1300, 1270, 1180, 1150, 1120, 1040, 950, 900, 850, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.62 (s, 2 H), 6.29 (s, 2 H), 4.53 (d, 2 H, J = 16.6 Hz), 4.29 (s, 2 H), 3.89 (d, 2 H, J = 16.6 Hz), 3.69 (s, 6 H), 2.38 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 155.8, 139.3, 134.2, 128.9, 115.3, 108.5, 68.0, 55.5, 55.2, 17.1; MS calcd for $C_{19}H_{22}N_2O_2$ m/e 310.16812, measured 310.16868.

Anal. Calcd for $C_{19}H_{22}N_2O_2$: C, 73.54; H, 7.09; N, 9.03. Found: C, 73.45; H, 7.12; N, 9.01.

2,8-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine-3,9-dimethanol (6h). To 491 mg (3.6 mmol) of 5-amino-2-methylbenzyl alcohol (**5h**) in 4 mL of 95% ethanol was added 1.8 mL (22.2 mmol) of 37% formalin solution. The mixture was cooled to 0 °C, and 15 mL of concentrated HCl (18.4 mmol) was added. The mixture was stirred at room temperature under nitrogen for 24 h. After 24 h, the reaction mixture was added whole to a 500-mL separatory funnel and worked up with the same quantities of base, CH_2Cl_2, and washes as for the general Tröger's procedure. The vacuum-dried brown solid/foam crude product mixture was combined with a small quantity of CH_2Cl_2 and left for 24 h. At the end of this time a white crystalline solid was deposited, and this was removed by filtration to yield 0.14 g of **6h**, a white solid (25%): mp 230–235 °C dec; IR (Nujol) 3350, 2750, 1320, 1300, 1210, 1170, 920, 720 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.02 (s, 2 H), 6.64 (s, 2 H), 4.95 (t, 2 H, J = 5.0 Hz), 4.51 (d, 2 H, J = 16.2 Hz), 4.35 (s, 4 H), 4.16 (s, 2 H), 3.98 (d, 2 H, J = 16.2 Hz), 2.06 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 145.4, 139.0, 129.8, 127.5, 125.7, 122.7, 66.6, 60.8, 57.9, 17.4; MS calcd for $C_{19}H_{22}N_2O_2$ m/e 310.16812, measured 310.16746.

Anal. Calcd for $C_{19}H_{22}N_2O_2$: C, 73.54; H, 7.09; N, 9.03. Found: C, 73.36; H, 7.15; N, 8.99.

1,2,4,7,8,10-Hexamethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine (6i). After the workup, the crude product (prepared by the general procedure) was purified by flash chromatography (4 × 12 cm column, SiO_2, 5% diethyl ether–CH_2Cl_2) to give 1.11 g (78%) of Tröger's base **6i**: R_f 0.43 (SiO_2, 5% diethyl ether–CH_2Cl_2); mp 187–190 °C; IR (Nujol) 3150, 2750, 1225, 1210, 975, 945, 870, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (s, 2 H), 4.42 (d, 2 H, J = 16.6 Hz), 4.23 (s, 2 H), 3.95 (d, 2 H, J = 16.9 Hz), 2.38 (s, 6 H), 2.14 (s, 6 H), 1.94 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 144.5, 131.4, 130.8, 130.4, 129.7, 126.3, 66.2, 54.4, 19.5, 16.8, 13.4; MS calcd for $C_{21}H_{26}N_2$ m/e 306.20959, measured 306.21018.

Anal. Calcd for $C_{21}H_{26}N_2$: C, 82.35; H, 8.49; N, 9.15. Found: C, 82.29; H, 8.56; N, 9.12.

