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Application of Tris(dipivaloylmethanato)europium(III) to the Assignments of Methyl Resonances of Triterpene Acetates related to β-Amyrin

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The nuclear magnetic resonance spectra of triterpene acetates related to β-amyrin were examined by use of the shift reagent $Eu(DPM)_3$ and assignments of their methyl resonances have been performed. Furthermore, the most favourable conformation of the co-ordinate complex was suggested based on the consideration of molecular models and the angle term in McConnel–Robertson equation.

Following Hinckley's discovery,[3] there have been reported a number of investigations[4] on the application of lanthanide shift reagents such as tris(dipivaloylmethanato)europium(III) ($Eu(DPM)_3$) to spectra simplification and signal assignment in nuclear magnetic resonance (NMR) spectra of alcohol and other lone pair containing organic compounds. Some of these were concerned with the assignments of methyl resonances of triterpenes,[5] but only a little work has been done with triterpene acetates in which the co-ordinating site of the metal ion is limited to the acetoxyl group.[6] We investigated the shift behaviors of methyl resonances of several 3-O-acetyl-triterpenes having geminal dimethyl group at the 4-position by use of $Eu(DPM)_3$ as the shift reagent and obtained some new information about the assignment of methyl signals and the location of co-ordinating europium ion.

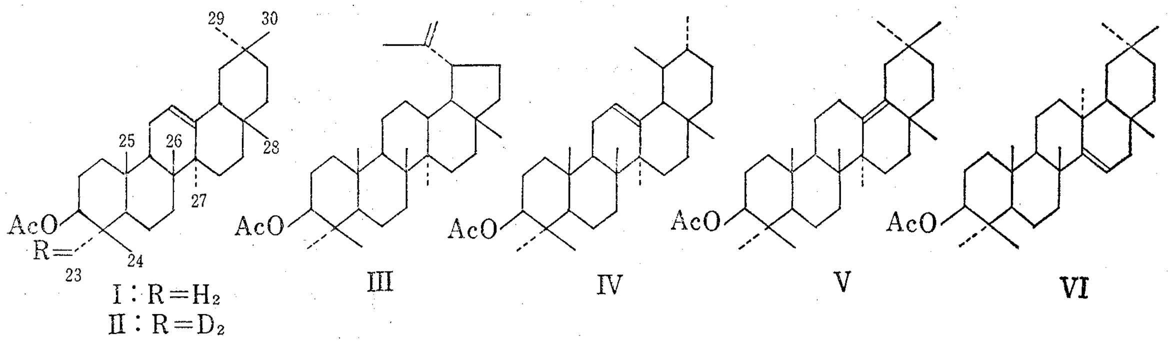

Chart 1

First, we examined the spectrum of β-amyrin acetate (I). On addition of $Eu(DPM)_3$, the spectrum was simplified and at a molar ratio 1: 3 of shift reagent to substrate all the methyl signals were separately recognized as illustrated in Fig. 1. The induced paramagnetic shifts of acetyl methyl, methine proton geminal to the acetoxyl group, C_2-hydrogens, and eight methyl groups in β-amyrin acetate (I) were given graphically in Fig. 2a, where the observed

