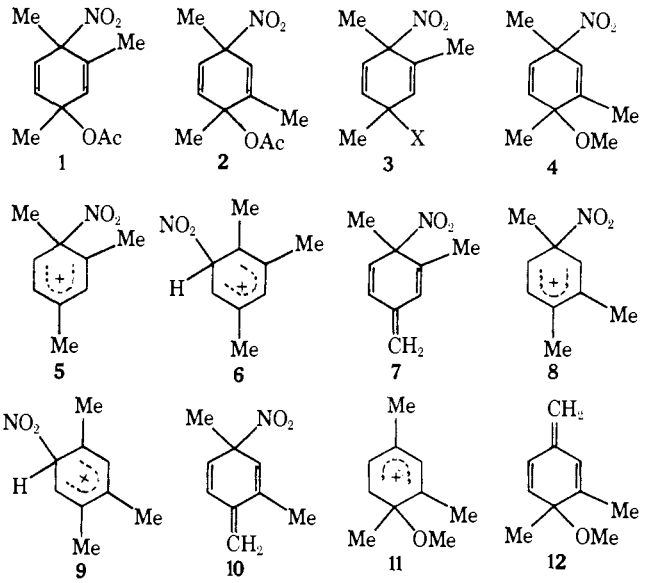

products. Methanol is a poorer leaving group than acetic acid and it is possible that in **4** the nitro group leaves to generate the more stable phenonium ion **11** in preference to the departure of the methoxy group which would give the less stable **8**. Deprotonation of **11** to **12**, followed by SN2′ substitution, would afford the observed product.

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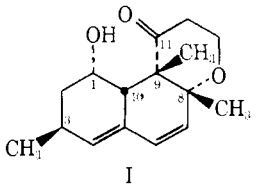
An Unusual Fungal Metabolite, LL-N313ζ

Sir:

In the course of screening for antifungal agents from soil microorganisms, we examined extracts from *Sporormia affinis* Sacc., Bomm and Rouss [Lederle culture N313], from which several novel polyketide derived compounds were obtained.[1] More recent fermentations of the fungus have provided yet another novel metabolite, LL-N313ζ, to which we assign the absolute stereostructure I. To our knowledge, this carbon



I

skeleton has not previously been observed in fungi.[2]

The *Sporormia* culture was fermented, harvested, and worked-up with carbon as previously described.[1] Chromatography of a concentrate over silica gel yielded, in addition to the other metabolites, the crystalline LL-N313ζ.

I is a crystalline material which melts at 172–173°, has $[\alpha]^{25}D$ −113° (MeOH) and the molecular formula $C_{16}H_{22}O_3$.[3] The ir spectrum of I shows hydroxyl absorption at 3440 cm^{-1} and a carbonyl band at 1680 cm^{-1}. The acetate of I, mp 114–115°, has acetyl carbonyl absorption at 1735 cm^{-1} with the ketonic band shifted to 1703 cm^{-1} indicating chelation of the hydroxyl and carbonyl groups in I. When the carbonyl of I is reduced using sodium borohydride, the resultant product, mp 130–131°, still has strong uv absorption at 239 nm (ε 20,000). Since I has a maximum at 242 nm (ε 19,900) clearly the carbonyl is not involved in the uv chromophore. This chromophore is destroyed by catalytic reduction with the uptake of either 1 or 2 mol of hydrogen; consequently, a conjugated diene is indicated.

The presence of the decalin system in I was demonstrated by heating the reduced product mp 130–131° mentioned above with 30% Pd/C to 280° in a nitrogen atmosphere to get 1,2,6-trimethylnaphthalene.

The pmr spectrum of I shows 3-proton singlets at δ 1.08 and 1.23 for tertiary methyls and a doublet at δ 1.02 for a secondary methyl group. An exchangeable signal at δ 1.96 indicates a hydroxyl proton. The remaining 12 protons of the molecule are labeled H_a through H_l according to decreasing chemical shift values. In the olefinic region H_a is a doublet at δ 6.20, H_b a singlet at δ 5.72, and H_c is another doublet at δ 5.47. H_a and H_c constitute a classic cis vinylic AB pair (J_{ac} = 9.5 Hz).

