Neutron Diffraction Structure of Melampodin: Its Role in the Reclassification of the Germacranolides

(cyclodeca-1,5-dienes/sesquiterpene lactones/cytotoxicity/chemotaxonomy/direct phase determination)

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Communicated by Maurice Goldhaber, April 20, 1973

ABSTRACT The precise crystal and molecular structure of melampodin, $C_{21}H_{34}O_2$, was determined from three-dimensional neutron diffraction data collected by counter techniques and phased by direct statistical methods. Crystals are orthorhombic, $P2_12_12_1$, $a = 8.990(9)$, $b = 14.352(14)$, $c = 16.294(16)$ Å, $V = 2102$ Å³, $d(\text{calc.}) = 1.328$ g·cm⁻³, $Z = 4$ molecules per unit cell. The structural model was refined by full matrix least-squares of 2303 observed independent reflections, with all 54 atoms treated anisotropically, to $R(F) = 5.0\%$. Hydrogen bonds link melampodin molecules together in the solid state. The conformation of the cyclodeca-1,5-diene ring is such that one intramolecular hydrogen atom interacts strongly with one double bond, but there is little or no transannular interaction between double bonds. Strain in the ten-membered ring and in the *trans*-fused lactone ring is discussed, as are chemical implications of the unsymmetric epoxide in the epoxyangelic acid side chain. The previously suggested reclassification of germacranolide sesquiterpene lactones into four subgroups is supported, and a new convention for configurational representations of the four subgroups is proposed.

A rapidly increasing number of different types of sesquiterpene lactones have been isolated from plants (1). Some have received considerable attention due to their antileukemic and tumor-inhibitory activities (2). While most of these terpenoids are *trans,trans*-cyclodeca-1,5-diene germacranolide sesquiterpene lactones (3), discovery of the naturally occurring *cis,trans*-cyclodeca-1,5-diene melampodin (4, 5) has necessitated reclassification of the germacranolides into four subgroups (5). All subgroups are characterized by cyclodecadienes with the double bonds in the biogenetically correct 1^A(10) and 4 positions; Fig. 1 depicts basic configurational structures (I-IV) and conformational representatives (V-VIII) for the four subgroups. The configurational representations shown are strongly recommended as standardized forms for the germacranolides.

The germacranolides (I) with 7,6-lactonic ring closure exist in a stable conformation with *syn*-orientation of the CA14 and CA15 methyl groups lying on the β -face of the medium ring, as shown for dihydrotamaulipin-A acetate (IV) (6). The *anti*-arrangement of CA14 and CA15 seems to be typical for the conformation of the melampolides (II) and heliangolides (III). The highly strained melampodin (VI) (4, 5, 7), polydalin (8), and enhydrin (9) adopt conformations with CA14 α and CA15 β to the plane of the medium ring (VI in Fig. 1). Typical derivatives of the heliangolides (CA14 β and CA15 α) are heliangine (VII) (5, 10), erioflorin (11), and woodhousin (12). The fourth subgroup contains *cis,cis*-cyclo-

deca-1,5-dienes (IV) and is not yet represented by a natural product, although eupacunin (13) and liatrin (14) seem to be biogenetic derivatives of this group. From inspection of Dreiding models, the *cis,cis*-germacranolide sesquiterpene lactones are expected to adopt a conformation with CA14 and CA15 both α to the plane of the medium ring, as indicated in VIII. Both the β -orientation and the two possible *anti*-arrangements of CA14 and CA15 seem to be less favored due to transannular hydrogen interactions.

Although there has been little direct study of the biosynthesis of medium ring compounds in plants, the germacranolides have been rationalized as the first biogenetic cyclization product of *trans,trans*-farnesyl pyrophosphate followed by biomodification reactions. The discovery of melampodin

FIG. 1. Configurational representations and conformational representatives of the four subgroups of germacranolides.

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raises the important question whether the *cis*-double bond is formed at an early biosynthetic stage or is generated later in a *trans-cis*-isomerization by spontaneous or enzyme-controlled processes. This question is of considerable interest since configurational and conformational differences in the medium rings are used as genetic markers in our biochemical systematic studies in the genus *Melampodium* in comparison to other genera and subfamilies in the family Compositae. For instance, the lack of melampolides in several species of the genus *Parthenium* (15) and the presence of sesquiterpene lactones typical for the Ambrosiinae provides further support for reclassification of the genus *Parthenium* from their present position in the Melampodiinae to the Ambrosiinae (16). We also expect fundamental information concerning the structural and conformational influences on cytotoxicity of sesquiterpene lactones to evolve from these studies.