2,3,8,9-Tetramethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine (6j) and 1,2,8,9-Tetramethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine (7j). The crude product was prepared by the general procedure and purified by flash chromatography (4 × 12 cm column, SiO_2, 10% diethyl ether–CH_2Cl_2) to give 1.051 g (80%) of a mixture of two structural isomers **6j** and **7j** with a ratio of 72:28, both having R_f 0.18 (SiO_2, 10% diethyl ether–CH_2Cl_2); mp 197–202 °C; IR (Nujol) 2750, 1610, 1550, 1210, 1170, 1100, 1075, 950, 915, 885 cm^{-1}; ^1H NMR ($CDCl_3$) **6j** (major component) δ 6.89 (s, 2 H), 6.64 (s, 2 H), 4.60 (d, 2 H, J = 16.2 Hz), 4.27 (s, 2 H), 4.08 (d, 2 H, J = 16.6 Hz), 2.16 (s, 6 H), 2.11 (s, 6 H); **7j** (minor component) δ 6.96 (d, 1 H, J = 7.9 Hz), 6.91 (d, 1 H, J = 3.9 Hz), 6.89 (s, 1 H, 6.65 (s, 1 H), 4.64–4.49 (m, 2 H), 4.26–4.15 (m, 4 H), 2.18 (s, 3 H), 2.15 (s, 3 H), 2.11 (s, 3 H), 1.97 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 145.8, 135.5, 132.2, 132.1, 128.6, 127.7, 126.0, 125.9, 125.8, 125.0, 122.3, 67.3, 66.8, 58.4, 58.0, 19.4, 19.0.

Anal. Calcd for $C_{19}H_{22}N_2$: C, 82.01; H, 7.91; N, 10.07. Found: C, 81.95; H, 8.01; N, 10.04.

Acknowledgment. Support for this work was provided by a grant from the National Institutes of Health.

Registry No. **2d**, 62133-07-7; **2e**, 555-21-5; **3d**, 7409-30-5; **3e**, 24954-67-4; **3h**, 1975-52-6; **4c**, 5339-26-4; **4d**, 111437-15-1; **4e**, 111437-16-2; **4h**, 2840-04-2; **5a**, 106-49-0; **5b**, 104-10-9; **5c**, 39232-03-6; **5d**, 111437-06-0; **5e**, 111437-08-2; **5f**, 95-68-1; **5g**, 102-50-1; **5h**, 111437-10-6; **5i**, 137-17-7; **5j**, 95-64-7; **6a**, 529-81-7; **6b**, 111437-05-9; **6c**, 101193-83-3; **6d**, 111437-07-1; **6e**, 111554-33-7; **6f**, 98883-82-0; **6g**, 111437-09-3; **6h**, 111437-11-7; **6i**, 111437-12-8; **6j**, 111437-13-9; **7j**, 111437-14-0; 4-$NO_2C_6H_4CH_2Cl$, 100-14-1; 4-$NO_2C_6H_4(CH_2)_2OH$, 100-27-6; formalin, 50-00-0.

Supplementary Material Available: General experimental methods, procedures, and data for the preparation of **2d**, **3d**, **4d**, **4e**, **4h**, **5b–e**, and **5h**, figures showing ORTEP plots and crystal packing diagrams for **6f**, **6h**, and **6j**, and tables of fractional coordinates, isotropic and anisotropic thermal parameters, bond lengths, bond angles, torsion angles, and dihedral angles from X-ray crystallographic analyses (36 pages). Ordering information is given on any current masthead page. Tables of observed and calculated structure factor amplitudes may be obtained from V. Lynch, Department of Chemistry, University of Texas at Austin, Austin, Texas 78712.

Enzymatic Hydrolysis of Alkyl 3,4-Epoxybutyrates. A New Route to (*R*)-(-)-Carnitine Chloride

Daniele Bianchi, Walter Cabri, Pietro Cesti, Franco Francalanci,* and Marco Ricci

Istituto G. Donegani S.p.A., 28100 Novara, Italy

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The enzyme-catalyzed hydrolysis of alkyl 3,4-epoxybutyrates to the corresponding epoxy acids is reported. By using esterases the reaction occurred with good stereoselectivity leading to optically active unreacted esters of *R* configuration. With proteases the stereoselectivity was reversed, and the *S* enantiomer of the unreacted ester was recovered, albeit in lower enantiomeric excess. Finally, upon preliminary optimization of the reaction conditions, a new synthesis of (*R*)-(-)-carnitine chloride by the successive use of a stereoselective and of a nonstereoselective enzymatic hydrolysis is shown.