All the signals mentioned so far were readily discernible upon examination of the original spectrum. The lines of the remaining protons were revealed only upon extensive spin decoupling work. H_d, H_f, H_h, and H_j located at δ 4.10, 3.92, 2.86, and 2.11, respectively, form a closed system not coupled to the remainder of the molecule. The coupling constants for this system have the following values (Hz): J_{hj} = 14.6, J_{df} = 11.5, J_{jf} = 3.5, J_{jd} = 1.2, J_{hf} = 11.2, and J_{hd} = 9.0. Clearly these protons form an ABXY system with AB and XY being geminal pairs. The chemical shift values indicate one pair, H_d and H_f, to be on a carbon bearing an oxygen and the other pair, H_h and H_j, to be adjacent to a carbonyl group.

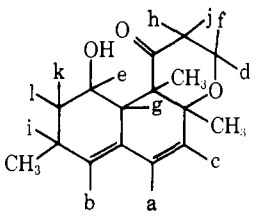
H_e is a broad signal at δ 3.95, hence on an oxygen-bearing carbon. In the spectrum of I, H_e is completely hidden by the H_d and H_f signals but in the spectrum of the acetate of I it is shifted to δ 5.03 and spin decoupling work on this derivative showed J_{eg} = 4.0 Hz. In the spectrum of I, H_g is a quartet at δ 3.42 with J_{gb} = 3.0 Hz. H_i gives a broad signal at δ 2.50 and is coupled to the secondary methyl group with J_{i,CH_3} = 7.0 Hz. The other coupling constants associated with H_i are J_{il} = 11.5 and J_{ik} = 5.0 Hz. H_k is located at δ 2.0 and with H_l which is mostly hidden under the tertiary methyl signal at δ 1.23 forms a geminal pair with J_{kl} = 13.0 Hz.

Using this information we were able to write the gross structure below. Confirmation of this structure was provided by the proton noise decoupled 22.6-MHz PFT-cmr spectrum of I taken in $CDCl_3$. Multiplicities were obtained by off-resonance decoupling studies

(1) The elaboration of three dihydroisocoumarins and three antifungal cyclopentenone antibiotics by the same culture has previously been reported: W. J. McGahren and L. A. Mitscher, *J. Org. Chem.*, 33, 1577 (1968); W. J. McGahren, J. H. van den Hende, and L. A. Mitscher, *J. Amer. Chem. Soc.*, 91, 157 (1969)

(2) W. B. Turner, "Fungal Metabolites," Academic Press, New York, N. Y., 1971.

(3) Satisfactory analyses (mass spectral and elemental) for I and its derivatives were obtained. Uv spectra were run in MeOH, ir spectra in KBr disks, and pmr spectra were taken at 100 MHz. A Bruker HFX 10 instrument was used for ^{13}C work by courtesy of Professor A. J. Bose, Stevens Institute of Technology, Hoboken, N. J. We thank our colleagues W. F. Fulmor and L. Brancone and staffs for spectral and analytical data, Miss P. Mullen formerly of Stamford Laboratories [now at Shulton, Clifton, N. J.) for the CD work and A. J. Shay and M. Dann and staffs for large scale fermentations and processing.



and chemical shifts were recorded relative to TMS. On the basis of chemical shift, multiplicity, and proximity to electronegative functionality, the assignments shown in Table I were made.[4-6]

Table I. ^{13}C Chemical Shifts of I in $CDCl_3$ Relative to TMS Together with Multiplicities and Assignments

δ_C	Multiplicity	Carbon no. assignment
195.9	s	11
135.5	d	4, 6, 7
132.1	d	
128.2	d	
130.0	s	5
78.7	s	8
66.8	d	1
60.0	t	13
57.0	s	9
41.7	d	10
39.3	t	12
38.6	t	2
25.3	d	3
21.1	q	methyls at 3, 8, and 9
20.7	q	
13.0	q	

The chirality of simple, optically active, transoid, heteroannular, conjugated dienes correlates well with the Cotton effects of their $\pi \rightarrow \pi^*$ transitions in the 230–280-nm region.[7]

The work of Beecham, *et al.*,[8,9] has shown that the presence of an allylic oxygen as in I dramatically affects the applicability of the basic diene helicity rule. According to Beecham, the "oxygen forming helix" or the C=CCO system determines the sign of the observed Cotton effect.[10] The CD curve of I is a double humped curve with $\Delta\epsilon_{242.5} = -21.9$ and $\Delta\epsilon_{212.5} = +21.9$; consequently, the stereochemistry at C8 is defined with the C=CCO system having left-handed helicity and the methyl group axial.