We have found that the availability of highly accurate structural data for model compounds is vital in interpretation of ^1H and ^{13}C magnetic resonance spectra of new compounds. Specifically, correlations of nuclear magnetic resonance (NMR) chemical shifts and coupling constants with torsional and dihedral angles derived from diffraction data in these strained medium ring compounds will be used in future work on elucidation of structure of melampodin analogues. Melampodin was therefore chosen as a bench mark compound for further studies on new and novel *cis-trans*-germacranolides.

EXPERIMENTAL

The crystal selected for neutron data collection, grown from an ethyl acetate solution, was bounded by fourteen faces belonging to the forms $\{110\}$, $\{111\}$, $\{011\}$, and $\{021\}$, and had a total volume of 21.46 mm³. The size and orientation of each face was accurately measured for use in the analytical absorption correction.

The crystal data published (5) are confirmed; melampodin, $\text{C}_{21}\text{H}_{24}\text{O}_9$, $M = 420.45$, crystallizes in the orthorhombic space group $\text{P}2_12_12_1$, $a = 8.990$, $b = 14.352$, $c = 16.294$ Å (all to 0.1% accuracy), $V = 2102$ Å³, $Z = 4$, $d(\text{calc.}) = 1.328$ g·cm⁻³, $\mu(\text{neutrons}) = 2.132$ cm⁻¹.

FIG. 2. Schematic diagram of melampodin. The numbering scheme shown is used throughout the text and is consistent with that found in ref. 5.

The crystal was mounted on an aluminum pin with Eastman 910 adhesive and Duco cement. The pin was then mounted on a computer-controlled four-circle diffractometer (17) at the Brookhaven National Laboratory High Flux Beam Reactor such that the $[100]$ direction was nearly parallel with the diffractometer ϕ axis. The thermal neutron flux at the crystal was approximately 4×10^6 n·cm⁻²·sec⁻¹, and a wavelength (λ) of 1.0142 Å was selected by means of a Ge(311) crystal monochromator. Intensity data were collected at $25 \pm 2^\circ\text{C}$, using a θ - 2θ step-scan technique to a maximum scattering angle (2θ) of 90° ($\sin \theta/\lambda = 0.7$). Each of 40 steps in a 2θ scan width of $1.4 + 11.2 \tan \theta$ degrees was counted for 4 sec. All reciprocal lattice points in the hkl octant and about 130 points in the hkl octant were investigated. Two standard reflections (4,2,7 and 1,3,6) were checked after every 31 intensity measurements throughout the entire data collection period. No systematic variation of the standard intensities was observed, and visual observation also revealed that no changes had occurred in the crystal during the diffraction experiment.

In the analytical absorption correction applied to each observed intensity, the transmission coefficient varied from 0.5713 to 0.7287. The data were then averaged over the two octants with a residual error of 4.3%. Of the 2884 independent structure amplitudes measured, 2303 were considered observed under the criterion $|F_0|^2 > 1.5\sigma_e^2$ where σ_e^2 is the estimated variance due to Poisson counting statistics alone. Each observed structure amplitude was assigned a variance $\sigma^2 = \sigma_e^2 + 0.045 |F_0|^2$. Structure factors were calculated, with

