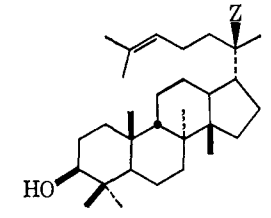
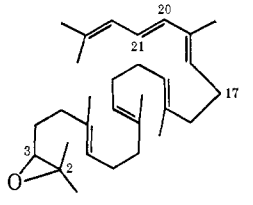
cyclase and 20,21-dehydro-2,3-oxidosqualene (**2**) which otherwise produced **4**. In addition, the rate of conversion of 2,3-oxidosqualene to lanosterol under anaerobic conditions with 2,3-oxidosqualene–sterol cyclase was decreased by the addition of the substrate **2**, *e.g.*, the rate of formation of lanosterol was depressed by *ca.* 30% when a mixture of 2,3-oxidosqualene and 5 molar equiv of **2** was incubated with 2,3-oxidosqualene–sterol cyclase. These results clearly indicate the involvement of this enzyme in the transformation of **2** to the cyclization product **4**. The stereochemistry of **4** can reasonably be assumed as shown on this basis.[10]

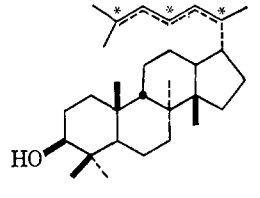
The synthesis of 20,21-dehydro-2,3-oxidosqualene (**2**) was accomplished from the aldehyde **8**[11] which was prepared by treatment of squalene with 1 equiv of ozone at −78° in methylene chloride solution, reduction with zinc–acetic acid, and purification by chromatography followed by fractional distillation. Reaction of **8** with N-bromosuccinimide–water–glyme afforded after tlc purification the bromohydrin corresponding to the addition of HOBr to the double bond farthest removed from the formyl group. This bromohydrin (10 mg) was labeled with tritium at the carbon α to the carbonyl by exposure to a mixture of tritiated water (10 μl, 1 mCi/mg) and tetrahydrofuran–triethylamine (0.5 ml, 18:1) at 50° for 14 hr. Reaction of the labeled or unlabeled bromohydrin with an excess of the Wittig reagent from triphenyl-6-methylhepta-3(*trans*),5-dien-2-ylphosphonium bromide and lithium diisopropylamide in tetrahydrofuran at 0° furnished 20,21-dehydro-2,3-oxidosqualene (**2**).[11,12]



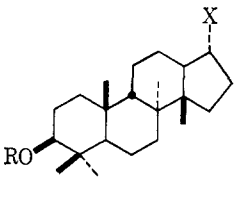
1, Z = +, enzymic or nonenzymic functional group



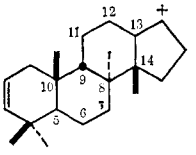
2



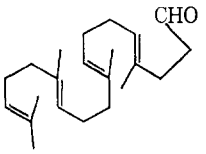
3, δ^+ at C*
4, HO at one C*



5, X = CH_3CO
7, R = H; X = COOR'



6, *m/e* 299



8

(10) A correlation of **4** with a naturally occurring protosterol derivative of known structure and stereochemistry [see S. Okuda, Y. Sato, T. Hattori, and H. Igarashi, *Tetrahedron Letters*, 4769 (1968)] will be reported in due course.

(11) Satisfactory spectroscopic (ir, nmr, mass) and analytical data were obtained for this product.

(12) The phosphonium salt was prepared from triphenylphosphonium bromide and 6-methylhepta-3(*trans*),5-dien-2-ol [J. Colonge and J. Varagnat, *Bull. Soc. Chim. France*, 1220 (1961)] in tetrahydrofuran at 25° for 20 hr.

A previous study,[3] closely related to that reported here, dealt with the enzymic formation of a bisnorprotosterol derivative lacking the methyl substituents at C-8 and C-14. The two systems now known which allow separation of the cyclization and rearrangement steps of mammalian steroidogenesis afford opportunities for the investigation of these steps in more detail. For example, reaction of the labeled ketone **5**, R = H, with 4-methylpent-3-enylmagnesium bromide affords the labeled protosterol **1**, Z = OH, an intermediate of great interest which is currently under investigation.[13]

(13) This work was supported by the National Institutes of Health, the National Science Foundation, and the Hoffmann-La Roche Co.