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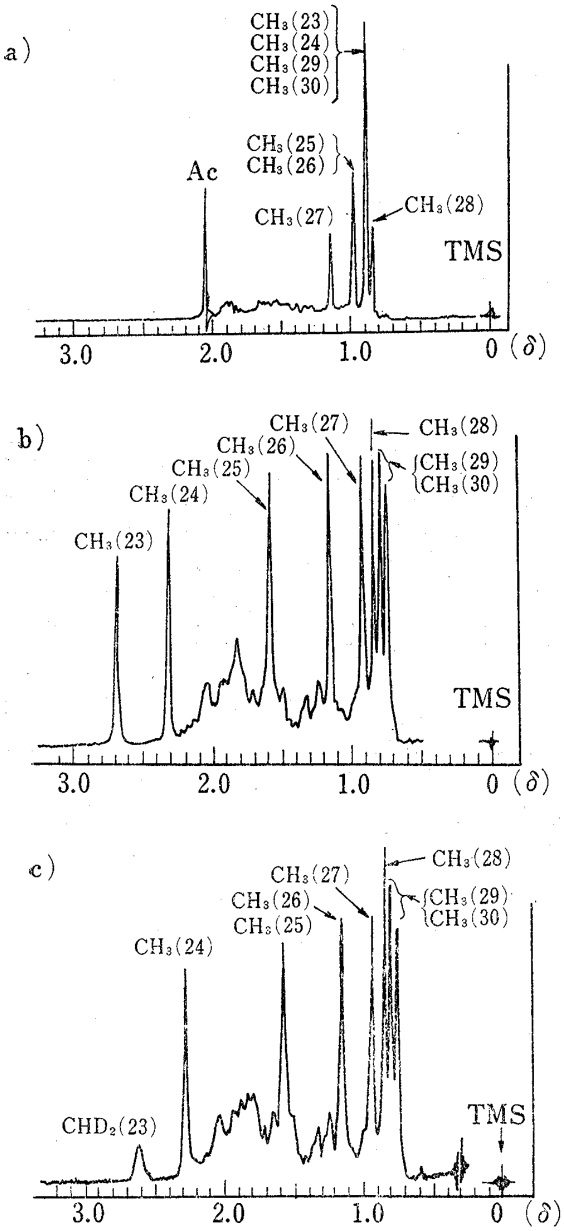

Fig. 1. 100 MHz NMR Spectra of a) β-Amyrin Acetate (I) in $CDCl_3$; b) β-Amyrin Acetate (I) in the Presence of Eu $(DPM)_3$ (0.35 mole equiv.); c) β-Amyrin Acetate-23,23-d_2 (II) in the Presence of Eu(DPM_3) (0.34 mole equiv)

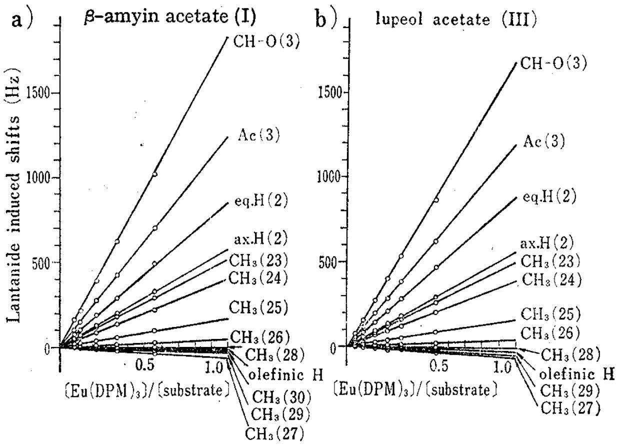

Fig. 2. Lanthanide induced Shifts of Some Signals of a) β-Amyrin Acetate (I) and b) Lupeol Acetate (III), plotted against the Molar Ratios of Eu $(DPM)_3$ to Substrate

shift values of each signal were plotted against the molar ratios of $Eu(DPM)_3$ to substrate. In this case, two methyl signals suffering the most significant downfield shift are assigned to the 23- and 24-methyl groups (or *vice versa*) and the next two methyl signals showing downfield shift to the 25- and 26-methyl groups, respectively, based on their distances from the co-ordinating metal atom.

In a recent publication,$^{6)}$ one of the present authors reported that in serraten-3β-ol acetate the methyl signal with the largest downfield shift would be assigned to the 24-methyl group. However, the concrete evidence was not present.

In order to examine the induced shift behaviors of 23- and 24-methyl signals we then measured the spectra of 23,23-dideuterated β-amyrin acetate (II), which had been synthesized from methyl hederagenate,$^{7)}$ at various ratios of shift reagent to substrate. As the result, it became clear that the methyl signal with the largest downfield shift is unambiguously assigned to the 23-methyl group as seen in Fig. 1c.

Next, to assign the signals of remaining four methyl groups, we compared the spectra of β-amyrin acetate (I) with those of lupeol acetate (III) which has only six tertiary methyls, since in the latter compound (III) the signals of the 27- and 28-methyl groups are expected to be easily discriminated from others (see Fig. 2b). Furthermore, 28,28-dideuterated β-amyrin acetate (IXb) was synthesized from methyl oleanolate (VII) according to the scheme$^{8)}$ shown in Chart 2, and its spectra obtained by co-ordination of the shift reagent were examined at various concentrations.

