Scholtz, *Monatshefte Chem.* **108**, 1401 (1977).

Acknowledgment. Support for this research was generously provided by the National Institutes of Health (Grant CA-16616), Chevron Research Co., and Eli Lilly Co., as well as (through department Equipment Grant CHE-76-05512) the National Science Foundation.

References and Notes

(1) National Science Foundation Predoctoral Fellow, 1973–1976.
(2) (a) E. Harri, W. Loeffler, H. P. Sigg, H. Stähelin, and Ch. Tamm, *Helv. Chim. Acta*, **46**, 1235 (1963); (b) V. Betina, L. Drobnica, P. Nemec, and M. Zemanova, *J. Antibiot., Ser. A*, 17, 93 (1964); (c) T. Hayashi, A. Takatsuki, and G. Tamura, *ibid.*, **27**, 65 (1974).
(3) G. Tamura, K. Ando, S. Suzuki, A. Takatsuki, and K. Arima, *J. Antibiot., Ser. A*, **21**, 160 (1968); A. Takatsuki, I. Yamaguchi, G. Tamura, T. Misato, and K. Arima, *ibid.*, **22**, 442 (1969); N. B. Finter, *Ann. N.Y. Acad. Sci.*, **173**, 131 (1970).
(4) V. Betina, *Neoplasma*, **16**, 23 (1969); V. Betina, K. Horáková, and Z. Baráth, *Naturwissenschaften*, **49**, 241 (1962).
(5) (a) V. L. Singleton, N. Bohonos, and A. L. Ullstrup, *Nature (London)*, **181**, 1072 (1958); (b) V. Betina, P. Nemec, J. Dobias, and Z. Baráth, *Folia Microbiol. (Prague)*, 7, 353 (1962); (c) R. N. Mirrington, E. Ritchie, C. W. Shoppee, S. Sternhell, and W. C. Taylor, *Aust. J. Chem.*, **19**, 1265 (1966); (d) V. Betina, J. Fuska, A. Kjaer, M. Kutková, P. Nemec, and R. H. Shapiro, *J. Antibiot., Ser. A*, **19**, 115 (1966); (e) R. G. Coombe, J. J. Jacobs, and T. R. Watson, *Aust. J. Chem.*, **21**, 783 (1968); (f) Y. Suzuki, H. Tanaka, H. Aoki, and T. Tamura, *Agric. Biol. Chem.*, **34**, 395 (1970); (g) E. Taniguchi, H. Yoshikawa, and K. Maekawa, *J. Fac. Agric., Kyushu Univ.*, 17, 129 (1973) [*Chem. Abstr.*, **79**, 76929 (1973)]; (h) S. Nishimura, *Jpn. Kokai*, **72** 25 385 (1972) [*Chem. Abstr.*, **78**, 41571 (1973)]; (i) I. D. Entwistle, C. C. Howard, and R. A. W. Johnstone, *Phytochemistry*, **13**, 173 (1974); (j) B. E. Cross and P. Hendley, *J. Chem. Soc., Chem. Commun.*, 124 (1975).
(6) V. L. Singleton and N. Bohonos, *Agric. Biol. Chem.*, **28**, 77 (1964); V. Betina, P. Nemec, Š. Kováč, A. Kjaer, and R. H. Shapiro, *Acta Chem. Scand.*, **19**, 519 (1965).
(7) H. P. Weber, D. Hauser, and H. P. Sigg, *Helv. Chim. Acta*, **54**, 2763 (1971).
(8) H. P. Sigg, *Helv. Chim. Acta*, **47**, 1401 (1964).
(9) R. G. Coombe, P. S. Foss, and T. R. Watson, *Chem. Commun.*, 1229 (1967); U. Handschin, H. P. Sigg, and Ch. Tamm, *Helv. Chim. Acta*, **51**, 1943 (1968); R. G. Coombe, P. S. Foss, J. J. Jacobs, and T. R. Watson, *Aust. J. Chem.*, **22**, 1943 (1969); C. T. Mabuni, L. Garlaschelli, R. A. Ellison, and C. R. Hutchinson, *J. Am. Chem. Soc.*, **99**, 7718 (1977).
(10) (a) E. J. Corey and R. H. Wollenberg, *Tetrahedron Lett.*, 4701 (1976); (b) E. J. Corey and R. H. Wollenberg, *ibid.*, 4705 (1976); (c) E. J. Corey, R. H. Wollenberg, and D. R. Williams, *ibid.*, 2243 (1977).
(11) R. Baudouy, P. Crabbé, A. E. Greene, C. Le Drian, and A. F. Orr, *Tetrahedron Lett.*, 2973 (1977).
(12) P. A. Bartlett, *J. Am. Chem. Soc.*, **98**, 3305 (1976).
(13) R. Pappo and P. W. Collins, *Tetrahedron Lett.*, 2627 (1972).
(14) J. Fried and J. C. Sih, *Tetrahedron Lett.*, 3899 (1973).
(15) H. Meerwein, K. Bodenbenner, P. Borner, F. Kunert, and K. Wundelich, *Justus Liebigs Ann. Chem.*, **632**, 38 (1960).
(16) H. E. Zimmerman and T. L. Cutshall, *J. Am. Chem. Soc.*, **81**, 4305 (1959), and references therein. See also S. K. Malhotra and F. Johnson, *ibid.*, **87**, 5493 (1965).
(17) G. Zweifel and C. C. Whitney, *J. Am. Chem. Soc.*, **89**, 2753 (1967); G. Zweifel and R. B. Steele, *ibid.*, **97**, 2754 (1967).
(18) J. Hooz and R. B. Layton, *Can. J. Chem.*, **51**, 2098 (1973).
(19) F. R. Green III, unpublished results. See also K. Bernady and M. J. Weiss, *Tetrahedron Lett.*, 4083 (1972).
(20) G. H. Posner, C. E. Whitten, and J. J. Sterling, *J. Am. Chem. Soc.*, **95**, 7788 (1973).
(21) J. J. Eisch and S.-G. Rhee, *J. Am. Chem. Soc.*, **96**, 7276 (1974).
(22) O. Mitsunobu and M. Eguchi, *Bull. Chem. Soc. Jpn.*, **44**, 3427 (1971); A. K. Bose, B. Lal, W. A. Hoffman, III, and M. S. Manhass, *Tetrahedron Lett.*, 1619 (1973).
(23) J. Staunton and E. J. Eisenbraun, in "Organic Syntheses", Collect. Vol. V, Wiley, New York, N.Y., 1973, p 8.
(24) M. Pailer and P. Bergthaller, *Monatshefte*, **99**, 103 (1968).
(25) (a) K. Auwers, *Ber. Dtsch. Chem. Ges.*, **26**, 364 (1893); (b) K. Kurosawa and H. Obara, *Bull. Chem. Soc. Jpn.*, **39**, 525 (1966); L. J. Dolby, S. Esfandiari, C. A. Ellinger, and K. S. Marshall, *J. Org. Chem.*, **36**, 1277 (1971).
(26) P. A. Bartlett, F. R. Green III, and E. H. Rose, *J. Am. Chem. Soc.*, preceding paper in this issue.
(27) T. H. Applewhite, J. S. Nelson, and L. A. Goldblatt, *J. Am. Oil Chem. Soc.*, **40**, 101 (1963).
(28) R. Bloch, P. Le Perchec, F. Rouessac, and J.-M. Conia, *Tetrahedron*, **24**, 5971 (1968).
(29) K. Fuji, S. Nakano, and F. Fujite, *Synthesis*, 276 (1975).
(30) N. Kunieda, Y. Fujiwara, J. Nokami, and M. Kinoshita, *Bull. Chem. Soc. Jpn.*, **49**, 575 (1976).
(31) T. Mukaiyama, M. Usui, and K. Saigo, *Chem. Lett.*, 49 (1976).
(32) We thank Professors E. J. Corey and R. H. Wollenberg for spectra and Professor Corey and Dr. A. von Wartburg (Sandoz, Ltd.) for samples of natural brefeldin A.
(33) S. Masamune, Y. Hayase, W. Schilling, W. K. Chan, and G. S. Bates, *J. Am. Chem. Soc.*, **99**, 6756 (1977); S. Masamune, S. Kamata, and W. Schilling, *ibid.*, **97**, 3515 (1975).
(34) E. J. Corey, K. C. Nicolaou, and L. S. Melvin, Jr., *J. Am. Chem. Soc.*, **97**, 654 (1975).
(35) For a similar case, see Y. Fukuyama, C. L. Kirkemo, and J. D. White, *J. Am. Chem. Soc.*, **99**, 646 (1977).
(36) E. J. Corey and L. S. Melvin, Jr., *Tetrahedron Lett.*, 929 (1975).

Synthesis of Anthopleurine, the Alarm Pheromone from *Anthopleura elegantissima*

James A. Musich and Henry Rapoport*

Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received December 12, 1977*

Abstract: Three of the four possible stereoisomers, **6**, **7**, and **8**, of (3-carboxy-2,3-dihydroxy-*N,N,N*-trimethyl)-1-propanaminium chloride, the proposed structure of anthopleurine, the alarm pheromone from the sea anemone *Anthopleura elegantissima*, were prepared from the corresponding 4-amino-2,3-dihydroxybutyric acids, **3**, **4**, and **5**, by betaine formation with *O*-methyl-*N,N'*-diisopropylisourea (**2**). Amino acids **3**, **4**, and **5** were prepared from D-glucose, L-tartaric acid, and D-tartaric acid, respectively. Biological assay of betaine hydrochlorides **6**, **7**, and **8** on the sea anemone, and comparison of physical and spectral data, establishes the structure of anthopleurine as 4-amino-4-deoxy-L-threonic acid betaine hydrochloride (**7**).

