in the decomposition of a simple 1-pryazoline.[5c]

The results are in agreement with Fukui's earlier suggestions[20] and product studies with racemic **1**.[9] Regardless of the detailed nature of the involved intermediates and similar to the stereomutation of (−)-**2t**,[21] (+)-**1** must decompose via chiral intermediates (avoiding a planar 0,0 trimethylene);[3] attractive candidates[6] are 90,90 (face to face[6d,7,22] or pyramidal[6c,23]) biradicals.

We are presently investigating systems where, according to Fukui's suggestion,[20] single inversion should be expected and others where the cyclopropane work[2] suggests 0,0 trimethylene intermediates.

Acknowledgment. We are grateful to the Deutsche Forschungsgemeinschaft for the financial support of this work and the Fonds der Chemischen Industrie for a stipend for H.B. We also thank Professor J. A. Berson, Yale University, for stimulating discussions during the progress of this work. Helpful advice regarding the separation of enantiomers by Professor A. Mannschreck and Dr. M. Mintas, University Regensburg, is gratefully acknowledged.

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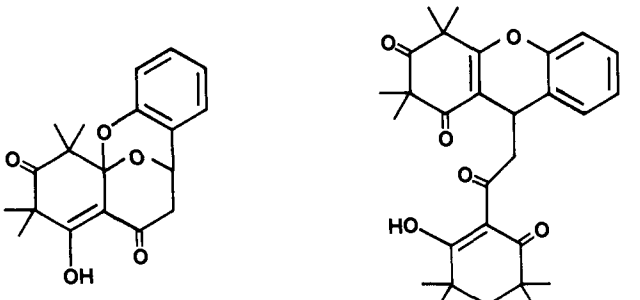
Vafzelin and Uvafzelin, Novel Constituents of *Uvaria Afzelii*

Sir:

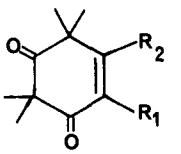
Higher plants of the genus *Uvaria* continue to be an interesting source of biologically active secondary metabolites.[1,2] During our recent studies, we noted that an ethanolic extract of the stems of *Uvaria afzelii* Scot Elliot (Annonaceae) showed significant antimicrobial activity.[3] Fractionation of the extract was guided by an antimicrobial assay and resulted in concentration of the activity in the ethyl acetate soluble fraction of an ethyl acetate–water partition. Chromatography of the active ethyl acetate fraction over silicic acid yielded a number of fractions, and we now wish to report the structures of two novel constituents. We have given these constituents the trivial names vafzelin (**1**) and uvafzelin (**2**).[4]

Vafzelin (**1**) was obtained as colorless prisms, mp 136–138 °C (*n*-hexane). High-resolution mass spectrometry and combustion analysis established the molecular formula of **1** as $C_{19}H_{20}O_5$. The UV spectrum showed λ_{max} (dioxane) to be 283 (ϵ 4.51 × 10^3), 250 sh (ϵ 2.62 × 10^3), and 218 nm (ϵ 3.93 × 10^3) while the IR showed bands at ν_{max} (KBr) 3415 (OH), 1725 (C=O), 1620 br (C=O), and 1595 cm^{-1} (C=C). The ^1H NMR ($CDCl_3$, 60 MHz) spectrum showed a four-proton multiplet (δ 7.27–6.53), an ABX pattern [δ 5.25 (dd, J = 1, 6 Hz), 3.20 (dd, J = 6, 18 Hz), 2.55 (dd, J = 1, 18 Hz)], and signals for four-methyl groups [δ 1.42 (6 H, s), 1.37 (3 H, s), and 1.17 (3 H, s)]. The ABX pattern and the multiplet in the aromatic region of the ^1H NMR spectrum are similar to those observed in the (*o*-hydroxylbenzyl)flavanones previously reported in *U. chamae*.[5] The ^{13}C NMR ($CDCl_3$, 15 MHz) spectrum also suggested an ortho-oxygenated alkyl substituted aromatic ring with signals at 150.2 s, 129.3 d, 125.5 d, 122.7 s, 121.4 d, and 117.1 d ppm similar to those previously reported.[6,7] Other signals in the ^{13}C NMR spectrum (211.4 s, 198.2 s, 183.7 s, 105.6 s, 53.0 s, 52.0 s, 26.1 q, 23.3 q, and 15.6 q ppm) were similar to those for syncarpic acid (**3**) and its *O*-methyl (**4**) and *C*-acetyl (**5**) derivatives.[8] The remaining three signals in the ^{13}C NMR spectrum appeared at 68.4 d (C-2), 39.2 t (C-3), and 98.7 s (C-8a) ppm. The assignment of this latter signal to a ketal carbon seemed reasonable from chemical shift theory and the fact that vafzelin (**1**) showed only three carbonyls (see **5**). The collective spectroscopic data suggested that vafzelin was composed of an *o*-hydroxycinnamoyl moiety and syncarpic acid (**3**). Since the structural evidence for **1** was largely presumptive and incomplete, a single-crystal X-ray diffraction experiment was performed.