TABLE 1. Intramolecular bond lengths and selected bond angles

Bond lengths (Å)			
CA01-CA02	1.490(3)	CA12-OX03	1.203(3)
CA01-CA10	1.336(3)	CA13-HY09	1.085(6)
CA01-HY01	1.095(7)	CA13-HY10	1.085(7)
CA02-CA03	1.486(4)	CA14-OX05	1.196(4)
CA02-OX01	1.447(4)	CA14-OX06	1.324(4)
CA02-HY02	1.082(6)	CA15-HY11	1.062(9)
CA03-CA04	1.482(3)	CA15-HY12	1.055(10)
CA03-OX01	1.432(4)	CA15-HY13	1.073(8)
CA03-HY03	1.102(6)	CA16-OX06	1.441(4)
CA04-CA05	1.347(3)	CA16-HY14	1.017(10)
CA04-CA15	1.497(4)	CA16-HY15	1.046(14)
CA05-CA06	1.476(3)	CA16-HY16	0.970(17)
CA05-HY04	1.099(6)	CA17-CA18	1.504(3)
CA06-CA07	1.549(3)	CA17-OX07	1.341(3)
CA06-OX02	1.460(3)	CA17-OX08	1.193(4)
CA06-HY05	1.098(6)	CA18-CA19	1.455(4)
CA07-CA08	1.522(3)	CA18-CA21	1.496(5)
CA07-CA11	1.497(3)	CA18-OX09	1.410(4)
CA07-HY06	1.104(5)	CA19-CA20	1.483(6)
CA08-CA09	1.547(3)	CA19-OX09	1.448(5)
CA08-OX07	1.451(3)	CA19-HY17	1.075(9)
CA08-HY07	1.099(5)	CA20-HY18	1.085(20)
CA09-CA10	1.511(3)	CA20-HY19	1.055(14)
CA09-OX04	1.412(3)	CA20-HY20	1.106(15)
CA09-HY08	1.098(5)	CA21-HY21	1.102(10)
CA10-CA14	1.494(3)	CA21-HY22	1.062(15)
CA11-CA12	1.490(3)	CA21-HY23	1.010(17)
CA11-CA13	1.327(3)	OX04-HY24	0.976(7)
CA12-OX02	1.349(3)		
Bond angles (degrees)			
CA05-CA06-CA07...	111.2(2)	CA06-CA07-CA08...	117.0(2)
CA05-CA06-HY05...	113.3(3)	CA06-CA07-CA11...	100.0(2)
CA05-CA06-OX02...	111.9(2)	CA06-CA07-HY06...	106.7(3)
HY05-CA06-CA07...	109.6(3)	CA08-CA07-CA11...	118.3(2)
HY05-CA06-OX02...	107.2(3)	CA08-CA07-HY06...	106.4(3)
OX02-CA06-CA07...	103.0(2)	HY06-CA07-CA11...	107.6(3)

Associated standard deviations in parentheses.

