more recent approximate methods have put it at $pK_E = 7.2 \pm 0.9$[2a] and 8.5 ± 0.3.[1]

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Total Synthesis of Bongkrekic Acid

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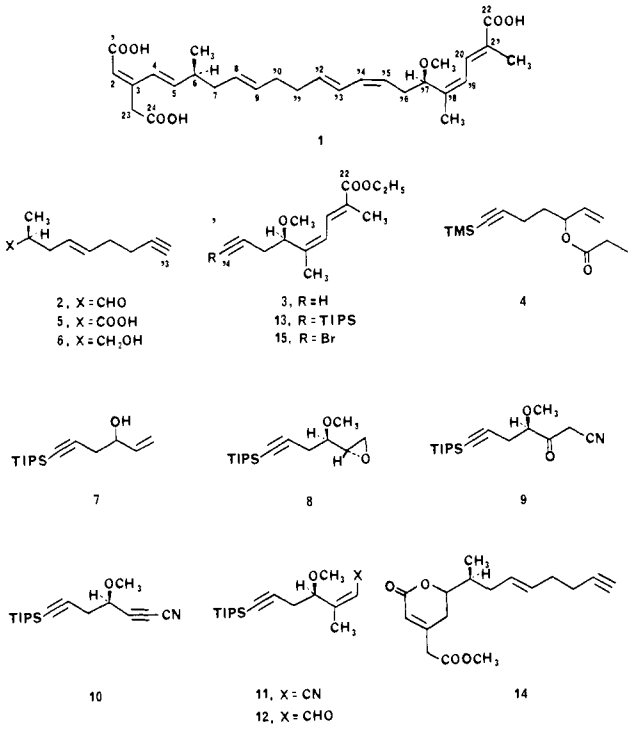
Adenosine triphosphate (ATP) from the oxidative phosphorylation of ADP in mitochondria can power eukaryotic cells only with the help of an ATP/ADP translocator protein that resides in the mitochondrial inner membrane.[1] Bongkrekic acid (**1**),[2,3]



a toxin produced by the microorganism *Pseudomonas cocovenenans*, has a sufficiently high affinity for the translocator on the inside of this membrane to block effectively the export of ATP.[1b] Reported herein is the first chemical synthesis of **1**, a substance only difficultly available by fermentation, which serves as a useful biochemical reagent and which is of interest also from a structural and biosynthetic point of view.

Retrosynthetic analysis led to the recognition of β-methylglutaconate (a possible biosynthetic component[4]), acetylenic aldehyde **2**, and acetylenic ester **3** as potentially useful synthetic precursors of **1**. The synthesis of **2**, corresponding to the C(5)–C(13) segment, was carried out as follows. 5-(Trimethylsilyl)(Me$_3$Si))-4-pentynal[5] and vinylmagnesium bromide in tetrahydrofuran (THF) at −78 °C gave a vinylcarbinol which was acylated with propionyl chloride–pyridine in methylene chloride at 0 °C to form **4** (80% overall). Propionate **4** was transformed into the acetylenic acid **5** (95% overall) by Claisen rearrangement of the *tert*-butyldimethylsilyl enol ether[6] at 50 °C followed by desilylation with 48% aqueous hydrofluoric acid in acetonitrile at 23 °C. The acid **5** was resolved as the ester with (*S*)-(+)-methyl mandelate by preparative HPLC on silica gel. The less polar diastereomer was reduced to the *l*-alcohol **6**, $[\alpha]^{23}_D$ −3.4° (*c* 3, $CHCl_3$), which was oxidized by 1.4 equiv of pyridinium chlorochromate in methylene chloride in the presence of neutral alumina at 23 °C for 1.5 h to the aldehyde **2**, $[\alpha]^{23}_D$ +16.2° (*c* 3.2, $CHCl_3$).[7]