1

2



3, R_1 = H; R_2 = OH
4, R_1 = H; R_2 = OCH_3
5, R_1 = $COCH_3$; R_2 = OH

Vafzelin crystallized in the monoclinic crystal system with a = 12.55 (2), b = 15.487 (2), c = 8.544 (1) Å, and β = 97.6 (1)°. Systematic extinctions and density considerations were uniquely accommodated by space group $P2_1/c$ with one molecule of $C_{19}H_{20}O_5$ forming the asymmetric unit. This choice, which was fully verified by subsequent refinement, requires that vafzelin be either achiral or a racemic mixture. Intensity data were collected on a fully automated four-circle diffractometer by using graphite monochromated Cu Kα (1.54178 Å) X-rays and a 1° ω scan. All 2512 unique diffraction maxima ($2\theta \leq 114°$) were collected and after correction for Lorentz, polarization, and background effects, 1631 (65%) were judged observed ($|F_o| \geq 3\sigma(F_o)$). The structure was solved uneventfully by using an automatic sign determining procedure.[9] Full-matrix least-squares refinements with anisotropic

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(2) Edwards, J. M.; Raffauf, R. F.; LeQuesne, P. W. *J. Nat. Prod.* **1979**, *42*, 85–91 and references therein.
(3) The extract showed activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium tuberculosis* when assayed as previously described: Hufford, C. D.; Funderburk, M. J.; Morgan, J. M.; Robertson, L. W. *J. Pharm. Sci.* **1975**, *64*, 789.
(4) A total of 150 mg of **1** and 600 mg of **2** were obtained from 43 g of the ethyl acetate fraction using benzene and 2% ether–benzene as eluant, respectively.
(5) Lasswell, W. L., Jr.; Hufford, C. D. *J. Org. Chem.* **1977**, *42*, 1295.
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(8) Syncarpic acid (**3**) was also identified as a constituent of the title plant. The *O*-methyl (**4**) and *C*-acetyl (**5**) derivatives were prepared as described by Hodgson et al. [Hodgson, D.; Ritchie, E.; Taylor, W. C.; *Aust. J. Chem.* **1960**, *13*, 385]. ^{13}C NMR **3** (pyridine-d_6 ppm) 214.6 s, 189.5 s, 101.3 d, 51.95 s, and 24.9 q; **4** ($CDCl_3$) 213.2 s, 199.1 s, 178.4 s, 99.2 d, 56.3 q, 55.3 s, 48.2 s, 25.0 q, and 24.3 q; **5** ($CDCl_3$) 209.9 s, 201.6 s, 199.3 s, 196.7 s, 109.6 s, 56.8 s, 52.2 s, 27.2 q, 24.4 q, 23.9 q.

