Detection of Boat Conformations in the Triterpene Friedelin by Methyl-to-Methyl Nuclear Overhauser Enhancements

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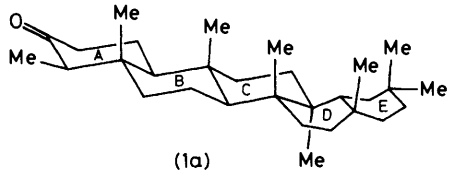
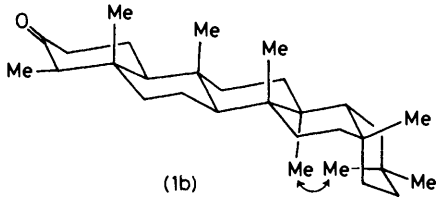
All the methyl-proton resonances of friedelin (1) have been assigned by n.O.e.-difference spectroscopy. The observation of substantial enhancements between Me-26 and Me-28, and between Me-28 and Me-30 can be explained by invoking boat conformations for rings D and E, in agreement with the crystal structure of a related compound. Methyl assignments were independently confirmed by lanthanide-shift experiments. Methyl spin–lattice relaxation times reflect steric crowding by other methyl groups and may provide a useful assignment tool.

We have recently shown that ^{1}H n.m.r. double-resonance-difference techniques can be used, alone or in combination with two-dimensional experiments, to determine complete spectroscopic,[1-3] conformational,[3] and structural[4] assignments in steroids and terpenes. All the compounds studied in this way to date have contained several electronegative functional groups. The resulting spread of proton chemical shifts at 400 MHz gives spectra which are highly complex but essentially first-order and ultimately completely capable of analysis.

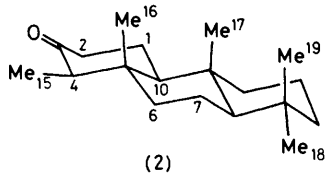
This happy result cannot be expected from less highly functionalised molecules. A single oxygen substituent will affect only a few neighbouring protons and the only other major shift influence will be the intrinsic difference between axial protons (δ *ca.* 1.2) and equatorial protons (δ *ca.* 1.6);[5] total resolution and analysis are clearly inaccessible at present. More promising, however, are the angular methyl groups whose signals are usually found well separated from each other and from the methylene envelope. In this paper we demonstrate conformational analysis using methyl-to-methyl nuclear Overhauser enhancements (n.O.e.) in the triterpene friedelin (1);[6] some confirmatory spin–lattice relaxation and lanthanide-shift experiments are also presented. We also report corresponding results for the closely related tricyclic ketone (2). For ease of comparison the structure (2) shown is the enantiomer of that actually used.[7]



(1a)



(1b)



(2)

Results

Figure 1 shows the resolution-enhanced 400-MHz spectrum of friedelin (1). Apart from a few downfield resonances the methylene envelope is concentrated (as expected) between δ 1.2—1.6, and the methyl signals are well resolved between δ 0.7—1.2. The spectrum of the tricyclic ketone (2) is essentially identical below δ 1.7, and shows a similar separation of the methylene envelope and methyl signals. In both molecules, assignment of the ring-A protons is straightforward using decoupling- and n.O.e.-difference spectroscopy (Table 1). Also readily assignable in friedelin is the 6-H_β, both by the appearance of its signal and by n.O.e. experiments described below. In the ketone (2) most of the ring-B protons are easily identified in a solvent-shifted spectrum, starting from the 1,3-diaxial n.O.e.s visible from 2-H_α and 4-H_α to the signals for 10-H and 6-H_α (Table 1). Repeated attempts to assign additional ring protons by double-resonance experiments were fruitless or gave highly ambiguous results in both compounds.

The methyl groups of friedelin (apart from Me-23 which is easy to locate) were assigned initially by n.O.e. experiments, some results of which are illustrated in Figure 2. Thus, irradiation of 1-H_β enhances peaks due to both Me-24 and -25, whilst irradiation of 6-H_β enhances peaks due to Me-23 and -24. Given these starting points, a series of irradiation experiments on the methyl groups themselves enhanced

Table 1. Ring-A and -B chemical shifts in friedelin (1) and the ketone (2) [a]

	Ketone (2)				Friedelin (1)	
	($CDCl_3$–C_6D_6) (2 : 1)		($CDCl_3$)		($CDCl_3$)	
Proton	δ_{ax}	δ_{eq}	δ_{ax}	δ_{eq}	δ_{ax}	δ_{eq}
1α		1.81		1.95		1.96
1β	1.51		1.69		1.69	
2α	2.08		2.27		2.30	
2β		2.29		2.39		2.39
4α	2.03		2.23		2.25	
10α	1.26				1.52	
6α	1.09				1.27	
6β		1.61		1.75		1.75
7α		1.39				

[a] For 0.02M solutions in the solvent indicated.