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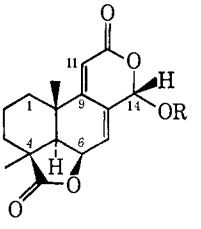
Received February 15, 1969

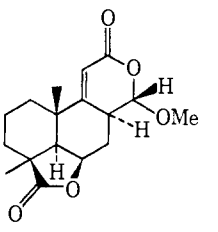
Structure of a C_{17} Antifungal Terpenoid from an Unidentified Acrostalagmus Species

Sir:

We report on the structure of a new antifungal[1] and biogenetically significant mold metabolite, LL-Z1271α (I), mp 214–216°, $[\alpha]_D$ −203° (*c* 0.29, MeOH), obtained from an unknown Acrostalagmus species known as culture LL-Z1271 in these laboratories. A minor metabolite is shown to be the corresponding lactol, LL-Z1271γ (II), mp 238–240°, $[\alpha]_D$ −259° (*c* 0.52, MeOH).



I, R = Me
II, R = H
III, R = Ac



IV

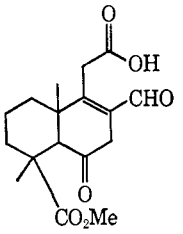
Mass spectral and elemental analysis[2] indicated the molecular formula of I to be $C_{17}H_{20}O_5$. The 60-Mc nmr spectrum showed the presence of two tertiary C-methyl's (δ 1.16 and 1.33) and a methoxy group at δ 3.70 in addition to four deshielded protons which from their chemical shifts must be attached to double bonds and carbon atoms bearing oxygen. The infrared spectrum suggested the presence of a γ-lactone (1775

(1) I possessed significant antifungal activity *in vitro* against a number of fungi and *in vivo* against some experimental ringworm infections in guinea pigs.

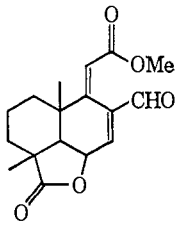
(2) Satisfactory analyses (elemental or mass spectral or both) were obtained for all compounds reported; ultraviolet spectra were taken in methanol and are reported in mμ (ϵ); infrared spectra were taken in KBr pellets and are reported in cm^{-1} for the hydroxyl and carbonyl regions only. Nmr spectra were measured at 60 and 100 Mc in deuteriochloroform (unless otherwise stated) with in some cases a small amount of DMSO added; shifts are expressed as δ values (parts per million) from tetramethylsilane as internal standard and coupling constants (*J*) are expressed in cycles per second (Hz). We thank W. Fulmor and L. Brancone and associates for the spectral and analytical data, Dr. J. Lancaster of the Stamford Laboratories for the spin-decoupling experiments, and Drs. J. Karliner and G. Van Lear for the mass spectra. We also thank A. C. Dornbush and G. S. Redin and associates for the *in vitro* and *in vivo* antifungal testing, Dr. P. Shu and associates for the fermentations and initial processing, and Dr. H. Tresner for the identification of the culture.

cm^{-1}) and another carbonyl group with absorption at 1730 cm^{-1}. The fact that I did not react with sodium borohydride indicated the lack of a ketone or aldehyde grouping and suggested that the uv maximum at 257 mμ (ε 13,500) was likely due to some type of diene chromophore. Hydrogenation of I with 5% Rh–Al_2O_3 in acetic acid gave among other products the dihydro derivative IV, $C_{17}H_{22}O_5$, mp 205° dec, ν_{max} 1770 and 1730 cm^{-1}, whose uv maximum at 218 mμ (ε 9950) was characteristic of an α,β-unsaturated lactone or ester and pointed to the presence of a diene lactone or ester grouping in the original chromophore.

The pseudo ester nature of the metabolite was indicated by mild basic hydrolysis in methanol which provided the lactol II, $C_{16}H_{18}O_5$, mp 238–240°, ν_{max} 3450, 1780, and 1680 cm^{-1}, λ_{max} 257 mμ (ε 15,400), and a small yield of the ester aldehyde V, $C_{17}H_{20}O_5$, mp 105° dec with bubbling, ν_{max} 1730 br and 1660 cm^{-1}, λ_{max} 237 mμ (ε 7800) (reduced by $NaBH_4$). Methylation of II with diazomethane in ether–methanol regenerated I and in addition gave the aldehyde ester VI, $C_{17}H_{20}O_5$, mp 190–196°, ν_{max} 1770, 1720, and 1690 cm^{-1}, λ_{max} 257 mμ very broad (ε 9100). Acetylation of II with acetic anhydride–pyridine gave the lactol acetate III, $C_{18}H_{20}O_6$, mp 205–209°, ν_{max} 1775 sh, 1760, and 1725 cm^{-1}, H-14 signal at δ 7.00, λ_{max} 257 mμ (ε 14,000).

