Table I. ^{13}C Chemical Shifts (δ)[a] for Protonated Carbons

compd	OCH_3 subst	OCH_3	OCH_3	OCH_3	phenyl
1	1,4	55.7	55.7		114.7 (C_2, C_3, C_5, C_6)
2	1,3	55.2	55.2		100.5 (C_2), 106.2 (C_4, C_6); 129.9 (C_5)
3	1,2	55.8	55.8		111.5 (C_3, C_6), 120.9 (C_4, C_5)
4	1,2,3	56.0	60.7	56.0	105.3 (C_4, C_6), 123.6 (C_5)

[a] Downfield from internal Me_4Si.

Table II. ^{13}C Spin-Lattice Relaxation Times (T_1, s)[a] for Protonated Carbons

compd	OCH_3 subst	OCH_3	OCH_3	OCH_3	phenyl
1	1,4	4.5	4.5		5.5 (C_2, C_3, C_5, C_6)
2	1,3	3.7	3.7		5.4 (C_2), 4.5 (C_4, C_6), 5.3 (C_5)
3	1,2	3.6	3.6		4.4 (C_3, C_6), 4.8 (C_4, C_5)
4	1,2,3	3.6	6.1	3.6	2.9 (C_4, C_6), 3.1 (C_5)

[a] Measurements were carried out at 15.1 MHz, with degassed solutions, 1 M in $CDCl_3$ at 30 °C. T_1 values were determined by using the inversion recovery method[9] and estimated from peak intensities by using the Bruker T_1 Program/II. They are the average of four determinations (±5%).

C_4 chemical shifts. Indeed their chemical shifts are $\delta_{^{13}C}$ 120.9 and 120.1, respectively. On the other hand, in **4** the carbon para to the *out-of-plane* 2-OCH_3 (C_5), which is expected to experience less shielding, resonates about 3 ppm downfield ($\delta_{^{13}C}$ 123.6).

We used T_1 values as indices of molecular motion for individual methoxy carbons in each molecule. In previous work we described the dramatic variation in T_1 values between planar and out-of-plane methoxy groups. This was explained[3] by invoking hindered rotation for the in-plane methoxy by the ortho protons of the ring. In contrast, methoxy groups that are perpendicular to the plane of the aromatic ring are unhindered by their ortho substituents and experience considerably faster rotation, as reflected by their longer T_1 values. The present data (Table II) support the conclusion that OCH_3 groups in the three dimethoxybenzenes (**1-3**) exist predominantly in the planar conformation while **4** has its 2-OCH_3 group in the out-of-plane conformation and the other two methoxy groups planar.

During the interpretation of the ^{13}C T_1 values for OCH_3 carbons two uncertainties were considered: (a) the possibility that spin-rotation relaxation may have a significant contribution in the overall relaxation rate; (b) the possibility that anisotropic tumbling of the entire molecule may be affecting differently the relaxation rates of the various OCH_3 carbons in the dimethoxybenzenes and may therefore not allow legitimate comparisons between their T_1 values. The first uncertainty was resolved through the measurement of $^{13}C-^1H$ NOE values for all protonated carbons. These uniformly showed maximum NOE values ($\eta \approx 2.0$),[7] indicating that $^{13}C-^1H$ dipolar relaxation is the only significant ^{13}C relaxation mechanism with no substantial contribution from spin-rotation. The second uncertainty was more difficult to deal with in a quantitative fashion. Examination of the T_1 values showed some variation among the protonated phenyl carbons in those molecules in which not all the CH aromatic carbons are equivalent, indicating some anisotropic character in the motion for the phenyl ring. However, careful consideration of the data indicated that this relatively modest anisotropic behavior does not alter the interpretation of our results.

Each arylmethoxy group is subject to two possible rotational processes, one around the Ar-O bond and the second around the O-CH_3 bond. Of these, rotation around the Ar-O bond is expected to be the slowest because of a considerably higher rotational barrier, a result of the partial double-bond character of this bond. Theoretical calculations have shown this to be the case.[8] Although both rotational processes should influence the spin-lattice relaxation of each methoxy carbon, the measured T_1 values are expected to be more sensitive to changes in the faster O-CH_3 rotations. The considerably longer T_1 value (6.1 s) of the 2-OCH_3 group in **4** is, thus, a reflection of freer rotation of an O-CH_3 bond perpendicular to the plane of the aromatic ring. On the other hand, the slightly higher OCH_3 T_1 value in *p*-dimethoxybenzene is more likely to be a reflection of a higher degree of anisotropy in the overall reorientation of this molecule.

