the ester products. Alcohols can be prepared with high values of ee by carrying hydrolyses to low conversions.

This enzyme-catalyzed reaction for preparation of chiral epoxy alcohols has advantages and disadvantages relative to transition-metal-catalyzed asymmetric epoxidation. Its major advantage is that it is experimentally the simpler procedure when applicable. A disadvantage of kinetic resolutions is that the theoretical *maximum* yield of chiral product is usually 50% based on racemic starting material (prochiral compounds such as **21** have a theoretical maximum yield of 100%).

Registry No. 1, 60456-24-8; (±)-**1**, 92418-55-8; **2**, 60456-25-9; (±)-**2**, 92418-56-9; **3**, 60456-26-0; (±)-**3**, 92418-57-0; **4**, 92418-73-0; (±)-**4**, 92315-14-5; **5**, 60456-27-1; (±)-**5**, 92418-58-1; **6**, 92418-60-5; (±)-**6**, 92315-15-6; **7**, 92315-26-9; (±)-**7**, 92419-24-4; **8**, 92418-61-6; (±)-**8**, 92315-16-7; **9**, 92418-62-7; (±)-**9**, 92315-17-8; **10**, 92418-63-8; (±)-**10**, 92315-18-9; **11**, 92418-64-9; (±)-**11**, 92315-19-0; **12**, 92418-65-0; (±)-**12**, 92345-46-5; **13**, 92418-66-1; (±)-**13**, 92315-20-3; **14**, 92418-67-2; (±)-**14**, 92315-21-4; **15**, 92418-68-3; (±)-**15**, 92315-22-5; **16**, 92418-69-4; (±)-**16**, 92345-47-6; **17**, 92418-70-7; (±)-**17**, 92315-23-6; (±)-**18**, 92345-48-7; (±)-**19**, 92315-24-7; (±)-**20**, 92418-59-2; (±)-**21**, 92315-25-8; E.C. 3.1.1.3, 9001-62-1; glycidol, 57044-25-4; 3-propylglycidol, 92418-71-8; 2-methylglycidol, 86884-89-1; (*R*)-3-methylglycidol, 58845-50-4; (*S*)-3-methylglycidol, 92418-72-9; 2,3-dimethylglycidol, 92315-27-0; oxiraneethanol, 76282-48-9; (*R*)-3-ethyloxiraneethanol, 91603-22-4; (*S*)-3-ethyloxiraneethanol, 91603-21-3.

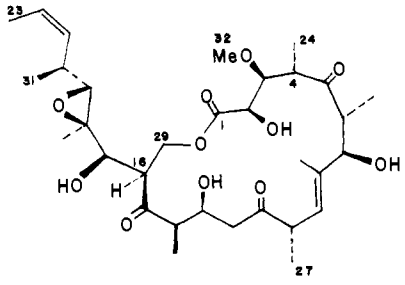
Tedanolide: A Potent Cytotoxic Macrolide from the Caribbean Sponge *Tedania ignis*

Francis J. Schmitz,* Sarath P. Gunasekera, Gopichand Yalamanchili, M. Bilayet Hossain, and Dick van der Helm*

Department of Chemistry, University of Oklahoma Norman, Oklahoma 73019

Received June 11, 1984

Tedania ignis is a common, widely distributed sponge in the Caribbean, colloquially known as the fire sponge[1] because contact with the skin is reported to cause a localized burning sensation and varying degrees of dermatitis for some individuals.[2] Our interest in *Tedania ignis* was stimulated by the fact that sponge extracts showed cytotoxicity and in vivo tumor inhibition. Earlier, we reported[3] the isolation of a marginally cytotoxic metabolite and several inactive compounds from this sponge. In this paper we report isolation of a potent cytotoxic macrolide designated tedanolide.



