Chem. Pharm. Bull. 25(8)2078—2081(1977)

UDC 547.597.02.08 : 543.422.25.06

Application of Homonuclear Internuclear Double Resonance Technique in Triterpene Field. I. Nuclear Overhauser Effects between Methyl Groups

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(Received December 25, 1976)

Homonuclear internuclear double resonance technique was applied to β-amyrin acetate (Ia) and the related compounds and this technique was proved to be useful for the qualitative measurements of the nuclear Overhauser effects between methyl-methyl protons. Assignments of methyl resonances of β-amyrin (Ib) and β-amyrone (IIa) were also carried out by the use of this technique.

Keywords——NMR; homonuclear INDOR technique; triterpenes; methyl-methyl NOE; NMR shift reagent; methyl signal assignment

Homonuclear INDOR (internuclear double resonance) spectroscopy, a nuclear magnetic double resonance technique, is a very powerful tool for determination of precise frequencies of hidden proton signals in complicated nuclear magnetic resonance (NMR) spectra. It was first introduced by Baker[2] and its effectiveness in the structure elucidation of complex organic molecules has recently been demonstrated by several groups of workers.[3] Previously, we also applied this method in the structure analyses of friedelin-type triterpenes and found that the nuclear Overhauser effect (NOE) between methyl and methylene or methine protons can be detected conveniently, although qualitatively, by the use of this method.[4]

It is generally recognized that the NOE's are operative between methyl groups located in spatial proximity, but the increases of signal intensities in these case are usually too small to be measured directly by the conventional technique. We recently investigated this problem taking advantage of the INDOR method and the result obtained will be reported in the present paper.

First, β-amyrin acetate (Ia) was chosen as a model compound, since the methyl resonances in this compound had been fully assigned by means of pseudo-contact shift using a lanthanide shift reagent, tris(dipivaloylmethanato)europium(III) ($Eu(DPM)_3$).[5] We used the same reagent here in order to separate the methyl signals from one another sufficiently enough for the double resonance experiments.

The NMR spectrum of Ia in the presence of $Eu(DPM)_3$ (*ca.* 0.6 mol. eq. to the substrate) is given in Fig. 1, which shows the methyl signals at δ 4.51, 3.75, 2.19, 1.31, 0.83, 0.69 ($2\times CH_3$), and 0.61 corresponding to the C–23, C–24, C–25, C–26, C–28, C–27 and C–29, and C–30 methyl groups, respectively.

1) Location: *a) 3190 Gofuku, Toyama; b) Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto; c) Ikawadani, Tarumi-ku, Kobe.*
2) E.B. Baker, *J. Chem. Phys.*, **37**, 911 (1962).
3) O. Sciacovelli, W. von Philipsborn, C. Amith, and D. Ginsburg, *Tetrahedron*, **26**, 4589 (1972); D.C. Ayres, J.A. Harris, P.N. Jenkins, and L. Phillips, *J. Chem. Soc.* (Perkin I), **1972**, 1343.
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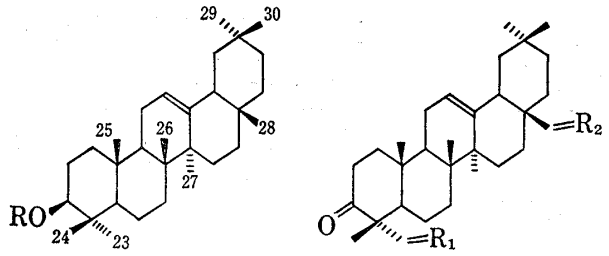

Ia: R=Ac
Ib: R=H

IIa: $R_1=R_2=H_2$
IIb: $R_1=D_2$, $R_2=H_2$
IIc: $R_1=H_2$, $R_2=D_2$

Chart 1

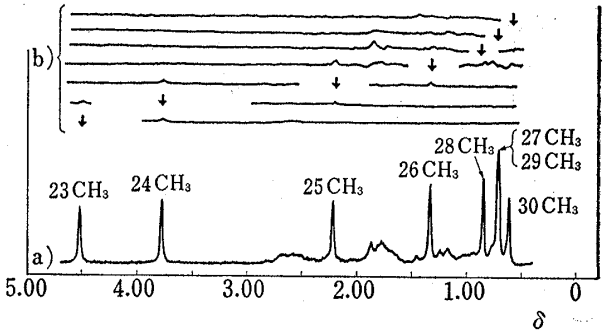

Fig. 1. INDOR Experiments of β-Amyrin Acetate (Ia)

a) NMR spectrum of Ia in the presence of $Eu(DPM)_3$.
b) INDOR spectra.