The vinylcarbinol **7**, obtained by reaction of lithiated 1-(triisopropylsilyl (TIPS))propyne[8] with acrolein, was subjected to Sharpless epoxidation with kinetic resolution (1.0 equiv of titanium tetraisopropoxide, 1.1 equiv of diisopropyl D-(−)-tartrate and 1.5 equiv of *tert*-butylhydroperoxide at −20 °C for 16 h) to afford after chromatography on silica gel 80% (theoretical yield, 40% wt yield) of hydroxy epoxide of >90% optical purity,[9] $[\alpha]^{23}_D$ +26.2° (*c* 1.3, EtOH), which upon treatment with sodium hydride–methyl iodide in THF at 20 ° yields 96% of *erythro*-methyl[9] epoxy ether **8**, $[\alpha]^{23}_D$ +21.5° (*c* 1.3, EtOH). Reaction of **8** with 5 equiv. of sodium cyanide in ethanol at 35 °C for 10 h resulted in S_N2 displacement at methylene to form a single cyanohydrin (81% yield), which upon oxidation with diisopropylcarbodiimide (1.5 equiv)–excess dimethyl sulfoxide (Me$_2$SO)–dichloroacetic acid (0.5 equiv) at 0 °C for 0.5 h produced the keto nitrile **9** (93% yield), $[\alpha]^{23}_D$ +42.7° (c 3.3, $CHCl_3$). The acetylene **10** was prepared from **9** by a novel two-step procedure: (1) reaction with sodium hydride at 23 °C in ether followed by 1.5 equiv of triflic anhydride (0 °C, 10 min) to form the enol triflate, (2) elimination (sodium hydride–ether–Me$_2$SO) at 0 °C for 0.5 h, overall yield of **10**, 65%, $[\alpha]^{23}_D$ −60.5° (*c* 2, $CHCl_3$). Reaction of **10** with 2 equiv of dimethylcopperlithium in THF at −78 °C for 10 min followed by quenching (−78 °C) and isolation gave stereospecifically the (*Z*)-α,β-olefinic nitrile **11**, $[\alpha]^{23}_D$ +118° (*c* 3, $CHCl_3$) (86%), which upon treatment with 1 equiv of diisobutylaluminum hydride in methylene chloride at −78 °C for 5 min afforded the *Z* aldehyde **12**, $[\alpha]^{23}_D$ +73° (*c* 2.2, $CHCl_3$) (88%). Condensation of **12** with ethyl 2-triphenylphosphoranylidenepropionate in THF at 23 °C for 4 h produced **13**, $[\alpha]^{23}_D$ +125.5° (*c* 2, $CHCl_3$) (96%) which was desilylated (1.2 equiv of tetrabutylammonium fluoride in THF at 23° for 1 hr) to form **3**, $[\alpha]^{23}_D$ +32° (*c* 2, $CHCl_3$) (99%).

Elaboration of the aldehyde **2** to a predecessor of the C(1)–C(13) segment was accomplished by using a new method based

(1) (a) Klingenberg, M. In "The Enzymes of Biological Membranes: Membrane Transport"; Martonosi, A. N., Ed.; Plenum Press: New York, 1976; Vol. 3, (b) Klingenberg, M. *Trends Biochem. Sci.* **1979**, *4*, 249.

(2) Structure: de Bruijn, J.; Frost, D. J.; Nugteren, D. H.; Gaudemer, A.; Lijmbach, G. W. M.; Cox, H. C.; Berends, W. *Tetrahedron* **1973**, *29*, 1541.

(3) Absolute configuration: Zyblir, J.; Gaudemer, F.; Gaudemer, A. *Experientia* **1973**, *29*, 648.

(4) Although the mode of biosynthesis of **1** has not been demonstrated, it is surmised that the two end segments, C(1)–C(4) and C(19)–C(22), originate from β-methylglutaconate (or equivalent) with C(5)–C(6) and C(17)–C(18) deriving from propionate and C(8)–C(16) deriving from five acetate units. In this scheme one carboxyl from β-methylglutaconate must be lost, leaving C(22) as terminal.

(5) Obtained from 4-pentyn-1-ol in 64% overall yield by the sequence (1) reaction with 2 equiv of *n*-butyllithium and then 2 equiv of Me$_3$SiCl, (2) O-desilylation with aqueous acid–THF, (3) oxidation with pyridinium chlorochromate in methylene chloride.

(6) Ireland, R. E.; Mueller, R. H.; Willard, A. K. *J. Am. Chem. Soc.* **1976**, *98*, 2868.

(7) The alcohol **6** obtained either directly from the mandelate ester or from the aldehyde **2** by reduction with sodium borohydride was shown to be of >90% optical purity by PMR analysis of the (−)-α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA) ester: Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543. The configurational assignment for **6** was based on conversion to the ditrityl derivative[3] of 2-methyl-1,4-butanediol.

(8) Corey, E. J.; Rücker, Ch. *Tetrahedron Lett.* **1982**, *23*, 719.

(9) See: Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237 for procedure and analytical method.

(10) Corey, E. J.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1969**, *91*, 1851.