Anthopleurine, the alarm pheromone of the sea anemone *Anthopleura elegantissima*, has been identified by chemical and spectroscopic methods as the quaternary ammonium ion, (3-carboxy-2,3-dihydroxy-*N,N,N*-trimethyl)-1-propanaminium, isolated and characterized as the crystalline chloride **1**. Released from wounded anemones, the pheromone evokes a characteristic contraction in nearby conspecifics at a median effective concentration, for the crystalline pheromone, of 3.5 $\times 10^{-10}$ M.[1] The response of the anemone to anthopleurine is mediated by the through-conducting system[2] and is competitively inhibited by L-proline and certain analogues thereof.[3] Anthopleurine has been subsequently shown to evoke an alarm response, visually indistinguishable from that of *A. elegantissima*, in the sea anemone *A. xanthogrammica.*[2]

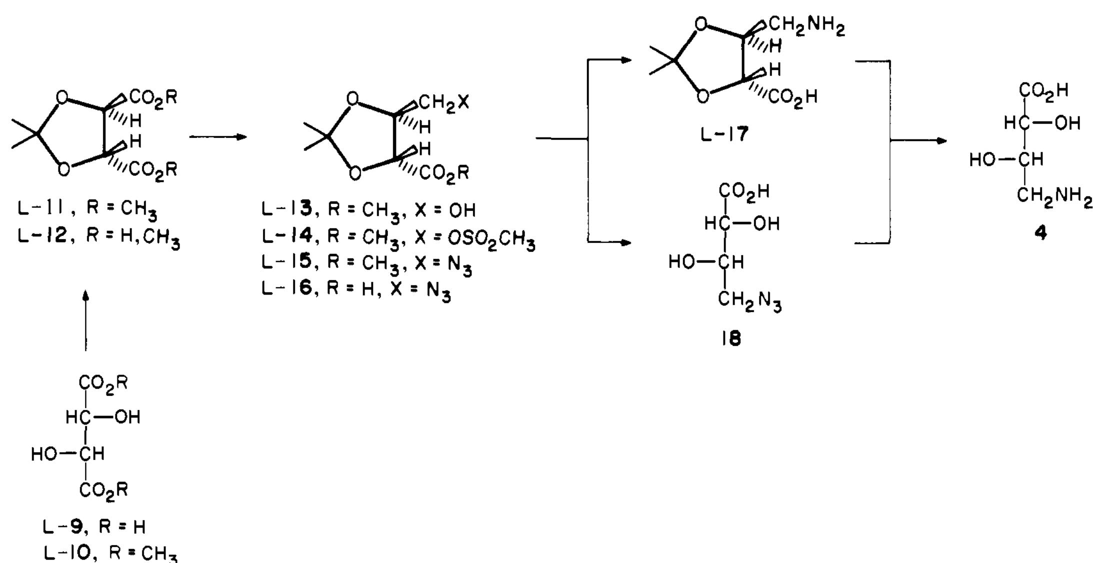
$Cl^-(CH_3)_3N^+CH_2CHOHCHOHCO_2H$

1

To further investigate the utility of *O*-methyl-*N,N'*-diisopropylisourea (**2**) in the conversion of amino acids to their corresponding betaines[4] and to confirm the structure and as-

0002-7863/78/1500-4865$01.00/0 © 1978 American Chemical Society

Scheme I. Preparation of 4-Amino-4-deoxy-L-threonic Acid (4) via 2,3-*O*-Isopropylidene-L-tartrate



sign the absolute stereochemistry of anthopleurine, three of the four possible stereoisomers, **6**, **7**, and **8**, of the proposed structure were prepared from the corresponding 4-amino-2,3-dihydroxybutyric acids, **3**, **4**, and **5**, of known stereochemistry and tested for pheromone activity.

Results and Discussion

Preparation of Amino Acids. With the expectation that the direction of rotation of the amino acid and corresponding betaine hydrochloride would be the same, as is true for 2,4-dideoxy-4-amino-L-threonic acid and its betaine, L-carnitine, the closest analogy available,[5,6] the two known dextrorotatory 4-amino-2,3-dihydroxybutyric acids, 4-amino-4-deoxy-D-erythronic acid (**3**)[7] and 4-amino-4-deoxy-L-threonic acid (**4**)[8,9] were the first synthetic objectives.

Except for certain modifications, noted in the Experimental Section, **3** was prepared in 8% overall yield from D-glucose according to known procedures.[7,10-12] The amino acid had properties consistent with those reported for **3** obtained from natural sources[7] and was homogeneous and clearly distinguishable from threo isomers **4** and **5** by standard automatic amino acid analysis.

The synthesis of 4-amino-4-deoxy-L-threonic acid (**4**) was accomplished in 8% overall yield from L-tartaric acid (L-**9**) according to Scheme I. Esterification of L-**9** using 3A molecular sieves to remove water from the reaction mixture gave dimethyl L-tartrate (L-**10**). Conversion of L-**10** to L-**11** by acid-catalyzed reaction with acetone in petroleum ether under reflux with simultaneous azeotropic removal of the water formed, according to the procedure of Feit,[13] resulted in significant racemization.[14] This racemization was eliminated by changing the solvent to pentane and using molecular sieves to remove the water; however, yields were only moderate. More effectively, acetonide formation with 2,2-dimethoxypropane in benzene under reflux using 4A molecular sieves to remove methanol afforded optically pure dimethyl 2,3-*O*-isopropylidene-L-tartrate (L-**11**) in 97% yield.

Partial hydrolysis of the diester with methanolic potassium hydroxide at room temperature produced methyl hydrogen 2,3-*O*-isopropylidene-L-tartrate (L-**12**). Selective reduction of the carboxylic acid function with excess borane in tetrahydrofuran at room temperature gave methyl 2,3-*O*-isopropylidene-L-threonate (L-**13**) which was converted to methyl *O*-methanesulfonyl-2,3-*O*-isopropylidene-L-threonate (L-**14**) with methanesulfonyl chloride in methylene chloride containing triethylamine. Displacement of methanesulfonate by azide proceeded smoothly in dimethylformamide at 100 °C to yield methyl 4-azido-4-deoxy-2,3-*O*-isopropylidene-L-threonate (L-**15**).

Catalytic hydrogenation of the azido ester was not fruitful because of polymerization of the resulting amino ester at room temperature, and it was necessary to first hydrolyze the ester of L-**15** with methanolic potassium hydroxide to give 4-azido-4-deoxy-2,3-*O*-isopropylidene-L-threonic acid (L-**16**). Catalytic hydrogenation of this azido acid in methanol afforded 4-amino-4-deoxy-2,3-*O*-isopropylidene-L-threonic acid (L-**17**). Hydrolysis of the acetonide in aqueous hydrochloric acid followed by ion-exchange desalting yielded the desired 4-amino-4-deoxy-L-threonic acid (**4**).

The amino acid was also obtained by reversing the order of the final two steps in the sequence. Hydrolysis of azido acid L-**16** in aqueous hydrochloric acid gave 4-azido-4-deoxy-L-threonic acid (**18**) which was catalytically hydrogenated in methanolic hydrochloric acid to yield **4**. By amino acid analysis, **4**, was homogeneous and clearly distinguishable from the D-erythro isomer (**3**). The yields and specific rotations of the compounds in Scheme I are listed in Table I.

In contrast to the specific rotations in water of +42° [8] and +43° [9] reported previously, the amino acid prepared in this manner has a specific rotation of +4.6° (+4.2 to +4.9°). To determine whether this discrepancy between our observed values and the reported values is the result of racemization of **4** prepared according to Scheme I, an error in the reported specific rotation, or an inversion of configuration at C-3 in the literature synthesis leading to **3**, with a reported specific rotation of +36.9°,[7] rather than **4**, we also prepared the amino acid according to the literature procedure (Scheme II).[8,9]

The amino acid precursor, methyl L-*threo*-3-cyano-2,3-diacetoxypropionate (methyl 3-cyano-2(*R*),3(*S*)-diacetoxypropionate (**19**)) was prepared, with minor modifications, according to known procedures[8,9,15] from dimethyl L-tartrate (L-**10**) in 17% yield. Amino acid **4** has been previously prepared by hydrogenation of **19** and the ethyl ester analogue **20** at elevated temperature and pressure with Raney cobalt[9] and platinum oxide[8] catalysts, respectively (Scheme II).

In our hands, hydrogenation of the nitrile at elevated temperature and pressure in the presence of Raney cobalt[9,16] in methanol gave, following acid hydrolysis and ion-exchange desalting, **4** contaminated (Table II) with a small amount of 4-amino-2-hydroxybutyric acid (**21**) of unknown configuration. Chromatography and recrystallization gave **4** with a specific rotation of +4.9° (Table I). A less efficient procedure

Table I. Yields and Specific Rotations of Amino Acids with Threo Configuration

L series				D series			
reactant	product	yield, %	$[\alpha]^{20}_D$, deg (g/100 mL, solvent)	reactant	product	yield, %	$[\alpha]_D$, deg (g/100 mL, solvent)
9	**10**	69	+4.8 (24.9, acetone)	**9**	**10**	83	−4.8 (26.0, acetone)
10	**11**	97	−53.7 (neat)	**10**	**11**	96	+53.0 (neat)
11	**12**	58	−53.7 (0.52, CH_3OH)	**11**	**12**	63	+51.1 (0.54, CH_3OH)
12	**13**	44	−19.2 (0.55, CH_3OH)	**12**	**13**	53	+18.7 (0.70, CH_3OH)
13	**14**	94	−25.7 (0.82, acetone)	**13**	**14**	99	+26.3 (1.20, acetone)
14	**15**	87	−96.3 (0.49, CH_3OH)	**14**	**15**	88	+94.7 (0.59, CH_3OH)
15	**16**	94	−98.5 (0.40, CH_3OH)	**15**	**16**	94	+95.4 (0.45, CH_3OH)
16	**17**	91	+20.5 (1.12, 60% aq acetone)	**16**	**17**	81	−21.7 (1.04, 60% aq acetone)
16	**18**	82	+4.6 (0.64, H_2O, −14.6 (0.69, 1 M HCl)	**17**	**5**	92	−4.8 (1.44, H_2O), +14.5 (0.72, 1 M HCl)
17	**4**	78					
18	**4**	79	+4.2 (0.59, H_2O)				
19	**4**	47	+4.9 (0.71, H_2O)				

for desalting the hydrolysis product involved precipitation of **4** from methanol with pyridine.[9] By amino acid analysis, TLC, NMR, melting point, and specific rotation, **4** prepared according to Scheme I and according to the literature procedure (Scheme II) are identical, suggesting that the reported specific rotations are in error.