TABLE 2. Torsion angles in degrees*

A	B	C	D	PHI	A	B	C	D	PHI	A	B	C	D	PHI	D	C	B	A	PHI
CA01	CA02	CA03	CA04	-13.6	CA07	CA11	CA12	CA02	15.3	CA17	CA18	CA19	CA20	-0.2	CA20	CA19	CA18	CA17	-0.2
CA01	CA02	CA03	CA04	94.8	CA07	CA11	CA12	CA02	-163.5	CA17	CA18	CA19	CA20	102.2	CA20	CA19	CA18	CA17	102.2
CA01	CA02	CA03	CA04	-162.8	CA07	CA11	CA12	CA02	-179.2	CA17	CA18	CA19	CA20	154.0	CA20	CA19	CA18	CA17	154.0
CA01	CA02	CA03	CA04	-123.0	CA07	CA11	CA12	CA02	0.2	CA17	CA18	CA19	CA20	-178.5	CA20	CA19	CA18	CA17	-178.5
CA01	CA02	CA03	CA04	-124.6	CA08	CA07	CA06	CA05	164.1	CA17	CA18	CA19	CA20	-60.1	CA20	CA19	CA18	CA17	-60.1
CA01	CA02	CA03	CA04	110.9	CA08	CA07	CA06	CA05	50.2	CA17	CA18	CA19	CA20	61.7	CA20	CA19	CA18	CA17	61.7
CA01	CA02	CA03	CA04	-3.4	CA08	CA07	CA06	CA05	-158.7	CA17	CA18	CA19	CA20	111.8	CA20	CA19	CA18	CA17	111.8
CA01	CA02	CA03	CA04	171.8	CA08	CA07	CA06	CA05	18.4	CA17	CA18	CA19	CA20	-4.9	CA20	CA19	CA18	CA17	-4.9
CA01	CA02	CA03	CA04	-10.6	CA08	CA07	CA06	CA05	57.1	CA17	CA18	CA19	CA20	96.2	CA20	CA19	CA18	CA17	96.2
CA02	CA01	CA10	CA14	-4.4	CA08	CA09	CA04	CA10	-51.9	CA18	CA19	CA20	CA21	-146.7	CA20	CA19	CA18	CA17	-146.7
CA02	CA01	CA10	CA14	173.8	CA08	CA09	CA04	CA10	-166.2	CA18	CA19	CA20	CA21	-23.4	CA20	CA19	CA18	CA17	-23.4
CA02	CA01	CA10	CA14	53.7	CA08	CA09	CA04	CA10	11.9	CA18	CA19	CA20	CA21	120.7	CA20	CA19	CA18	CA17	120.7
CA02	CA01	CA10	CA14	-119.5	CA09	CA08	CA07	CA11	-168.9	CA18	CA19	CA20	CA21	-122.5	CA20	CA19	CA18	CA17	-122.5
CA02	CA01	CA10	CA14	110.8	CA09	CA08	CA07	CA11	-67.7	CA18	CA19	CA20	CA21	-120.2	CA20	CA19	CA18	CA17	-120.2
CA02	CA01	CA10	CA14	-107.3	CA09	CA08	CA07	CA11	111.3	CA18	CA19	CA20	CA21	123.7	CA20	CA19	CA18	CA17	123.7
CA02	CA01	CA10	CA14	55.9	CA09	CA08	CA07	CA11	-178.2	CA18	CA19	CA20	CA21	-121.4	CA20	CA19	CA18	CA17	-121.4
CA03	CA02	CA01	CA10	-129.9	CA09	CA10	CA04	CA14	-9.8	CA18	CA19	CA20	CA21	-123.0	CA20	CA19	CA18	CA17	-123.0
CA03	CA02	CA01	CA10	-155.4	CA09	CA10	CA04	CA14	122.8	CA18	CA19	CA20	CA21	-115.6	CA20	CA19	CA18	CA17	-115.6
CA03	CA02	CA01	CA10	7.6	CA10	CA01	CA02	CA06	-109.5	CA19	CA18	CA17	CA21	-85.9	CA20	CA19	CA18	CA17	-85.9
CA03	CA02	CA01	CA10	177.9	CA10	CA01	CA02	CA06	-109.5	CA19	CA18	CA17	CA21	-23.5	CA20	CA19	CA18	CA17	-23.5
CA03	CA02	CA01	CA10	54.1	CA10	CA01	CA02	CA06	165.0	CA19	CA18	CA17	CA21	94.7	CA20	CA19	CA18	CA17	94.7
CA03	CA02	CA01	CA10	-59.7	CA10	CA01	CA02	CA06	-78.1	CA19	CA18	CA17	CA21	-143.5	CA20	CA19	CA18	CA17	-143.5
CA03	CA02	CA01	CA10	109.1	CA10	CA01	CA02	CA06	75.8	CA19	CA18	CA17	CA21	119.9	CA20	CA19	CA18	CA17	119.9
CA03	CA02	CA01	CA10	-108.3	CA10	CA01	CA02	CA06	-178.3	CA19	CA18	CA17	CA21	-122.7	CA20	CA19	CA18	CA17	-122.7
CA03	CA02	CA01	CA10	151.3	CA11	CA07	CA06	CA05	35.0	CA19	CA20	CA21	CA22	-118.7	CA20	CA19	CA18	CA17	-118.7
CA03	CA02	CA01	CA10	101.3	CA11	CA07	CA06	CA05	-78.9	CA19	CA20	CA21	CA22	125.1	CA20	CA19	CA18	CA17	125.1