The potential of enzymes as catalysts in synthetic organic chemistry has received much attention in recent years.[1] Because enzymes can simultaneously display high chemical, regiochemical, and stereochemical selectivity, their use can be suitable when two or more reactive groups are present in the same substrate as in the case of the enantioselective hydrolysis of epoxy esters. This reaction was reported by Whitesides for the resolution of 2,3-epoxy alcohol carboxylic esters[2] which represents an alternative

(1) (a) Whitesides, G. M.; Wong, C. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (b) Chibata, I.; T.; Tosa, T.; Sato, T. *J. Mol. Catal.* **1986**, *37*, 1. (c) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (d) Klibanov, A. M. *Science (Washington, D.C.)* **1983**, *219*, 722.

(2) Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250.

0022-3263/88/1953-0104$01.50/0

Table I. Enzymatic Hydrolysis of (*R*,*S*)-Alkyl 3,4-Epoxybutyrates 1a–e[a]

entry	alkyl	enzyme (mg)	time, h	conv,[b] %	unreacted ester ee, %[c]	unreacted ester config
1	*i*-C_4H_9	steapsin (700)	17	61	75	*R*
2		steapsin (700)	21	65	84	*R*
3		steapsin (700)	27	70	>95	*R*
4		pancreatin (450)	9	54	59	*R*
5		pancreatin (450)	15	67	82	*R*
6		pancreatin (450)	17	70	86	*R*
7		*C. cylindracea* (450)	15	57	74	*R*
8		pig liver esterase (5)	8	52	62	*R*
9		novo SP 225 (1000)	22	70	4	*R*
10		LPL amano (50)	3	100		
11		*Chromobacterium* (50)	5	100		
12		*Strept. griseus* (450)	150	54	50	*S*
13		subtilisin BPN′ (150)	8	66	6	*S*
14		alcalase 2.0 T (700)	25	51	3	*S*
15		alcalase 2.0 T (1000)[d]	15	100		
16		subtilisin Carlsberg (300)[e]	3	60	2	*S*
17		papain (3500)	24	45	17	*S*
18		*Aspergillus sojae* (3500)	9	49	26	*S*
19	*n*-C_4H_9	steapsin (700)	26	60	92	*R*
20	*t*-C_4H_9	steapsin (700)	3	0	0	
21	*n*-C_8H_{17}	steapsin (700)	24	60	>95	*R*
22	$CH_2C_6H_5$	steapsin (700)	7	62	58	*R*

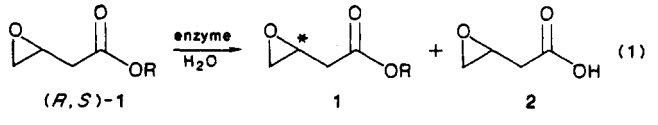
[a] Unless otherwise stated, all reactions were carried out at 20 °C in phosphate buffer (0.05 N; 100 mL) at pH 7.0 on 70 mmol of substrate. [b] Based on NaOH consumed. [c] Determined by ^1H NMR in the presence of Eu(hfc)$_3$. [d] Temperature 35 °C, pH 8.0. [e] pH 7.5.

to the asymmetric Sharpless epoxidation of allylic alcohols.[3] In our attempts to find a convenient synthesis of (*R*)-(−)-carnitine chloride **3**, a biological molecule of current interest as a therapeutic agent in the treatment of myocardial ischaemia,[4] we were interested in preparing (*R*)-3,4-epoxybutyrates, already used in racemic form as starting material for the synthesis of (*R*,*S*)-carnitine.[5] No simple chemical method is however available for the synthesis of esters of optically active epoxy acids.[6]

We found that several hydrolytic enzymes catalyze the stereoselective hydrolysis of the ester moiety of alkyl 3,4-epoxybutyrates **1a–e** leaving the oxirane ring virtually unaffected and giving epoxy esters with good enantiomeric excesses (ee's). These compounds were used as chiral building blocks for the synthesis of (*R*)-(−)-carnitine chloride.