tedanolide

2*R*, 3*S*, 4*S*, 6*R*, 7*R*, 10*S*, 13*S*, 14*R*, 16*R*, 17*R*, 18*R*, 19*R*, 20*S*

Sponge specimens, collected at Summerland Key, FL, and frozen for shipment, were soaked successively in $CHCl_3$–MeOH (1:1) and $CHCl_3$, and the combined, concentrated extracts were partitioned between hexane and 10% aqueous methanol. The alcohol layer was diluted to 30% water and extracted with chloroform, and the chloroform solubles were chromatographed over Sephadex LH-20 [$CHCl_3$–MeOH (1:1)], monitored by bioassay (KB system).[4] Further purification involved (a) chromatography over deactivated silica gel[5] ($CHCl_3$ to 5% MeOH–$CHCl_3$), (b) HPLC (5-μm SiO_2, 4% MeOH/$CHCl_3$), and (c) HPLC [reverse phase C-18, H_2O–MeOH (35:65)]. Recrystallization of tedanolide from benzene–chloroform (9:1)[6] yielded white crystals (yield, ~1 × 10^{-4}% of dry weight), mp 193–194 °C dec, $[\alpha]$ +18.7° (*c* 0.08, $CHCl_3$). High-resolution FABMS, *m/e*, confirmed the formula $C_{32}H_{50}O_{11}$. The IR spectrum showed absorptions at 3600, 1750, and 1705 cm^{-1} compatible with hydroxyl, ester, and ketone groups. The ^{13}C NMR spectrum[7] confirmed the presence of three saturated ketone groups, one ester, and two double bonds. The ^1H NMR spectrum[8] revealed the presence of five secondary methyl groups, an oxygen-deshielded quaternary methyl group, one methoxyl group, and two vinyl methyl groups. Through decoupling and difference decoupling experiments, all the protons of tedanolide could be assigned to five partial structures, but these were separated by carbonyl groups or quaternary carbons, and no unequivocal structure could be deduced.

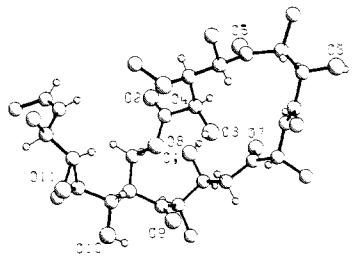


Figure 1. Perspective view of tedanolide. Protons on methyl groups have been left out for clarity.

The structure of tedanolide was determined by X-ray diffraction. Tedanolide crystallizes in the orthorhombic space group $P2_12_12_1$, with cell dimensions (138 K) a = 16.084 (7) Å, b = 29.850 (20) Å, and c = 6.671 (4) Å. All 3792 unique reflections with $2\theta < 150°$ were measured on an automatic diffractometer at 138 K using Cu Kα radiation [2996 reflections larger than $2\sigma(I)$] using methods described previously.[9] The structure was solved by direct methods[10] and Fourier syntheses. All the hydrogen

(1) See, for example: de Laubenfels, M. W. *Trans. Zool. Soc. London* **1950**, *No. 27*, 1.

(2) Yaffee, H. L. S. L.; Sturgardter, F. *Arch. Dermatol.* **1963**, *87*, 601.

(3) Schmitz, F. J.; Vanderah, D. J.; Hollenbeak, K. H.; Enwall, C. E. L.; Gopichand, Y.; SenGupta, P. K.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1983**, *48*, 3741.

(4) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3* (2), 1–103. Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations in μg/mL of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an $ED_{50} < 20$ μg/mL. KB refers to a cell culture of a human carcinoma of the nasopharynx. PS refers to in vitro lymphocytic leukemia.

(5) Slurried prior to use in CH_3OH–H_2O (95:5).

(6) Fortuitous crystallization during an NMR experiment in C_6D_6–$CDCl_3$ (9:1) yielded crystals suitable for X-ray analysis, ending nearly 2 years of recrystallization attempts which all yielded microcrystals.

(7) ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.2, 10.6, 11.4, 13.4, 14.3, 15.3, 16.6, 18.5 (all q), 44.8 (t, CH_2CO), 31.1, 45.5, 48.4, 49.6, 52.1, 53.3 (all d, CHCO), 60.4 (s, OCH_3), 62.9 (>C—O), 63.9 (CH_2O), 66.7, 68.3, 72.2, 77.0, 79.6, 83.0 (all d, CHO), 125.2, 129.2, 130.0 (all d, C=CH), 136.4 (s, =C), 171.4 (s, OC=O), 212.7, 214.2, 215.5 (s, C=O).

