the cleavage of a tiglic acid ester (7). In molephantin the base peak is at *m/e* 69, due to the methacrylate side chain. Acetylation of molephantinin with acetic anhydride in pyridine gave an acetate (II), $C_{22}H_{26}O_7$, *m/e* 402.1670 ($M^+$), mp 131°, which also showed comparable IR and NMR spectra to those of molephantin acetate (V).

Extensive NMR decoupling (100 MHz) led to the following assignment of protons, which was consistent with the structure of molephantinin acetate as depicted in II: δ 6.92 (1H, br m, H-19), 6.57 (1H, s, H-5), 6.36 (1H, s, H-1), 6.34 (1H, d, $J$ = 2.5 Hz, H-13), 6.04 (1H, br s, H-3), 5.77 (1H, d, $J$ = 2.0 Hz, H-13), 5.21 (1H, dt, $J$ = 4.0, 10.5 Hz, H-8), 4.34 (1H, d, $J$ = 3.5 Hz, H-6), 3.40 ([H, m, H-7), 2.62 (2H, m, H-9), 2.07 (3H, s, $OCOCH_3$), 1.98 (3H, d, $J$ = 1.5 Hz, H-15), 1.83 (6H, m, H-18 and H-20), and 1.80 (3H, d, $J$ < 1.0 Hz, H-14). For example, irradiation of the multiplet centered at δ 6.92 caused the six-proton multiplet at δ 1.83 to collapse to a singlet. Thus, the multiplet at δ 1.83 could be assigned to the methyl groups at C-18 and C-19.

Irradiation of the broad singlet at δ 6.04 (H-3) caused a three-proton doublet at δ 1.98 to collapse to a singlet, which could thus be assigned as the C-4 methyl signal, as was also observed in the decoupling experiment of molephantin acetate. Irradiation of the C-6 proton at δ 4.34 caused only a sharpening in the multiplet at δ 3.40, which was assigned to the C-7 proton, suggesting that the protons at C-5 and C-6 are not coupled to each other. Drieding models of this compound indicated the feasibility of this suggestion since the dihedral angle between H-5 and H-6 is approximately 90°.

Oxidation of molephantinin with Jones reagent afforded dehydromolephantinin (III), mp 136°, $C_{20}H_{22}O_6$, whose spectral data were in accord with the assigned Structure III. The circular dichroism curve of molephantinin shows a strong positive Cotton effect at 244 nm, indicating that molephantinin possesses the same stereochemistry and absolute configuration as molephantin.

The foregoing evidence leads to the formulation of the complete structure of molephantinin as I. Studies on the structure–activity relationships among molephantinin-related sesquiterpene lactones are currently in progress.

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*Kuo-Hsiung Lee* [x]
*Toshiro Ibuka*

Department of Medicinal Chemistry
School of Pharmacy
University of North Carolina
Chapel Hill, NC 27514

*Huan-Chang Huang*

School of Pharmacy
Kaohsiung Medical College
Kaohsiung, Taiwan
Republic of China

*David L. Harris*

Department of Chemistry
University of North Carolina
Chapel Hill, NC 27514

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[x] To whom inquiries should be directed.

## $^{13}$C-NMR Study of Aqueous Glutaraldehyde Equilibria

**Keyphrases** □ Glutaraldehyde—aqueous equilibria determined using $^{13}$C-NMR, estimation of free aldehyde, relationship to pH, antibacterial activity □ Antibacterial activity—glutaraldehyde free aldehyde content determined by $^{13}$C-NMR □ NMR spectroscopy—determination, aqueous glutaraldehyde equilibria, estimation of free aldehyde, relationship to pH

*To the Editor:*

Recently the insensitivity to concentration and pH of the free aldehyde content of aqueous glutaraldehyde was reported (1). The percentage of free aldehyde was calculated on the basis of the absence (2) of dialdehyde and found to be around 47% from integral ratios of $^1$H-NMR peaks. This amount is twice as large as estimates based on $^{13}$C-NMR spectra. Moreover, the $^{13}$C-NMR results of Whipple and Ruta (3) and our own results indicate clearly two types of free aldehyde carbonyl groups, which must be due to the free dialdehyde and the hemihydrate, respectively. Our study covered a pH range of 1–8 and concentrations of 25–3% and confirmed previous studies that the equilibria among the dialdehyde, the hemihydrate, the dihydrate, and the cyclic hemiacetal are not greatly affected by pH or concentration.