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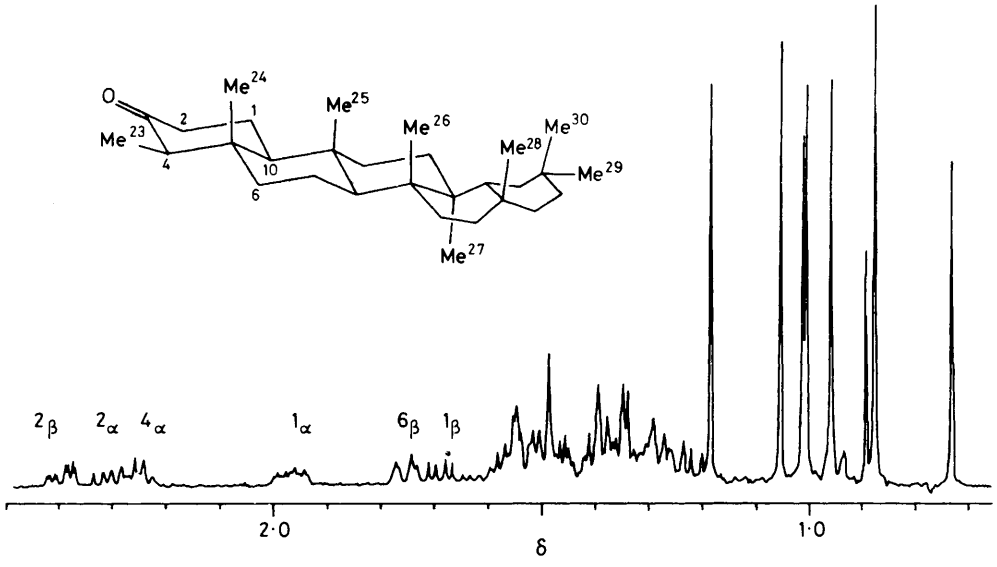

Figure 1. 400-MHz resolution-enhanced spectrum of friedelin (1), 0.02M in $CDCl_3$.

Table 2. Spectroscopic properties of the methyl groups of friedelin (1) [a]

Protons	$\delta_{obs.}$ (±0.003)	T_1/s	Relative Eu-induced shifts Obs.	Calc.	$\delta_{calc.}$ [b] (± error)	Methyl-group signals enhanced in n.O.e. difference spectrum
23	0.879	0.80	1.5		0.879 [c]	24 [d]
24	0.726	0.81	1.0 [c]	1.0 [c]	0.73 (<0.01)	23, 25
25	0.870	0.47	0.30	0.42	0.88 (0.01)	24, 26 [d]
26	1.008	0.52	0.18	0.20	1.06 (0.05)	—
27	1.051	0.64	0.15	0.16	1.04 (−0.01)	None
28	1.182	0.66	0.08	0.10	1.14 (−0.04)	26, (30) [e]
29	1.002	0.62	0.05	0.07	0.99 (−0.01)	—
30	0.954	0.41	0.05	0.06	0.95 (<0.01)	28

[a] For 0.02M solutions in $CDCl_3$. [b] From extrapolation of lanthanide-induced shifts. [c] By definition. [d] Single experiment, signals due to methyl groups as 23 and 25 are coincident. [e] Very small enhancement: see Figure 2.

signals due to neighbouring methyl groups (Figure 2) and allowed us to construct a complete set of assignments (Table 2), even though some methyl signals were too close together to allow any possibility of observations of n.O.e.s between them.

These assignments demanded that there be substantial n.O.e.s between Me-26 and Me-28, and between Me-28 and Me-30. Such n.O.e.s would not be expected for the all-chair conformation (1b) but are entirely consistent with the boat conformations for rings D and E shown in structure (1a). The similarity in size of the Me-25 ⟶ Me-26 and Me-28 ⟶ Me-26 enhancements suggested that these methyl–methyl distances are comparable. The absence of methyl enhancements to or from the signal assigned to Me-27 also supported structure (1a) rather than (1b).

Support for these assignments was sought in two additional sets of experiments, also summarised in Table 2. First, spin–lattice relaxation rates indicated which were the sterically most hindered methyl groups (see Discussion). Second, and perhaps more convincingly, the effect of $Eu(fod)_3$ [europium tris-(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionate] shift reagent was investigated. Addition of the shift reagent caused both rapid downfield shifts and the severe line broadening to be expected at 400 MHz.[8] Also, as expected,[8] line broadening became less serious at high shift-reagent concentrations. The relative shifts of the methyl groups were compared with those predicted from distance measurements made on a Dreiding model, assuming the lanthanide ion binds collinearly with the carbonyl group with a Eu–O distance of 3 Å.