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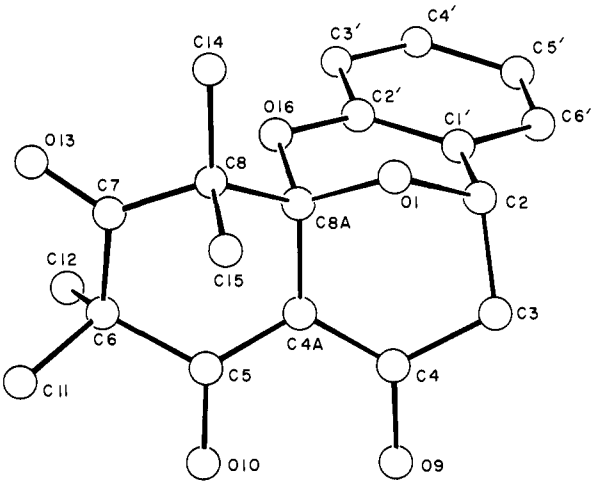

Figure 1. A computer-generated perspective drawing of vafzelin (**1**). Hydrogens are omitted for clarity and the material is a naturally occurring racemate.

heavy atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.086 for the observed data. Additional crystallographic details can be found in the supplementary material. A computer-generated perspective drawing of vafzelin (**1**) is given in Figure 1. The β-diketone system at C(4) and C(5) is in the enol form.

Uvafzelin (**2**) was obtained as colorless needles, mp 138–140 °C (MeOH). High-resolution mass spectrometry and combustion analysis established the molecular formula as $C_{29}H_{32}O_7$. The UV, IR, and ^{1}H NMR spectral data showed some similarities with those of **1**.[10] The significant differences were the presence of additional carbonyl signals in the IR spectrum and the presence of additional methyl signals and position of the ABX pattern in the ^{1}H NMR spectrum. The ^{13}C NMR ($CDCl_3$, 15 MHz) spectrum of **2** showed similarities with **1** and confirmed the presence of an ortho-oxygenated alkyl-substituted aromatic ring (150.6 s, 129.2 d, 128.1 d, 125.5 d, 124.6 s, and 116.2 d ppm), carbonyl groups (212.1 s, 209.8 s, 201.5 s, 198.8 s, 197.6 s, and 196.5 s ppm), methyl groups (25.0, 24.8, 24.5, 24.2, 24.0, and 23.8 ppm), aliphatic carbons (56.9 s, 55.4 s, 51.8 s, 47.5 s ppm), and olefinic carbons (110.5 s and 110.1 s ppm). The significant differences between the ^{13}C NMR spectra of **2** and **1** are apparent in the position of a doublet signal at 30.0 ppm (68.4 ppm in **1**) and a triplet signal at 48.4 ppm (39.2 ppm in **1**), the presence of a signal at 168.8 s ppm (C-5a), and the absence of a signal for a ketal carbon. The spectral data suggested that uvafzelin (**2**) contained an *o*-hydroxycinnamoyl moiety and two syncarpic acid moieties, and since the linkages between these fragments were not readily apparent, a single-crystal X-ray diffraction experiment was performed.

Uvafzelin (**2**) crystallized in the monoclinic crystal class with $a = 6.061$ (1), $b = 25.632$ (2), $c = 16.547$ (1) Å, and $\beta = 93.82$ (1)°. Systematic extinctions uniquely indicated space group $P2_1/c$ and density considerations suggested one molecule of $C_{29}H_{32}O_7$ per asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were recorded on a four-circle diffractometer using graphite monochromated Cu Kα (1.54178 Å) X-rays and a variable speed, 1° ω scan. Of the 3956 reflections surveyed, 2869 (73%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects. A phasing model was arrived at by a multisolution weighted sign-determining procedure.[9] Full-matrix least-squares refinement with anisotropic temperature factors for carbon and oxygen and assigned isotropic temperature factors for hydrogen have converged to a final unweighted crystallographic residual of 0.066 for the observed reflections. Additional details can be found in the supplementary material. Figure 2 is a computer-generated perspective drawing of the final X-ray model of uvafzelin (**2**). The β-diketone at C(2′) and C(8′) is in the enol form.