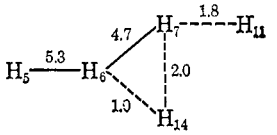


V



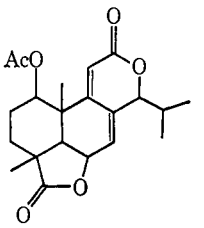
VI

Spin-decoupling experiments in deuteriobenzene (with a little deuteriochloroform added to aid solution)[3a] revealed clearly the relationship between the γ-lactone, diene, and pseudo ester groupings. A one-proton triplet (broad) at δ 4.75 is assigned to the C-6 proton on the lactone ether terminus and is vicinally coupled to the C-5 hydrogen at δ 1.55 ($J_{5,6}$ = 5.3 Hz) which is partially obscured by the methylene envelope.[3b] Also, H-6 is coupled to H-7 at δ 6.45 ($J_{6,7}$ = 4.7 Hz) and is weakly coupled to H-14 (one-proton triplet at δ 5.50; $J_{6,14}$ = 1.0 Hz) in the COOCHOMe grouping. The chemical shift of the H-7 signal at δ 6.47 is consistent for a hydrogen on the terminus of a diene ester system and is 1,4 coupled to H-11 whose signal occurs at δ 6.70 as a one-proton doublet ($J_{7,11}$ = 1.8 Hz). In addition, H-7 experiences allylic coupling to H-14 by 2.0 Hz. These couplings are summarized as shown below.

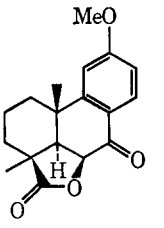


The presence of two carbocyclic rings is dictated on consideration of the molecular formula, the functional groups noted, and the lack of additional unsaturation in I. The almost identical nature of the uv spectrum of I with that of VII[4] and the assumption that we were dealing with a terpene (two tertiary C-methyl's), together with the nmr and chemical evidence, led us to conclude that only expression I is consistent with the above data.

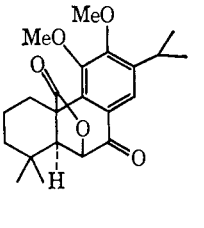
The γ-lactone was placed between C-4 and C-6 (as opposed to C-10 and C-6) in a 1,3 diaxial arrangement on the basis of the coupling constant between H-5 and H-6 (5.3 Hz) which is in accord with other diterpenoids of known stereochemistry such as the podocarpic acid derivative VIII where $J_{5,6}$ = 5.8 Hz.[5] Also, the $J_{6,7}$ of 4.7 Hz in I supports the quasiequatorial nature of H-6.[6] The $J_{5,6}$ value for the carnosol-related lactone IX is less than 1 Hz, indicating a nearly 90° dihedral angle between the C-5 and C-6 protons.[5] Finally, stereochemical considerations with the help of Dreiding models in conjunction with the stereochemistry of H-5 and H-6 as dictated by the nmr evidence just mentioned strongly suggest the relative configuration of C-4, C-5, C-6, and C-10, as shown in I. Assignment of the C-14 stereochemistry is not readily obtained from the



VII



VIII



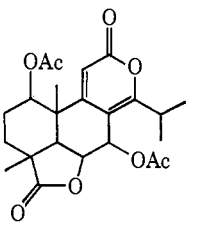
IX

magnitude of the allylic and homoallylic couplings between H-14, H-6, and H-7 (1.0 and 2.0 Hz, respectively). However, in the dihydro derivative IV the H-14 signal is a sharp doublet at δ 4.99 with $J_{8,14}$ = 9.0 Hz indicative of a diaxial arrangement of these two protons. Examination of models shows that reduction of the C-7,C-8 double bond would most certainly occur from the side opposite to the γ-lactone and C-10 methyl group and hence the axial H-14 must be *cis* to these groups in IV and of course in I.