Hydrogenation of cyano ethyl ester **20** at elevated temperature and pressure in the presence of platinum oxide in acetic acid containing sulfuric acid, desalting with lead(II) acetate, acid hydrolysis, and desalting with silver(I) oxide are reported to give **4** with a specific rotation of +42° (H_2O) in 14% yield and 4-amino-2-hydroxybutyric acid (**21**) in 21% yield by fractional recrystallization.[8] Examination of the reaction product in our hands reveals a more complex mixture. Hydrogenation of cyano methyl ester **19** under the same conditions but using ion-exchange desalting gave **4** and **21**, as reported;[8] however, 4-aminobutyric acid (**22**) was the major product. The same results were obtained in a reaction performed at room temperature and low pressure.

If the hydrolysis mixture is desalted according to the prescribed silver(I) oxide procedure,[8] glycine (**23**), apparently formed at the expense of **4** by oxidative cleavage of the diol function,[17] is also present in the product mixture (Scheme II, Table II). A satisfactory separation of **4** and **21** by either fractional recrystallization[8] or silica gel chromatography was not realized.

To more conclusively determine the source of the glycine (**23**), aqueous solutions of the hydrochlorides of analytically pure 4-amino-4-deoxy-D-erythronic acid (**3**) and 4-amino-4-deoxy-D-threonic acid (**5**) were treated with excess silver(I) oxide on the steam bath for periods of 15 min to 1.5 h. In each case, glycine (**23**) was formed in amounts of 8 to 11%. No attempt was made to increase the conversion to glycine by employing vigorous stirring or by using a greater excess of silver(I) oxide. Clearly, desalting with silver(I) oxide is hazardous; similar oxidative cleavage of diols in carbohydrates with silver(I) oxide has been previously observed.[17]

Preparation of Betaine Hydrochlorides. The reaction of *O*-methyl-*N,N'*-diisopropylisourea (**2**)[18] with α-amino acids affords a mild and effective conversion to the corresponding betaines. Furthermore, etherification of hydroxyl groups does not occur and the method is nonracemizing.[4] Therefore, this preparative method was applied to the 4-amino-2,3-dihydroxybutyric acids. Treatment of either 4-amino-4-deoxy-D-erythronic acid (**3**) or 4-amino-4-deoxy-L-threonic acid (**4**) with excess **2** in methanol under reflux resulted in complete consumption of the amino acid and intermediate methylated compounds within a few hours. TLC analysis of the reaction mixture showed two major products, one corresponding in R_f to the betaine and the other remaining at the origin. This polar product is probably a trimethylammonium salt, resulting from an intramolecular displacement of trimethylamine by the carboxylate ion of the betaine. An analogous thermal decomposition of 4-aminobutyric acid betaine has been described.[19]

Ion exchange chromatography and subsequent recrystallization afforded 4-amino-4-deoxy-D-erythronic acid betaine hydrochloride (**6**) and 4-amino-4-deoxy-L-threonic acid betaine hydrochloride (**7**) in 43 and 28% yields, respectively. The melting points and specific rotations of **6** and **7**, as well as those reported for anthopleurine, are shown in Scheme III.

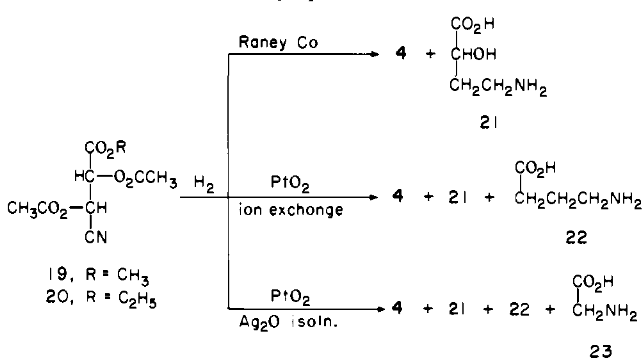
Although the specific rotation of **7** corresponds in neither direction nor magnitude to that reported for anthopleurine, the melting point of **7** does correspond to the reported value. Since the pheromone is reported to be dextrorotatory and since both dextrorotatory isomers were desired for biological testing, the preparation of 4-amino-4-deoxy-D-threonic acid betaine hydrochloride (**8**), the enantiomer of **7**, became necessary.

Table II. Hydrogenation of Methyl 3-Cyanopropionate (**19**). Amino Acid Analysis of Product Mixture

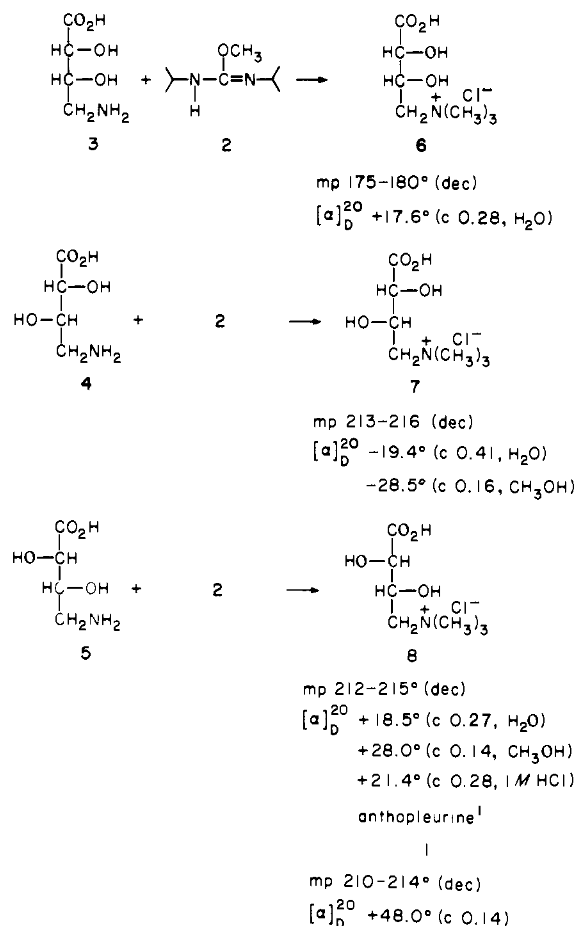
hydrogenation catalyst	desalting procedure	amino acid analysis,[a] % (rel amount)			
		4[b]	**21**[b]	**22**[c]	**23**[b]
Raney Co	ion exchange	94 (1.00)	3 (0.04)		
Raney Co	Ag_2O	57 (1.00)	3 (0.06)		6 (0.11)
PtO_2[d]	ion exchange	25 (1.00)	24 (0.98)	37 (1.48)	
PtO_2[e]	ion exchange	(1.00)	(1.02)	(1.48)	
PtO_2[e]	Ag_2O	9 (1.00)	18 (2.00)	25 (2.80)	4 (0.49)

[a] Performed with a Beckman Model 120C amino acid analyzer, using citrate buffer throughout. [b] Retention times: 138.5 min (**4**), 144.5 min (**21**), 86.5 min (**23**), using a column 55 cm × 9 mm, Resin AA-15, 0.2 N buffers at pH 3.2 and 4.2. [c] Retention time: 12 min on a column 5.5 cm × 9 mm, Resin AA-27, 0.35 N buffer at pH 5.26. [d] At 20 °C and 36-psi H_2 pressure. [e] At 70 °C and 1000-psi H_2 pressure.

Scheme II. Preparation of 4-Amino-4-deoxy-L-threonic Acid (4) via 3-Cyano-2(*R*),3(*S*)-diacetoxypropionate



Scheme III. Preparation and Properties of Betaine Hydrochlorides



Beginning with D-tartaric acid (D-**9**), 4-amino-4-deoxy-D-threonic acid (**5**) was prepared in 16% overall yield according to Scheme I. Yields and specific rotations of the synthetic intermediates in the scheme are listed in Table I. The D-*threo*-amino acid was homogeneous, identical with its enantiomer **4**, and distinguishable from the D-*erythro*-amino acid (**3**) by amino acid analysis, and had a specific rotation of −4.8° (Table I). Treatment of **5** with excess isourea **2** in aqueous methanol afforded the desired 4-amino-4-deoxy-D-threonic acid betaine hydrochloride (**8**) in 42% yield. In a somewhat more tedious procedure, similar to that employed in the synthesis of carnitine,[5,20] involving two ion-exchange desalting steps, **8** could also be prepared by treating **5** with methyl iodide and potassium hydroxide in aqueous methanol. Because of the susceptibility of the diol function of the amino acids to oxidative cleavage with silver(I) oxide, the methyl iodide–silver(I) oxide method of preparing betaines was not investigated. As expected, **8** is dextrorotatory with a rotation having the same magnitude as that of **7**; both have the same melting point as that reported for anthopleurine.