CA03	CA02	CA01	CA10	-144.1	CA11	CA07	CA06	CA05	71.5	CA19	CA20	CA21	CA22	-117.7	CA20	CA19	CA18	CA17	-117.7
CA03	CA02	CA01	CA10	-22.7	CA11	CA07	CA06	CA05	-47.4	CA19	CA20	CA21	CA22	120.4	CA20	CA19	CA18	CA17	120.4
CA03	CA02	CA01	CA10	123.4	CA12	CA11	CA10	CA09	80.7	CA19	CA20	CA21	CA22	-124.1	CA20	CA19	CA18	CA17	-124.1
CA03	CA02	CA01	CA10	127.8	CA12	CA11	CA10	CA09	176.9	CA19	CA20	CA21	CA22	-113.7	CA20	CA19	CA18	CA17	-113.7
CA03	CA02	CA01	CA10	-118.1	CA12	CA11	CA10	CA09	87.6	CA19	CA20	CA21	CA22	102.0	CA20	CA19	CA18	CA17	102.0
CA03	CA02	CA01	CA10	-126.5	CA13	CA12	CA11	CA10	-102.2	CA20	CA21	CA22	CA23	37.3	CA20	CA19	CA18	CA17	37.3
CA03	CA02	CA01	CA10	119.7	CA13	CA12	CA11	CA10	-162.1	CA20	CA21	CA22	CA23	-36.8	CA20	CA19	CA18	CA17	-36.8
CA03	CA02	CA01	CA10	-16.4	CA13	CA12	CA11	CA10	19.1	CA20	CA21	CA22	CA23	37.3	CA20	CA19	CA18	CA17	37.3
CA03	CA02	CA01	CA10	-157.2	CA13	CA12	CA11	CA10	0.0	CA20	CA21	CA22	CA23	-38.4	CA20	CA19	CA18	CA17	-38.4
CA03	CA02	CA01	CA10	5.3	CA14	CA13	CA12	CA11	-67.3	CA20	CA21	CA22	CA23	36.8	CA20	CA19	CA18	CA17	36.8
CA03	CA02	CA01	CA10	-118.5	CA14	CA13	CA12	CA11	178.4	CA20	CA21	CA22	CA23	-38.4	CA20	CA19	CA18	CA17	-38.4
CA03	CA02	CA01	CA10	127.7	CA14	CA13	CA12	CA11	160.6	CA20	CA21	CA22	CA23	102.0	CA20	CA19	CA18	CA17	102.0
CA03	CA02	CA01	CA10	-75.8	CA14	CA13	CA12	CA11	-162.1	CA20	CA21	CA22	CA23	-37.3	CA20	CA19	CA18	CA17	-37.3
CA03	CA02	CA01	CA10	43.1	CA14	CA13	CA12	CA11	178.4	CA20	CA21	CA22	CA23	36.8	CA20	CA19	CA18	CA17	36.8
CA03	CA02	CA01	CA10	-147.6	CA15	CA14	CA13	CA12	-67.3	CA20	CA21	CA22	CA23	-38.4	CA20	CA19	CA18	CA17	-38.4
CA03	CA02	CA01	CA10	16.9	CA15	CA14	CA13	CA12	179.9	CA20	CA21	CA22	CA23	36.2	CA20	CA19	CA18	CA17	36.2
CA03	CA02	CA01	CA10	-48.3	CA15	CA14	CA13	CA12	-40.9	CA20	CA21	CA22	CA23	-37.7	CA20	CA19	CA18	CA17	-37.7
CA03	CA02	CA01	CA10	-167.2	CA15	CA14	CA13	CA12	40.9	CA20	CA21	CA22	CA23	39.0	CA20	CA19	CA18	CA17	39.0
CA03	CA02	CA01	CA10	146.6	CA15	CA14	CA13	CA12	-37.8	CA20	CA21	CA22	CA23	-39.0	CA20	CA19	CA18	CA17	-39.0
CA03	CA02	CA01	CA10	8.6	CA15	CA14	CA13	CA12	-30.5	CA20	CA21	CA22	CA23	-63.1	CA20	CA19	CA18	CA17	-63.1
CA03	CA02	CA01	CA10	-172.5	CA16	CA15	CA14	CA13	-0.8	CA21	CA22	CA23	CA24	102.5	CA20	CA19	CA18	CA17	102.5
CA03	CA02	CA01	CA10	-61.9	CA16	CA15	CA14	CA13	34.9	CA21	CA22	CA23	CA24	-100.3	CA20	CA19	CA18	CA17	-100.3
CA03	CA02	CA01	CA10	-28.1	CA16	CA15	CA14	CA13	37.9	CA21	CA22	CA23	CA24	52.7	CA20	CA19	CA18	CA17	52.7
CA03	CA02	CA01	CA10	43.5	CA16	CA15	CA14	CA13	34.9	CA21	CA22	CA23	CA24	-76.9	CA20	CA19	CA18	CA17	-76.9
CA03	CA02	CA01	CA10	169.1	CA16	CA15	CA14	CA13	-35.8	CA21	CA22	CA23	CA24	-69.3	CA20	CA19	CA18	CA17	-69.3
CA03	CA02	CA01	CA10	-78.0	CA16	CA15	CA14	CA13	-37.9	CA21	CA22	CA23	CA24	47.6	CA20	CA19	CA18	CA17	47.6
CA03	CA02	CA01	CA10	-123.3	CA16	CA15	CA14	CA13	35.8	CA21	CA22	CA23	CA24	-120.8	CA20	CA19	CA18	CA17	-120.8