The aim of this particular experiment was merely spectroscopic assignment rather than conformational analysis, and within the precision of our measurements and binding model no distinction was attempted or, indeed, was possible between structures (1a) and (1b). Agreement between observed and predicted shifts (Table 2) relative to Me-24 was sufficiently good to give assignments of all the *shifted* methyl signals, apart from those of Me-29 and -30 which could not be distinguished; knowing the largest absolute lanthanide-induced shift (Me-23) and all the relative shifts, we calculated the initial positions of all methyl groups to give independent confirmation of the n.O.e.-derived assignments. The precision of all predicted shifts was better than 0.05 p.p.m.

For completeness and comparison, corresponding results for the ketone (2) are given in Table 3.

Discussion

The detection of boat conformations in friedelin (1) is, perhaps, not surprising since in the all-chair conformation (1b) the steric interactions between Me-27 and Me-29 would be

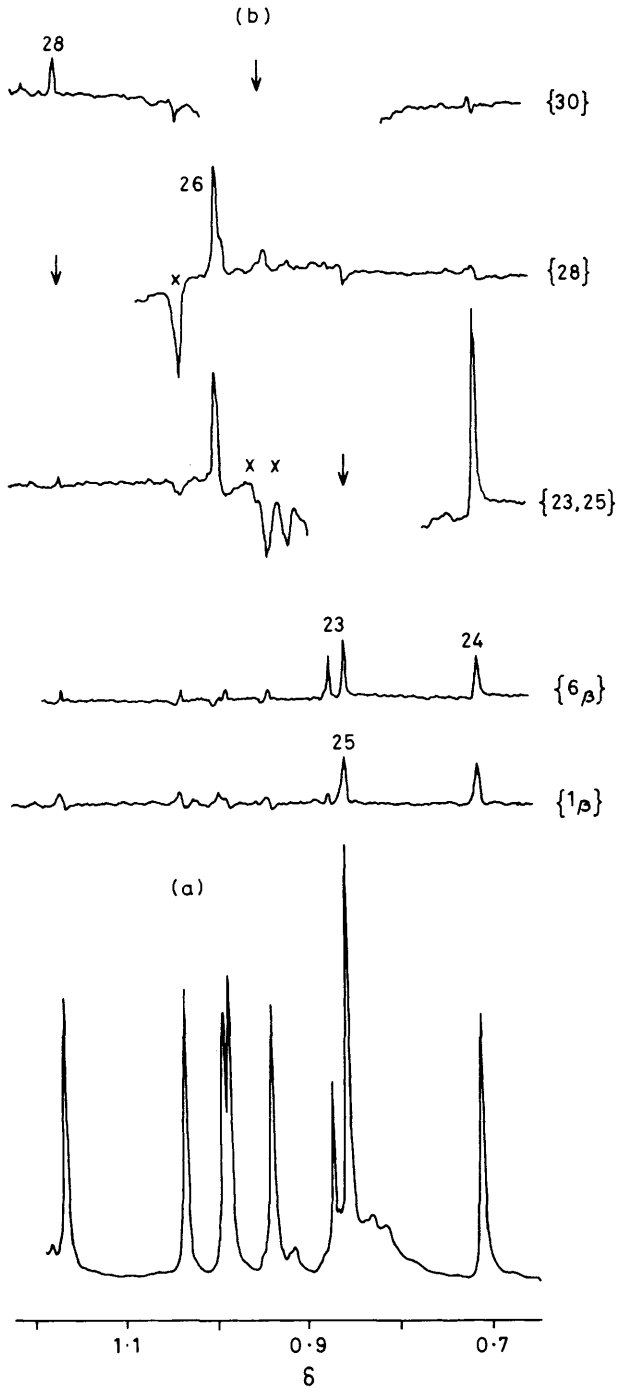

Figure 2. Partial 400-MHz spectra of friedelin (1). (a), Control spectrum with irradiation far off resonance. (b), N.O.e. difference spectra arising from irradiation of the protons designated { }. Difference spectra are × 16 vertical display scale. X marks partial saturation of a signal arising from adjacent irradiation.

very severe. Indeed, in the closely related epifriedelinol the same boat–boat gross conformation has been found by *X*-ray crystallography.[9] Nevertheless, it is remarkable that we are able to draw these qualitative conclusions from n.m.r. effects in protons which are some five rings and ten bonds removed from the nearest functional group.

