ing of curves, superpositioning, and adding of superposed curves.

Of course, one could fit a compartment model and proceed in a similar manner, but I have already expressed a preference for using simple empirical techniques (1). What is required to achieve the curve matching expeditiously is a small digital computer with an oscilloscope-type display[1]. One sets up the controlled-release blood level curve as background on the oscilloscope and then tries to match it by trial and error, which usually requires 5–10 min.

At this point, by *theoretical* pharmacokinetic computations, a regimen of plain-release doses has been derived which is predicted to match the controlled-release formulation with respect to its blood level curve. The next step is, of course, to verify the validity of these theoretical calculations with an actual comparative trial in human subjects. Figure 5 shows the actual mean blood level curves of phenylpropanolamine obtained in a crossover trial in 12 subjects. A controlled-release formulation of 150 mg was compared with the following regimen:

hours	dose, mg
0	52.4
1	21.4
2	23.8
3	23.8
5	9.6
6	9.6
7	9.4

This regimen was derived from pharmacokinetic computations of the type described. The blood level curves obtained in Fig. 5 appear to show a good match; apparently this regimen of divided doses does give rise to essentially the same blood level profile as the controlled-release formulation.

The question still exists as to whether the divided dose regimen is sufficiently dispersed over time to merit the title controlled release for the formulation that it appears to mimic. As an extreme example, the controlled-release description would probably not be merited for a formulation that appears to be equivalent to a divided dose regimen of 145 mg at zero hr and 5 mg at 1 hr. Obviously, some arbitrary rules are needed. Nevertheless, the proposed method offers a reasonable way of handling the problem of demonstrating controlled release.

So that no misunderstanding occurs, it should be noted that no claim is made that the divided dose regimen is a model of how the controlled-release formulation behaves *in vivo* but rather that it gives rise to blood levels equivalent to those generated by the controlled-release formulation.

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[1] A PDP-12 has been found ideal for this purpose.

Antitumor Agents XIV: Molephantinin, a New Potent Antitumor Sesquiterpene Lactone from *Elephantopus mollis*

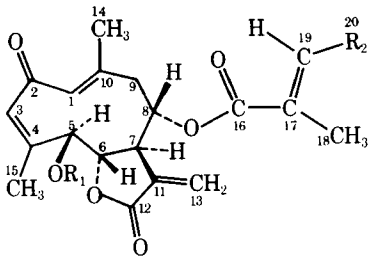
Keyphrases □ *Elephantopus mollis* H.S.K.—isolation and structure determination of molephantinin, antitumor activity □ Molephantinin—isolation, structure determination, and antitumor activity □ Antitumor agents, potential—molephantinin, constituent of *Elephantopus mollis* H.S.K. □ Structure-activity relationships—sesquiterpene lactones as antitumor agents

To the Editor:

We recently reported the isolation and structure determination of two novel cytotoxic germacranolides, molephantin (IV) (1) and phantomolin (2), from *Elephantopus mollis* H.S.K. This report[1] involves the isolation of an additional new sesquiterpene lactone, molephantinin (I), from the winter collection of this same plant. Molephantinin showed significant (T/C ≥ 125%) inhibitory activity against the Walker 256 carcinosarcoma in rats (T/C = 397%) at the 2.5-mg/kg level[2].

Molephantinin was isolated from the mother liquor after the removal of molephantin and phantomolin by silica gel column chromatography. Molephantinin, mp 223–225°, has the composition $C_{20}H_{24}O_6$ and shows an IR spectrum very similar to that of molephantin, indicating the presence of a hydroxy group (3420 cm^{-1}), a γ-lactone (1775 cm^{-1}), and the conjugated enone system (1713 and 1650 cm^{-1}). The NMR spectrum of molephantinin is superimposable with that of molephantin, except for the signal patterns in the acid portion of the ester. These differences were observed in molephantinin as the broad vinyl multiplets at δ 6.92 (1H, H-19) and the vinyl methyl multiplets at δ 1.83 (6H, H-18 and H-20), which are the typical signals for the tigloyl group (5, 6).

Added confirmation for these assignments was obtained by a comparison of the mass spectra of molephantinin and molephantin. The base peak in the mass spectrum of molephantinin is at *m/e* 83, due to



I: R_1 = H, R_2 = CH_3
II: R_1 = $COCH_3$, R_2 = CH_3
III: =O replaces R_1O ⟋ H, R_2 = CH_3
IV: R_1 = H, R_2 = H
V: R_1 = $COCH_3$, R_2 = H

[1] For Part XIII, see Ref. 3. For Part XII, see Ref. 2.
[2] Antitumor activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, by a literature method (4).

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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^{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