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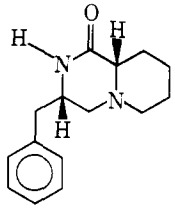
Structure and Synthesis of Verruculotoxin, a New Mycotoxin from *Penicillium verruculosum* Peyronel

Sir:

The fungus *Penicillium verruculosum* Peyronel, isolated from green peanuts, produces a toxic metabolite with an LD_{50} of 20 mg/kg (oral, 1-day-old cockerel).[1] Acute toxicity was characterized by ataxia, prostration, and complete lack of muscular coordination. In spite of these severe signs, the animals' eyes were open, and they appeared to be otherwise alert for the next hour. Isolation procedures, ir, NMR, MS, and uv data, for this metabolite have been reported in a preliminary paper, and the trivial name verruculotoxin was proposed.[1] We wish now to report the absolute stereostructure of verruculotoxin (**1**) from a combination of x-ray diffraction analysis and synthesis.



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Verruculotoxin (**1**) crystallizes from CH_2Cl_2–heptane as large needles in the common and unambiguously determined space group $P2_12_12_1$ with a = 14.340 (1), b = 12.211 (1), and c = 7.896 (1) Å, and one molecule of $C_{15}H_{20}N_2O$ per asymmetric unit. All unique data with $2\theta \leq 114°$ were collected on a fully automated four-circle diffractometer using Cu radiation (λ 1.5418 Å). A total of 1048 reflections were measured and after correction for Lorentz, background, extinction, and polarization effects a total of 925 (88%) were judged observed ($F_o^2 \geq 3\sigma(F_o^2)$). A multiple solution tangent formula approach[2] clearly showed 12 out of the 18 nonhydrogen atoms. A three-dimensional electron density synthesis revealed the remaining nonhydrogen atoms. Hydrogen atoms were located in a subsequent difference synthesis. Full-matrix least-squares refinements with anisotropic temperature factors for the nonhydrogen atoms and isotropic temperature factors for the hydrogen atoms lowered the conventional crystallographic discrepancy index to 0.036 for the observed reflections.[3] Figure 1 is a computer generated drawing of the final x-ray model.

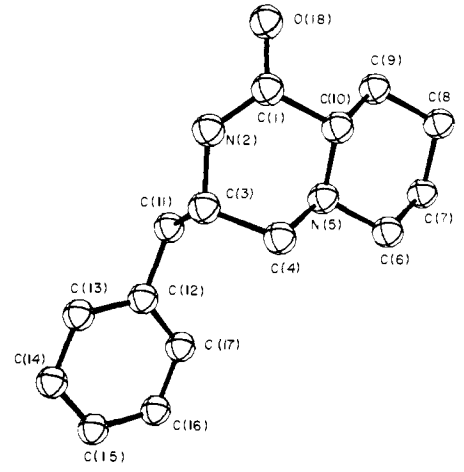
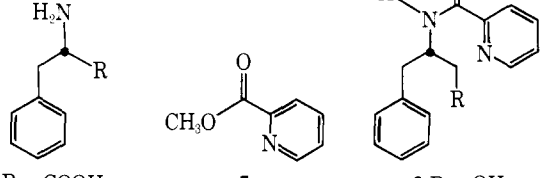


Figure 1. A computer generated perspective drawing of verruculotoxin **1**. The absolute configuration is as shown. Hydrogen atoms have been omitted for clarity.

Verruculotoxin (**1**) represents the first naturally occurring example of the octahydro-2*H*-pyrido[1,2-*a*]pyrazine system.[4] The piperazine ring of verruculotoxin (**1**) exists in a flattened chair conformation with the benzyl group in an axial position. Atoms C(10), C(1), O(18), N(2), and C(3) form a plane. The piperidine ring is also in a chair conformation and the bridgehead N(5) is puckered to give a trans ring junction. The phenyl group is planar, and no hydrogen bonds were detected in the crystal structure. Tables I–V which can be found in the supplementary material contain the fractional coordinates, important bond distances, important bond angles, temperature factors, and observed and calculated structure factors, respectively.

Verruculotoxin (**1**) can be viewed as a modified cyclic dipeptide of pipecolic acid and phenylalanine. A straightforward synthetic approach based on this dissection was undertaken. Phenylalinol (**4**)[5] was prepared from L-phenylal-

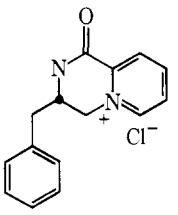


2, R = COOH
3, R = $COOCH_3$
4, R = CH_2OH

5

6, R = OH
7, R = Cl

anine (**2**) by quantitative esterification with thionyl chloride in MeOH according to the procedure of Brenner and Huber[6] and reduction of the resulting methyl ester hydrochloride (**3**) with $NaBH_4$ in 50% aqueous EtOH (68%). A neat solution of equimolar amounts of phenylalinol (**4**) and methyl picolinate (**5**) was heated at 150° for 1 hr under a nitrogen atmosphere. The resulting amide **6** crystallized in near quantitative yield when placed under a vacuum for 12 hr. Recrystallization from hexane–ethyl acetate yielded a sample whose NMR, ir, and high resolution mass spectra were consistent with structure **6**. Alcohol **6** was smoothly transformed into chloride **7** by treatment with thionyl chloride in methylene chloride (96%). The NMR of **7** was consistent with the assigned structure but no further characterization was attempted. Chloride salt **8** was produced by refluxing a DMF solution of **7** for 9 hr and then precipitating **8** with acetone (64%). Hydrogenation of **8** in methanol



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using PtO_2 and 40 psi of hydrogen yielded verruculotoxin (**1**) and its C(10) epimer in 98% yield. Thin layer chromatography (silica gel-PF_{254}, $CHCl_3$–acetone 1:1) and visualization with ninhydrin showed two light yellow spots of which the major (~80%, R_f = 0.64) was identical with natural verruculotoxin (**1**). Recrystallization of the crude reaction mixture from benzene gave pure **1** which possessed physical and biological properties identical with natural **1**. In contrast the C(10) epimer had no observable biological effect at 25 mg/kg (oral, 1-day-old cockerel) dose levels.