* Angles refer to the enantiomorph of the absolute configuration (5), and conform to the convention proposed by the IUPAC-IUB Commission (29).

FIG. 3. Stereopair diagram of melampodin with 50% probability envelopes for all thermal ellipsoids. The enantiomer shown was chosen arbitrarily due to the insensitivity of the neutron diffraction experiment to molecular configuration. Subsequently, it was discovered to be the mirror image of the absolute configuration determined by x-ray analysis (5).

FIG. 4. Stereopair diagram of two melampodin molecules linked in the solid state by a hydrogen bond.

the neutron scattering lengths (18) $b(\text{C}) = 0.665 \times 10^{-12}$, $b(\text{H}) = -0.378 \times 10^{-12}$, and $b(\text{O}) = 0.575 \times 10^{-12}$ cm.

A report has already appeared (7) concerning the direct-methods solution of the melampodin structure from these neutron data. The atomic positions for carbon and oxygen derived from the E-map were very close to those derived from the x-ray study. With these positions, approximate hydrogen positions, and isotropic thermal parameters, the residual factors ($R_1 = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, $R_2 = \{ \Sigma \sigma^{-2} (|F_o| - |F_c|)^2 / \Sigma \sigma^{-2} |F_o|^2 \}^{1/2}$), which were initially 30%, converged in 6 cycles of full matrix least-squares to $R_1 = 11.5\%$ and $R_2 = 11.7\%$. Further refinement with anisotropic thermal parameters for all 54 atoms reduced the residuals to their final values of $R_1 = 0.050$ and $R_2 = 0.041$ with an error of fit ($\Sigma \sigma^{-2} (|F_o| - |F_c|)^2 / \{ \text{number of observations} - \text{number of variables} \}$) of 1.082. Due to the large number of parameters (487), two blocks of variables were refined separately. The first block contained parameters for all atoms in or attached directly to the two fused rings (Fig. 2), while the second block contained parameters relative to the two side chains.[†] Table 1 contains all bonding internuclear distances and selected bond angles, and Table 2 contains all torsion angles. The following computer programs were used: PROCH (initial

data processing); TOMPA (Lorentz, polarization, absorption, and extinction); ECSORTH (sorting and averaging of data); MULTAN (statistical phase determination); JIM-DAP (local version of Zalkin Fourier summation); LINUS (local version of ORFLS) (19); ORFFE (function and error) (20); PLANET (least-squares planes) (21); ORTEP (molecular plotting) (22); and TORSION (torsional angles).

DISCUSSION

Although the absolute configuration of melampodin was not determined from the neutron diffraction data, the relative configurations at the eight chiral centers agree with the assignments of Neidle and Rogers (5). Furthermore, all bond lengths and torsion angles involving carbon and oxygen atoms derived from both neutron and x-ray experiments agree within experimental error.

In addition to heavy atom positions, precise positional and thermal parameters for all hydrogen nuclei were obtained from these neutron diffraction data in contrast to the hydrogen parameters from the x-ray diffraction experiments, which have large uncertainties associated with them. The quality of the data presented here may be judged, e.g., by noting the size and orientation of the thermal ellipsoids for all 12 methyl protons (Fig. 3). These ellipsoids accurately represent the hindered rotational or extreme librational motion expected of these groups in the solid state, from which we infer that there is little or no systematic error in these data.

Two equivalent intermolecular hydrogen bonds link each melampodin molecule to two neighboring molecules in the solid state. The two neighboring molecules are related to the first by two screw axes, and the atoms involved are the hydroxyl group (OX04-HY24) and the carbonyl oxygen of the

[†] A listing of the positional and thermal parameters of the atoms and a table of the observed and calculated structure amplitudes may be obtained from ASIS/NAPS, c/o Microfiche Publications, 305 East 46 St., New York, N.Y. 10017. Remit with order \$1.50 for microfiche or \$5.00 for photocopies. Make checks payable to Microfiche Publications. Note: The NAPS document number was not available at press time and will be given in a subsequent issue of the Proceedings.

lactone ring (OX03). The hydrogen bond (Fig. 4) is unsymmetric [O—H = 0.976(7) Å, H···O = 1.888(7) Å] and non-linear (O—H···O = 158°). This is similar to hydrogen bonds found in oxalic acid dihydrate (23).