The data provided in this communication give two independent types of evidence indicating that at least in solution, *o*-dimethoxybenzene has its two methoxy groups predominantly in a conformation in which the O-CH_3 bonds are coplanar with the phenyl ring. These results are in agreement with the majority of the X-ray crystallographic data[8] on molecules with aromatic *o*-dimethoxy substitution. Our results differ from the previously reported conformational analysis of *o*-dimethoxybenzene in the gas phase and in solution.[1] It is possible to explain the differences between the results described here and those obtained from gas-phase[1] measurements on the basis of a solvent effect. Indeed, large differences were found in the rotational barrier of OCH_3 in anisole when this was measured in the gas phase and in the liquid phase.[10] However, the discrepancy between our data and the conclusions drawn in the previous investigation for the preferred conformations in solution should point out the dangers involved in the use of partition coefficient measurements for conformational analysis.

Registry No. *p*-Dimethoxybenzene, 150-78-7; *m*-dimethoxybenzene, 151-10-0; *o*-dimethoxybenzene, 91-16-7; 1,2,3-trimethoxybenzene, 634-36-6.

(7) Freeman, R.; Hill, H. D.; Kaptein, R. *J. Magn. Reson.* **1972**, *7*, 327.

(8) Hofer, O. *Monatsh. Chem.* **1978**, 109, 405.

(9) Vold, R. L.; Waugh, J. S.; Klein, M. P.; Phelps, E. E. **1968**, *48*, 3331.

(10) Allen, G.; S. Fewster. In "Internal Rotation in Molecules"; Orville-Thomas, W. J., Ed.; Wiley-Interscience: New York, 1974; p 276.

Pseudopterolide, an Irregular Diterpenoid with Unusual Cytotoxic Properties from the Caribbean Sea Whip *Pseudopterogorgia acerosa* (Pallas) (Gorgonacea)

Maury M. Bandurraga and William Fenical*

Institute of Marine Resources
Scripps Institution of Oceanography
La Jolla, California 92093

Stephen F. Donovan and Jon Clardy*

Department of Chemistry—Baker Laboratory
Cornell University, Ithaca, New York 14853

Received June 14, 1982

Chemical investigations of marine soft corals of the order Gorgonacea (Cnidaria, Alcyonaria), the sea whips and fans, have yielded several metabolites possessing potent biological activities.[1] Our investigations of these beautiful marine invertebrates have been guided by the in situ evaluation of cytotoxicity using appropriate cell lines such as the fertilized sea urchin egg.[2] During

(1) (a) Weinheimer, A. J.; Matson, J. A.; Hossain, M. B.; van der Helm, D. *Tetrahedron Lett.* **1977**, 2933. (b) Weinheimer, A. J.; Matson, J. A.; van der Helm, D.; Poling, M. *Ibid.* **1977**, 1295. (c) Weinheimer, A. J.; Matson, J. A. *Lloydia* **1975**, *38*, 387. (d) Fenical, W.; Okuda, R. K.; Bandurraga, M. M.; Culver, P.; Jacobs, R. S. *Science (Washington, D.C.)* **1981**, *212*, 1512.

(2) Cytotoxicity was assayed by utilizing the fertilized egg of the California sea urchin *Lytechinus pictus* (Verrill). The pharmacological aspects of this assay have been described: Jacobs, R. S.; White, S.; Wilson, L. *Fed. Proc. Am. Soc. Exp. Biol.* **1981**, *40*, 26.

0002-7863/82/1504-6463$01.25/0

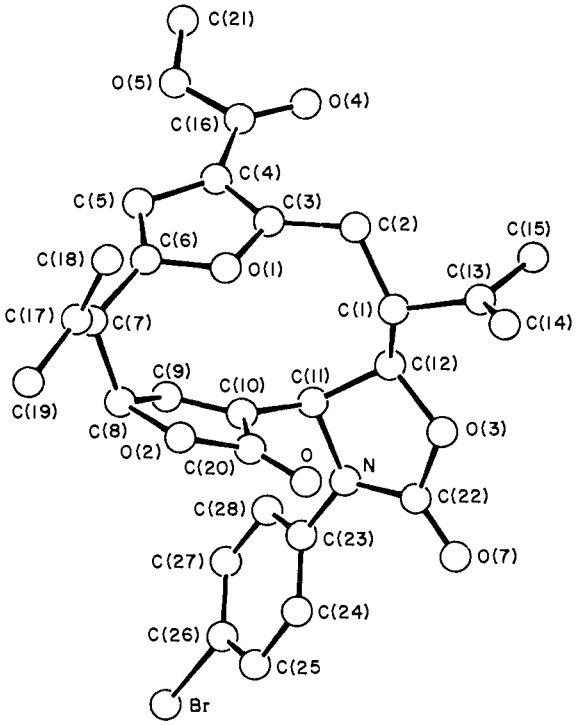
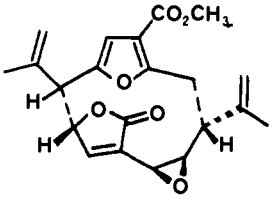