Results and Discussion

Several commercially available enzyme preparations were tested in the hydrolysis of alkyl 3,4-epoxybutyrates (eq 1).



a: R = *i*-C_4H_9
b: R = *n*-C_4H_9
c: R = *t*-C_4H_9
d: R = *n*-C_8H_{17}
e: R = $CH_2C_6H_5$

Esters of primary aliphatic alcohols were good substrates for the enzymatic resolution. The stereoselectivity was low with the benzyl ester **1e**, while no reaction was observed with the *tert*-butyl ester **1c** (Table I). Choosing (*R*,*S*)-**1a** as a model compound, a survey of reactivity of enzymes showed that most esterases preferentially hydrolyzed the *S* enantiomer with moderate to good selectivity. On the contrary, proteases, when reactive,[7] preferentially hydrolyzed the *R* enantiomer, but the stereoselectivity was much lower. According to the theory of kinetic resolution,[8] the stereoselectivity depends on the extent of conversion; therefore, to get satisfactory enantiomeric excesses it was necessary to increase the degree of conversion at the expense of decreased yield. We investigated in some detail the influence of pH and temperature on the hydrolysis rate of (*R*,*S*)-**1a** catalyzed by steapsin. As shown in Figure 1, best conditions were 20 °C and pH 7.0. It is worth noting that, at pH 7.0, both an increase and a decrease of temperature resulted in a lower reaction rate, suggesting that

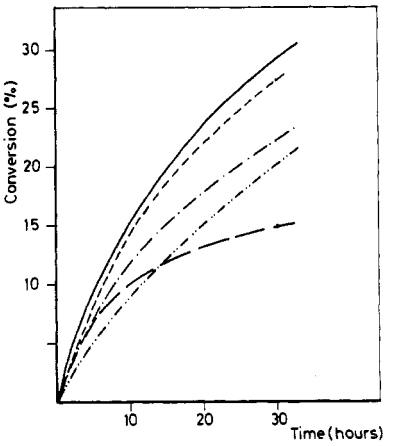
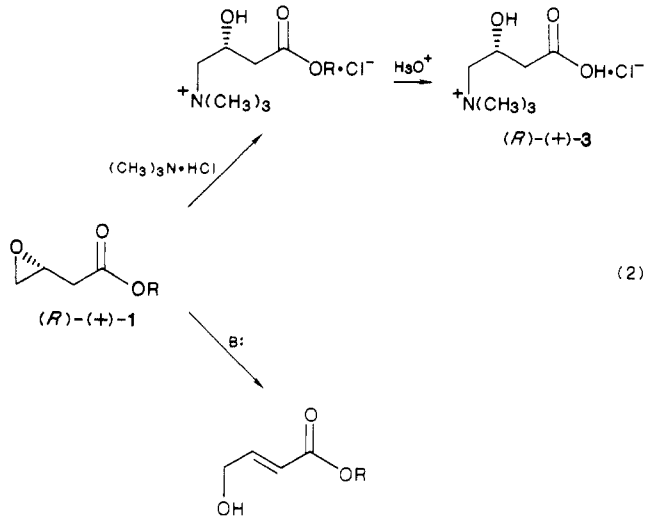


Figure 1. Influence of temperature and pH on the hydrolysis rate of (*R*,*S*)-**1a** in the presence of steapsin (**1a**, 30 mmol; steapsin, 100 mg; 0.05 N phosphate buffer, 40 mL): (—) 20 °C, pH 7.0; (- - -) 20 °C, pH 6.5; (-·-) 20 °C, pH 7.5; (-···-) 10 °C, pH 7.0; (– –) 30 °C, pH 7.0.

(3) (a) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974. (b) Pfenninger, A. *Synthesis* **1986**, 89.
(4) Woster, P. M.; Murray, W. J. *J. Med. Chem.* **1986**, *29*, 865.
(5) Boots, S. G.; Boots, M. R. *J. Pharm. Sci.* **1975**, *64*, 1262.
(6) (a) Rossiter, B. E.; Sharpless, K. B. *J. Org. Chem.* **1984**, *49*, 3707. (b) Hanessian, S.; Bedeschi, A.; Battistini, C.; Mongelli, N. *J. Am. Chem. Soc.* **1985**, *107*, 1438.
(7) No reaction was observed in the presence of α-chymotrypsin, thermolysin, and protease from *Streptomyces caespitosus*.
(8) (a) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. (b) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237.

at temperatures higher than 20 °C, some deactivation of the enzyme occurred.

The (*R*)-(+)-alkyl 3,4-epoxybutyrates thus obtained were used as chiral synthons for the synthesis of (*R*)-(−)-carnitine chloride.[9] The most straightforward synthesis of (*R*)-(−)-carnitine chloride from (*R*)-(+)-alkyl 3,4-epoxybutyrates seemed to be the opening of the oxirane ring by methanolic trimethylamine hydrochloride followed by acid hydrolysis of the ester mojety[5] (eq 2).