Treatment of I in dry methanol with 1 equiv of sodium methoxide gave the pyrone X, $C_{17}H_{20}O_5$, ν_{max} 1770, 1720, 1640, and 1560 cm^{-1}, λ_{max} 291 mμ (ε 5900), formed by Michael addition of methoxide at C-7 and subsequent elimination of the methoxy at C-14. Analysis of the 100-Mc nmr spectrum of X showed the H-6 and H-7 signals at δ 4.70 and 4.60, respectively, with $J_{6,7}$ = 3.5 Hz, $J_{5,6}$ = 5.5 Hz, and $J_{7,14}$ = 2.0 Hz. The H-11 and

(3) (a) In deuteriochloroform the H-11 and H-14 signals are superimposed on each other to give a two-proton signal at δ 5.80; (b) in deuteriochloroform the H-5 signal is seen clearly as a sharp doublet at δ 1.99.

(4) VII is formed from i by sodium borohydride reduction. The latter is the diacetate of the corresponding diol obtained from the seeds and leaves of *Podocarpus nagi*, Zoli, and Moritzi: Y. Hayashi, S. Takahashi, H. Ona, and T. Saken, *Tetrahedron Letters*, 2071 (1968).

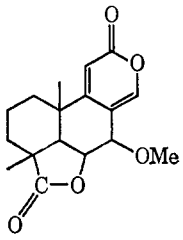


i

(5) E. Wenkert, A. Fuchs, and J. D. McChesney, *J. Org. Chem.*, **30**, 2931 (1965).

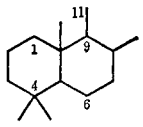
(6) E. W. Garbisch, *J. Amer. Chem. Soc.*, **86**, 5561 (1964).

H-14 signals were observed at δ 6.00 and 7.60, respectively, with $J_{11,14}$ = 1.0 Hz.



X

C_{16} terpenoids appear to be unique in nature, and the isolation of I and II raises interesting biogenetic questions. Inspection of structure I suggests a possible origin from a C_{20} precursor by microbiological degradation although one might also consider derivation from a C_{15} precursor (XI) by the addition of a C_1 unit to C-11.



XI

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Equilibrium Constant for Allyl Radical Recombination. Direct Measurement of "Allyl Resonance Energy"

Sir:

We report the direct measurement of the equilibrium constant, $K_{r,d}$, for reaction 1 and, thereby, a direct measurement of the allyl resonance energy (ARE).[1]

$$\underset{A}{M + 2\text{allyl}} \underset{k_d}{\overset{k_r}{\rightleftharpoons}} \underset{BA}{1,5\text{-hexadiene}} + M \qquad (1)$$

Values of $K_{r,d}$ have been determined at 913 and 1063°K (Table I), and these, combined with the known entropy change (Tables I and II) for the reaction, yield a value of the enthalpy change which, corrected to room temperature (Table II), is $\Delta H°_{r,d}$ = −62.2 kcal/mol. Since

$$\Delta H°_{r,d}(T°K) = \Delta H°_{f,T}(BA) - 2\Delta H°_{f,T}(A) \qquad (2)$$

and since $\Delta H°_{f,298}(BA)$ = 20.2 kcal/mol (Table II), this leads to $\Delta H°_{f,298}(A)$ = 41.2 kcal/mol.

Table I. Experimental Results

	913°K	1063°K
k_d, sec^{-1}	4.3	5.5×10^2
k_r, M^{-1} sec^{-1}	7.4×10^9	5.0×10^9
$K_{r,d}$ M^{-1}	1.8×10^9	9.4×10^6
$\Delta S°_{r,d}$ gibbs/mol[a]	−34.8	−34.7
$\Delta E°$, kcal/mol[b]	−61.0	−59.5
$\Delta H°$, kcal/mol	−62.8	−61.6

[a] Table II. [b] $\ln K_{r,d}\ (M^{-1}) = [\Delta S° - \Delta nR(1 + \ln (R'T))]/R - (\Delta E°/RT)$, where the superscript refers to a standard state of 1 atm and R' distinguishes the gas constant in units of l.-atm/mol °K from units of cal/mol °K.