Figure 1. Computer-generated perspective drawing of the final X-ray model of urethane **3**. The stereochemical designators are as follows: (1*S*), (7*R*), (8*R*), (11*S*), (12*R*).

a recent Caribbean expedition on board the research vessel *Calanus*,[3] we were attracted to the common red sea whip *Pseudopterogorgia acerosa* (Pallas), extracts of which showed considerable cytotoxic activity. We now report the isolation and structure determination of an interesting irregular diterpenoid, pseudopterolide (**1**), which is a major metabolite of *P. acerosa*. Pseu-

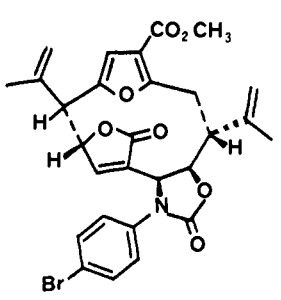


1

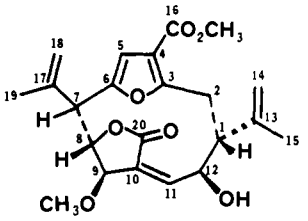
dopterolide inhibits overall cell cleavage but does not inhibit nuclear division in the fertilized urchin egg assay. This effect, while still under investigation, appears similar to the multinucleation effects produced by the fungal cytotoxin cytochalasin D.[4]

Pseudopterolide (**1**) was isolated from the extract of *P. acerosa* (collected Florida Keys, June 1980) as an amorphous solid, by conventional chromatographic methods (0.7% dry weight).[5] A molecular formula of $C_{21}H_{22}O_6$ was established for **1** by high-resolution mass spectrometry, and this composition was fully supported by both ^1H and ^{13}C NMR. Signals at δ 163.8 (s), 160.3 (s), 150.4 (s), 114.8 (s), 111.5 (d), and 51.4 (q) in the ^{13}C NMR spectrum, coupled with an infrared absorption at 1712 cm^{-1}, and two singlets in the ^1H NMR spectrum at δ 6.44 (1 H) and 3.82 (3 H), indicated **1** possessed an α,α'-disubstituted β-carbomethoxyfuran constellation.[6] A second carbonyl absorption at 1767 cm^{-1}, along with a one-proton band at δ 7.12 in the ^1H NMR spectrum, and bands at δ 171.9 (s), 151.3 (d), 129.0 (s), and 79.7 (d) in the ^{13}C NMR spectrum were assigned to an α-substituted α,β-unsaturatred γ-lactone functionality. The remaining oxygen atom in **1** was confidently assigned to a cis-disubstituted epoxide on the basis of ^{13}C NMR bands observed at δ 59.9 (d) and 52.6 (d) and ^1H NMR bands at δ 3.65 (1 H, d, J = 4 Hz) and δ 2.99 (1 H, dd, J = 10, 4 Hz).

Consideration of the olefinic and functional group unsaturation in pseudopterolide led to the conclusion that **1** was monocarbocyclic. Proton NMR characteristics, however, excluded the common terpenoid possibilities for this compound since **1** contained the equivalent of six methyl groups, in the form of two isopropenyl groups, one ester, and one lactone, rather than the five methyls of most diterpenoids. Proton NMR decoupling studies and computer simulation experiments allowed the assignment of pseudopterolide to the basic carbon skeleton as in **1**. However, the configuration at five asymmetric centers and the position of the carbomethoxy ester at either C(4) or C(5) remained undefined. Since **1** failed to crystallize or yield suitable crystalline derivatives, we turned to a related noncrystalline compound, the adduct **2**,