(8) ^1H NMR (300 MHz, $CDCl_3$) δ 1.10 (3 H, dd, J = 6.7, 1.7 Hz, H-31), 1.12 (6 H, d, J = 6.5 Hz, H-28 and -27), 1.24 (3 H, d, J = 7.2 Hz, H-24), 1.29 (3 H, d, J = 6.8 Hz, H-25), 1.39 (3 H, s, H-30), 1.56 (1 H, d, J = 2.5 Hz, 7-OH), 1.61 (3 H, dd, J = 7.4, 1.7 Hz, H-23), 1.63 (3 H, d, J = 1.4 Hz, H-26), 2.20 (1 H, d, J = 3.6 Hz, 17-OH), 2.45 (1 H, ddq, J = 10.8, 9.4, 6.9 Hz, H-20), 2.53 (1 H, dd, J 16.9, 3.8 Hz, H-12), 2.58 (1 H, dd, J = 16.9, 9.0 Hz, H-12), 2.65 (1 H, d, J = 9.4 Hz, H-19), 2.83 (1 H, d, J = 8.7 Hz, 2-OH), 3.03 (1 H, dq, J = 6.5, 6.6 Hz, H-14), 3.04 (1 H, dq, J = 9.9, 6.8 Hz, H-6), 3.24 (1 H, dd, J = 9.5, 3.9 Hz, H-17), 3.25 (1 H, dq, J = 8.6, 7.2 Hz, H-4), 3.30 (3 H, s, H-32), 3.37 (1 H, d, J = 3.2 Hz, 13-OH), 3.42 (1 H, dq, J 10.8, 6.6 Hz, H-10), 3.54 (1 H, ddd, J = 11.6, 9.5, 4.1 Hz, H-16), 3.68 (1 H, dd, J = 8.6, 1.7 Hz H-3), 3.87 (1 H, dd, J = 8.7, 1.7 Hz H-2), 4.11 (1 H, dd, J = 11.6, 11.6 Hz, H-29), 4.11 (1 H, dd, J = 9.9, 2.5 Hz H-7), 4.26 (1 H, dd, J = 11.6, 4.1 Hz H-29), 4.31 (1 H, dddd, J = 9.0, 6.5, 3.3, 3.2 Hz, H-13), 5.24 (1 H, ddq, J = 10.8, 10.8, 1.7 Hz, H-21), 5.46 (1 H, dq, J = 10.8, 7.4 Hz, H-22), 5.47 (1 H, dq, J = 10.8, 1.4 Hz, H-9).

(9) Eng-Wilmot, D. L.; Rahman, A.; Mendenhall, J. V.; Grayson, S. L.; van der Helm, D. *J. Am. Chem. Soc.* **1984**, *106*, 1285.

(10) Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. M., MULTAN 78, a system of computer programs for the automatic solution of crystal structures from X-ray diffraction data, University of York, York, England, 1978.

0002-7863/84/1506-7251$01.50/0

atoms were located from a difference Fourier map. The full-matrix least-squares[11] refinement using anisotropic temperature factors[12] (isotropic for H atoms) converged to an R of 0.045. The absolute configuration was determined by using the anomalous dispersion of oxygen[13] and measuring the 18 most sensitive Friedel pairs repeatedly. Differences for 15 pairs are in agreement with the enantiomer shown in Figure 1.[14] In the crystal structure tedanolide is shaped like an elongated disk (length 9.0 Å, width 5.5 Å) with the hydroxy groups at C-2 and -13 directed to the interior of the ring and the carbonyl oxygens at C-5, -11, and -15 oriented perpendicular to the plane of the ring. There is one probable intramolecular H bond [O(10)···O(9) 2.824 (4) Å] and two intermolecular H bonds [O(3)···O(7) and O(8)···O(4)].