Biological Testing. The aggregating form of *Anthopleura elegantissima* was collected at low tide from the exposed intertidal rocks of a jetty at Doran Park near the Bodega Marine Laboratory, Bodega Bay, Calif. Anemones from a single clone[21] were transferred to glass bowls and maintained in running sea water prior to testing. Each bowl of anemones was removed from the holding tank of running sea water ~15 min before testing. The compounds tested for pheromone activity were amino acids **3**, **4**, and **5**, betaine hydrochlorides **6**, **7**, **8**, and 4-aminobutyric acid betaine hydrochloride (**24**).[19,22]

To a bowl of anemones was introduced via syringe an aliquot of the distilled water solution of the compound being tested.[23] The number of anemones responding with at least one rapid flexure within ~30 s of the time of addition were recorded. The intensity of each response was also characterized and recorded as weak, if the response involved only the convulsive, radially symmetrical flexures of the tentacles toward the base of the column, or strong, if the response eventually involved contraction of the mesenterial retractor muscles and of the marginal sphincter.[1,2]

The median effective concentration (EC_{50}) is defined as that concentration of pheromone which evokes an alarm response in 50% of the anemones tested, and the EC_{50} of crystalline anthopleurine is reported to be 7.4×10^{-8} g/L (±14%) or 3.5×10^{-10} M.[1] Our data clearly show that only 4-amino-4-deoxy-L-threonic acid betaine hydrochloride (**7**) exhibits pheromone activity consistent with that ascribed to anthopleurine. Its EC_{50} is in the concentration range from 3.8×10^{-9} to 3.8×10^{-11} M. Furthermore, of the compounds tested, only **7** evoked strong alarm responses from the anemones.

Of interest, also, is 4-amino-4-deoxy-D-erythronic acid betaine hydrochloride (**6**) which is active down to 3.8×10^{-7} M. At no concentration, however, did **6** evoke a strong response. Whether this activity is due to inherent pheromone activity of **6** or to contamination with **7** is not known. Since **6** and **7** differ in configuration only at C-3, and since even an amount too small to be chemically detected of **7** in **6**, resulting from C-3 epimerization, would cause a sample of **6** to show activity, the latter possibility cannot be dismissed. Only very weak and infrequent responses were elicited by even the most concentrated solutions of **8**. No activity whatsoever was shown by the other compounds tested.

Specific Rotation Discrepancy. Although the pheromone activity and melting point of 4-amino-4-deoxy-L-threonic acid betaine hydrochloride (**7**) correspond to those reported for anthopleurine and the NMR spectra are identical by direct comparison, there is clearly a discrepancy between the reported specific rotation of +48° (*c* 0.14)[1] and our observed rotation in water of −19.4° (*c* 0.41) which was independent of concentration over a factor of 4. Erroneous reporting of the specific rotation or contamination of the isolated natural product with a highly dextrorotatory material could possible account for the discrepancy. However, there is no evidence supporting either of these "error" hypotheses, and we attempted to find an explanation for this discrepancy in optical rotation.

Though assumed to be water, the solvent in which the specific rotation of anthopleurine was measured is not specified. The magnitude of the rotation of the synthetic material increases moderately as the solvent is changed from water to 1 M HCl to methanol, but the direction of rotation remains positive (Scheme III). Furthermore, the specific rotation of the free betaine **25**, obtained from **8**, is +11.7° in water, indicating that the free betaine and betaine hydrochloride have the same direction of rotation. Therefore, dependence of the direction of rotation upon solvent does not account for the discrepancy.

Treatment of betaine hydrochloride **8** in methanol under reflux for 144 h resulted in partial conversion to a compound with higher R_f on TLC. A singlet at δ 3.77 in the NMR spectrum of the product mixture was suggestive of methyl ester formation. However, the specific rotation of the mixture, +18.1°, was very similar to that of the starting betaine hydrochloride, +18.5°, suggesting that prolonged treatment of the pheromone with boiling methanol, as might occur in a digestion or recrystallization step, is not likely to account for the specific rotation discrepancy. The free betaine **25**, derived from **8**, is inert to such treatment.

Conditions required to thermally decompose 4-aminobutyric acid betaine to butyrolactone are known to be dependent upon whether the betaine is hydrated or anhydrous; lower temperatures are required if the betaine is hydrated.[19] Such decomposition of **7** would yield L-threono-1,4-lactone, a known

compound with a specific rotation reported to be +30.7° in water[24] and +46.7,[25] +47.0,[24] and +51.2° [26] in methanol. Contamination, then, of **7** with significant amounts of the lactone could effect both the magnitude and direction of the specific rotation of **7**. To determine the viability of this hypothesis, **8**, the enantiomer of biologically active betaine hydrochloride **7**, and **25**, the free betaine obtained from **8**, were heated near their melting points, the temperatures necessary to cause decomposition. The predominant product in each case was identical by TLC with authentic L-threono-1,4-lactone[25] and was concluded to be D-threono-1,4-lactone (**26**), its enantiomer. Whereas both **8** and **25** are dextrorotatory, the decomposition product mixtures were levorotatory, consistent with the hypothesis that thermal decomposition of **7** to L-threono-1,4-lactone, as might occur in a rigorous drying procedure, could affect both the magnitude and direction of the specific rotation of **7** and might conceivably account for the discrepancy between the observed and reported specific rotations.

Subsequent to the completion of our experiments, the specific rotation found for the natural anthopleurine sample was revealed to be −48° rather than +48°, as published.[27] With the direction of optical rotation no longer in conflict, the only remaining discrepancy between the properties ascribed to the natural product and those observed for the synthetic material is the magnitude of rotation. In regard to this, it should be noted that the magnitudes of the specific rotations of **7**, prepared by the isourea method, and its enantiomer **8**, prepared by three different procedures, agree within experimental error and no change in rotation results upon repeated exposure to the conditions of the isourea reaction. Thus we conclude that the correct rotation of anthopleurine is that which we find for **7**.

Conclusion

The correspondence between the melting points, NMR spectra, and alarm pheromone activities of the natural product and synthetic **7** establishes the complete structure of anthopleurine as 4-amino-4-deoxy-L-threonic acid betaine hydrochloride (**7**).

Experimental Section

General. *O*-Methyl-*N,N'*-diisopropylisourea (**2**) was prepared as described.[18] All melting points are uncorrected. Microanalyses and amino acid analyses (using a Beckman 120C instrument) were performed by the Analytical Laboratory, University of California, Berkeley. NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) on a Varian T-60 spectrometer with internal Me_4Si (organic solvents) or sodium γ-trimethylsilylpropanesulfonate (D_2O), mass spectra were obtained on an MS12 spectrometer, and IR spectra were recorded in $CHCl_3$ (unless otherwise noted) on a Perkin-Elmer 137 spectrophotometer. Optical rotations of solutions were measured on a Bendix Ericsson ETL-NPL automatic polarimeter, Type 143A; rotations of neat liquids were measured on a Zeiss photoelectric precision polarimeter (±0.005°). Thin layer chromatography was performed on MN Polygram Sil G/UV_{254} pre-coated plastic sheets and Analtech Uniplate silica gel GF pre-coated glass slides and column chromatography was with Merck silica gel (0.063–0.2 mm). Gas chromatography was done with an Aerograph Autoprep Model A-700 using a 10 ft by 0.25 in. column packed with 5% SE-30 on Chrom W, 80/100 mesh, at a flow rate of 100 mL/min. Solutions were dried over $MgSO_4$, and evaporations were under reduced pressure using a Berkeley rotary evaporator. Unless otherwise indicated, all reactions were performed in a nitrogen atmosphere.

Ion-Exchange Desalting of Amino Acid Hydrochlorides. Bio-Rad AG50W-X8 cation-exchange resin, 100/200 mesh, was converted from the NH_4^+ to the H^+ form by washing first with H_2O and then with 2 M HCl until the effluent was pH 2, followed by H_2O until the effluent was pH 3–4. The hydrochloride salt in H_2O was absorbed onto the column, and the H_2O wash was continued until the effluent was neutral. The amino acid was then obtained by eluting with 2 M NH_4OH until the effluent was pH 9. Alkaline fractions containing the amino acid were combined and evaporated at 50 °C, the residue was dissolved in H_2O three times, and the solvent was evaporated as before to yield the amino acid.

Isolation of Betaine Hydrochlorides by Ion Exchange. The crude product, dissolved in 0.5 M HCl, was adsorbed onto a column (1.8 × 28 cm) of Bio-Rad AG50W-X8 cation-exchange resin, 100/200 mesh, in the H^+ form that had been thoroughly washed free of all traces of NH_4Cl or NaCl with 0.5 M HCl. The betaine hydrochloride was eluted in fractions of 8 mL each at a flow rate of ~4 mL/min with gradually increasing concentrations (0.5 to 1.5 M) of aqueous HCl,[1] analyzing the column effluent by TLC on silica gel (consecutive developments with $CH_3OH/CHCl_3$/concentrated NH_3, 6:2:1, and C_2H_5OH/H_2O, 7:3). The betaine was visualized with Tollens reagent by heating the slides for several minutes on a hot plate. Fractions containing the desired product were combined and evaporated to dryness.

2,3-*O*-Isopropylidene-D-erythrono-1,4-lactone. Using a procedure similar to that described,[11] the reaction of D-erythrono-1,4-lactone[10] (2.10 g, 17.8 mmol) in acetone (200 mL, dried over 3A molecular sieves) containing concentrated HCl (20 drops) proceeded at room temperature for 6 h, after which the solution was neutralized (pH 8, Na_2CO_3). Filtration, evaporation of the filtrate to dryness, distribution of the residue between H_2O (25 mL) and $CHCl_3$ (3 × 50 mL), drying of the combined $CHCl_3$ extracts, evaporation of the $CHCl_3$, and sublimation at 55 °C (0.03 mm) gave 2.24 g (80%) of a white powder: mp 67–68.5 °C (lit. mp 68–68.5,[11] 65–67.5 °C[28]); $[\alpha]^{20}_D$ −120° (*c* 0.50, H_2O) (lit. $[\alpha]^{20}_D$ −112° (*c* 1.5, H_2O),[11] $[\alpha^{22}_D$ −116° (*c* 1, $H_2O)$[28]).