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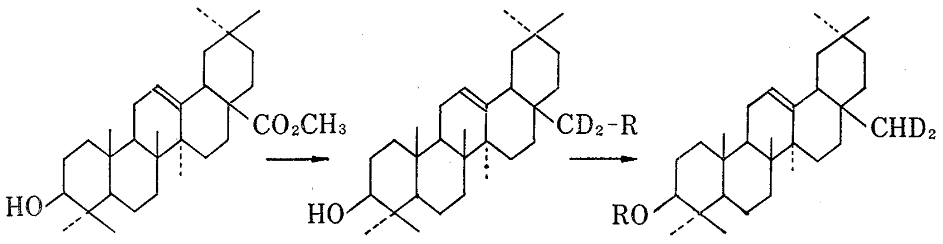

VII

VIIIa : R=OH
VIIIb : R=OTs
VIIIc : R=I

IXa : R=H
IXb : R=Ac

Chart 2

From these experiments, it was clearly demonstrated that the signal showing the largest upfield shift is assigned to the 27-methyl and the signal which suffered no significant shift to the 28-methyl group, respectively. Also it is noteworthy that the olefinic 12-hydrogen shifted slightly to higher field on addition of the shift reagent as seen in Fig. 2.

Recently some examples of upfield shift of proton signal due to Eu(DPM)$_3$ have been reported[9] and since then the important contribution of the angle (θ) between the ligand-field axis of the complex and the radius vector from the metal atom to the observed proton has been emphasized. According to the McConnel-Robertson equation,[10] paramagnetic shift due to the co-ordination of Eu(DPM)$_3$ apparently depends on the distances from the metal atom to the proton and the angle θ. In this equation the pseudo-contact term becomes negative when the term ($3\cos^2\theta$-1) is negative; namely, θ is in the range of 54.736°—125.264°.

From the consideration of this angle factor and the detailed examination of molecular model, it was concluded that the most favourable conformation of the co-ordinate complex may be represented by the structure depicted in Fig. 3 where the metal ion locates at the α-side of the molecule. Thus the conformation reported previously by one of the present authors[6] should be revised. With the new conformation (Fig. 3), the shift behaviors of 2-methylene and 12-olefinic hydrogens, 23-, 27-, and 28-methyl groups are consistently explained.

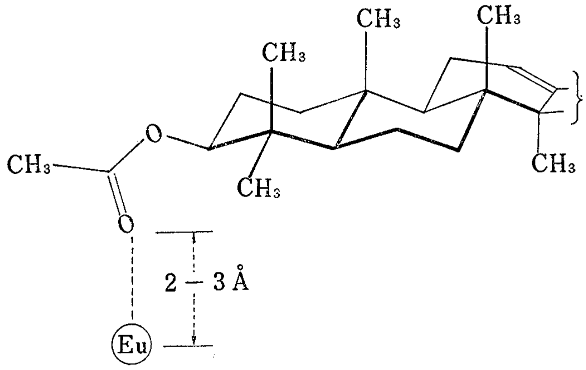

Fig. 3. The Preferred Conformation of Co-ordinate Complex

The remaining two methyl signals showing a small upfield shift were assigned to the 29- and 30-methyl groups, respectively, in the order of shift value, simply based on the consideration of distances from the co-ordinating metal ion.[11]

Then the NMR spectra of other 3-O-acetyl-triterpenes (α-amyrin acetate, δ-amyrin acetate, taraxerol acetate) were measured at various ratios of Eu(DPM)$_3$ to substrate and the signal assignments were performed in analogy with I. The result obtained was summarized in Table

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[9] B.L. Shapiro, J.R. Hlubucek, G.R. Sullivan, and L.F. Johnson, *J. Am. Chem. Soc.*, **93**, 3281 (1971); M. Kishi, K. Tori, T. Komeno, and T. Shingu, *Tetrahedron Letters*, **1971**, 3525.

[10] H.M. McConnel and R.E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).

[11] In this connection it should be noted that the assignments of methyl resonances of a series of β-amyrin type triterpenes have already been carried out by Tursch, *et al.* and Itoh, *et al.* on the basis of the substituent effects on chemical shifts. Our present assignments for β-amyrin acetate (I) agree well with those reported by them. See B. Tursch, R. Savoir, R. Ottinger, and G. Chiurdoglu, *Tetrahedron Letters*, **1967**, 539; S. Itoh, M. Kodama, M. Sunagawa, T. Oda, and H. Hikino, *Tetrahedron Letters*, **1969**, 2905.