Table II. Thermochemical Quantities

Molecule	$\Delta H°_{f,298}$[a]	$S°_{298}$[b]	$C°_{p,300}$[b]	$C°_{p,800}$[b]	$C°_{p,1000}$	$C°_{p,1500}$
1,5-Hexadiene[c]	20.2	89.4	28.8	57.9	64.4	74.3
Allyl	41.4 ± 1.1[d]	62.1[f]	14.6[f]	28.8[f]	31.9[f]	36.8[f]
	41.2[e]					

[a] kcal/mol. [b] Gibbs/mol. [c] S. W. Benson, "Thermochemical Kinetics," John Wiley & Sons, Inc., New York, N. Y., 1968; S. W. Benson, *et al.*, *Chem. Rev.*, in press. [d] Reference 3a [amended slightly by D. M. Golden and S. W. Benson, *Chem. Rev.*, **69**, 125 (1969); the original value was 40.6 kcal/mol]. [e] This work. We apologize for the fortuitous closeness of the two results. [f] H. E. O'Neal and S. W. Benson, *Int. J. Chem. Kin.*, **1**, 217 (1969); ref *c*.

The above value of $\Delta H°_{f,298}(A)$ yields $DH°_{298}$(allyl–H) = 88.4 kcal/mol, $DH°_{298}(n\text{-}C_3H_7\text{–H})$ = 98 kcal/mol;[2] thus ARE = 9.6 ~ 10 kcal/mol.

Controversy abounds as to the correct value of ARE. Values quoted range from 10 to 25 kcal/mol.[3] The correct value is important, not only for knowledge of the BDE's of allyl-weakened bonds, but the actual numerical value must surely be fundamental for any theory which attempts to quantitatively describe chemical bonding.

Free-radical heats of formation are generally measured by kinetic methods. The difference between the activation energy in forward and reverse directions for any reaction involving the radical of interest will yield its heat of formation if the heats of formation of all the other species involved are known.

In practice, it has rarely been possible to measure activation energies in both directions, so that certain assumptions are made about the activation energy in a given direction. In ref 3a and 3b, E_3 is measured and the assumption is made that the activation energy E_{-3} = 1 ± 1 kcal/mol when R is allyl and methylallyl

$$RH + I \underset{-3}{\overset{3}{\rightleftharpoons}} R + HI \qquad (3)$$

radical, respectively. In ref 3c–g, the measured rate constant is for homolytic bond scission and the assumption is that radical recombination, the back reaction, has zero activation energy.

Both assumptions seem to be reasonably well founded for simple alkyl radicals, but it has not been clear that they apply to allyl radicals. In addition, the bond

(1) Defining the bond dissociation energy (BDE) for any bond A–B

$$DH°_T(A\text{–}B) = \Delta H°_{f,T}(A) + \Delta H°_{f,T}(B) + \Delta H°_{f,T}(AB)$$

we may define the stabilization energy in the allyl radical, commonly called the allyl resonance energy (ARE), as

$$DH°_{298}(n\text{-}C_3H_7\text{–H}) - DH°_{298}(\text{allyl–H}) \equiv \text{ARE}$$

(2) J. A. Kerr, *Chem. Rev.*, **66**, 465 (1966).

(3) See, for example: (a) D. M. Golden, A. S. Rodgers, and S. W. Benson, *J. Am. Chem. Soc.*, **88**, 3196 (1966); (b) K. W. Egger, D. M. Golden, and S. W. Benson, *ibid.*, **86**, 5420 (1964); (c) R. J. Akers and J. J. Throssel, *Trans. Faraday Soc.*, **63**, 124 (1967) [the value of $\Delta H°_{f,298}$(allyl) quoted here is derived from an incorrect value of $\Delta H°_f$(1,5-hexadiene); see ref 3d or Table II for the correct value]; (d) J. B. Homer and F. P. Lossing, *Can. J. Chem.*, **44**, 2211 (1966); (e) W. von E. Doering and V. Toscano, unpublished work [quoted by W. von E. Doering, *et al.*, *Tetrahedron*, **23**, 3943 (1967)]; (f) C. Walling, "Free Radicals in Solution," John Wiley & Sons, Inc., New York, N. Y., 1950, p 50 [the value of ARE is referenced to $DH°(CH_3\text{–H})$ = 102 kcal/mol]; (g) J. A. Berson and E. J. Walsh, Jr., *J. Am. Chem. Soc.*, **90**, 4730 (1968).