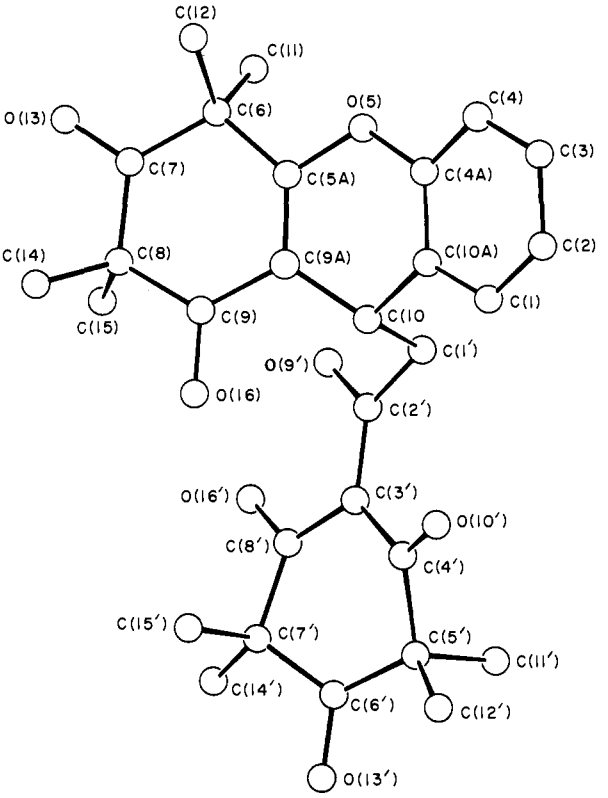

Figure 2. A computer-generated perspective drawing of uvafzelin (**2**). Hydrogens are omitted for clarity and the material is a naturally occurring racemate.

The structures of vafzelin (**1**) and uvafzelin (**2**) are unusual, and no similar metabolites are reported in the literature. Vafzelin (**1**) could be rationalized as a flavanone in which the A ring or the triacetate precursor of the A ring was extensively methylated. Flavanones with alkylated A rings are common constituents of *Uvaria* extracts. A putative afzelin precursor, syncarpic acid (**3**), is a constituent of *Uvaria afzelli* extracts.[8] Formally vafzelin (**1**) can be derived from the C-acylation of syncarpic acid (**3**) by an *o*-hydroxycinnamoyl moiety followed by conjugate additions. Uvafzelin (**2**) could be made by a similar process: the acylation of syncarpic acid (**3**) by an *o*-hydroxycinnamoyl moiety, the conjugate addition of syncarpic acid (**3**), and dehydration. It should be noted that vafzelin (**1**) and uvafzelin (**2**) are naturally occurring racemates and thus may be assembled without enzymic assistance. The plausibility of these ideas is currently being investigated.

Vafzelin (**1**) and uvafzelin (**2**) were both tested for antimicrobial activity but only uvafzelin was active.[11]

Acknowledgment. This investigation was supported in part by grants from the National Cancer Institute, CA15590 (C.D.H.)

(9) All crystallographic calculations were done on a Prime 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE, data reduction programs (M. E. Leonowicz, Cornell University, 1978), BLS, block-diagonal least-squares refinement (K. Hirotsu, Cornell University, 1978), ORFLS (modified), full-matrix least squares (W. R. Busing, K. O. Martin, and H. S. Levy, Oak Ridge, ORNL-TM-305), ORTEP, crystallographic illustration program (C. Johnson, Oak Ridge, ORNL-3794), MULTAN-76, direct methods and fast Fourier transform (G. Germain, P. Main, and M. Woolfson, University of York).