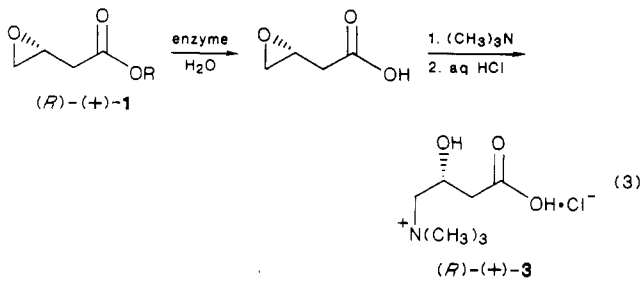
(2)

Unfortunately, the above reaction was accompanied by an extensive (ca. 50%) isomerization of the epoxide to the corresponding allyl alcohol. An observed increase of the pH from 6 up to 10 during the first minutes of the reaction suggested that some hydrochloric acid, in equilibrium with triethylamine hydrochloride, reacted with the epoxide ring to give a 3-hydroxy-4-chlorobutyrate.[10] The liberated base was then able to catalyze the above rearrangement.[11] This side reaction could, however, be suppressed by using the carboxylic acid as substrate. The conversion of an epoxy ester into the corresponding carboxylic acid is difficult to do by conventional chemical methods, but in this case it could be easily performed by a second enzymatic, not stereoselective hydrolysis (Table I, entries 10, 11, 14–16). In particular we found that alcalase (Novo Industri, Denmark), a very cheap enzyme commonly used as a detergent additive, was able to hydrolyze the ester moiety under very mild conditions, leaving the oxirane ring unaffected (Table I, entries 14, 15).

At the end of the reaction, the mixture was heated in the presence of aqueous trimethylamine. After treatment with hydrochloric acid and evaporation of water, an oil was obtained from which (*R*)-(−)-carnitine chloride was recovered by extraction with boiling isopropyl alcohol in 75–80% yield from (*R*)-(+)-1 (eq 3). The ee's of (*R*)-(−)-carnitine chloride were fully consistent with those of the starting (*R*)-(+)-epoxybutyrates, thus showing that no racemization took place during the second enzymatic hydrolysis or the successive reactions.

Experimental Section

^{1}H NMR spectra were recorded in $CDCl_3$ solution [$(CH_3)_4Si$ internal standard] on a Bruker AM 300 instrument. Optical rotations were measured neat or as water solutions with a Perkin-Elmer 241 polarimeter. All hydrolytic reactions were performed with a Metrohm pH-stat. Lipase from *Chromobacterium viscosum* (1500 U/mg), steapsin (11 U/mg), lipase from *Candida cylindracea* (500 U/mg), pig liver esterase (200 U/mg), α-chymotrypsin (58 U/mg), thermolysin (46 U/mg), substilisin BPN′ (10 U/mg), subtilisin Carlsberg (12 U/mg), papain (2.9 U/mg), and proteases from *Streptomyces caespitosus* (0.7 U/mg), *Streptomyces griseus* (5.2 U/mg), and *Aspergillus sojae* (0.44 U/mg) were purchased from Sigma Chemical Co.; pancreatin (57 U/mg) was purchased from Unibios (Italy); lipase SP 225 and alcalase 2.0 T (2.16 U/g) were obtained from Novo Industri (Denmark); lipoprotein lipase (1120 U/mg) was purchased from Amano Chemical Co. (Japan).



(3)

Synthesis of Racemic Alkyl 3,4-Epoxybutyrates 1a–e. Alkyl 3,4-epoxybutyrates **1a–e** were prepared by epoxydation of the corresponding 3-butenoates[12] by *m*-chloroperoxybenzoic acid. In a typical experiment, 2.8 g (ca. 16 mmol) of *m*-chloroperoxybenzoic acid in 40 mL of chloroform was added dropwise to a stirred solution of 1.42 g (10 mmol) of isobutyl 3-butenoate in 6 mL of chloroform. After the addition was complete, the resulting solution was stirred 50 h and then washed with saturated sodium sulfite (3 × 10 mL) and sodium bicarbonate solutions, dried ($CaCl_2$), filtered, and evaporated under vacuum. The residue was purified by chromatography (silica gel, Et_2O/*n*-hexane as the eluent) obtaining isobutyl 3,4-epoxybutyrate (**1a**) (1.31 g, 83%); ^{1}H NMR δ 3.93 (d, 2 H), 3.31 (m, 1 H), 2.86 (m, 1 H), 2.58 (m, 3 H), 1.96 (m, 1 H), 0.96 (d, 6 H).

Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.54; H, 8.80.

***n*-Butyl 3,4-epoxybutyrate (1b)**: 81% yield; ^{1}H NMR δ 4.13 (t, 2 H), 3.30 (m, 1 H), 2.84 (m, 1 H), 2.55 (m, 3 H), 1.64 (m, 2 H), 1.40 (m, 2 H), 0.95 (t, 3 H).

Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.52; H, 8.76.

***n*-Octyl 3,4-epoxybutyrate (1d)**: 80% yield; ^{1}H NMR δ 4.12 (t, 2 H), 3.28 (m, 1 H), 2.85 (m, 1 H), 2.56 (m, 3 H), 1.64 (m, 2 H), 1.17–1.51 (m, 10 H), 0.88 (t, 3 H).

Anal. Calcd for $C_{12}H_{22}O_3$: C, 67.25; H, 10.35. Found: C, 66.96; H, 10.19.

Benzyl 3,4-epoxybutyrate (1e): 83% yield; ^{1}H NMR δ 7.34–7.37 (m, 5 H), 5.17 (s, 2 H), 3.31 (m, 1 H), 2.84 (m, 1 H), 2.61 (m, 2 H), 2.56 (m, 1 H).

Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.51; H, 6.11.

Enzymatic Hydrolysis of (*R*,*S*)-Alkyl 3,4-Epoxybutyrates 1a–e. The following procedure is representative. To a magnetically stirred mixture of (*R*,*S*)-isobutyl 3,4-epoxybutyrate (**1a**) (11.06 g, 70 mmol) in 0.05 N phosphate buffer (100 mL) at 20 °C and pH 7.0, steapsin (700 mg, 7700 units) was added and the pH maintained at 7.0 with 1 N aqueous sodium hydroxide by using a pH-stat. The hydrolysis was allowed to proceed to 70% conversion (27 h). The reaction mixture was extracted with ether (3 × 70 mL), and the organic layer was dried (Na_2SO_4) and evaporated to dryness. 1-Butanol was removed from the reaction crude by azeotropic distillation with *n*-heptane (100 mL). The residue was then distilled to give 2.4 g of (*R*)-(+)-*n*-butyl 3,4-epoxybutyrate (**1b**), bp 116–118 °C (40 mm); $[\alpha]^{20}_D$ +10.5° (neat, d^{20}_4 1.003); integration of the $COOCH_2$ signals in the ^{1}H NMR spectrum recorded in the presence of Eu(hfc)$_3$ indicated that the product was of 95% ee [racemate, δ 4.18 (dt); *R* isomer, δ 4.17 (t)].

(9) The epoxy acid formed during the enzymatic hydrolysis was recovered in 60% yield (based on the theoretical amount) by extraction (5×) with ether of the acidified (pH <2.0) aqueous solution after its saturation with ammonium sulfate.

(10) The presence of 3-hydroxy-4-chlorobutyrates was detected by GLC–MS analysis.

(11) (a) McClure, J. D. *J. Org. Chem.* **1967**, *32*, 3888. (b) Degenhardt, C. R. *J. Org. Chem.* **1980**, *45*, 2763.

(12) Alkyl 3-butenoates were prepared as described in ref 5 for the *tert*-butyl ester.

Synthesis of (*R*)-(−)-Carnitine Chloride (3) (*R*)-(+)-isobutyl 3,4-epoxybutyrate (3.3 g, 20.9 mmol) of 95% ee was suspended in 30 mL of 0.003 N phosphate buffer at 35 °C and pH 8 and treated with alcalase 2.0 T (0.4 g, 0.86 unit). The pH was maintained at 8 with 1 N aqueous NaOH by using a pH-stat until the consumption of base stopped (15 h). The reaction mixture was extracted with methylene chloride (2 × 10 mL) and reacted for 2 h at 45 °C with 5 N trimethylamine (5 mL, 25 mmol). After evaporation to dryness to remove unreacted trimethylamine, concentrated hydrochloric acid (3 mL) was added, and the resulting solution was evaporated to dryness. The residue was crystallized from isopropyl alcohol to give 3.28 g of (*R*)-(−)-carnitine chloride (3) (79% yield); $[\alpha]^{25}_D$ −22.2° (*c* 1, H_2O). The ee, determined by comparison of the optical activity with the literature value ($[\alpha]^{25}_D$ −23.7°),[13] was 94%.