When I was subjected to the Horeau procedure,[11] the recovery of (−)-α-phenylbutyric acid (R-acid) determines the configuration at C1 to be S. In addition, since the hydroxyl and carbonyl groups are chelated and since protons H_e and H_g are cis to one another (J_{eg} = 4.0 Hz) the stereochemistry at C1 and C9 is defined so that the methyl group is axial and the pyranone ring is in the chair conformation.

In the pmr spectrum of I, taken in pyridine, the broad signal of H_i is located at δ 2.65 which is a deshielding shift of δ 0.15 from that observed in $CDCl_3$. This means that this proton is 1,3-diaxial relative to the hydroxyl group[12] and the secondary methyl is thus equatorial as expected. Hence the stereochemistry of I is completely defined.

The unusual constitution of I may be formally derived from a polyketide intermediate which consists of five acetate and two propionate units.[13]

The presence of a tertiary methyl group such as that at C9 is rare in polyketide derived mold metabolites. To our knowledge, the only example is portentol,[2] although it seems reasonable to suggest that diplodiatoxin[6] is another example.

(4) E. Wenkert and B. L. Buckwalter, *J. Amer. Chem. Soc.*, **94**, 4367 (1972).
(5) J. Polonsky, Z. Baskevitch, N. Cagnoli-Bellavita, P. Ceccherelli, B. L. Buckwalter, and E. Wenkert, *J. Amer. Chem. Soc.*, **94**, 4369 (1972).
(6) P. S. Steyn, P. L. Wessels, C. W. Holzapfel, D. J. J. Potgieter, and W. K. A. Louw, *Tetrahedron*, **28**, 4775 (1972).
(7) E. Charney, H. Ziffer, and U. Weiss, *Tetrahedron*, **21**, 3121 (1965).
(8) A. F. Beecham, *Tetrahedron*, **27**, 5207 (1971).
(9) A. F. Beecham, A. McL. Mathieson, S. R. Johns, J. A. Lamberton, A. A. Sioumis, T. J. Batterham, and I. G. Young, *Tetrahedron*, **27**, 3725 (1971).
(10) Dreiding models of I indicate that with the half-chair conformations of the decalin rings the diene system is virtually planar.
(11) A. Horeau and H. B. Kagan, *Tetrahedron*, **20**, 2431 (1964).
(12) P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, **90**, 5480 (1968).
(13) It is entirely possible that the C3 and C9 methyls are introduced from the one carbon pool. This would preserve the notion that no fungal product incorporates propionate within the chain as pointed out by Turner (see ref 2, p 363).

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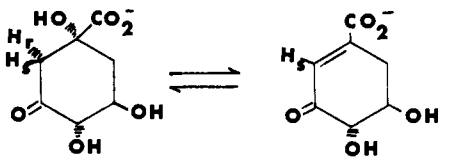
Mechanism of Dehydroquinase Catalyzed Dehydration. I. Formation of a Schiff Base Intermediate

Sir:

The conversion of dehydroquinic acid (**1**) to dehydroshikimic acid (**2**), is catalyzed by the enzyme dehydro-



1 **2**

lyase (5-dehydroquinate hydrolyase, E C 4.2.1.10). Dehydroquinase was first isolated and partially purified by Mitsuhashi and Davis,[1] and was subsequently used in the classic determination of the absolute stereochemical course of citric acid biosynthesis by Hanson and Rose.[2] These researchers also established that the elimination of water from **1** proceeds in a syn manner so that the prochiral *R* proton is eliminated under equilibration conditions in contrast to the anti elimination most frequently observed in other carbon–oxygen lyase systems.[3] Rose has suggested that these rarely observed enzymatic syn eliminations can be reasonably explained in terms of carbanion intermediates.[2,3] We present evidence here that this syn dehydration involves Schiff base formation between the enzyme and its substrate **1**.

Dehydroquinase was isolated from *E. coli* 83-2[4] and purified according to published procedures.[1] Succes-

(1) S. Mitsuhashi and B. D. Davis, *Biochim. Biophys. Acta*, **15**, 54 (1954).
(2) K. R. Hanson and I. A. Rose, *Proc. Nat. Acad. Sci. U. S.*, **50**, 981 (1963).
(3) I. A. Rose in "The Enzymes," Vol. II, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1970, p 309.
(4) An initial strain of these bacteria was kindly furnished by Professor B. D. Davis and P. C. Tai.