(10) UV λ_{max} (MeOH) 215 (1.56×10^4), 283 (1.93×10^4), and 305 sh nm (1.13×10^4); IR ν_{max} (KBr) 3400, 1722, 1710, 1670, 1640, and 1585 cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz) 7.67–7.07 (4 H, m), 4.67 (1 H, dd, $J = 5, 8$ Hz), 3.63 (1 H, dd, $J = 5, 13$ Hz), 2.93 (1 H, dd, $J = 8,13$ Hz), 1.67, 1.55, 1.47, 1.42, 1.38, and 1.37 (s, 24 H).

(11) Other active fractions are being investigated.

(12) Uvafzelin (**2**) had minimum inhibitory concentration (MIC) values of 6.3, 3.1, and 3.1 μg/mL against *S. aureus*, *B. subtilis*, and *M. smegmatis*, respectively. The MIC values were determined as reported previously.[3]

and CA24487 (J.C.), the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, and an NIH Training Grant to Cornell University (D.V.E.).

Supplementary Material Available: Fractional coordinates, thermal parameters, bond distances, bond angles, and observed and calculated structure factors for vafzelin (**1**) and uvafzelin (**2**) (32 pages). Ordering information is given on any current masthead page.

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Chiral Discrimination in the Quenching of an Enzyme-Bound Fluorescent NAD^+ Analogue by an Optically Active Quencher

Sir:

The first stage in protein–ligand interaction involves a bimolecular association process. The experimentally observed rate constants for this reaction are in some cases diffusion controlled, one example being the interaction between superoxide dismutase and the superoxide ion whose rate constant was found to be 2.4×10^9 M^{-1} s^{-1}.[1] However, in most cases the associations between proteins and ligands are considerably slower, and the observed "on" rate constants for small ligands are typically between 10^7 and 10^8 M^{-1} s^{-1}.[2] These experimental values deviate largely from the results of calculations based on the Smoluchowski equation,[3] and models that use modifications of this equation have been developed in which the existence of a restricted target area on the enzyme surface is assumed.[4-7] A capture window model which also takes into account the size of the entering ligand was used by Szabo to correlate k_{on} values for the interaction between a series of ligands and hemoglobin with the ligand sizes.[5] Obviously the capture window model, which assumes a circular opening on the surface of the enzyme and no specific interactions with the ligand, is a crude approximation. Indeed, the rate constant obtained by using this model for the interaction between hemoglobin and O_2 was about tenfold larger than the experimental one, proving that the fit between ligand and protein is crucial for the efficiency of the association reaction. Thus the stereochemistry of a ligand, in addition to its size, may play a major role in the interaction.

In the present communication, we report what we believe to be the first direct observation of stereoselectivity in the interaction between an enzyme and a chiral molecule which is not a substrate and whose site of interaction is removed from the active site. Nevertheless this interaction may serve as a model for the first step of association between enzyme and ligand. The system studied was that of liver alcohol dehydrogenase (LADH) interacting with the two enantiomers of methionine, and the rate of association was determined from the quenching of fluorescence of a coenzyme analogue bound to the enzyme.

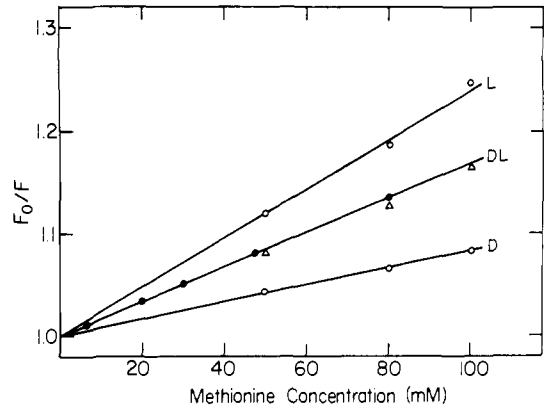

Figure 1. Stern–Volmer plots for quenching of fluorescence of the LADH–ϵNAD^+–pyrazole complex by L-, D-, and DL-methionine. The ternary complex was prepared in 0.05 M phosphate buffer (pH 7.3) and contained 1.2×10^{-5} M of both LADH and ϵNAD^+ and 1.7 mM pyrazole. (○) Quenching by L- or D-methionine. (●) Quenching by DL-methionine. (△) F_0/F values obtained by averaging the corresponding values of D- and L-methionine.