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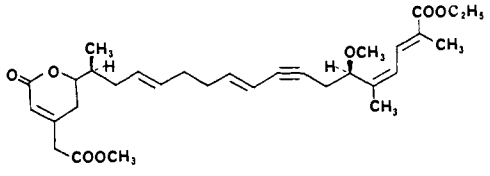
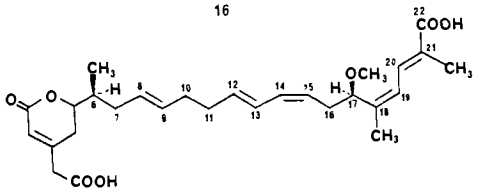
partly on biogenetic considerations. The aldehyde **2** was added to the dilithio derivative of dimethyl 3-methylglutaconate[11] (1.5 equiv) in THF at −40 °C initially and then at −40–0 °C for 20 min to give after extractive workup and chromatography on silica gel 60% of **14** as a mixture of *S,S* and *R,S* diastereomers. The coupling of **14** with the C(14)–C(22) precursor, bromo acetylene **15** (formed from **3** in 96% yield by sequential treatment with 1 equiv of silver trifluoroacetate and 1.2 equiv of triethylamine in methylene chloride at 20 °C and 1 equiv of bromine at −78 °C), to give enyne **16**, $[\alpha]^{23}_D$ +56° (*c* 2.2, $CHCl_3$), was effected in 78%



16



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yield by the sequence: (1) hydroboration of **14** with 1.1 equiv of disiamylborane in THF at 0 °C for 2 h, (2) addition to 3.4 equiv of sodium methoxide in THF at 20 °C (10 min) and conversion to a mixed cuprate with 1.2 equiv of cuprous cyanide in THF at −20 °C for 2 h, (3) reaction with **15** for 8 h at −20 °C followed by quenching with ammonia–ammonium chloride, extraction with ether, treatment of the extract with excess acetic acid at 23 °C for 10 min,[12] and chromatographic purification on silica gel. Lindlar reduction of the triple bond of **16** gave the corresponding *cis*-olefin (73%) along with some overreduction product. Saponification of this *cis*-olefin with 10 equiv of tetra-*n*-butylammonium hydroxide in 1:1 methanol–water at 23 °C for 30 min afforded after acidification diacid **17**, which was directly treated with potassium methoxide (20 equiv) in 9:1 THF–methanol at 0 °C for 5 min to give after acidification, extractive isolation, and preparative reversed phase (RP) chromatography 65% of bongkrekic acid (**1**), identical with an authentic sample by RP-HPLC and UV spectral comparison of aqueous solutions. Because the free acid **1** is unstable in neat form, it was characterized after conversion (ethereal diazomethane) to the trimethyl ester. Identity of synthetic and naturally derived trimethyl esters of **1** was confirmed by NMR, IR, UV, HPLC, and optical rotatory comparison. Rotations ($[\alpha]^{23}_D$) observed for synthetic and naturally derived **1** trimethyl ester were +80 ± 2 and +85 ± 2°, respectively.

The synthesis of bongkrekic acid described herein in stereocontrolled, convergent, and sufficiently effective to provide a good source of this valuable substance.[14]

Registry No. 1, 11076-19-0; **1** (trimethyl ester), 42415-59-8; **2**, 88303-96-2; **3**, 88303-97-3; (±)-**4**, 88303-98-4; (±)-**4**-ol, 88304-13-6; (±)-**4** (*tert*-butyldimethylsilyl enol ether), 88304-14-7; (±)-**5**, 88303-99-5; **6**, 88304-00-1; (±)-**7**, 88304-01-2; **8**, 88304-02-3; **8**-ol, 88304-15-8; **8** (cyanohydrin), 88304-16-9; **9**, 88304-03-4; 9 (enol triflate), 88304-17-0; **10**, 88304-04-5; **11**, 88304-05-6; **12**, 88304-06-7; **13**, 88304-07-8; (*S,S*)-**14**, 88304-08-9; (*R,S*)-**14**, 88304-09-0; **15**, 88304-10-3; **16** (isomer 1), 88304-11-4; **16** (isomer 2), 88335-53-9; **17** (isomer 1), 88304-12-5; **17** (isomer 2), 88335-55-1; **17** (diester, isomer 1), 88304-19-2; **17** (diester, isomer 2), 88335-54-0; 5-(trimethylsilyl)-4-pentynal, 68654-85-3; vinyl bromide, 593-60-2; lithio-1-(triisopropylsilyl)propyne, 82192-58-3; acrolein, 107-02-8; ethyl 2-triphenylphosphoranylidenepropionate, 5717-37-3; dimethyl dilithio-3-methylglutaconate, 88304-18-1.