Note added in proof: After submission of this manuscript for publication, a highly enantioselective PLE-catalyzed hydrolysis of methyl 3,4-epoxybutyrate was reported (Mohr, P.; Rösslein, L.; Tamm, C. *Helv. Chim. Acta* **1987**, *70*, 142).

(13) Strack, E.; Lorenz, J. *Z. Physiol. Chem.* **1960**, *318*, 129.

Solution and Solid-State Conformations of Ascidiacyclamide, a Cytotoxic Cyclic Peptide from Ascidian

Toshimasa Ishida,* Masayuki Tanaka, Michiko Nabae, and Masatoshi Inoue

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

Shinji Kato, Yasumasa Hamada, and Takayuki Shioiri

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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The solution and solid-state conformations of ascidiacyclamide, a cytotoxic cyclic peptide from ascidian, were determined by ^1H NMR spectroscopy and X-ray diffraction. The measurement of solvent and temperature dependences showed that the peptide NH protons of C_2-symmetric ascidiacyclamide are all solvent-shielded, suggesting the locations of these protons in the interior of the ring structure of ascidiacyclamide. On the basis of coupling constants and the known stereochemical preferences, a saddle-shaped conformation was proposed as being favored in solution. X-ray diffraction analysis revealed a C_2-symmetric saddle-shaped conformation, almost compatible with the NMR data. Two benzene molecules per one ascidiacyclamide molecule were co-crystallized and stabilized by the van der Waals contact with D-valine and L-isoleucine side chains, respectively. No hydrogen bond was observed in the crystal packing. The analysis of the thermal behavior of the crystals, with the aid of their IR spectra, showed the thermal stability of the ring conformation observed in the crystal. The conformation for the related cyclic peptides, along with its biological implications, was discussed on the basis of the present results.

Introduction

Lipophilic cyclic peptides from marine organisms have been receiving increasing interest, due largely to the high potency of their antineoplastic and/or cytotoxic activities.[1,2] Several cyclic peptides that contain unusual thiazole and oxazoline amino acids have been isolated from ascidian (Chart I): ulithiacyclamide (1),[3] ulicyclamide (2),[3a,4] patellamides A (3),[5] B (4),[4,6] and C (5),[4,6a,b] and ascidiacyclamide (6).[7] These cyclic peptides, which exhibit potent cytotoxic activites,[5a,7a] all have a common (1, 3–6) or related (2) ring structure. Therefore this ring formation may be necessary for the cytotoxic activity, although up to now little has been known about the mechanism of its biological action.

Study of the active conformation of the biologically important molecule is very useful for understanding the mechanism of action at the atomic level. As part of a program to elucidate the active forms of these cyclic

Table I. NMR Parameters of NH and $C_\alpha H$ Resonances

		δ (ppm) at 24 °C	$d\delta/dT \times 10^4$ (ppm/deg)	$J_{HNC_\alpha H}$ (Hz) at 24 °C	$J_{HNC_\alpha H}$ (Hz) at 61 °C
NH(1)	C_6D_6	8.330	17	7.58	7.91
	$CDCl_3$	7.951	−7	7.91	7.91
	$(CD_3)_2SO$	7.904	5	8.71	8.24
NH(2)	C_6D_6	7.587	−2	9.89	9.81
	$CDCl_3$	7.380	−15	10.55	10.22
	$(CD_3)_2SO$	7.323	10	9.85	10.29

		$J_{\alpha\beta}$ (Hz) at 24 °C	$J_{\alpha\beta}$ (Hz) at 61 °C
H5	C_6D_6	7.25	7.36
	$CDCl_3$	5.94	6.37
	$(CD_3)_2SO$	6.61	6.56
H14	C_6D_6	4.45	4.10
	$CDCl_3$	5.90	5.30
	$(CD_3)_2SO$	5.50	4.45

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