strength on proton resonances due to aromatic solvents used is usually weaker than that due to shift reagent discussed above, the ASIS are favourable in view of the simple procedure and easy recovery of samples.

1. ASIS of sterols and triterpene alcohols

In compounds with a C-3β-hydroxyl group (I-X, table 4) a significant difference was observed between the spectra measured in both $CDCl_3$ and C_5H_5N. Thus, the 30- and 31-methyl protons occupying position vicinal to the hydroxyl group were deshielded considerably ($\Delta_{C_5H_5N}$, 0.20 to 0.34 ppm) in C_5H_5N relative to $CDCl_3$. The magnitude of the deshielding was almost independent of *axial* or *equatorial* configuration of the methyls, indicating that the two methyl groups have equal geometrical relationship against the hydroxyl group [16]. Although other methyl protons presented in table 1 also exhibited the consistent ASIS, the magnitude of the induced shift values for these protons was relatively small. The ASIS with C_5H_5N can therefore be used as a simple and sensitive method for confirming the presence of the C-4-methyl groups in naturally occurring 4-methyl sterols and triterpene alcohols.

The ASIS behaviors of a proton situated geminally to the C-3-β-hydroxyl group are also of interest, because the direction of the shifts is influenced by the nature of aromatic solvent used: the C-3α-methine proton suffers downfield shifts ($\Delta_{C_5H_5N}$, ca. 0.20 ppm) in C_5H_5N, but it shifted to the opposite direction (to upfield) in C_6H_6 ($\Delta_{C_6H_6}$, ca. −0.18 ppm) and C_6F_6 ($\Delta_{C_6F_6}$, ca. −0.10 ppm).

2. ASIS of keto derivatives

In C-3-keto derivatives (XI–XX, table 5), the ASIS behaviors of protons much diff-

Table 4

ASIS data on sterols and triterpene alcohols examined[a].

Compounds		30-CH_3[b]	31-CH_3[b]	19-CH_3[b,f]	18-CH_3[b]	32-CH_3[b]	21-CH_3[c]	26,27-di-CH_3[c]	3α-H[d]
I	ΔC_5H_5	0.07		−0.06	0.02		0.08	0.06	−0.23
	ΔC_5H_5N	0.27		0.03	0.03		0.05	0.02	0.15
	ΔC_6F_5	0.01		0.07	0.06		0.05	0.02	−0.08
II	ΔC_5H_5		0.06	−0.05	0.03		0.11	0.07	−0.17
	ΔC_5H_5N		0.29	0.07	0.05		0.09	0.03	0.26
	ΔC_6F_5		0.03	0.09	0		0.07	0.02	−0.10
III	ΔC_5H_5	0.05	0.03	−0.06	0.02		0.10	0.05	−0.16
	ΔC_5H_5N	0.23	0.23	0.08	0.06		0.09	0.04	0.15
	ΔC_6F_5	0	0	0.08	0.07		0.06	0.04	−0.12
IV	ΔC_5H_5	0.12	0.11	0.03	0.04		0.09	0.08	−0.11
	ΔC_5H_5N	0.31	0.31	0.13	0.07		0.07	0.05	0.27
	ΔC_6F_5	0	0	0.08	0.10		0.06	0.04	−0.06
V	ΔC_5H_5	0.02	0.06	−0.01	0.09		0.09	0.08	−0.24
	ΔC_5H_5N	0.20	0.25	0.03	0.07		0.06	0.03	0.11
	ΔC_6F_5	0.04	0.08	0.05	0.07		e	0.05	−0.09
VI	ΔC_5H_5	0.04	0.03	−0.04	0.08	0.07	0.09	0.02	−0.20
	ΔC_5H_5N	0.24	0.26	0.06	0.09	0.10	0.08	0.02	0.20
	ΔC_6F_5	0.04	0.04	0.07	0.10	0.09	e	0.02	−0.04
VII	ΔC_5H_5	0.04	0.02	−0.03	0.10	0.06	e	0.07	−0.18
	ΔC_5H_5N	0.26	0.26	0.10	0.12	0.09	e	0.04	0.22
	ΔC_6F_5	0.04	0.01	0.09	0.10	0.06	e	0.04	−0.07