Of the three intraannular hydrogen atoms, only the hydrogen atom attached to CA09 is in near intramolecular contact**. These contacts are with a methyl proton (HY08···HY12 = 2.24 Å), an oxygen atom (HY08···OX07 = 2.49 Å), and intimate transannular contact with the π -electron cloud associated with the *trans* double bond (HY08···CA04 = 2.42 Å). We attribute the observed ¹H magnetic resonance signal for HY08 at δ = 3.98 (4) to shielding by the CA04–CA05 π -electron system. This signal is in marked contrast to the proton chemical shifts for similarly situated hydrogen atoms in tamaulipin-A (δ = 4.7) (25), tamaulipin-B (δ = 4.5) (26), chihuahuain (δ = 4.42) (27), and heliangine (δ = 4.47) (28).

The two double bonds of the *cis,trans*-cyclodecadiene ring of melampodin may be considered to be in contact (center-to-center distance 3.2 Å) and hence some π – π interaction might be expected. However, it is obvious from Fig. 3 that the relative orientation of the two π -electron systems is not conducive to appreciable interaction; indeed, the angle between the two nodal plane normals is 46.2°. Hence, the conformation of this melampolide ring system inhibits the transannular interaction normally observed in germacrolides (6).

Of the six atoms associated with each double bond, four of these define precisely the nodal plane††; the two π -bonded ring carbon atoms and the two unconstrained nonring atoms lie within 0.0005 Å of this plane, but the two attached ring carbon atoms both lie above the plane. Thus, the strain inherent in the ten-membered ring does not manifest itself as a simple torsion about the internuclear line between double-bonded atoms as might be expected. Instead, the strain pulls the carbon atoms of the ring out of the nodal planes, which themselves remain intact. The largest strain of this type is at CA05, and should produce a noticeable effect in the ¹³C magnetic resonance spectrum. A further point of strain appears at CA07, one of the two fusion points between the two rings. The other fusion point, CA06, retains near-normal tetrahedral bond angles (Table 2), but the angles about CA07 are clearly nontetrahedral. The indicated strain undoubtedly contains contributions from the ten-membered ring and the five-membered lactonic ring, but we are unable to assess the relative contributions due to a lack of supporting structural data.

Finally, we point out the dissimilarity between the two epoxide groups in melampodin. The ring epoxide is symmetric in the sense that the carbon-oxygen bond lengths are statistically equivalent. However, the epoxide group on the epoxyangelic acid side chain is nonsymmetric: the CA18–OX09 bond length of 1.410 (4) Å is significantly shorter than the

CA19–OX09 length of 1.448(4) Å. Further, the three longest carbon-oxygen epoxide bonds average 1.442(7) Å, again significantly longer than the CA18–OX09 bond. This effect is probably due to the electron-withdrawing carbonyl attached to CA18, and indicates that nucleophilic attack should occur preferentially at the β -carbon (CA19) in epoxyangelic acid and its analogues.

We thank S. Neidle and D. Rogers for allowing us to see their x-ray data before its publication, and Linda and Terri Lore and Helga Fischer for plant collection and extraction. A portion of this research was done under the auspices of the U.S. Atomic Energy Commission.

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** Estimated van der Waals radii are: hydrogen, 1.2; oxygen, 1.4; half-width of aromatic (π) molecule, 1.7 Å (24).

†† The equation of least-squares plane 1, defined by atoms CA14, CA01, CA10, and HY01, is $0.857x + 12.736y + 7.348z = 8.783$, where x , y , z are fractional coordinates. Atoms CA02 and CA09 lie above this plane by 0.119 and 0.038 Å, respectively. Atoms CA15, CA04, CA05, and HY04 define plane 2, $7.046x + 8.183y + 4.012z = 3.761$, and atoms CA03 and CA06 lie 0.171 and 0.356 Å, respectively, above this plane. Bonds CA01–CA02 and CA10–CA09 make angles of 4.6° and 1.5°, respectively, with plane 1, while bonds CA04–CA03 and CA05–CA06 make angles of 6.7° and 14.0°, respectively, with plane 2.