Tedanolide is of mixed acetate–propionate biogenesis (acetate units at C-1,2 and -11,12). It differs from other macrolides in that the site of lactonization is not near the end of the carbon skeleton.[15] Tedanolide is highly cytotoxic, exhibiting an ED_{50} or 2.5×10^{-4} in KB and 1.6×10^{-5} in PS.[4] Cell-flow cytofluorometry[16] analysis revealed that tedanolide causes accumulation of cells in the S phase at concentrations as low as 0.01 μg/mL. In vivo tumor inhibition evaluation of tedanolide is in progress.

Only a few other macrolides have been isolated from marine organisms: the bryostatins,[17] aplysiatoxin,[18] debromoaplysiatoxin,[18] oscillatoxin A and brominated analogues,[19] and aplasmomycin.[20] The extremely low yield in which tedanolide is obtained indicates that it may be a metabolite of some microorganism as was found in the case of okadaic acid.[21] We are searching for such a source.

Acknowledgment. This investigation was supported by Grants CA-17256 and CA-17562 awarded by the National Cancer Institute, DHEW, and Grants NA83AA-D-00011 and NA80AA-D00084, Department of Commerce, NOAA, Office of Sea Grant. We thank Dr. Robert E. Schroeder for assistance in specimen collection, Dr. Paul Schmidt, Oklahoma Medical Research Foundation, Oklahoma City, OK, for use of a 300-MHz NMR spectrometer, Drs. K. L. Rinehart, Jr., and J. C. Cook, Jr., University of Illinois, Urbana, IL, for a high-resolution FAB mass spectrum, and Dr. C. Costello for low-resolution FAB and high-resolution EI mass spectra. We thank Dr. G. B. Boder, Lilly Research Labs, Indianapolis, IN, for providing the cell cytofluorometry data.

Registry No. Tedanolide, 92471-87-9.

Supplementary Material Available: Tables of atomic positional and thermal parameters, bond distances, bond angles, and selected torsion angles and a list of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

(11) All least-squares refinements and Fourier calculations were carried out by using program system SHELX 76. Sheldrick, G. M., SHELX 76, program for crystal structure determination, University Chemical Laboratory, Cambridge, England.
(12) The final atomic parameters (positional and thermal) are available as supplementary material.
(13) Bijvoet, J. M.; Peerdeman, A. F.; van Bounnel, A. J. *Nature (London)* **1951**, *168*, 271. Ealick, S. E.; van der Helm, D.; Weinheimer, A. J. *Acta Crystallogr., Sect. B* **1975**, *B31*, 1618.
(14) Table giving Bijvoet differences are available as supplementary material.
(15) Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 585.
(16) Cf.: Horan, P. K.; Wheeless, L. L., Jr. *Science (Washington, D. C.)* **1977**, *198*, 149.
(17) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846. Pettit, G. R.; Herald, C. L.; Kamano, Y.; Gust, D.; Aoyagi, R. *J. Nat. Prod.* **1983**, *46*, 528. Pettit, G. R.; Herald, C. L.; Kamano, Y. *J. Org. Chem.* **1983**, *48*, 5354.
(18) Kato, Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 2245.
(19) Mynderse, J. S.; Moore, R. E. *J. Org. Chem.* **1978**, *43*, 2301. Moore, R. E. In "Marine Natural Products: Chemical and Biological Perspectives"; Scheuer, P. J. Ed.; Academic Press: New York, 1981; Vol. IV, Chapter 1.
(20) Nakamura, H.; Iitaka, Y.; Kitahara, T.; Okazaki, T.; Okami, Y. *J. Antibiot.* **1977**, *30*, 714.
(21) See ref 16, in: Tachibana, K.; Scheuer, P. J.; Tsukitani, Y.; Kikuchi, H.; Van Engen, D.; Clardy, J.; Gopichand, Y.; Schmitz, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 2469.