The INDOR experiment monitoring the singlet line of C–23 methyl group at δ 4.51 gave a small peak at δ 3.75, which may be interpreted as an intensity increase of the monitor signal due to an NOE of the C–24 methyl group. There was observed no peak in the region of other methyl resonances. In turn, when the singlet signal of C–24 methyl group at δ 3.75 was monitored, two peaks were observed at δ 4.51 and 2.19, which can be reasonably ascribed to NOE's between the C–24 methyl group and the geminal C–23 and 1,3-diaxial C–25 methyl groups, respectively.

Similarly, INDOR experiments monitoring the C–25 and C–26 methyl signals at δ 2.19 and 1.31 afforded two small peaks at δ 3.75 and 1.31 in the former case and a small peak at δ 2.19 in the latter case as illustrated in Fig. 1. These were also considered to originate from the NOE's between methyl groups concerned. On the contrary, monitoring the other methyl signals (δ 0.83, 0.69, and 0.61) gave no significant INDOR peak except for a few broad peaks which were probably due to NOE's between methyl-methylene or methyl-methine protons.

From the above observation, it was concluded that the INDOR method is particulary useful for the qualitative measurement of NOE's between methyl-methyl protons.

Then we applied this method to a study of NMR spectrum of β-amyrin (Ib) in combination with the pseudo-contact shift method. Assignments of methyl resonances of Ib have already been made by several workers, but there is some confusion in the literature. For instance, Tursch, *et al.*[6a] and Itoh, *et al.*[6b] have reported that the C–23 and C–24 methyl groups resonate at δ 0.99 and 0.79, respectively, while the reverse assignment of these signals has recently been reported by ApSimon, *et al.*[6c] based on the pseudo-contact shift study. It is considered that the INDOR method is suitable for making a choice of the correct assignment of these signals.

Upon addition of $Eu(DPM)_3$, all the methyl signals in the NMR spectrum of Ib shifted in varying degree towards down-field. The induced paramagnetic shifts of methyl groups were given graphically in Fig. 2, where the observed chemical shifts were plotted against the molar ratios of $Eu(DPM)_3$ to the substrate. At the molar ratio of *ca.* 0.2 ([$Eu(DPM)_3$]/[substrate]), eight methyl signals were recognized separately at δ 5.35, 5.21, 2.75, 1.71, 1.53, 1.10, 0.96, and 0.92, as shown in Fig. 3.

When the INDOR experiments were performed using each of these signals as monitor lines in the same manner as described for Ia, there were observed NOE peaks between the signals at δ 5.35 and 2.75 and between those at δ 2.75 and 1.71 (Fig. 3), whereas no significant NOE peak could be observed between the signals at δ 5.35 and 5.21 which are attributa-

6) *a*) B. Tursch, R. Savoir, R. Ottinger, and H. Hikino, *Tetrahedron Lett.*, **1967**, 539; *b*) S. Itoh, M. Kodama, M. Sunagawa, T. Oda, and H. Hikino, *Tetrahedron Lett.*, **1969**, 2905; *c*) J.W. ApSimon, H. Beierbeck, and A. Fruchier, *Can. J. Chem.*, **50**, 2725 (1972).

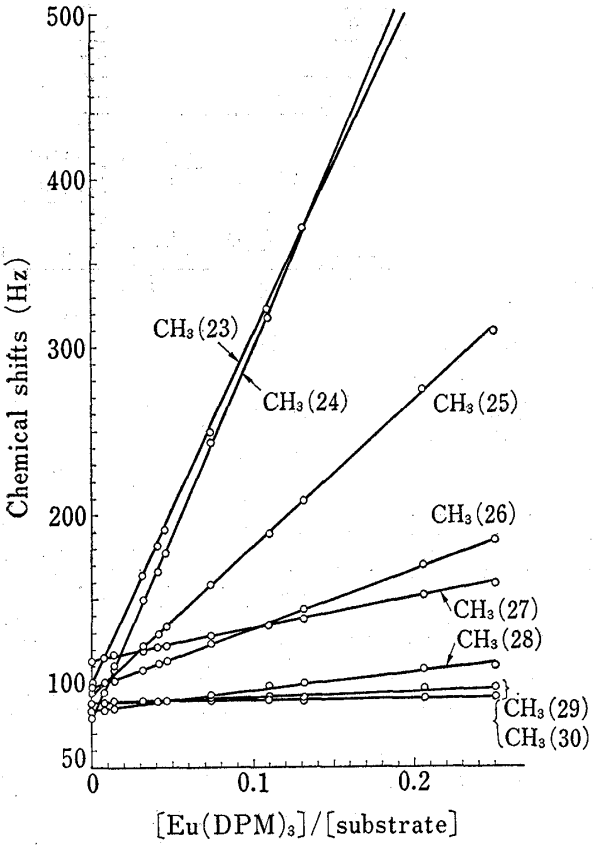

Fig. 2. Lanthanide induced Shifts of β-Amyrin (Ib), plotted against the Molar Ratios of $Eu(DPM)_3$ to Substrate