VIII	ΔC_6H_6	0.05	0.05	(−0.11,−0.19)	0.05	0.02	e	0.01	−0.20
	ΔC_5H_5N	0.26	0.27	(−0.01,−0.05)	0.07	0.06	e	0.03	0.20
	ΔC_6F_6	0.04	−0.02	(0.05, 0.03)	0.09	0.06	e	0	−0.14
IX	ΔC_6H_6	0.07	0.07	(−0.09,−0.09)	0.05	0.04	e	0.07	−0.16
	ΔC_5H_5N	0.24	0.28	(0.02, 0.01)	0.08	0.07	e	0.04	0.22
	ΔC_6F_6	0.02	−0.02	(0.05, 0.07)	0.09	0.04	e	0.03	−0.15
X	ΔC_6H_6	0.04	0.07	(−0.06,−0.16)	0.04	0.01	e	0.02	−0.16
	ΔC_5H_5N	0.24	0.28	(0.05,−0.02)	0.06	0.06	e	0.02	0.22
	ΔC_6F_6	0.03	−0.02	(0.11, 0.07)	0.10	0.06	e	0.01	−0.09

[a] ASIS values are defined as $\delta_{\text{aromatic solvent}} - \delta_{CDCl_3}$; negative value represents an upfield shift of a proton in aromatic solvent relative to $CDCl_3$.

[b] Singlet.

[c] Doublet (J, 6.3 – 7.2 Hz).

[d] Multiplet.

[e] Not determined due to signal overlapping.

[f] Values in parentheses show individual centers of the pair of doublet (J, ca. 4,5 Hz) of 9β, 19-cyclopropane methylene on cycloaratane triterpenes.

Table 5
ASIS data on keto derivatives examined[a].

Compounds		30-CH_3[b]	31-CH_3[b]	19-CH_3[b,f]	18-CH_3[b]	32-CH_3[b]	21-CH_3[c]	26,27-di-CH_3[c]
XI	ΔC_6H_6	0.07		0.29	−0.02		0.10	0.05
	ΔC_5H_5N	0.07		−0.06	0		0.08	0.02
	ΔC_6F_6	−0.04		0.18	0.11		0.08	0.04
XII	ΔC_6H_6		−0.15	−0.28	−0.01		0.10	0.08
	ΔC_5H_5N		−0.04	−0.10	0		0.08	0.03
	ΔC_6F_6		0.05	0.15	0.10		0.08	0.04
XIII	ΔC_6H_6	0.08	−0.10	−0.25	0		0.09	0.07
	ΔC_5H_5N	0.08	−0.01	−0.08	0.02		0.07	0.05
	ΔC_6F_6	0	0.04	0.12	0.10		0.07	0.03
XIV	ΔC_6H_6	0.18	−0.06	−0.09	0.03		0.09	0.07
	ΔC_5H_5N	0.18	0.05	0	0.01		0.07	0.04
	ΔC_6F_6	−0.01	0.06	0.09	0.12		0.07	0.04
XV	ΔC_6H_6	0	−0.12	−0.19	0.04		0.07	0.06
	ΔC_5H_5N	0.03	0	−0.05	0.05		e	0.03
	ΔC_6F_6	−0.14	0.09	0.14	0.08		0.08	0.05
XVI	ΔC_6H_6	0.02	−0.08	−0.19	0.03	0.03	0.08	0.03
	ΔC_5H_5N	0.06	0	−0.05	0.03	0.06	0.09	0.04
	ΔC_6F_6	0	0.06	0.15	0.12	0.12	e	0.02
XVII	ΔC_6H_6	0.01	−0.07	−0.17	0.05	0.05	e	0.07
	ΔC_5H_5N	0.09	−0.02	−0.04	0.07	0.05	e	0.05
	ΔC_6F_6	−0.02	0.05	0.15	0.12	0.12	e	0.04
XVIII	ΔC_6H_6	0.05	−0.11	(−0.11, −0.01)	0	−0.04	e	0
	ΔC_5H_5N	0.04	0	(0.10, 0.27)	0.04	0.01	e	0.01
	ΔC_6F_6	−0.05	0.09	(e , 0.52)	0.13	0.03	e	−0.02
XIX	ΔC_6H_6	0.05	−0.09	(−0.07, 0.02)	0.02	−0.02	e	0.05
	ΔC_5H_5N	0.05	0.02	(0.11, 0.29)	0.05	0.01	e	0.04
	ΔC_6F_6	−0.04	0.10	(e , 0.53)	0.13	0.03	e	0.04
XX	ΔC_6H_6	0.05	−0.11	(−0.08, −0.02)	0.02	−0.05	e	0.05
	ΔC_5H_5N	0.05	0.01	(0.10, 0.26)	0.05	0.01	0.05	0.05
	ΔC_6F_6	−0.04	0.10	(e , 0.51)	0.15	0.02	e	0.03

ered from those found in their parent hydroxyl compounds. Inspection of table 5 revealed that the direction and magnitude of the ASIS of protons lying in the vicinity of a C-3-carbonyl group can be generally estimated by applying reference plane rules formerly proposed [13c, 15, 18a].

