^{13}C NMR OF TETRAHYMANOL

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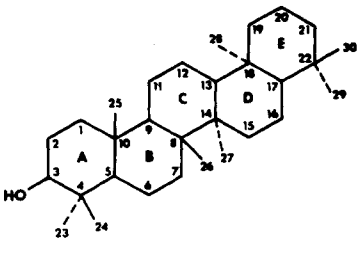
Triterpenoids can be divided into those which are biosynthesized *via* a non-oxidative cyclization of squalene, e.g., tetrahymanol, and those which proceed under aerobic conditions *via* 2,3-oxidosqualene, *e.g.*, lanosterol and lupeol (1,2). Since certain triterpenoids may replace sterols as membrane components (1,3) but not as regulators of sexual expression (4), their structural elucidation is of interest for phylogenetic and functional reasons (5). The purpose of the present communication is to assign the ^{13}C nmr resonances for the saturated pentacycle tetrahymanol **1** (cf. fig. 1), confirming the structure (6,7).

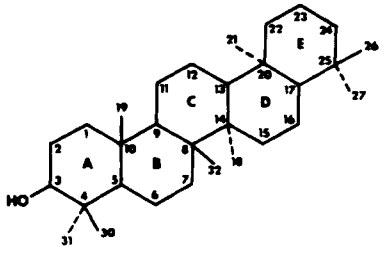
RESULTS AND DISCUSSION

Based on the work of Wenkert *et al.* (8), to assign the signals for all but two carbon atoms of tetrahymanol, lupeol **2** and lupene **3** (cf. fig. 2) were used as model compounds; a lanthanide shift reagent was also used. Since the A, B, and C rings of **1** are analogous to the A, B, and C rings of **2** and the E, D, and C rings of **1** are the same as the A, B, and C rings of **3**, the assignments of the carbon atoms in **1** follow directly from those of **2** and **3**. Because the 3β-hydroxyl group in **1** destroys what would otherwise be a symmetrical molecule about the dashed line in fig. 2, many pairs of peaks, i.e., 5–17, 6–16, 7–15, 8–14, 9–13, 10–18, 11–12, 25–28, 26–27 (triterpenoid numbering system, fig. 1 (9)), have similiar but not identical chemical shifts. To make the assignments for all but one of these pairs, the lanthanide shift reagent Yb $(dpm)_3$ was used. The downfield shifts induced by the shift reagent are shown in fig. 3. For all additions of shift reagent, the signals for pair 8–14 were obscured, thus not permitting their differentiation.

The ^{13}C chemical shift for C–3 of **1** agrees with that of the 3β-hydroxyl-substituted carbon of lupeol **2**. Further confirmation of the orientation of the hydroxyl in **1** comes from the 200 MHz ^1H nmr spectrum where H–3 at 3.195 is a clearly resolved double-doublet with couplings of 10.2 Hz and 5.9 Hz, in contrast to the 90 Hz MHz spectrum where the signal is an unresolved multiplet. The 10 Hz coupling can only arise from a vicinal diaxial configuration, indicating that the hydroxyl must be oriented equatorially or β.



TRITERPENOID NUMBERING SYSTEM



STEROID NUMBERING SYSTEM

FIG. 1. Structure of tetrahymanol with the triterpenoid and steroid numbering system.