The absolute configuration was established by observing that both natural and synthetic verruculotoxin (**1**) had a positive Cotton effect for the 220-nm band (θ = +3300). Since L-phenylalanine was used as a starting material and since epimerization was considered unlikely in the synthesis, the absolute configuration of verruculotoxin is as shown in Figure 1. This is reasonable as verruculotoxin is most probably derived biogenetically from the two L-amino acids, phenylalanine and pipecolinic acid.

Supplementary Material Available: fractional coordinates (Table I), important bond distances (Table II), important bond angles (Table III), temperature factors (Table IV), observed and calculated structure factors (Table V) (9 pages). Ordering information is given on any current masthead page.

References and Notes

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(7) Camille and Henry Dreyfus Teacher-Scholar Grant Awardee, 1972–1977 and Fellow of the Alfred P. Sloan Foundation 1973–1975.

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Synergism of the Effect of Solvent and of General Base Catalysis in the Hydrolysis of a Schiff Base

Sir:

The kinetics and mechanism of the formation and hydrolysis of Schiff bases have been the subject of intensive study for quite some time.[1] A major impetus for these investigations has been the intermediacy of these compounds in the catalytic mechanism of several enzymes, including acetoacetate decarboxylase,[2] dehydroquinase,[3] 2-keto-3-deoxy-L-arabonate dehydratase,[4] δ-aminolevulinic acid dehydratase,[5] D-4-deoxy-5-oxoglucarate hydrolyase,[6] and various aldolases.[7-9] Although the rates of formation and hydrolysis of many Schiff bases are very rapid,[10-12] their interconversion with the corresponding aldehydes or ketones is often several orders of magnitude too slow to account for observed enzymatic rates.[13]

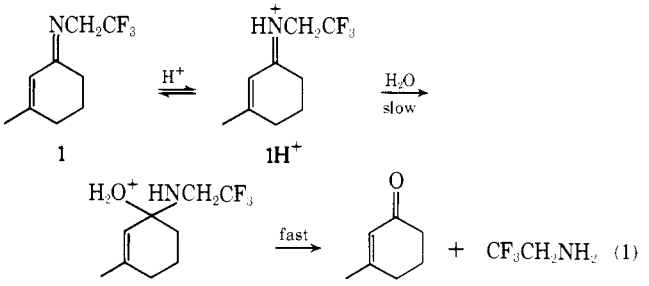
Table I. Solvent Effects on the Hydrolysis of 2,2,2-Trifluoro-*N*-(3-methyl-2-cyclohexenylidene)ethylamine in Dioxane–Water Solutions at 25.0 ± 0.1°[a]

	k^{obsd} (sec^{-1})	
% dioxane[b]	(0.1 *M* HCl)	(0.01 *M* HCl)
0	3.47×10^{-4}	3.61×10^{-4}
20	7.08×10^{-4}	
50	2.13×10^{-3}	2.27×10^{-3}
60	2.83×10^{-3}	
70	3.70×10^{-3}	4.05×10^{-3}
80	4.37×10^{-3}	
90	3.92×10^{-3}	6.43×10^{-3}[c]

[a] The reactions were monitored spectrally as previously described (ref 18). [b] Solutions are volume/volume. No other ions were added to the solutions to control the ionic strength. [c] 6.80×10^{-3} sec^{-1} at 0.001 *M* HCl.

Previous investigations have shown that these reactions are subject to general acid–base catalysis,[16,17] but it does not appear that general catalysis alone can account for this discrepancy. We now wish to report that lowering the solvent polarity also accelerates Schiff base hydrolysis. Furthermore, a combination of general base catalysis and a reduced solvent polarity is much more effective than would be predicted from the magnitude of these effects acting individually, i.e., a synergism exists between the two effects. Since both of these methods of facilitating Schiff base hydrolysis are potentially available to enzymes, our results may provide a basis for understanding the corresponding enzymatic reactions.

In the pH range 0–6, 2,2,2-trifluoro-*N*-(3-methyl-2-cyclohexenylidene)ethylamine (**1**) hydrolyzes by general base assisted rate-determining attack of water on the conjugate acid (**1H**$^+$) to produce a carbinolamine which decomposes to products (eq 1).[18] Addition of increasing amounts of dioxane to aqueous HCl solutions produces a marked increase in the rate of hydrolysis (Table I). For example, the rate constant for the hydrolysis of **1** in 90% dioxane (0.01 *N* HCl) is 18-fold larger than in pure water (0.01 *N* HCl) even though the concentration of water is ten times lower in 90% dioxane. Since the pK_a of **1H**$^+$ is 6.77[19] virtually all of **1** is present as **1H**$^+$ in this pH range so the observed rate constant refers to water attack on **1H**$^+$. Consequently, it appears that the actual rate constant for attack of water on the protonated Schiff base (**1H**$^+$) is 180-fold greater in 90% dioxane than in water.



(1)

The effect of changing the polarity of the solvent on the rate of hydrolysis is even more dramatic for the general base catalyzed reaction in chloroacetate buffers (Table