4-Azido-4-deoxy-2,3-*O*-isopropylidene-D-erythronic Acid. In a modification of the described procedure,[12a] a mixture of 2,3-*O*-isopropylidene-D-erythrono-1,4-lactone (3.95 g, 25 mmol) and sodium azide (5.72 g, 88 mmol, freshly activated) in DMF (24 mL; dried over 4A molecular sieves) was stirred in a 110 °C bath for 30 h. After the cooled suspension was filtered, the DMF was removed at 35 °C (1.0 mm), the residue was distributed between H_2O (125 mL) and $CHCl_3$ (200 mL), the aqueous layer was further extracted with ether (200 mL), the combined organic extracts were dried and evaporated, and the residue was sublimed at 55 °C (0.05 mm) to give 921 mg (23%) of recovered lactone.

The aqueous layer was acidified to pH 2 with 1 M H_2SO_4, saturated with NaCl, and extracted with ether (4 × 300 mL). The combined ether extracts were dried and evaporated to give 3.62 g of residue which was triturated and washed with 2,2,4-trimethylpentane. Sublimation at 55 °C (0.05 mm) yielded first a small quantity of liquid condensate, followed by 2.43 g (48%) of the azide: mp 56–61 °C (lit.[12] mp 55–56 °C); $[\alpha]^{20}_D$ +74.7° (*c* 0.48, acetone) (lit.[12] $[\alpha]_D$ +72° (*c* 0.47, acetone)).

4-Amino-4-deoxy-2,3-*O*-isopropylidene-D-erythronic Acid. In a modification of the described procedure,[12a] 4-azido-4-deoxy-2,3-*O*-isopropylidene-D-erythronic acid (2.00 g, 10 mmol) was hydrogenated in CH_3OH (200 mL) in the presence of 10% Pd/C (200 mg) at room temperature and 36-psi hydrogen pressure. After 4 h, the catalyst was removed by filtration and washed with H_2O (40 mL), the combined filtrate was concentrated at 50 °C to 15 mL, and the cloudy solution was diluted successively with 2-propanol (50 mL) and acetone (25 mL) to give 1.42 g (81%) of the extremely hygroscopic amino acid: mp 164–167 °C (lit.[12a] $[\alpha]_D$ +92° (*c* 1.02, 60% aqueous acetone).

Amino acid analysis: two peaks with retention times of 62 min, corresponding to the protected amino acid, and 117 min, corresponding to **3**, from partial hydrolysis of the acetonide during the amino acid analysis.

4-Amino-4-deoxy-D-erythronic Acid (3). A solution of 4-amino-4-deoxy-2,3-*O*-isopropylidene-D-erythronic acid (2.16 g, 12.3 mmol) in 1 M HCl (48 mL) was heated under reflux for 1.5 h and evaporated at 45 °C to give 2.32 g of a pale yellow oil. Ion-exchange desalting and dissolution in a minimum volume of hot H_2O (7.5 mL) and slow dilution with 2-propanol (15 mL) gave crystals which, after being washed with acetone and drying, resulted in 1.49 g (89%) of the amino acid: mp 217–219 °C dec (lit.[7] mp 215–217 °C dec; $[\alpha]^{20}_D$ +32.4° (*c* 0.28, H_2O) (lit.[7] $[\alpha]^{20}_D$ +36.9° (*c* 0.25, H_2O), calculated from ORD curve); homogeneous by amino acid analysis, retention time 117.5 min, retention time relative to threo isomers **4** and **5**, 0.85; NMR (D_2O) δ 2.98–3.17 (m appearing as br d with J = 5 Hz, 2 H), 3.97–4.23 (m, 2 H).

Dimethyl L- (and D-) Tartrate (L-10 and D-10). A mixture of L-(+)-tartaric acid (L-**9**, 75.0 g, 0.50 mol), methanol (162 mL), and concentrated H_2SO_4 (25 mL) was placed under a Soxhlet extractor containing conditioned 3A molecular sieves (100 g). The extractor was filled to capacity with an additional volume of CH_3OH (160 mL) and the reaction mixture was heated under reflux (internal temperature 67–68 °C) for 18 h. The cooled reaction mixture was neutralized with anhydrous $NaCO_3$ (4.77 g) and filtered, the methanol was evaporated, and the residue was Kugelrohr distilled (115 °C, 0.75 mm)) to give 61.5 g (69%) of **10:** $[\alpha]^{20}{}_D$ +4.8° (*c* 24.9, acetone), $[\alpha]_{578}$ +19.1° (*c* 25.1, H_2O), $[\alpha]_{546}$ +20.9° (*c* 25.1, H_2O) (lit.[29] $[\alpha]^{20}D$ +4.6° (*c* 25, acetone), $[\alpha]_{578}$ +19.3° (*c* 25, H_2O), $[\alpha]_{546}$ +21.2° (*c* 25, H_2O)).

In similar fashion, dimethyl D-tartrate (D-**10**) was prepared from D-(−)-tartaric acid (D-**9**) in an 83% yield: $[\alpha]^{20}{}_D$ −4.8° (*c* 26.1, acetone); NMR and IR spectra of D-**10** are identical with those of L-**10**; $[\alpha]$ of D-**10** equal and opposite to that of L-**10**.

Dimethyl 2,3-*O*-Isopropylidene-L- (and D-) tartrate (L-11 and D-11). A mixture of dimethyl L-tartrate (L-**10**, 35.6 g, 0.20 mol), 2,2-dimethoxypropane (31.4 g, 0.302 mol), and *p*-toluenesulfonic acid monohydrate (121 mg, 0.64 mmol) in benzene (80 mL) was heated under reflux under a Soxhlet extractor containing freshly conditioned 4A molecular sieves (20 g) for 2.25 h. The sieves were replaced at 1.5 h with a freshly conditioned batch. Anhydrous K_2CO_3 (210 mg) was added to the cooled reaction mixture which was stirred at room temperature for 4 h, filtered, and evaporated. The residue was taken up in ether (400 mL), the ether solution was washed with saturated aqueous borax ($Na_2B_4O_7 \cdot 10H_2O$, 50 mL), H_2O (25 mL), and saturated NaCl (25 mL), dried, and evaporated, and the residue was Kugelrohr distilled at 80 °C (0.1 mm), yielding 38.5 g (88%) of L-**11**: $[\alpha]^{20}{}_D$ −53.7° (neat) (lit.[30] $[\alpha]^{20}{}_D$ −53.1° (neat)).

In a similar fashion, dimethyl D-tartrate (D-**10**) was converted to dimethyl 2,3-*O*-isopropylidene-D-tartrate (D-**11**) in 96% yield: $[\alpha]^{20}{}_D$ +53.0° (neat); NMR and IR of D-**11** are identical with those of L-**11**; $[\alpha]$ of L-**11** and D-**11** equal and opposite.

Diethyl 2,3-*O*-isopropylidene-L-tartrate was first prepared as described.[13] However, the product, obtained in 69% yield, showed $[\alpha]^{20}{}_D$ −34.4° (neat) compared with a reported[31] value of $[\alpha]^{15}{}_D$ −51.2° (neat). Therefore the literature procedure[13] was modified as follows.

A solution of diethyl L-tartrate (20.0 g, 97 mmol) in acetone (60 mL) and petroleum ether (100 mL, 30–60 °C) containing concentrated H_2SO_4 (0.035 mL) was heated under reflux (bath temperature 60 °C) and the solvent cycled through a Soxhlet extractor containing conditioned 4A sieves (20 g) for 72 hr, with the sieves being replaced by freshly conditioned sieves every 24 h. By GC analysis (170 °C), the reaction had reached ~50% completion, it was evaporated, and the residue was taken up in Et_2O (160 mL), washed with H_2O (20 mL) and saturated NaCl (20 mL), dried, and evaporated to give 19 g of residue which was chromatographed on silica gel (600 g) with $CHCl_3$/ethyl acetate (9:1) as eluent. The diethyl 2,3-*O*-isopropylidene-L-tartrate fraction was Kugelrohr distilled at 80 °C (0.05 mm) and gave 8.5 g (35%) of acetonide: $[\alpha]^{20}{}_D$ −48.8° (neat) (lit.[31] $[\alpha]^{15}{}_D$ −51.2° (neat)).

From the chromatography was also recovered by Kugelrohr distillation (80–85 °C (0.05 mm)) 9.5 g (47%) of diethyl L-tartrate: $[\alpha]^{20}{}_D$ +8.6° (neat) (lit.[32] $[\alpha]_D$ +7.8° (neat)).

Methyl Hydrogen 2,3-*O*-Isopropylidene-L- (and D-) tartrate (L-12 and D-12). To a solution of dimethyl 2,3-*O*-isopropylidene-L-tartrate (L-**11**, 2.18 g, 10 mmol) in CH_3OH (3 mL) was added a solution of KOH (657 mg, 10 mmol) in CH_3OH (5 mL) over 1 h. The reaction mixture was stirred an additional hour and evaporated to give a residue which was distributed between H_2O (10 mL) and Et_2O (3 × 20 mL). The combined Et_2O extracts were dried and evaporated to give 207 mg (9.5%) of recovered L-**11**. The aqueous portion was acidified to pH 3.5 with 2 M HCl, saturated with NaCl, and extracted with Et_2O (6 × 20 mL), readjusting the pH to 3.5 following each extraction. The combined Et_2O extracts were dried and evaporated and the residue was Kugelrohr distilled at 75–80 °C (0.02 mm) to give 1.18 g (58%) of L-**12**: $[\alpha]^{20}{}_D$ −53.3° (*c* 0.52, CH_3OH); NMR δ 1.52 (s, 6 H), 3.85 (s, 3 H), 4.85 (s, 2 H), 8.53 (br s, 1 H). Anal. ($C_8H_{12}O_6$) C, H.

In analogous fashion, dimethyl 2,3-*O*-isopropylidene-D-tartrate (D-**11**, 46 g, 211 mmol) gave 27 g (63%) of methyl hydrogen 2,3-*O*-isopropylidene-D-tartrate (D-**12**): $[\alpha]_D$ +51.1° (*c* 0.54, CH_3OH); NMR and IR of D-**12** are identical with those of L-**12**. Anal. ($C_8H_{12}O_6$) C, H.