Could these conclusions be more quantitative? Distance measurements on models show that (assuming similar rotation rates for all methyls) in the pure, all-chair form (1b) the n.O.e. from Me-28 to Me-26 would be no more than a tenth of that from Me-25 to Me-26, where for compound (1) they are experimentally very similar. The enhancement of the Me-30 signal by Me-28 is very small but this does not necessarily indicate a long distance between them: the enhancement of the Me-28 by Me-30 is somewhat larger (Figure 2). This result occurs because Me-30 is also efficiently relaxed by the very close Me-29, reducing the n.O.e. from Me-28. This explanation is supported by the T_1 values: Me-30 is the fastest relaxing group of all those present. The next fastest relaxing methyl groups are Me-25, which has 2 diaxial methyl and 3 diaxial proton neighbours, and Me-26 which has similar neighbours. By contrast the ring-A methyls are relatively devoid of neighbours and relax slowly.

Table 3. Spectroscopic properties of the methyl groups in the ketone (2) [a]

Protons	$\delta_{obs.}$	T_1/s	Relative Eu-induced shift	$\delta_{calc.}$
15	0.87	1.3	1.5	0.87
16	0.74	1.4	1.0	0.74
17	0.89	1.0	0.30	0.88
18	0.83	1.0	0.14	0.84
19	0.90	0.8	0.16	0.89

[a] For solutions in 0.02M in $CDCl_3$.

Identical effects are seen in the ketone (2) (Table 3), but a the relaxation times are longer because the molecule is smaller and tumbles more rapidly than does friedelin. We are able, therefore, to rationalise observed relaxation-rate variations between methyl groups at least in part through proximity effects of other methyl protons. It should be pointed out that there is evidence both from carbon-[10] and proton-T_1 [11] studies on steroids to indicate that 1,3-diaxial interactions of methyl groups with protons (rather than with other methyl groups) *increase* rotation rates and reduce relaxation efficiency. If friedelin's T_1 values *do* reflect rotation rates then the opposite effect is operating, the most hindered methyls being those which relax more efficiently. Presumably, ^{13}C n.m.r. measurements could clearly establish methyl correlation times, and allow quantitative methyl-to-methyl distance measurements to be made by kinetic n.O.e. methods.[12,13]

The methyl-to-methyl n.O.e.s observed in this work are remarkably large: they are in the 0.5—5% range for methyl saturation levels of considerably less than 50%. It is clear that, given adequate chemical-shift dispersion, the n.O.e.s will be powerful probes of the conformations of terpenes and steroids, although it seems unlikely that distance measurements will become accurate enough to detect the characteristic bowing of the skeleton.[9]

Two aspects of the chemical shifts in this type of molecule deserve comment. First, the effective separation of the methylene envelope from the methyl-group signals is only obtainable at very high frequencies since axial protons (δ 1.2) with multiplet widths of 30—40 Hz will intrude into the methyl region at field strengths below *ca.* 300 MHz. Second, it is clear from compounds (1) and (2) that highly characteristic methyl chemical shifts are observed, most notably for Me-24 and the methyl at the fusion of the two boat rings, Me-28, but the generality of such shifts remains to be established.

Experimental

Friedelin (1) was a gift from Dr. D. H. Williams (Cambridge), and the ketone (2) from Dr. P. Albrecht (Institut de Chimie,

ULP, Strasbourg). Solutions for n.m.r. study were 0.02M in $CDCl_3$, and were not degassed.

^1H N.m.r. spectra were obtained at 400 MHz using a Bruker WH400 instrument. Chemical shifts are referenced to $SiMe_4$ ($\delta = 0$). Spin–lattice relaxation rates were measured by non-linear least-squares fitting of observed inversion-recovery data to a best single exponential. The resulting *relative* T_1 values are certainly accurate to better than 5% but the *absolute* precision is undoubtedly worse. N.O.e.- and decoupling-difference spectra were acquired using previously described microprograms.[2] The methyl-to-methyl n.O.e. experiments were only successful when extremely low irradiating power levels (35—40 dB below 0.2 W) were used, leading to 10—30% saturation, and methyl n.O.e.s of 0.5—5%. The excessive bandwidth of higher power levels precluded useful methyl-to-methyl observations. 600—1 000 transients were acquired in n.O.e. experiments, and 2 Hz exponential line broadening was employed.

Acknowledgements

We are grateful to J. D. Mersh for a preliminary experiment and for computing help, to the S.E.R.C. and Hickinbottom Bequest (Royal Society of Chemistry) for financial support to J. K. M. S. and to the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq, Brazil) for a Fellowship for F. R. de A. N.

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Received 17*th June* 1982; *Paper* 2/1020