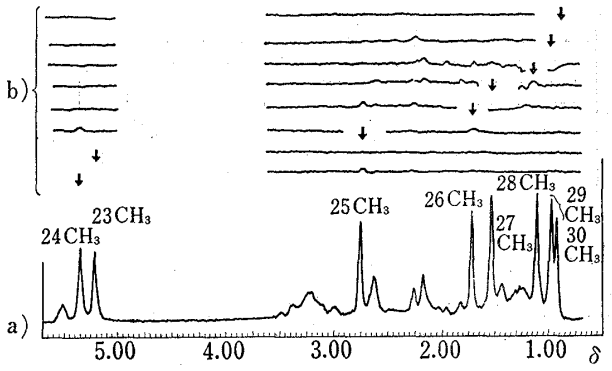

Fig. 3. INDOR Experiments of β-Amyrin (Ib)

a) NMR spectrum of Ib in the presence of $Eu(DPM)_3$.
b) INDOR spectra.

ble to the C–23 and C–24 methyl groups or *vice versa* on the basis of their shift behavior. This is because the chemical shift difference between those signals was too small to effect the double resonance experiments and it was not enlarged sufficiently by increasing the amount of the shift reagent.

However, it is evident from the above findings that the signals at δ 5.35, 2.75, and 1.71 are ascribable to the C–24, C–25, and C–26 methyl groups, respectively, which are disposed mutually in 1,3-diaxial relation. Threfore, the one at δ 5.21 is assigned to the C–23 methyl group and this was further confirmed by direct comparison of the spectrum with that of synthetic sample of β-amyrin-23,23-d_2.[7]

It must be emphasized that the C–24 methyl protons show the most significant down-field shift by the shift reagent. This suggests that the coordinating metal ion may be located in the upper side of the substrate molecule and is closer to the C–24 methyl than the C–23 methyl, in contrast to the case of β-amyrin acetate (Ia) reported previously.[5] Assignments of the remaining four methyl signals were made on the basis of induced shift values which depend on the distances from the coordinating metal ion and of the comparison of the spec-

Table I. Assignments of Methyl Resonances of β-Amyrin (Ib) and β-Amyrone (IIa)

	β-Amyrin (Ib)	β-Amyrone (IIa)
CH_3 (23)	δ 1.000	δ 1.096[a]
CH_3 (24)	0.790	1.058
CH_3 (25)	0.940	1.066
CH_3 (26)	0.970	1.024
CH_3 (27)	1.135	1.145
CH_3 (28)	0.830	0.845[b]
CH_3 (29)	0.875	0.875
CH_3 (30)	0.875	0.875

a, *b*) These values were confirmed by the spectral comparison with synthetic β-amyrone-23,23-d_2 (IIb) and -28,28-d_2 (IIc), respectively.

7) T. Kikuchi, M. Niwa, and T. Yokoi, *Chem. Pharm. Bull.* (Tokyo), **21**, 1378 (1973).

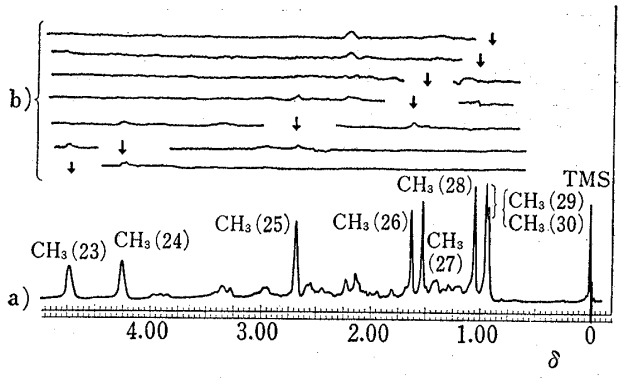

Fig. 4. INDOR Experiments of β-Amyrone (IIa)

a) NMR spectrum of IIa in the presence of $Eu(DPM)_3$.
b) INDOR spectra.