3



2

which had been isolated earlier from specimens of *P. acerosa* stored in methanol.[7] Compound **2** was also prepared from **1** by treatment with sulfuric acid or *p*-toluenesulfonic acid in methanol. Treatment of **2** with *p*-bromophenyl isocyanate in benzene/pyridine yielded the crystalline urethane **3**. An X-ray investigation of **3** revealed the monocarbocyclic structures of this group of compounds and also showed that an apparent S_N2' elimination of methoxide had occurred to produce the cyclic urethane. Figure 1 shows the final perspective drawing of this urethane (**3**) depicting the absolute stereochemistry.[8]

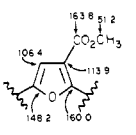
(3) Our utilization of the research vessel *Calanus* was afforded by NSF support to the University of Miami.

(4) Tannenbaum, S. W., Ed. "Cytochalasins: Biochemical and Cell Biological Aspects"; North Holland: New York, 1978.

(5) Pseudopterolide (**1**): $[\alpha]^{26}_D$ +96.3° (*c* 1.9, $CHCl_3$); UV (Et_2O) λ_{max} 223 nm (ϵ 8000); IR ($CHCl_3$) 1767, 1712 cm^{-1}; HR MS: M^+ obsd 370.1409, calcd 370.1416 for $C_{21}H_{22}O_6$; ^1H NMR (360 MHz, $CDCl_3$) δ 7.12 (1 H, br s, J = 1.5 Hz), 6.44 (1 H, s), 5.49 (1 H, dd, J = 4.5, 1.5 Hz), 5.06 (1 H, br s), 5.03 (1 H, br s), 5.00 (1 H, br s), 4.84 (1 H, br s), 3.85 (1 H, br d, J = 4.5 Hz), 3.82 (3 H, s), 3.74 (1 H, dd, J = 15.5, 13 Hz), 3.65 (1 H, d, J = 4 Hz), 3.13 (1 H, ddd, J = 13, 10, 3.5 Hz), 2.99 (1 H, dd, J = 10, 4 Hz), 2.80 (1 H, dd, J = 15.5, 3.5 Hz), 1.97 (3 H, br s), 1.96 (3 H, br s); ^{13}C NMR (20 MHz, $CDCl_3$) 171.9 (s), 163.8 (s), 160.3 (s), 151.3 (d), 150.4 (s), 145.3 (s), 140.5 (s), 129.0 (s), 114.8 (s), 114.8 (t), 112.5 (t), 111.5 (d), 79.7 (d), 59.9 (d), 52.6 (d), 51.4 (q), 48.9 (d), 42.6 (d), 28.1 (t), 21.4 (q), 20.8 (q) ppm.

(6) Missakian, M. G.; Burreson, B. J.; Scheuer, P. J. *Tetrahedron* **1975**, *31*, 2513.

(7) Pseudopterolide–methanol adduct: $[\alpha]^{26}_D$ −115.4° (*c* 2.4, $CHCl_3$); UV (MeOH) λ_{max} 275 nm (ϵ 2000), 235 (ϵ 4000); IR ($CHCl_3$) 3500, 1740, 1710 cm^{-1}; HR MS: M^+ obsd 402.1657, calcd 402.1678 for $C_{22}H_{26}O_7$; ^1H NMR (360 MHz, $CDCl_3$) δ 6.39 (1 H, s), 5.93 (1 H, d, J = 8 Hz), 5.10 (1 H, br s), 5.09 (1 H, d, J = 4 Hz), 5.00 (1 H, br s), 4.98 (1 H, br s), 4.89 (1 H, br s), 4.48 (1 H, d, J = 12 Hz, D_2O exchangeable), 3.98 (1 H, ddd, J = 12, 10, 8 Hz), 3.82 (3 H, s), 3.65 (1 H, s), 3.65 (1 H, m, J = 10, 10, 2 Hz), 3.61 (1 H, dd, J = 15, 10 Hz), 3.57 (1 H, br d, J = 4 Hz), 3.16 (3 H, s), 2.64 (1 H, dd, J = 15, 2 Hz), 1.93 (3 H, br s), 1.82 (3 H, br s); ^{13}C NMR (20 MHz, $CDCl_3$) 171.4 (s), 163.5 (s), 160.7 (s), 151.3 (s), 145.8 (s), 143.5 (d), 139.9 (s), 130.4 (s), 116.5 (s), 115.6 (t), 113.8 (t), 112.2 (d), 86.5 (d), 81.7 (d), 70.3 (d), 54.6 (q), 51.2 (q), 51.0 (d), 47.9 (d), 29.8 (t), 21.9 (q), 18.9 (q) ppm.