An Organic Chemical Model of the Acyl-α-chymotrypsin Intermediate

Ishwar M. Mallick, Valerian T. D'Souza, Motowo Yamaguchi, James Lee, Phillip Chalabi, Robert C. Gadwood,* and Myron L. Bender*

Departments of Chemistry and Biochemistry Northwestern University, Evanston, Illinois 60201

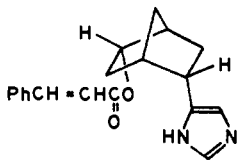
Received July 18, 1984

Chymotrypsin is a large (MW 24 800) and complicated (245 amino acids) enzyme. For many years we have been working on an organic model of it. This is feasible because the constituents of the active site are only three,[1] and their stereochemistry (distances and angles with respect to one another) is known through X-ray analysis.[2]

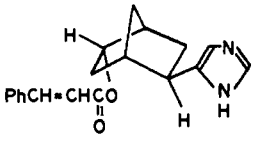
Several models of enzymes have already been synthesized, mainly based on cyclomayloses. These include models of chymotrypsin,[3,4,8] ribonuclease,[5] carbonic anhydrase,[6] and transaminase.[7] Previous models of chymotrypsin synthesized either by us[3,4] or by others[8] have suffered from the fact that they have used the binding ability of the cycloamyloses but have not incorporated all three known catalytic groups of chymotrypsin. Chymotrypsin is the archetype of about 20 serine proteases and is thus an important enzyme. All of the serine proteases proceed through an acyl-enzyme intermediate.

We started on models of the acyl-enzyme intermediate (summarized in Table I) since its reactions do not have to consider binding. We first attached two of the three known constituents of the active site to a completely rigid backbone whose stereochemistry could be regulated easily.

Thus we synthesized *exo*-imidazolyl-*endo*-hydroxyl- and *endo*-imidazolyl-*endo*-hydroxylnorbornane, which we converted to the corresponding cinnamates, *endo,endo*-(**1**) and *exo,endo*-5-[4(5)-imidazolyl]bicyclo[2.2.1]hept-2-yl *trans*-cinnamate (**2**).[9]



1



2

We found that the imidazolyl group in the endo,endo compound, **1**, but not in the exo,endo compound, **2**, participated in the hydrolysis of the ester linkage across the bicyclic ring system. This was apparent from the kinetics of hydrolysis, which showed dependence on the ionization of the imidazolyl group in the endo,endo compound but not in the exo,endo compound.[10]

We then found that the participation by the imidazolyl group in the endo–endo compound **1** was due to a base catalysis by the imidazolyl group since a k_{H_2O}/k_{D_2O} effect of 3.0 occurs.[10] This was mechanistically interesting since the deacylation of cinnamoyl–chymtrypsin proceeds by imidazole acting as a base with a k_{H_2O}/k_{D_2O} effect of 2.5[11] and since imidazole in all other

(1) Bender, M. L. "Mechanisms of Homogeneous Catalysis from Protons to Proteins"; Wiley-Interscience: New York, 1971; pp 505–514.
(2) Blow, D. M.; Birktoft, J. J.; Hartley, B. S. *Nature (London)* **1969**, *221*, 337–340.
(3) VanEtten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242–3253.
(4) Iwakura, Y.; Toda, I.; Onozuka, S.; Hattori, K.; Bender, M. *J. Am. Chem. Soc.* **1975**, *97*, 4432–4434.
(5) Breslow, R.; Doherty, J.; Guillot, G.; Lipsey, C. *J. Am. Chem. Soc.* **1978**, *100*, 3227–3228.
(6) Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152–1153.
(7) Breslow, R.; Czarnik, A. *J. Am. Chem. Soc.* **1983**, *105*, 1390–1391.
(8) Cramer, F.; Mackensen, G. *Angew. Chem.* **1966**, *78*, 641.
(9) Utaka, M.; Takeda, A.; Bender, M. L. *J. Org. Chem.* **1974**, *39*, 3772–3777.
(10) Komiyama, M.; Roesel, T. R.; Bender, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 23–25.

0002-7863/84/1506-7252$01.50/0