The 19- and 31-methyl protons were appreciably shielded in C_6H_6 ($\Delta_{C_6H_6}$ values of −0.17 to −0.29 ppm and −0.06 to −0.15 ppm, respectively), while the 30-methyl proton was either scarcely affected or deshielded slightly, as the formers lie behind a plane passing through the C-3-carbon atom at right angle to the C=O bond and the latter lies approximately in the plane [13a–13c]. Compounds XIV showed somewhat small $\Delta_{C_6H_6}$ value (−0.09 ppm) of the 19-methyl and large $\Delta_{C_6H_6}$ value (0.18 ppm) of the 30-methyl in comparison with the others, presumably due to the introduction of Δ^5-bond in the steroid skeleton.

On the other hand, C_6F_6 caused downfield shifts of the 18-, 19-, and 31-methyl protons as well as two doublets of 9β, 19-cyclopropane methylene proton (in compounds XVIII–XX). The results are consistent with the previous generalization [17, 18]: the direction of C_6F_6-induced shifts is opposite to that of C_6H_6-induced shifts. Since in general the chemical shifts of the 19-, 30- and 31-methyl groups in triterpene ketones are very similar, simultaneous use of C_6H_6-and C_6F_6-induced shifts are particularly available for differentiation between them. In addition, Δ^8-lanostene type of compounds (XVI and XVII) had consistently large $\Delta_{C_6F_6}$ value (0.12 ppm) of the 32-methyl proton, compared with cycloartane type of compounds (XVIII–XX, 0.02–0.03 ppm). A similar relationship was also found on the corresponding proton in the C_6F_6-induced shift spectra of C-3β-acetate derivatives of those compounds (see table 6). The observations may be a useful measure to distinguish the two types of triterpenes.

As expected, the 19-methyl proton in compounds XI–XVII had negative $\Delta_{C_5H_5N}$ values (−0.04 to −0.10 ppm) as it lies behind a plane passing through the C-2- and C-4-carbon atoms adjacent to the C-3-carbonyl group [15]. It is, however, noticed here that in compounds XVIII–XX, the 9β, 19-cyclopropane methylene proton lying behind the plane shows unexpected positive $\Delta_{C_5H_5N}$ values (0.10 to 0.29 ppm).

3. ASIS of acetate derivatives

With some pentacyclic triterpene acetates related to oleanene and lupane series, Willson and Williams [13d] have reported that an *axial* 31-methyl proton situated in vicinal position to a C-3β-acetoxyl group suffers an appreciable upfield shift on passing from $CDCl_3$ to C_6H_6. In tetracyclic triterpene acetates and closely related compounds examined here (XXI–XXIX, table 6), however, all of the methyl protons exhibit downfield shifts in each aromatic solvent used, with the exceptions of C-3-acetyl- and 19-methyl protons which are shielded in C_6H_6 [13a]. The present assignment was supported by comparison of the spectrum of 4α-methyl-5α-cholest-7-en-3β-ylacetate (XXI) with that of 4,4-dimethyl analog (XXIV). Another feature observed for the C-3β-acetate derivatives was that the C-3α-methine proton was deshielded in both C_6H_6 [13d] and C_5H_5N [16] ($\Delta_{C_6H_6} \cong \Delta_{C_5H_5N}$, ca. 0.20 ppm), whereas it was shielded in C_6F_6 ($\Delta_{C_6F_6}$, ca. −0.10 ppm).