Methyl 2,3-*O*-Isopropylidene-L- (and D-) threonate (L-13 and D-13). To a solution of methyl hydrogen 2,3-*O*-isopropylidene-L-tartrate (L-**12**, 4.5 g, 22 mmol) in THF (65 mL) at 0 °C was added over 10 min a 1 M solution of BH_3 in THF (33 mL, 33 mmol). The bath was removed, the reaction mixture was stirred at room temperature for 24 h and evaporated, and the residue was distributed between H_2O (40 mL) and Et_2O (4 × 150 mL), with the H_2O portion being saturated with NaCl. The combined Et_2O extracts were washed with 0.5 M $NaHCO_3$ (30 mL) and saturated NaCl (30 mL), dried, and evaporated to a residue which was Kugelrohr distilled at 80–85 °C (0.1 mm) giving 1.85 g (44%) of distillate: $[\alpha]^{20}{}_D$ −19.2° (*c* 0.55, CH_3OH); NMR δ 1.47 (s, 3 H), 1.50 (s, 3 H), 2.03 (br s, 1 H), 3.82 (s, 3 H), 3.77–4.33 (m, 2 H), 4.40 (t, 2 H, J = 7 Hz). Anal. ($C_8H_{14}O_5$) C, H.

In an analogous fashion, methyl hydrogen 2,3-*O*-isopropylidene-D-tartrate (D-**12**, 25.5 g, 125 mmol) gave, after 9 h of stirring at room temperature, 12.5 g (53%) of methyl 2,3-*O*-isopropylidene-D-threonate (D-**13**): $[\alpha]^{20}{}_D$ +18.7° (*c* 0.70, CH_3OH); NMR and IR of D-**13** are identical with those of L-**13**. Anal. ($C_8H_{14}O_5$) C, H.

Methyl 4-*O*-Methanesulfonyl-2,3-*O*-isopropylidene-L- (and D-) threonate (L-14 and D-14). To a solution of methyl 2,3-*O*-isopropylidene-L-threonate (L-**13**, 352 mg, 1.85 mmol) and Et_3N (281 mg, 2.8 mmol) in CH_2Cl_2 (10 mL) at −10° was added over 5 min, methanesulfonyl chloride (172 μL, 255 mg, 2.22 mmol). The reaction mixture was stirred at this temperature an additional 10 min, at which time GC analysis (170 °C; relative retention times 1.0 (L-**13**), 3.23 (L-**14**)) indicated complete reaction. The reaction mixture was diluted with ice-cold CH_2Cl_2 (80 mL) and washed successively with ice-cold H_2O (8 mL), 2 M HCl (8 mL), 0.5 M $NaHCO_3$ (8 mL), and saturated aqueous NaCl (8 mL). The CH_2Cl_2 was dried and evaporated and the residue was kugelrohr distilled at 100 °C (0.03 mm) to give 464 mg (94%) of L-**14**: $[\alpha]^{20}{}_D$ −25.7° (*c* 0.82, acetone); NMR δ 1.47 (s, 3 H), 1.50 (s, 3 H), 3.10 (s, 3 H), 3.83 (s, 3 H), 4.45 (s, 4 H); IR (neat) ν_{max} 1175 and 1350 (s, sulfonate ester), 1740 and 1760 cm^{-1} (s, CO_2CH_3); mass spectrum (rel intensity) *m/e* 254 ($M^+ - CH_2$, 10), 253 ($M^+ - CH_3$, 100), 209 ($M^+ - CO_2CH_3$, 20), 151 ($M^+ - CO_2CH_3 - (CH_3)_2CO$, 56), 115 ($M^+ - (CH_3)_2CO_2 - CH_3SO_2$, 49), 79 ($CH_3SO_2$, 85), 73 ($(CH_3)_2CO_2$, 78), 59 ($(CH_3)_2COH$, 93), 43 ($C_3H_7$, 100). Anal. ($C_9H_{16}O_7S$) C, H.

In analogous fashion, methyl 2,3-*O*-isopropylidene-D-threonate (D-**13**, 11.9 g, 63 mmol) gave 16.5 g (99%) of methyl 4-*O*-methanesulfonyl-2,3-*O*-isopropylidene-D-threonate (D-**14**): $[\alpha]^{20}{}_D$ +26.3° (*c* 1.2, acetone); NMR and IR of D-**14** are identical with those of L-**14**. Anal. ($C_9H_{16}O_7S$) C, H.

Methyl 4-Azido-4-deoxy-2,3-*O*-isopropylidene-L- (and D-) threonate (L-15 and D-15). A mixture of L-**14** (2.13 g, 7.9 mmol) and activated sodium azide (778 mg, 12 mmol) in DMF (20 mL) was heated at 100 °C for 50 min, at which time GC analysis (170 °C; relative retention times 1.0 (L-**14**), 0.41 (L-**15**)) indicated complete reaction. The cooled reaction mixture was distributed between H_2O (100 mL) and Et_2O (3 × 200 mL) and the combined Et_2O extracts were washed with saturated aqueous NaCl (50 mL), dried, and evaporated. The residue was chromatographed on silica gel (90 g) with CH_2Cl_2 as the eluent and the azide was kugelrohr distilled at 70 °C (0.25 mm) to give 1.48 g (87%) of L-**15**: $[\alpha]^{20}{}_D$ −96.3° (*c* 0.49, CH_3OH); NMR δ 1.45 (s, 3 H), 1.52 (s, 3 H), 3.18–3.85 (m, 2 H), 3.82 (s, 3 H), 4.27–4.53 (m, 2 H); IR (neat) ν_{max} 1735 and 1765 (s, CO_2CH_3), 2105 cm^{-1} (s, N_3). Anal. ($C_8H_{13}N_3O_4$) C, H, N.

In analogous fashion, D-**14** (15.4 g, 57 mmol) afforded 10.9 g (88%) of distilled methyl 4-azido-4-deoxy-2,3-*O*-isopropylidene-D-threonate (D-**15**): $[\alpha]^{20}{}_D$ +94.7° (*c* 0.59, CH_3OH); NMR and IR of D-**15** are identical with those of the L-**15**. Anal. ($C_8H_{13}N_3O_4$) C, H, N.

4-Azido-4-deoxy-2,3-*O*-isopropylidene-L- (and D-) threonic Acid (L-16 and D-16). To a solution of L-**15** (1.07 g, 5 mmol) in CH_3OH (10 mL) was added a 2 M solution of KOH in CH_3OH (2.5 mL, 5 mmol) over 1 h at room temperature. The reaction mixture was evaporated and the residue was distributed between H_2O (25 mL) and Et_2O (2 × 50 mL); evaporation of the ether extracts gave 91 mg (8.5%) of recovered L-**15**. The aqueous portion was acidified to pH 3 with 2 M HCl, saturated with NaCl, and extracted with Et_2O (5 × 50 mL). The combined Et_2O extracts were dried and evaporated to give 943 mg (94%) of an oil which slowly solidified: mp 49–52 °C; $[\alpha]^{20}{}_D$ −98.5° (*c* 0.40, CH_3OH); NMR δ 1.47 (s, 3 H), 1.55 (s, 3 H). 3.20–3.90 (AB part of δ_A 3.72 and δ_B 3.38, J_{AB} = 14 Hz, $\Delta\nu_{AB}$ = 20.7 Hz, J_{AX} = 2 Hz, J_{BX} = 3 Hz, 2 H), 4.20–4.60 (m, 2 H), 9.35 (br s, 1 H); IR ν_{max} 1725 (s, CO_2H), 2105 cm^{-1} (s, N_3). Anal.

($C_7H_{11}N_3O_4$) C, H, N.

In analogous fashion, D-**15** (3.66 g, 17 mmol) was hydrolyzed to give 3.21 g (94%) of pure 4-azido-4-deoxy-2,3-*O*-isopropylidene-D-threonic acid (D-**16**): mp 47-52 °C; $[\alpha]^{20}{}_D$ +95.4° (*c* 0.45, CH_3OH); NMR and IR of D-**16** are identical with those of the L-**16**. Anal. ($C_7H_{11}N_3O_4$) C, H, N.

4-Amino-4-deoxy-2,3-*O*-isopropylidene-L- (and D-) threonic Acid (L-17 and D-17). L-**16** (1.41 g, 7 mmol) was hydrogenated in the presence of 10% Pd/C (147 mg) in CH_3OH (150 mL) at room temperature and a hydrogen pressure of 28 psi. After 6 h, the catalyst was removed by filtration and washed with H_2O (25 mL) and the combined filtrates were evaporated. The residue was crystallized by dissolving the solid in hot H_2O (4 mL), concentrating the solution in vacuo at 50 °C until crystals began to appear, and diluting the warm suspension with 2-propanol (15 mL) to give 1.12 g (91%) of the amino acid L-**17**: mp 225-226 °C dec; $[\alpha]^{20}{}_D$ +20.5° (*c* 1.2, acetone/H_2O, 60:40); NMR (D_2O) δ 1.47 (s, 6 H), 3.20-3.37 (m, 2 H), 4.13-4.30 (m, 2 H). Anal. ($C_7H_{13}NO_4$) C, H, N.

In analogous fashion, hydrogenation of D-**16** (3.1 g, 15.5 mmol) yielded, after 4.5 h, 2.20 g (81%) of D-**17**: mp 231-233 °C dec; $[\alpha]^{20}{}_D$ −21.7° (*c* 1.04, acetone/H_2O, 60:40); NMR spectrum of D-**17** is identical with that of the L-**17**. Amino acid analysis: two peaks with retention times 82.5 min, corresponding to D-**17**, and 138.5 min, corresponding to **5** from hydrolysis of D-**17** during the amino acid analysis. Anal. ($C_7H_{13}NO_4$) C, H, N.