TABLE I. Some Chemical Shifts of 3-O-acetyl-triterpenes in δ-Value Downfield from Tetramethylsilane using a 100 MHz Instrument and the Relative Shift Values with Respect to the Induced Shift of Acetyl Methyl

	β-Amyrin acetate (I) S_{Ac}[a] = 1248.3 Hz		α-Amyrin acetate (IV) S_{Ac} = 953.3 Hz		δ-Amyrin acetate (V) S_{Ac} = 1300.0 Hz		Taraxerol acetate (VI) S_{Ac} = 970.0 Hz		Lupeol acetate (III) S_{Ac} = 1200.0 Hz	
	δ	shift ratio	δ	shift ratio	δ	shift ratio	δ	shift ratio	δ	shift ratio
Ac (3)	2.041	1.00	2.033	1.00	2.042	1.00	2.033	1.00	2.033	1.00
CH-O (3)	4.500	1.47	4.500	1.40	4.525	1.42	4.467	1.44	4.467	1.41
ax. H (2)[b]	(1.617)	0.47	(1.533)	0.47	(1.350)	0.53	(1.567)	0.46	(1.550)	0.47
eq. H (2)[b]	(1.817)	0.69	(1.583)	0.74	(1.685)	0.74	(1.733)	0.75	(1.650)	0.74
CH_3 (23)	0.875	0.42	0.867	0.42	0.850	0.42	0.875	0.42	0.850	0.42
CH_3 (24)	0.875	0.33	0.867	0.33	0.850	0.33	0.875	0.33	0.850	0.33
CH_3 (25)	0.967	0.14	0.975	0.13	0.850	0.14	0.950	0.13	0.850	0.14
CH_3 (26)	0.967	0.04	1.001	0.03	0.850	0.04	1.092	0.04	1.033	0.04
CH_3 (27)	1.133	−0.05	1.067	−0.05	1.150	−0.05	0.908	−0.07	0.942	−0.05
CH_3 (28)	0.833	0.00	0.792	0.00	1.008	0.00	0.825	−0.01	0.792	0.00
CH_3 (29)	0.875	−0.03	0.800	−0.06	0.708	−0.03	0.950	−0.03	1.692	−0.04
CH_3 (30)	0.875	−0.02	0.908	−0.03	0.933	−0.01	0.902	−0.01	—	—
Olefinic H	5.183	−0.01	5.117	−0.02	—	—	5.533	0.00	4.617	−0.02

a) S_{Ac} is the induced paramagnetic shift for the acetyl methyl group at the ratio 1:1 of $Eu(DPM)_3$ to substrate.
b) Values in parentheses were obtained by simple extrapolation method.

I, where the shift values were recorded in term of relative value with respect to that of acetyl methyl. The observed shift values of corresponding signals in the five compounds examined are remarkably similar to each other. Therefore, the location of the metal ion in the complexes would be the same throughout these compounds.

Experimental

All the melting points were measured with a Kofler-type apparatus and are uncorrected. Infrared (IR) spectra were measured for solutions in chloroform with a Hitachi KPI spectrometer. Measurements of NMR were made for deuterated chloroform solutions ranging in concentration from 0 to 0.7 mole equiv. with increasing amounts of $Eu(DPM)_3$ with Varian A-60 and HA-100D NMR Spectrometers. The line positions of signals are given on the δ-scale with reference to tetramethylsilane as the internal standard. The molar ratio of $Eu(DPM)_3$ to the substrate was estimated by integrating the area of tertiary methyl groups and the area of the pivaloyl group of $Eu(DPM)_3$. Mass spectral determinations were performed with a Hitachi RMU-6D mass spectrometer with a direct inlet system. Preparative thin-layer chromatography was performed on Merck Kieselgel GF_{254} with chloroform and plates were examined under ultraviolet (UV) light (for UV absorption materials on GF_{254} plates). For extraction of substances from the kieselgel methylene chloride was used as solvent.