Since modifications of C(1), C(7), C(8), and C(12) are not involved in the conversion **1** → **2** → **3**, these centers in **1** are fully defined. To determine the stereochemistry at C(9) through C(11), however, requires careful interpretation of the stereospecific chemical processes involved. Construction of molecular models indicates the stereochemical relationships between **1**, **2**, and **3**. Due to the extremely hindered "back" face of C(8) to C(12) in these molecules, solvolysis and elimination must involve substituents on the "front" face. The allylic displacements (S_N2') of epoxide **1** and of methoxy derivative **2** must involve the predicted syn orientation of displacing and leaving groups.[11] The ^1H NMR spectra of **1-3** support this contention. As a consequence of this reasoning, the epoxide stereochemistry at C(11) was established as *S*.

The structure of pseudopterolide[12] represents a novel monocyclic skeleton related only in part to cubitene,[13] a 12-membered ring with two isopropenyl groups oriented 1,3 instead of 1,7. While pseudopterolide can be dissected symmetrically into two geranyl units in two possible ways, perhaps suggesting a biogenesis involving dimerization, the prevalence of the 14-membered ring cembrenoids in marine soft corals suggests a mechanism involving ring contraction.

Acknowledgment. Research at the Scripps Institution was supported by the National Science Foundation Oceanography Section, under Grant OCE 80-14167. Research at Cornell University was supported by the National Institutes of Health under Grant CA 24487. We thank Dr. Frederick M. Bayer, Smithsonian Institution, for identification of *Pseudopterogorgia acerosa* (Pallas). We express our appreciation to Professor Robert Jacobs for his collaboration in evaluating the pharmacological properties of pseudopterolide.

Supplementary Material Available: Tables of fractional coordinates, bond distances, and bond angles for urethane **3** (5 pages). Ordering information is given on any current masthead page.

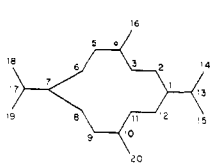
(8) Preliminary X-ray photographs of the urethane **3** showed orthorhombic symmetry and lattice constants of a = 12.470 (2) Å, b = 24.001 (2), and c = 9.356 (3) Å were determined by a least-squares fit of 15 moderate 2θ values measured on a diffractometer. Systematic extinctions, crystal density, and the presence of chirality were uniquely accommodated by space group $P2_12_12$ with a unit of $C_{28}H_{28}BrNO_7 \cdot H_2O$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a four-circle diffractometer using graphite monochromated Cu K$\bar{\alpha}$ (1.541 78 Å) radiation and a variable-speed 1° ω scan. Of the 2189 reflections surveyed, 2041 (93%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects. A phasing model was achieved by standard heavy-atom methods.[9] Full-matrix least-squares refinements using anisotropic non-hydrogen atoms, isotropic, fixed hydrogens, and anomalous scattering corrections for bromine have converged to a current residual of 0.095 for the structure shown and 0.099 for the enantiomer.[10]

(9) All crystallographic calculations were performed on a Prime 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were as follows: REDUCE and UNIQUE, data reduction programs (Leonowicz, M. E., Cornell University, 1978); BLS78A, anisotropic block-diagonal least-squares refinement (Hirotsu, K.; Arnold, E., Cornell University, 1980); XRAY76 ("The X-ray System of Crystallographic Programs"; Stewart, J. M., Ed.; University of Maryland, Technical Report TR-445, March 1976); ORTEP, crystallographic illustration program (Johnson, C. K., Oak Ridge, TN, ORNL-3794); BOND, molecular metrics program (Hirotsu, K., Cornell University, 1978); MULTAN 78 ("A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data"; University of York, England, Principal author P. Main). For literature description of MULTAN see: Germain, G.; Main, P.; Woolfson, M. W. *Acta Crystallogr., Sect. B* **1970**, *B26*, 274-285. Woolfson, M. M. *Acta Crystallogr., Sect. A* **1977**, *B33*, 219-225.

(10) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.

(11) Magid, R. M. *Tetrahedron* **1980**, *36*, 1901.

(12) We propose the nomenclature "Pseudopterane" (i) to define this new ring system and the numbering sequence shown.



i

(13) Prestwich, G. D.; Wiemer, D. F.; Meinwald, J.; Clardy, J. *J. Am. Chem. Soc.* **1978**, *100*, 2560.