Supplementary Material Available: Spectroscopic data are given for the synthetic intermediates depicted in the chart as well as bongkrekic methyl ester (3 pages). Ordering information is given on any current masthead page.

(11) Preparation of dimethyl 3-methylglutaconate: Henrick, C. A.; Willy, W. E.; Baum, J. W.; Baer, T. A.; Garcia, B. A.; Mastre, T. A.; Chang, S. M. *J. Org. Chem.* **1975**, *40*, 1. The dilithio derivative was formed from the glutaconate ester and 2 equiv of lithium diisopropylamide in THF containing hexamethylphosphoric triamide initially at −78 °C and then at 0 °C for 1 h.

(12) This crucial operation destroys residual boranes, which otherwise cause decomposition of **16** during isolation.

(13) We are indebted to Drs. D. H. Nugteren and A. Gaudemer for kindly providing reference samples of bongkrekic acid ammonium salt in aqueous solution.

(14) This work was supported by the National Institutes of Health.

Structures of Nickel(II) and Cobalt(II) Carboxypeptidase A

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As part of a series of structural studies of metallocarboxypeptidase A,[1] we report here X-ray diffraction results to 1.7-Å resolution which show that the Co^{+2} enzyme and the Ni^{+2} enzymes have only one nonprotein ligand, namely, H_2O. In the Zn^{2+}, Co^{2+}, and Ni^{2+} enzymes, the protein ligands are ND1 of His-69, ND1 of His-196, and both oxygens (OE1 and OE2) of Glu-72. The detailed geometries of the Zn^{2+} and Co^{2+} sites are the same within experimental error, while relative shifts of about 0.5 Å have occured for Ni^{2+} and H_2O in the Ni^{2+} enzyme (Figure 1).

If one counts both oxygens of Glu-72 as ligands the coordination number of the metal is five in all three of these metallocarboxypeptidases. These results are in agreement with an electronic spectral and magnetic susceptibility study[2] of the Co^{2+} enzyme but not with the octahedral geometry assigned to the Ni^{2+} enzyme.[2] However, the relative shifts that occur for Ni^{2+} and H_2O in our X-ray diffraction results do approximate an octahedral metal site *in which the sixth position is vacant.*

The structure of the native (Zn^{2+}) enzyme at pH 7.5 is that of a recent study to 1.54 Å resolution,[3] which has been refined to a crystallographic *R* value of 0.17. The values of the temperature factor are 6 Å^2 for OE1 and OE2 of Glu-72, 3 Å^2 for the ND1 nitrogens of the two histidines, and 15 Å^2 for the Zn^{2+}-bound H_2O molecule at an occupancy of 0.7. Hence, there is reduced occupancy or slight positional disorder of this H_2O, or a combination of both. Nevertheless, there is no more than one nonprotein ligand to the Zn^{2+} ion in this structure.

In order to prepare the Co^{2+} and Ni^{2+} enzymes, the native enzyme (Sigma) was demetalized with *o*-phenanthroline and then reconstituted with the appropriate metal.[4] Metals at 99.998% purity were obtained from Johnson Matthey, Inc. Single crystals were obtained, at pH 7.5 buffered with 20 mM cacodylate in microdialysis tubing (Spectropor), by reducing the concentration of NaCl from 1 to about 0.2 M. All glassware was prewashed with acid, and plastic laboratory ware was washed with buffers that contained *o*-phenanthroline. All water was deionized and then double distilled. X-ray diffraction data for Ni^{2+} enzyme were collected from eight crystals, which yielded one data set complete to 1.80 Å and one set to 1.68 Å. For the Co^{2+} enzyme, nine crystals yielded one data set to 1.85 Å and two sets to 1.7 Å. These multiple data sets for each metallo derivative were reduced, averaged, and then scaled against the data for the native enzyme.[3] Starting from coordinates for the Zn^{2+} enzyme, structures of the Ni^{2+} and Co^{2+} enzymes were refined by the least-squares method

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(1) For reviews and general references, see: Hartsuck, J. A.; Lipscomb, W. N. *Enzymes* **1971**, *3*, 1–56. Lipscomb, W. N. *Proc. Natl. Acad. Sci., U.S.A.* **1980**, *77*, 3875–3878.

(2) Rosenberg, R. C.; Root, C. A.; Gray, G. B. *J. Am. Chem. Soc.* **1975**, *97*, 21–26.

(3) Rees, D. C.; Lewis, M.; Lipscomb, W. N. *J. Mol. Biol.* **1983**, *168*, 369–387.

(4) Latt, S. A.; Vallee, B. L. *Biochemistry* **1971**, *10*, 4263–4270.

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