Erythrodiol-28,28-d_2 (VIIIa)——Methyl oleanolate (VII) (180 mg) was treated with excess lithium aluminum deuteride (150 mg) in boiling ether (8 ml) for 8 hr. After the excess reagent was decomposed with water, the mixture was acidified with dil. HCl and extracted with CH_2Cl_2. The organic layer was washed with dil. Na_2CO_3, dried ($MgSO_4$), and evaporated. Recrystallization of the residue (158 mg) from CH_2Cl_2–MeOH gave colorless needles (VIIIa) (140 mg), mp 234—236°. Mass Spectrum *m/e*: 444 (M^+) ($C_{30}H_{48}D_2O_2$). IR ν_{max} cm^{-1}: 3600, 3450, 2200. NMR δ: 5.22 (1H, t, J=3.5 Hz, olefinic proton), 3.23 (1H, q, J=6.5, 9 Hz, C$\underline{H}$-OH), 1.18—0.80 (7×*tert*-CH_3).

β-Amyrin-28,28-d_2 (IXa)——To a mixture of erythrodiol-28,28-d_2 (VIIIa) (130 mg), pyridine (3 ml), and benzene (3 ml) was added a solution of *p*-toluenesulfonyl chloride (150 mg) in benzene (3 ml) and the mixture was gently refluxed for 18 hr. After cooled, the reaction mixture was diluted with water and extracted with ether. The organic layer was washed succesively with 3% HCl and dil. Na_2CO_3, dried ($MgSO_4$), and evaporated. Separation of the residue (253 mg) by preparative thin-layer chromatography (TLC) gave the starting material (34 mg) and a monotosylate (VIIIb) (84 mg). IR ν_{max} cm^{-1}: 3600, 3400, 2250, 1360, 1180. NMR δ: 7.78, 7.31 (4H, A_2B_2 q, J=8 Hz, aromatic protons), 5.10 (1H, t, J=3.5 Hz, olefinic proton), 3.12 (1H, q, J=6.5, 9 Hz, C$\underline{H}$-OH), 2.45 (3H, s, aryl-CH_3), 1.10—0.56 (7×*tert*-CH_3).

A mixture of the above monotosylate (VIIIb) (80 mg), NaI (110 mg), and abs. acetone (4 ml) was heated in a sealed tube at 160° for 39 hr. After the solvent was evaporated *in vacuo*, the product was taken up in

CH_2Cl_2. The CH_2Cl_2 solution was washed with dil. Na_2CO_3, dried ($MgSO_4$) and evaporated. Purification of the residue by preparative TLC afforded an iodide (VIIIc) (47 mg), mp 157—160°. IR ν_{max} cm^{-1}: 3600, 3450, 2260, NMR δ: 5.26 (1H, t, J=3.5 Hz, olefinic proton), 3.23 (1H, q, J=6, 9 Hz, C<u>H</u>-OH), 1.17—0.80 (7 × *tert*-CH_3).

The above iodide (VIIIc) (45 mg) was then hydrogenated over W-2 Raney nickel (1.8 g) in EtOH (5 ml) at room temperature and atmospheric pressure, until hydrogen uptake ceased. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to afford a crystalline product (43 mg), which was recrystallized from CH_2Cl_2–MeOH to afford colorless needles (IXa) (31 mg), mp 196—198°. Mass Spectrum *m/e*: 428 (M^+) ($C_{30}H_{48}D_2O$). IR ν_{max} cm^{-1}: 3600, 3450, 2200. NMR δ: 5.20 (1H, t, J=3.5 Hz, olefinic proton), 3.24 (1H, q, J=6, 9Hz, C<u>H</u>-OH), 1.15—0.80 (7 × *tert*-CH_3).

β-Amyrin Acetate-28,28-*d*$_2$ (IXb)——A solution of IXa (37 mg) in pyridine (1.5 ml) and acetic anhydride (1.5 ml) was allowed to stand overnight at room temperature. Working up of the mixture in the usual manner afforded a crystalline product (37 mg), which was recrystallized from CH_2Cl_2–MeOH to give β-amyrin acetate-28,28-d_2 (IXb) (31 mg) as colorless needles, mp 243—245°. Mass Spectrum *m/e*: 470 (M^+) ($C_{32}H_{50}D_2O_2$). IR ν_{max} cm^{-1}: 2200, 1720, 1250. NMR δ: 5.20 (1H, t, J=4 Hz, olefinic proton), 4.52 (1H, q, J=6.5, 9 Hz, C<u>H</u>-OAc), 2.04 (3H, s, Ac), 1.14—0.87 (7 × *tert*-CH_3).

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