Stereocontrolled Synthesis of (+)-Negamycin from an Acyclic Homoallylamine by 1,3-Asymmetric Induction

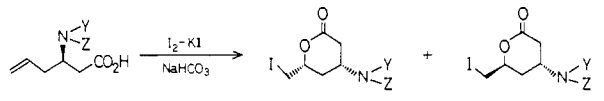
Yi-Fong Wang, Toshio Izawa, Susumu Kobayashi, and Masaji Ohno*

Faculty of Pharmaceutical Sciences
University of Tokyo, Hongo, Bunkyo-ku
Tokyo 113, Japan

Received June 28, 1982

Negamycin 2-[(3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoyl]-1-methylhydrazinoacetic acid (**1**), has attracted a great deal of synthetic study[1] since its isolation[2] and characterization,[3] because it possesses a strong inhibitory activity against Gram-negative bacteria, including *Pseudomonas* with low toxicity. However, none of the previous synthetic methods can afford the chiral β-amino acid effectively. We report here an efficient and enantioselective synthesis of negamycin **1** from the acyclic homoallylamine **3** in a highly stereocontrolled manner starting from methyl (*S*)-β-aminoglutarate (**4**).

A combination of enzymatic and chemical procedures was taken as our synthetic strategy as shown in Scheme I. The chiral homoallylamine **3** was considered to be a good intermediate for asymmetric induction, and the chiral half-ester **4** was chosen as the starting synthon, because it is now easily available in quantity by enzymatic hydrolysis of the prochiral precursor **5**.[4] Thus, the chiral half-ester **4** with *S* configuration was first converted to the chiral *tert*-butyl ester **6** with isobutene-H_2SO_4 (catalyst) in 88% yield, and then basic hydrolysis (0.25 N NaOH) afforded the chiral half-ester **7** with *R*-configuration quantitatively. The aldehyde **8** was prepared in 76% yield from **7** by treatment with dimethylpyrazole-DCC followed by reduction with $LiAlH_4$. Our key intermediate **9** was obtained in 80% yield by Wittig reaction of **8** with $Ph_3P{=}CH_2$ in THF at -78 °C. The compound **9** has a common double bond located at the δ,ε position and at the β,γ position for the carboxyl group and benzyloxycarbonyl (*Z*)-amino group, respectively. Therefore, asymmetric induction[5] is possible in two ways. Iodolactonization of **9a** and **9b** was first examined.



Treatment of **9a** with I_2-KI-$NaHCO_3$ in H_2O-CH_2Cl_2 at 0 °C for 4 h afforded a mixture of *cis*- and *trans*-iodo-δ-lactone (**10a**) in 94% yield, but the ratio was about 1.5:1 slightly in favor of the desired cis enantiomer. The ratio was improved to 6:1 *cis*-

(1) (a) Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H.; Ohno, M. *J. Am. Chem. Soc.* **1972**, *94*, 4353. (b) Pierdet, A.; Nēdēlec, L.; Delaroff, V.; Allais, A. *Tetrahedron* **1980**, *36*, 1763. (c) Pasquet, G.; Boucherot, D.; Pilgrim, W. R. *Tetrahedron Lett.* **1980**, *21*, 931. (d) Kondo, S.; Iinuma, K.; Yoshida, K.; Yokose, K.; Ikeda, Y.; Shimazaki, M.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 208. (e) Streicher, W.; Reinshagen, H. *Chem. Ber.* **1975**, *108*, 813.

(2) Hamada, M.; Takeuchi, T.; Kondo, S.; Ikeda, Y.; Naganawa, H.; Maeda, K.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1970**, *23*, 170.

(3) Kondo, S.; Shibahara, S.; Takahashi, S.; Maeda, K.; Umezawa, H.; Ohno, M. *J. Am. Chem. Soc.* **1971**, *93*, 6305.

(4) Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, Yi-F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405. Originally, pig liver esterase was used as the enzyme, but 4 is now prepared by the enzyme of microbial origin (*Flavobacterium lutescens*), and the detailed study of the enzymatic process will be reported elsewhere.

(5) (a) Bartlett, P. A. *Tetrahedron* **1980**, *36*, 2 and references cited therein. (b) Bartlett, P. A.; Jernstedt, K. K. *J. Am. Chem. Soc.* **1977**, *99*, 4829.

0002-7863/82/1504-6465$01.25/0