Lipase-Catalyzed Hydrolysis as a Route to Esters of Chiral Epoxy Alcohols[1]

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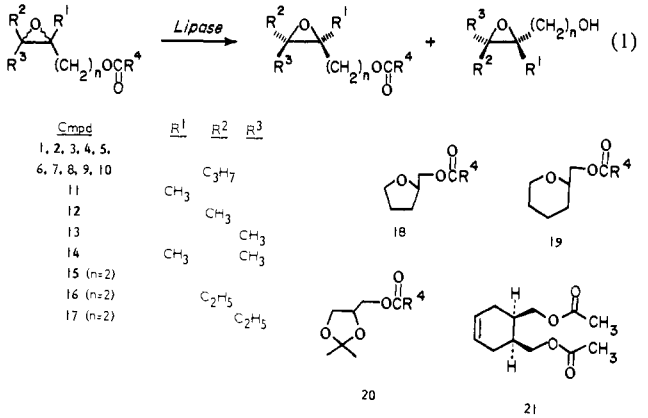
A current objective of organic chemistry is the development of methods for chiral synthesis.[3-6] This paper describes initial studies of an enantioselective, enzyme-catalyzed hydrolysis of esters of epoxy alcohols which provides an alternative to the Sharpless reaction as a route to these useful chiral synthons (eq 1; this



equation implies absolute stereochemistry only for **1**). The structure of R^4 is indicated for each compound in Figure 1. The substituents are hydrogens unless indicated otherwise. We are interested in this reaction partly because it offers a practical synthetic route to certain members of a useful class of chiral substances and partly because it provides an opportunity to compare the characteristics of enantioselective biological and abiological synthetic methods.

An initial survey of enzymes[7,8] that hydrolyze glycidol esters indicated that lipase (E.C. 3.1.1.3, Sigma Type II, from porcine pancreas) has the best combination of activity, selectivity, and cost.[9] Racemic esters of several epoxy alcohols and related substances were subjected to hydrolysis using this lipase.[10] To facilitate comparison, most reactions were carried to 60% completion (based on the quantity of acid released) under the same conditions (pH 7.8, T = 25 °C). Unhydrolyzed ester was recovered by extraction (recoveries ranged 60–90% of the amount estimated to be present based on acid released). Enantiomeric excesses of recovered ester were measured using Eu(hfc)[11] (Figure 1).

These data provide the basis for several observations bearing on the utility of this method. Lipase is not deactivated by reaction

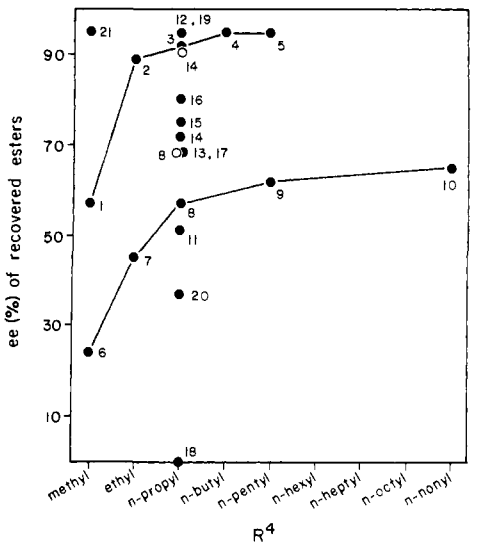


Figure 1. Observed enantiomeric excesses of esters isolated following partial hydrolysis of racemic esters with lipase. Filled points correspond to 60% hydrolysis of the total ester originally present (100% conversion would correspond to complete hydrolysis of *both* enantiomers of the racemic mixture); open points correspond to 80% hydrolysis.

with the epoxide moiety, and it hydrolyzes a wide variety of structures with useful enantioselectivity.[12] The enzyme is active at water–organic interfaces, and solubility of the organic substrate in water is not necessary. The enantioselectivity depends on the structure of the acid components of the ester (R^4, eq 1), with better results obtained with longer *n*-alkyl groups (although foaming and emulsification becomes an experimental problem for R^4 larger than C_5H_9). Since the values of enantiomeric excess increase with extent of conversion in a fashion that is, in principle, well understood,[13] simply extending reaction times and extent of conversion can be made to yield highly enantiomerically enriched product, albeit at the expense of decreased yield.