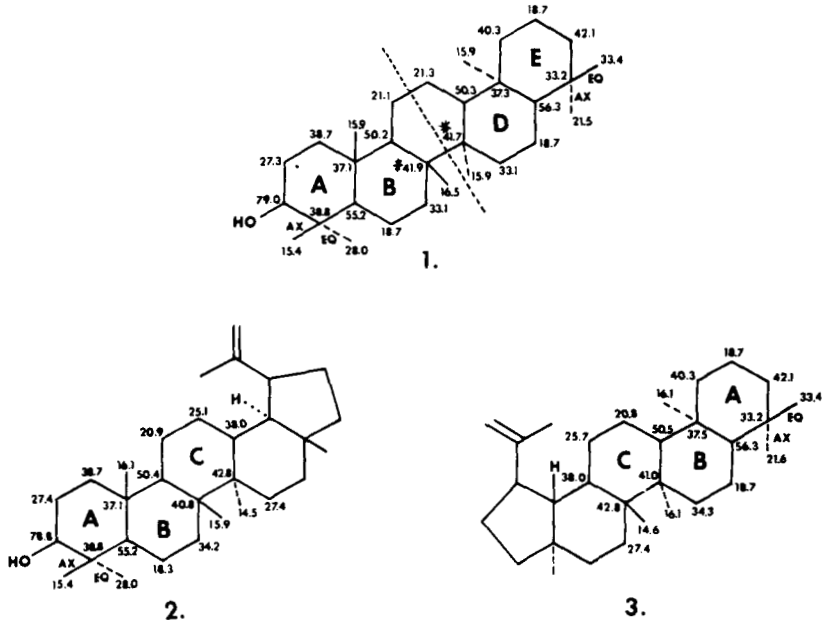


FIG. 2. ^{13}C nmr chemical shifts in $CDCl_3$ of 1, 2,[6] and 3.[6]
* shifts may be interchanged.

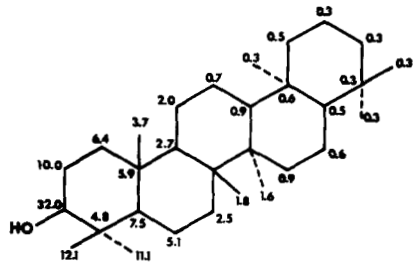


FIG. 3. Downfield shifts in ppm on 1 induced by Yb(dpm)$_3$, extrapolated to a unit ytterbium/substrate ratio.

EXPERIMENTAL

The ^{13}C nmr were recorded on a Jeol PS-100 operating at 25.03 MHz. The pulse sequence used in obtaining the Fourier transform spectra was the multiplicity separation sequence of Le Cocq and Lallemand (10) where the C and CH_2 signals and the CH and CH_3 signals are of opposite phase. Spectra were run with a 90° flip angle (16μs) at a repetition rate of 2s using 32K data points to cover a width of 10,000 Hz (this width used to suppress spurious responses). To more accurately determine the number of carbons at each shift, one spectrum was run at a repetition rate of 10s to allow full relaxation of the signals between pulses. This rate is more than five times the reported relaxation times of 1-1.5s for axial methyls in cholestane derivatives (11). A Varian EM390 operating at 90MHz and a Nicolet 200 operating in the FT mode at 200 MHz were used to obtain the ^1H nmr spectra, which were the same as those previously described (12,13). The downfield shifts were determined by four sequential additions of shift reagent and least squares extrapolation to unit ytterbium/substrate ratio.

Tetrahymena pyriformis, strain W, was cultured as previously described (10). The cells were lyophilized and the dried cells were extracted three times with chloroform-methanol (2:1 v/v) at 55°. After saponification of this total lipid fraction with 10% KOH in 95% aqueous methanol, the ether-soluble neutral lipid was chromatographed on an alumina column with increasing amounts of ether in hexane. The 4,4-dimethyl fraction was further purified to yield 1 by chromatography on a Lipidex-5000 column (14). Compound **1** was >99% pure according to glc and reversed-phase hplc (15,16). Electron ionization mass spectra were obtained on a 70/70 F double-focusing instrument (MicroMass, VF-Organic Ltd.)

by use of the direct probe and an ion-source temperature of 180 . The MS for **1** showed a m/z 428 (M^+, 70%), confirming its molecular weight and structurally diagnostic fragmentation pattern due to a reverse Diels-Alder rearrangement, giving rise to a base peak of m/z 191 (M^+, 100%).

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[1]Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.