Although $^{13}$C-NMR spectra are more easily interpreted than $^1$H-NMR spectra (because of larger shifts and absence of spin–spin coupling), the use of peak intensities in spectra obtained by the pulsed Fourier transform technique can be misleading. Possible problems arising from the processing of the

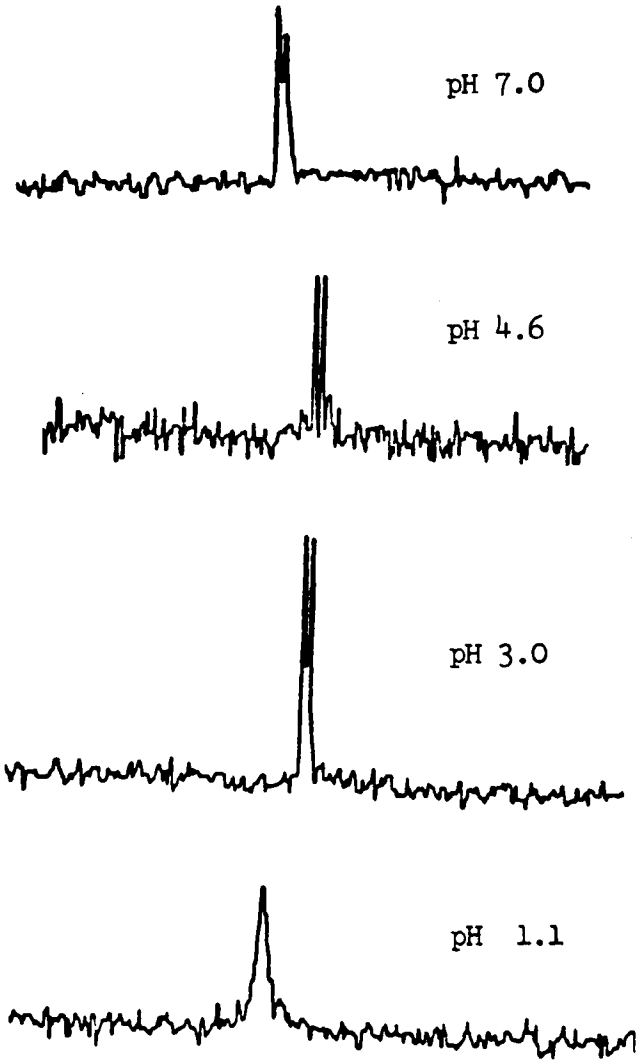


**Figure 1**—*$^{13}$C-NMR line shape dependence on pH of aqueous glutaraldehyde (carbonyl region).*

transient signal, such as sensitivity enhancement and digitization, can usually be avoided by peak area integration. However, equal numbers of carbon atoms in different sites can give dissimilar peak intensities due to a differing spin-lattice relaxation time, $T_1$, and nuclear overhauser enhancement due to proton decoupling (4). Whipple and Ruta (3) used a pulse delay and compared methylene carbon peaks to mitigate this problem. We used a smaller (2 μsec, 13°) pulse width with no delay and peak intensities of the carbons attached to oxygen at 209.2 and 208.4 (free aldehyde) and 91.0–95.2 (hydrated and cyclic forms) ppm.

Our results, at 38° in aqueous solution (with 10% $D_2O$ for the heteronuclear lock[1]), are consistent with those of Whipple and Ruta when differences in temperature are taken into account. Since the relevant carbonyl peaks were of almost equal area, we found twice as much monohydrate as dialdehyde. The total free aldehyde content was equivalent to 28 free aldehyde groups/100 molecules of glutaraldehyde, compared to 24% found by Whipple and Ruta (3) at the lower temperature of 23°. This finding is in agreement with their predictions of the effects of temperature on the equilibria involved.

In the absence of the raw data of King *et al.* (1), we recalculated the proton NMR data of Hardy *et al.* (2), using our dialdehyde–monohydrate ratio of 1:2. We found that their intensity ratios can be adequately explained on this basis to give, on an average, 4% dialdehyde, 8% monohydrate, 54% dihydrate[2], and 34% hemiacetal. These results give about 16% free aldehyde group instead of the 43% calculated by Hardy *et al.* (2), which is in better agreement with the now available $^{13}$C-NMR data. The 47% free aldehyde found by King *et al.* (1) would similarly decrease.

Finally, we wish to report preliminary evidence of pH-dependent linewidths in the $^{13}$C-NMR spectrum of glutaraldehyde (Fig. 1). Sharp resonances are observed around pH 3–5, while line broadening is evident above and below this range. We attribute this line broadening to an increase in the rate of interconversion of one form into another. Previously, Wolman (5) suggested that the effectiveness of neutral buffered formalin in histological and histochemical work was due to the rapid conversion at neutral and alkaline pH of the polymerized form of HCHO to the monomer. Although this theory met with some skepticism (6), we feel that similar kinetic factors might best explain the pH-dependent activity of glutaraldehyde in the light of our $^{13}$C-NMR findings.

Therefore, in view of the insignificance of pH and concentration dependence on the equilibria themselves, we suggest that the rates at which the equilibria are established (or maintained as the active components are depleted *in situ*) are important in considerations of antibacterial activity. This alternative interpretation of the pH dependence of antibacterial activity of commercial glutaraldehyde solutions is being investigated further.

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*C. E. Holloway* ˣ
Chemistry Department
York University
Downsview, Ontario, Canada
*F. H. Dean*
Ontario Research Foundation
Sheridan Park, Mississauga, Ontario, Canada

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ˣ To whom inquiries should be directed.

[1] Varian CFT 20 instrument.

[2] For comparison only with original data. Whipple and Ruta (3) indicated that peaks in this area have been misassigned to the dihydrate and should belong to *cis*- and *trans*-forms of the hemiacetal. This reassignment would not affect the analysis for free aldehyde.