A representative experimental procedure for an ester that hydrolyzes with good enantioselectivity is that for glycidyl butyrate (**3**). A mixture of 300 g of **3** (2.08 mol) and 300 mL of water was placed in a 1-L three-necked flask equipped with a pH electrode, and the two-phase mixture was stirred vigorously with a magnetic stirring bar. Addition of 7.5 g of crude lipase initiated the hydrolysis. The pH was kept at 7.8 by addition of 7 M NaOH using a pH controller. When 60% of the theoretical amount of base required for complete hydrolysis of **3** had been added (178 mL, 1.25 mol, 6-h reaction time) the reaction was poured into 1 L of dichloromethane. The phases were separated and the aqueous phase reextracted with two 200-mL portions of methylene chloride. The combined organic extracts were washed once with 300 mL of 10% $NaHCO_3$ and twice with 200-mL portions of water, dried ($MgSO_4$ containing a small amount of Na_2CO_3), and concentrated on a rotary evaporator. Distillation yielded 107 g of (*R*)-**3** (0.74 mol, 89% based on the theoretical yield of one enantiomer), bp 81–82 °C (12 torr), with ee = ≥92%.[11,14]

A similar procedure for **16** started with 32.5 g (0.174 mol) of **16** and carried the hydrolysis to 75% conversion. We obtained 7.1 g (38 mmol, 88% based on the theoretical yield of one enantiomer) of material with ee = ≥95%.[11]

Alcohols were recovered from the reactions summarized in Figure 1 in 30–80% yield by extraction; values of ee were low (30–65%) as expected for resolutions designed to give high ee for

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(10) Epoxy alcohols were prepared by use of the procedure of Sharpless (Sharpless, K. B.; Verhoefen, T. R. *Aldrichimica Acta* **1979**, *12*, 63–74) and esters by use of reaction with acid chloride in pyridine/Et_2O (Sonntag, N. O. V. *Chem. Rev.* **1953**, *52*, 237–416).
(11) The accuracy of the reported values of ee is ±5%, except for values >90%; for certain substances in this range, analysis indicated only a value between 90% and 100%.
(12) Lipases accept a wide range of substrates. Desnuelle, P. In "The Enzymes", 3rd ed.; Boyer, P. O., Ed.; Academic Press: New York, 1972; Vol VII, Chapter 19. Brockerhoff, H. "Lipolytic Enzymes"; Academic Press: New York, 1974.
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(14) The observed optical rotation for resolved **3** was $[\alpha]_D$ −26.96° (*c* 20.465, $CHCl_3$); lit. $[\alpha]_D$ −28.4° for (*R*)-**3**: Lok, C. M.; Ward, J. P.; van Dorp, D. A. *Chem. Phys. Lipids* **1976**, *16*, 115–122.

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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*

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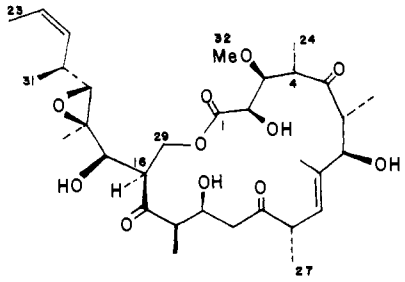
Tedania ignis is a common, widely distributed sponge in the Caribbean, colloquially known as the fire sponge[1] because contact with the skin is reported to cause a localized burning sensation and varying degrees of dermatitis for some individuals.[2] Our interest in *Tedania ignis* was stimulated by 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.

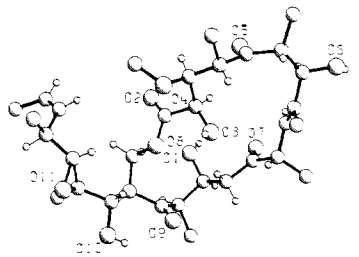


Figure 1. Perspective view of tedanolide. Protons on methyl groups have been left out for clarity.

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

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(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).

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