Table 6

ASIS data on acetate derivatives[a]

Compounds		30-CH_3[b]	31-CH_3[b]	19-CH_3[b]	18-CH_3[b]	32-CH_3[b]	21-CH_3[c]	26,27-di-CH_3[c]	3α-H[d]	Acetyl-CH_3[g]
XXI	ΔC_6H_6	0.03		−0.05	0.06		0.10	0.07	0.24	−0.25
	ΔC_5H_5N	0.07		0	0.06		0.09	0.04	0.23	0.04
	ΔC_6F_6	0.04		0.09	0.07		0.09	0.03	−0.09	0.02
XXII	ΔC_6H_6	0.04	0.05	−0.06	0.02		e	0.06	0.24	−0.28
	ΔC_5H_5N	0.05	0.07	0.02	0.02		e	0.04	0.24	0.01
	ΔC_6F_6	0.05	0.09	0.07	0.07		e	0.02	−0.22	0
XXIII	ΔC_6H_6	0.08	0.08	−0.03	0		0.08	0.06	0.20	−0.26
	ΔC_5H_5N	0.10	0.11	0.04	0.01		0.07	0.04	0.23	0.03
	ΔC_6F_6	0.02	0.04	0.09	0.09		0.08	0.04	−0.09	0.04
XXIV	ΔC_6H_6	0.01	0.03	−0.05	0.03		0.09	0.06	0.18	−0.29
	ΔC_5H_5N	0.04	0.04	0.02	0.03		e	0.03	0.25	0.01
	ΔC_6F_6	0.06	0.05	0.03	0.05		0.08	0.04	−0.12	0
XXV	ΔC_6H_6	0.10	0.10	−0.04	0.06	0.05	0.11	0.02	0.13	−0.28
	ΔC_5H_5N	0.07	0.10	0.01	0.05	0.07	e	0.02	0.11	0.01
	ΔC_6F_6	0.08	0.11	0.09	0.09	0.08	0.07	0	−0.11	0
XXVI	ΔC_6H_6	0.08	0.08	−0.05	0.07	0.05	0.11	0.07	0.17	−0.28
	ΔC_5H_5N	0.07	0.10	0.02	0.07	0.07	e	0.06	0.17	0.02
	ΔC_6F_6	0.08	0.11	0.11	0.13	0.07	e	0.05	−0.16	0
XXVII	ΔC_6H_6	0.06	0.08	(−0.17,−0.21)	0.02	0.03	e	0.04	0.16	0.25
	ΔC_5H_5N	0.07	0.09	(−0.08,−0.05)	0.02	0.04	e	0.03	0.16	0.04
	ΔC_6F_6	0.08	0.14	(0.05, 0.10)	0.08	0	e	0.01	−0.13	−0.01
XXVIII	ΔC_6H_6	0.07	0.08	(−0.14,−0.19)	0.04	0.03	e	0.05	0.16	−0.26
	ΔC_5H_5N	0.07	0.07	(−0.06,−0.05)	0.04	0.03	e	0.03	0.16	0.02
	ΔC_6F_6	0.08	0.13	(0.08, 0.11)	0.10	0	e	0.04	−0.13	−0.03
XXIX	ΔC_6H_6	0.06	0.08	(−0.14,−0.21)	0.02	0.02	e	0.05	0.20	−0.27
	ΔC_5H_5N	0.08	0.08	(−0.06,−0.07)	0.03	0.04	e	0.05	0.20	0
	ΔC_6F_6	0.09	0.11	(0.08, 0.07)	0.08	0	e	0.03	−0.10	0

[a–f] See in table 4.
[g] Singlet.

Appendix

The systematic names and the corresponding trivial names of sterols and triterpene alcohols used in this study are as follows: 4α-methyl-5α-cholestane-3β-ol, 4α-methylcholestanol; 4β-methyl-5α-cholestane-3β-ol, 4α-methylcholestanol; 4α-methyl-5α-cholest-7-en-3β-ol, 4α-methyl-Δ^7-cholestenol (lophenol); 4,4-dimethyl-5α-cholestan-3β-ol, 4,4-dimethylcholestanol; 4,4-dimethylcholest-5-en-3β-ol, 4,4-dimethylcholesterol; 4,4-dimethyl-5α-cholest-7-en-3β-ol, 4,4-dimethyl-Δ^7-cholestenol; 5α-lanost-8,24-dien-3β-ol, lanosterol; 5α-lanost-8-en-3β-ol, dihydrolanosterol; 9β,19-cyclo-5α-lanost-24-en-3β-ol, cycloartenol; 9β,19-cyclo-5α-lanostan-3β-ol, cycloartanol; 24-methylene-9β, 19-cyclo-5α-lanostan-3β-ol, 24-methylenecycloartanol.

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