4-Azido-4-deoxy-L-threonic Acid (18). A solution of L-**16** (100 mg, 0.5 mmol) in 0.1 M HCl (5 mL) was stirred at room temperature for 26 h. The reaction mixture was evaporated and the residue was chromatographed on silica gel (2 g) with benzene/CH_3OH/HOAc (45:8:4) as the eluent to give 66 mg (82%) of **18**: NMR (acetone-d_6) δ 3.40-3.52 (d, 2 H, J = 7 Hz), 3.97-4.47 (m, 2 H), 6.07 (br s, exchangeable with D_2O). Anal. ($C_4H_7N_3O_4$) C, H, N.

4-Amino-4-deoxy-D-threonic Acid (5). A solution of D-**17** (1.74 g, 10 mmol) in 1 M HCl (40 mL, 40 mmol) was heated under reflux for 1.5 h and evaporated and the residue desalted by ion exchange. Crystallization of the residue was effected by dissolving it in hot H_2O (7 mL) and slow dilution with 2-propanol (15 mL) while continuing to heat the mixture on the steam bath. After addition of the 2-propanol, the suspension was allowed to cool to room temperature, yielding 1.24 g (92%) of the amino acid: mp 227-230 °C dec; $[\alpha]^{20}{}_D$ +14.5° (*c* 0.72, 1 M HCl), $[\alpha]_D$ −4.8° (*c* 1.44, H_2O); NMR (D_2O) δ 3.07-3.22 (m, appearing as two doublets with downfield signal of each doublet coinciding with J = 5, 8 Hz, 2 H), 3.93-4.32 (m, 2 H). By amino acid analysis, the product was homogeneous, had a retention time of 138.5 min, and had a retention time relative to the D-erythro isomer **3** of 1.18. Anal. ($C_4H_9NO_4$) C, H, N.

Methyl L-Tartramate. Using the reaction[8] and isolation[15] procedures described, dimethyl L-tartrate (L-**10**, 82.4 g, 463 mmol) was converted to 25.9 g (34%) of methyl L-tartramate: mp 135-138 °C (lit. mp 136.5-140,[15] 132-135 °C[33]); $[\alpha]^{20}{}_D$ +63.5° (*c* 1.15, H_2O) (lit. $[\alpha]^{22.5}{}_D$ +62.9° (*c* 1.21, H_2O),[15] $[\alpha]_D$ +57.8° (H_2O)[33]).

Methyl 2,3-*O*-Diacetyl-L-tartramate. Using a procedure similar to that described,[8] methyl L-tartramate (8.16 g, 50 mmol) was converted to methyl 2,3-*O*-diacetyl-L-tartramate. Following the second filtration, the filtrate was evaporated and the residue was dissolved in boiling CH_3OH (50 mL). The solution was diluted with Et_2O (100 mL) at the boiling point, and the crystals deposited upon cooling were collected to give 8.6 g. The mother liquor yielded a second crop of 951 mg and total yield was 9.5 g (77%): mp 150-152 °C (lit.[9] mp 147-148 °C); $[\alpha]^{20}{}_D$ −33.9° (*c* 1.0, CH_3OH); NMR δ 2.12 (s, 3 H), 2.15 (s, 3 H), 3.73 (s, 3 H), 5.30-6.30 (br s, 2 H), 5.50-5.70 (AB q, δ_A 5.68, δ_B 5.52, J_{AB} = 2 Hz, $\Delta\nu_{AB}$ = 9.8 Hz, 2 H).

Methyl L-*threo*-3-Cyano-2,3-diacetoxypropionate (Methyl 3-Cyano-2(*R*),3(*S*)-diacetoxypropionate) (19). Using the described[9] procedure, methyl 2,3-*O*-diacetyl-L-tartramate (17.3 g, 70 mmol) was converted to **19**. Recrystallization from petroleum ether/benzene (2:1) and sublimation at 72 °C (0.2 mm) gave 10.2 g (64%) of **19**: mp 73-75 °C (lit.[9] mp 74-75.5 °C); $[\alpha]^{20}{}_D$ +46.3° (*c* 0.99, CH_3OH); NMR δ 2.17 (s, 3 H), 2.25 (s, 3 H), 3.80 (s, 3 H), 5.45-5.87 (AB q, δ_A 5.84, δ_B 5.48, J_{AB} = 3.5 Hz, $\Delta\nu_{AB}$ = 21.7 Hz). Anal. ($C_9H_{11}NO_6$) C, H, N.

4-Amino-4-deoxy-L-threonic Acid (4). A. By Hydrolysis of 17. A solution of L-**17** (727 mg, 4.2 mmol) in 1 M HCl (15 mL) was heated under reflux for 2 h and then evaporated and the residue was subjected to ion-exchange desalting. The resulting solid was dissolved in H_2O (6 mL) and filtered with charcoal, the filtrate was evaporated, and the residue was crystallized from H_2O/2-propanol (2:1) to give 440 mg (78%) of **4**: mp 221-225 °C dec (lit. mp 221-222 °C dec,[8] 222-224 °C dec[9]; $[\alpha]^{20}{}_D$ +4.6° (*c* 0.64, H_2O) (lit. $[\alpha]^{27}{}_D$ +42° (H_2O),[8] $[\alpha]^{25}{}_D$ +43° (*c* 0.7, H_2O)[9]), $[\alpha]^{20}{}_D$ −14.6° (*c* 0.68, 1 M HCl); NMR δ 3.05-3.20 (m, appearing as 2d, J = 5, 8 Hz, with downfield signal of each doublet coinciding, 2 H), 3.83-4.30 (m, 2 H); mass spectrum (rel intensity) *m/e* 117 ($M^+ - 2H_2O - CO$, 9), 60 ($M^+ - CO_2 - CH_2NH_3$, 29), 44 (CO_2, 46), 30 (CH_2O, CH_2NH_2, 100). Anal. ($C_4H_9NO_4$) C, H, N. Amino acid analysis: one peak, retention time 138.5 min.

When the hydrolysis of L-**17** was conducted in 0.1 M HCl at 20 °C for 26 h, an 85% yield of **4** was obtained, identical in all respects with the product from hydrolysis in refluxing 1 M HCl.

B. By Reduction of 18. 4-Azido-4-deoxy-L-threonic acid (**18**, 53 mg, 0.33 mmol) in CH_3OH (10 mL) containing 1 M HCl (0.37 mL) was hydrogenated in the presence of 10% Pd/C (7 mg) at room temperature and 27-psi hydrogen pressure. After 4 h, the catalyst was removed by filtration, the filtrate was evaporated, and the residue was subjected to ion-exchange desalting to give 35.0 mg (79%) of **4**, identical with the material prepared by hydrolysis of L-**17**, above.

C. From 19. Hydrogenation with Raney Cobalt Catalyst. Using a procedure similar to that described,[9] **19** (2.17 g, 9.5 mmol) was hydrogenated in CH_3OH (33 mL) at 70 °C for 6 h in the presence of Raney Co[16] (1.04 g) at a hydrogen pressure of 88 atm. The reaction mixture was filtered, the filtrate was evaporated, and the residue was boiled in 6 M HCl (13 mL) under reflux for 2.5 h. Removal of the solvent gave 1.56 g of crude hydrochloride. A 162-mg portion was subjected to ion-exchange desalting and gave 87 mg of a mixture. Amino acid analysis established the presence of two amino acids, **4**, with a retention time of 138 min, and 4-amino-2-hydroxybutyric acid (**21**), with a retention time of 144.5 min (in the ratio of 25:1, respectively, Table II).

To 1.40 g of the crude hydrochloride dissolved in CH_3OH (12 mL) was added pyridine (0.7 mL). The precipitate was dissolved in H_2O (7 mL), treated with charcoal, and filtered through Celite and the filtrate evaporated. Chromatography on silica gel (30 g) with $CH_3OH/CHCl_3$/concentrated NH_3 (6:2:1) as the eluent and crystallization from H_2O/2-propanol gave 404 mg (32%) of **4** identical in all respects with material prepared above. The most efficient purification procedure was to desalt the crude hydrochloride, treat with charcoal, and crystallize from H_2O/2-propanol. This gave pure **4** in 47% yield.

D. From 19. Hydrogenation with Platinum Catalyst. The nitrile **19** (470 mg, 2.1 mmol) was hydrogenated in glacial acetic acid (6 mL) containing concentrated H_2SO_4 (109 mg) in the presence of PtO_2 (51 mg) at 20 °C at a hydrogen pressure of 36 psi for 20 h. At this time, the catalyst had agglutinated, additional PtO_2 (50 mg) was added, and the hydrogenation was continued for another 48 h. The mixture was filtered, the filtrate was evaporated, the residue was dissolved in 6 M HCl, and the solution was heated under reflux for 2.5 h. Evaporation and ion-exchange desalting gave 223 mg of a mixture of three amino acids (**4, 21,** and **22**) having R_f values of 0.55, 0.42, and 0.32 on silica gel TLC ($CH_3OH/CHCl_3$/concentrated NH_3, 6:2:1, developed twice) (Table II).

Alternatively, we followed the described procedure[8] for hydrogenation of **19** in glacial acetic acid containing concentrated H_2SO_4 in the presence of PtO_2 at 70 °C and 1000-psi hydrogen pressure. Isolation through the lead acetate step, hydrolysis in 6 M HCl, and ion-exchange desalting gave a mixture of three amino acids: **4, 21,** and **22.** When the prescribed[8] silver oxide desalting step was used, a fourth amino acid, glycine (**23**), was also formed; **4** was present in 9% yield in this mixture of four amino acids (Table II).