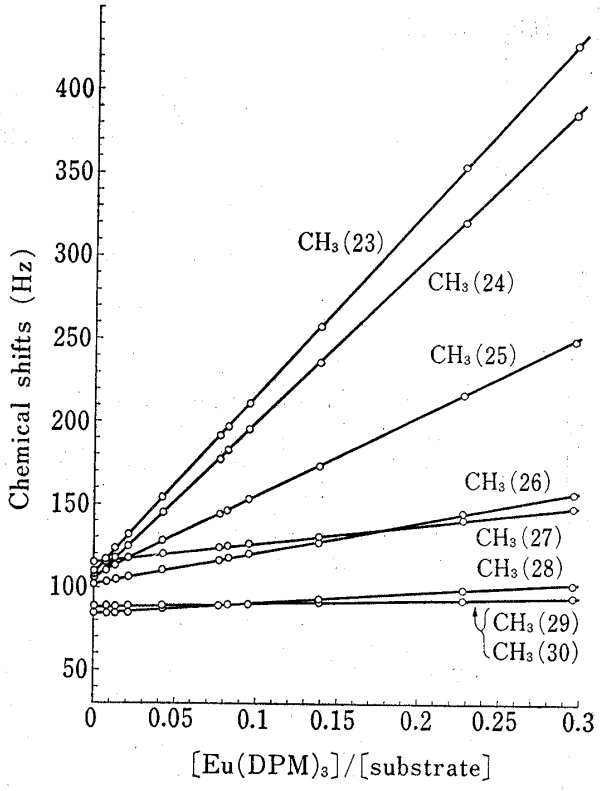

Fig. 5. Lanthanide induced Shifts of β-Amyrone (IIa), plotted against the Molar Ratios of $Eu(DPM)_3$ to Substrate

trum with that of synthetic β-amyrin-28,28-d_2.[5] The result thereby obtained is summerized in Table I. Our present result supports the assignments proposed by Tursch, *et al.*[6a] and Itoh, *et al.*[6b]

Finally, we carried out the assignments of methyl resonances of β-amyrone (IIa) in analogous manner and the NOE peaks observed in the INDOR experiments are shown in Fig. 4. The induced paramagnetic shifts and final assignments of its methyl signals are given in Fig. 5 and Table I. These assignments were confirmed by the comparative study with the spectra of synthetic β-amyrone-23,23-d_2 (IIb) and -28,28-d_2 (IIc).

Experimental

Melting points were determined with a Kofler-type apparatus and are uncorrected. Mass spectral (MS) determinations were performed with a Hitachi RMU-6D mass spectrometer with a direct inlet system. Infrared (IR) spectra were measured for solutions in chloroform with a Hitachi KPI spectrometer. Measurements of NMR were made for deuteriochloroform and chloroform solutions ranging in concentrations from 0 to *ca.* 0.6 mol. eq. with increasing amounts of $Eu(DPM)_3$ with Varian A-60 and/or HA-100D instruments. The line positions of signals are given on the δ scale with reference to tetramethylsilane as the internal standard. The molar ratios of $Eu(DPM)_3$ to the substrates were estimated by integrating the area of tertiary methyl groups and the area of the pivaloyl methyl groups of $Eu(DPM)_3$. INDOR spectra were measured with a Varian HA-100D instrument for INDOR experiments. Preparative thin-layer chromatography (TLC) was performed on Merck Kieselgel GF_{254} with chloroform and plates were examined under ultraviolet light (for UV-absorption materials on GF_{254} plates). For extraction of substances from the Kieselgel, methylene chloride was used as solvent.

β-Amyrone-28,28-d_2 (IIc)——To a mixture of CrO_3 (50 mg) and pyridine (2 ml) was added a solution of β-amyrin-28,28-d_2[5] (17 mg) in pyridine (1 ml). The reaction mixture was stirred in room temperature for 5 hr and then diluted with water and extracted with $CHCl_3$. The organic layer was washed successively with 3% HCl and dil. Na_2CO_3, dried ($MgSO_4$), and evaporated. The residue (16 mg) was purified by preparative TLC to afford β-amyrone-28,28-d_2 (IIc, 14 mg) which was recrystallized from CH_2Cl_2–MeOH to give colorless prisms, mp 175—178°. MS *m/e*: 426 (M^+, $C_{30}H_{46}D_2O$). IR ν_{max} cm^{-1}: 2250 (C–D), 1700 (C=O). NMR δ: 5.21 (1H, t, J=3 Hz, olefinic H), 1.15—0.88 (7×*tert*-CH_3).

β-Amyrone-23,23-d_2 (IIb)——β-Amyrin-23,23-d_2[7] (10 mg) was treated with CrO_3-pyridine in the same manner as above. Usual working-up gave β-amyrone-23,23-d_2 (IIb, 7 mg), which was purified by preparative TLC and then recrystallized from CH_2Cl_2–MeOH to give pure sample of IIb, mp 176—178°. MS *m/e*: 426 (M^+, $C_{30}H_{46}D_2O$). IR ν_{max} cm^{-1}: 2250 (C–D), 1700 (C=O). NMR δ: 5.21 (1H, t, J=3 Hz, olefinic H), 1.15—0.85 (7×*tert*-CH_3).