In a previous study we showed that the fluorescence of ϵNAD^+[8] bound to LADH is quenched both by iodide ions and by DL-methionine.[9] In the case of iodide the quenching was clearly dynamic, i.e., by collision between quencher and the fluorescent ethenoadenine ring, as evidenced by the excellent correspondence between the degree of quenching and the shortening of the fluorescence decay time.[9] Very similar behavior is observed when methionine is used as the quenching agent. The ratio between the fluorescence intensity of ϵNAD^+ bound to LADH in the absence of quencher and in the presence of 80 mM DL-methionine is 1.14. The fluorescence decay times of the coenzyme analogue in the same two samples were found to be 28.6 and 25.4 ns, respectively. Thus the shortening of the decay time (by a factor of 1.13) agrees well with the degree of fluorescence quenching, proving that the quenching by methionine is also solely dynamic. Since dynamic quenching occurs by direct contact between fluorophore and quencher, the latter must diffuse into the adenine binding site on LADH during the excited-state lifetime of the ϵNAD^+.

We found that while the rate constant for quenching of bound ϵNAD^+ by iodide was reduced only 1.6 times compared with that for the quenching of the free coenzyme analogue (i.e., from 3.9×10^9 to 2.5×10^9 M^{-1} s^{-1}), the ratio between the quenching rate constants of free and bound ϵNAD^+ by DL-methionine was above 12 (7.4×10^8 vs. 5.9×10^7 M^{-1} s^{-1}). Thus, while the "window" on the enzyme surface through which the ligands penetrate is large enough to allow fast diffusion of I^-, it considerably slows the diffusion of the bulky methionine. When the window and ligand are of comparable sizes, the rate of diffusion of the latter into the active site is determined to a large extent by thermal fluctuations in the protein which affect both the size and geometry of the window.[5] In such cases the rate of association between protein and ligand may depend not only on the size of the latter but also on its stereochemistry. Such chiral discrimination in the association is demonstrated in Figure 1 which presents Stern–Volmer plots for the quenching of the LADH–ϵNAD^+–pyrazole complex by D-, L-, and DL-methionine. The rate constants for fluorescence quenching of the ethenoadenine ring were calculated in each of the three cases by using the Stern–Volmer equation.[10] These constants were found to be $k_q^L = 8.4 \times 10^7$ M^{-1} s^{-1}; $k_q^D = 3.0 \times 10^7$ M^{-1} s^{-1}; and $k_q^{DL} = 5.9 \times 10^7$ M^{-1} s^{-1} (k_q^D, k_q^L, and k_q^{DL} being respectively the rate constants for quenching by D-, L-, and DL-methionine).

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(3) M. W. Smoluchowski, *Z. Phys. Chem.*, *92*, 124 (1917).
(4) (a) T. L. Hill, *Proc. Natl. Acad. Sci., U. S. A. 72*, 4918 (1975); (b) **73**, 679 (1976).
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(6) H. Nakatani and H. B. Dunford, *J. Phys. Chem.*, **83**, 2662 (1979).
(7) P. Richter and M. Eigen, *Biophys. Chem.*, **2**, 255 (1974).

(8) Abbreviations used: ϵNAD^+, nicotinamide $1,N^6$-ethenoadenine dinucleotide; LADH, horse liver alcohol dehydrogenase.
(9) A. Gafni, *Biochemistry* **18**, 1540 (1979).
(10) In the Stern–Volmer equation: $F_0/F = 1 + k_q\tau_a[Q]$, F_0, F are the fluorescence intensities in absence and presence of quencher respectively, k_q is the quenching rate constant, τ_a is the fluorescence decay time in the absence of quencher (28.6 ns, see ref 9), and [Q] is the concentration of quencher.

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