4-Amino-4-deoxy-D-erythronic Acid Betaine Hydrochloride (6). A mixture of **3** (541 mg, 4 mmol) and **2**[18] (3.8 g, 24 mmol) in CH_3OH (16 mL) was heated under reflux for 3.5 h, the mixture was evaporated, and the residue was distributed between H_2O (10 mL) and $CHCl_3$/2-propanol (9:1, 3 × 35 mL). The aqueous portion was evaporated and ion-exchange chromatography gave 382 mg (45%) of a solid hydrochloride. Recrystallization was effected by dissolving this hydrochloride in hot H_2O (1 mL) and diluting the solution successively with 2-propanol (5 mL) and acetone (10 mL) while continuing to heat the mixture on the steam bath. After the addition of the acetone, the cloudy mixture was allowed to cool to room temperature and further diluted with Et_2O (10 mL). After the mixture was allowed to stand at 0 °C for 36 h, the crystals were collected and dried to give 369 mg (43%) of the betaine hydrochloride: mp 175-180 °C

dec; $[\alpha]_D$ +17.6° (*c* 0.28, H_2O); NMR (D_2O) δ 3.25 (s, 9 H), 3.52–3.60 (m, appearing as 2d with upfield peak of each doublet coinciding, *J* = 4, 6 Hz, 2 H), 4.33–4.60 (m, 2 H); IR (KBr) ν_{max} 1715 cm^{-1}. Anal. ($C_7H_{16}NO_4Cl$) C, H, N.

4-Amino-4-deoxy-L-threonic Acid Betaine Hydrochloride (7). Treatment of 4-amino-4-deoxy-L-threonic acid (**4**, 406 mg, 3 mmol) with isourea **2**[18] (1.85 g, 11.7 mmol) in CH_3OH (12 mL) under reflux for 4.5 h and isolation as described above gave, following ion-exchange chromatography, 182 mg (28.3%) of a crystalline solid. Recrystallization of 157 mg was accomplished by dissolving the solid in hot H_2O (0.5 mL) and diluting the solution successively with 2-propanol (2.5 mL) and acetone (5 mL) while continuing to heat the mixture on the steam bath. After the acetone addition, the cloudy suspension was allowed to cool to room temperature and further diluted with Et_2O (5 mL). The precipitated white crystals were dried to give 144 mg of the betaine hydrochloride **7**: mp 213–216 °C dec (lit.[1] mp 210–214 °C dec for anthopleurine); $[\alpha]^{20}{}_D$ −19.4° (*c* 0.41, H_2O), $[\alpha]^{20}{}_D$ −28.5° (*c* 0.16, CH_3OH) (lit.[1] $[\alpha]^{16}{}_D$ +48° (*c* 0.14) for anthopleurine); IR (KBr) ν_{max} 1730 cm^{-1}; NMR (6% DCl in D_2O) δ 3.27 (s, 9 H), 3.53–3.65 (m appearing as 2d with downfield signal of each doublet coinciding, *J* = 5, 7 Hz, 2 H), 4.42 (d, *J* = 2 Hz, 1 H), 4.53–4.80 (m, 1 H), identical with the 60-MHz spectrum of anthopleurine by direct comparison.[27] Anal. ($C_7H_{16}NO_4Cl$) C, H, N.

When betaine hydrochloride **7** was desalted and resubjected to the isourea and isolation procedures, it was recovered with identical properties.

4-Amino-4-deoxy-D-threonic Acid Betaine Hydrochloride (8). A solution of 4-amino-4-deoxy-D-threonic acid (**5**, 101 mg, 0.75 mmol) and isourea **2**[18] (481 mg, 3 mmol) in H_2O (1 mL) and CH_3OH (2 mL) was stirred at room temperature in a stoppered flask for 11 days. Isolation as described above gave, following ion-exchange chromatography, 81.0 mg (50%) of hydrochloride which was recrystallized, as described for **7** using H_2O (0.2 mL), 2-propanol (1.1 mL), acetone (2.2 mL), and Et_2O (2.2 mL), giving 67 mg (42%) of the betaine hydrochloride: mp 212–215 °C dec; $[\alpha]^{20}{}_D$ +18.5° (*c* 0.30, H_2O), +21.4° (*c* 0.28, 1 M HCl), +28.0° (*c* 0.14, CH_3OH); IR and NMR identical with those of **7**. Anal. ($C_7H_{16}NO_4Cl$) C, H, N.

Silver Oxide Treatment of 4-Amino-4-deoxy-D-threonic Acid Hydrochloride (5 HCl). A solution of **5** (102 mg, 0.76 mmol) in 1 M HCl (3.8 ml) was allowed to stand at room temperature for 15 min and then evaporated to give 30 mg of the hydrochloride which was dissolved in H_2O (5 mL) and divided into two equal portions. To one portion was added Ag_2O (130 mg, 0.56 mmol) and the mixture was heated on a steam bath for 15 min and filtered. The filtrate was saturated with H_2S and filtered and the filtrate evaporated to give 35.0 mg of a tan solid. The second portion of the hydrochloride salt was treated in the same way, except that heating was continued for 1.5 h. Isolation as described above gave 25 mg of a tan solid. Both solids contained 7–8% of glycine (**23**).

Similar silver oxide treatment of **4-amino-4-deoxy-D-erythronic acid hydrochloride (3 HCl)** led to the formation of ~10% glycine (see Table II).

D-Threono-1,4-lactone (26). 4-Amino-4-deoxy-D-threonic acid betaine (**25**) was obtained by ion-exchange desalting of betaine hydrochloride **8**: mp 200.5–212 °C dec; $[\alpha]^{20}{}_D$ +11.7° (*c* 0.15, H_2O). When **25** was heated at 188 °C for 1 h and 210 °C for 3 h, an oil distilled to the cold-finger condenser in 66% yield. Redistillation gave material identical (except rotation) with authentic L-threono-1,4-lactone,[25] and it was concluded to be the enantiomorph, D-threono-1,4-lactone (**26**). Similar pyrolysis of the betaine hydrochloride **8** led to **26** but in poorer yield.

Acknowledgment is made to Dr. C. Hand of the Bodega Marine Laboratory for use of the laboratory facilities, to J. Allen for assistance in collecting sea anemones, and to T. F. Buckley for assistance in biological testing of the anemones. We thank Dr. N. R. Howe for his cooperation.

References and Notes

(1) N. R. Howe and Y. M. Sheikh, *Science*, **189**, 386 (1975).
(2) N. R. Howe, *J. Comp. Physiol. A.*, **107**, 67 (1976).
(3) N. R. Howe, *J. Exp. Biol.*, **65**, 147 (1976).
(4) J. A. Musich and H. Rapoport, *J. Org. Chem.*, **42**, 139 (1977).
(5) T. Kaneko and R. Yoshida, *Bull. Chem. Soc. Jpn.*, **35**, 1153 (1962).
(6) M. Tomita and Y. Sendju, *Hoppe-Seyler's Z. Physiol. Chem.*, **169**, 263 (1927).
(7) I. Chibata, K. Okumura, S. Takeyama, and K. Kotera, *Experientia*, **25**, 1237 (1969).
(8) A. Yokao and S. Akutagawa, *Bull. Chem. Soc. Jpn.*, **35**, 644 (1962).
(9) N. Takamura, N. Taga, T. Kanno, and M. Kawazu, *J. Org. Chem.*, **38**, 2891 (1973).
(10) R. Barker and D. L. MacDonald, *J. Am. Chem. Soc.*, **82**, 2301 (1960).
(11) D. L. Mitchell, *Can. J. Chem.*, **41**, 214 (1963).
(12) (a) S. Hanessian, *J. Org. Chem.*, **34**, 675 (1969); (b) S. Hanessian and T. H. Haskell, *J. Heterocycl. Chem.*, 1, 57 (1964).
(13) P. W. Feit, *J. Med. Chem.*, 7, 14 (1964).
(14) Formation of the acetonide of diethyl L-tartrate according to the procedure of Feit[13] was first investigated. A modified version of this procedure was then used to prepare L-11 (see Experimental Section).
(15) F. Nerdel and E. Henkel, *Chem. Ber.*, **85**, 1138 (1952).
(16) (a) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, N.Y., 1967, p 977; (b) R. Billica and H. Adkins, "Organic Syntheses", Collect Vol. III, Wiley, New York, N.Y., 1955, p 176.
(17) K. G. A. Busch, J. W. Clark, L. B. Genung, E. F. Schroeder, and W. L. Evans, *J. Org. Chem.*, 1, 1 (1936).
(18) E. Schmidt and F. Moosmuller, *Justus Liebigs Ann. Chem.*, **597**, 235 (1955).
(19) R. Willstatter, *Chem. Ber.*, **35**, 584 (1902).
(20) H. E. Carter and P. K. Bhattacharyya, *J. Am. Chem. Soc.*, **75**, 2503 (1953).
(21) L. Francis, *Biol. Bull.* (*Woods Hole, Mass.*), **144**, 64 (1973).
(22) (a) R. Krimberg, *Biochem. Z.*, **296**, 261 (1938); (b) W. Keil, W. Linneweh, and K. Poller, *Z. Biol.*, **86**, 187 (1927).
(23) N. R. Howe, personal communication, claims adding the test solution in one portion while stirring the water in the bowl yields optimum response.
(24) K. Gatzi and T. Reichstein, *Helv. Chim. Acta*, **20**, 1298 (1937).
(25) G. Nakaminami, H. Edo, and M. Nakagawa, *Bull. Chem. Soc. Jpn.*, **46**, 266 (1973).
(26) J. M. Perel and P. G. Dayton, *J. Org. Chem.*, **25**, 2044 (1960).
(27) N. R. Howe, personal communication.
(28) R. Schaffer, *J. Res. Natl. Bur. Stand. A.*, **65**, 507 (1961).
(29) T. M. Lowry and H. H. Abram, *J. Chem. Soc.*, **107**, 1187 (1915).
(30) Y. Tsuzuki, *Bull. Chem. Soc. Jpn.*, 11, 362 (1936).
(31) Y. Tsuzuki, *Bull. Chem. Soc. Jpn.*, **10**, 255 (1935).
(32) T. M. Lowry and J. O. Cutler, *J. Chem. Soc.*, **121**, 532 (1922).
(33) K. Mislow and C. Hamermesh, *J. Am. Chem. Soc.*, **77**, 1590 (1955).