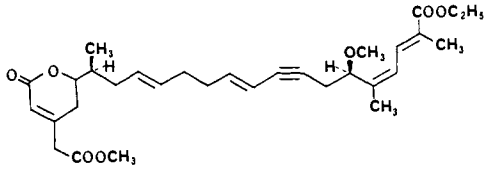
partly on biogenetic considerations. The aldehyde **2** was added to the dilithio derivative of dimethyl 3-methylglutaconate[11] (1.5 equiv) in THF at −40 °C initially and then at −40–0 °C for 20 min to give after extractive workup and chromatography on silica gel 60% of **14** as a mixture of *S,S* 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%



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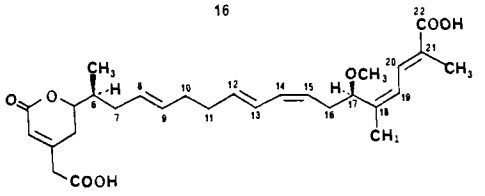

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yield by the sequence: (1) hydroboration of **14** with 1.1 equiv of disiamylborane in THF at 0 °C for 2 h, (2) addition to 3.4 equiv of sodium methoxide in THF at 20 °C (10 min) and conversion to a mixed cuprate with 1.2 equiv of cuprous cyanide in 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.

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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.

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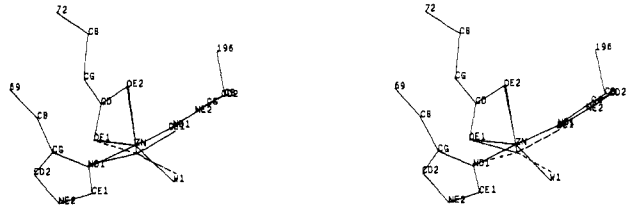

Figure 1. Stereogram of the metal binding site of carboxypeptidase A. The solid lines represent the side chains of His-69, His-196, and Glu-72 and bond to each ligand, including the water molecule (W1), for ZnCPA. The dashed lines are the bonds between Ni^{2+} and the Ni^{2+} ligands, superimposed on the Zn^{2+} structures.

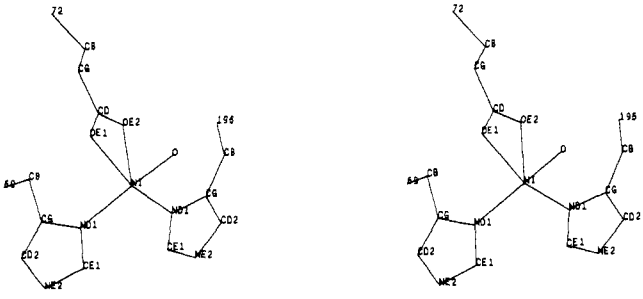

Figure 2. Model of the Ni enzyme in the same orientation as Figure 3. The water is marked O.

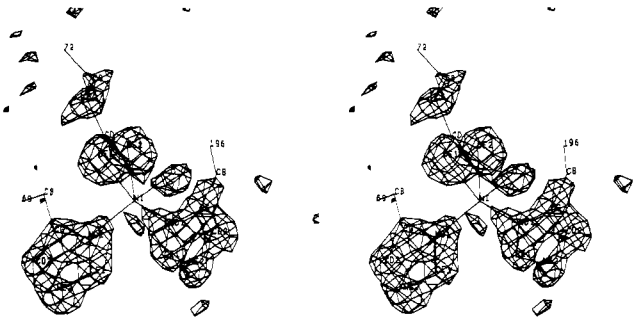

Figure 3. Difference electron density map of the Ni^{2+} enzyme, where the calculated structure factors were determined from the difference between the refined NiCPA coordinates, minus the side chain atoms of His-69, His-196, Glu-72 and all water molecules within 6 Å of the metal, and the observed amplitudes for NiCPA. It is clear from these figures that although the Ni^{2+} and water have moved to give a more nearly octahedral geometry, the sixth ligand position is empty.

of Agarwal.[5] The number of refinement cycles was 19 for the Ni^{2+} enzyme and 13 for the Co^{2+} enzyme and included three cycles of geometric regularization evenly spaced throughout the refinement for each metallo derivative. The final regularized models yielded standard deviations of 0.02 Å for bond lengths and 4° for bond angles and R values of 0.14 for the Ni^{2+} enzyme and 0.15 for the Co^{2+} enzyme. Unconstrained bond lengths are probably accurate to within 0.2 Å. (Table I).

A few detailed lengths are as follows. The protein structures of all three metalloenzymes are the same within experimental error except as noted. For example, root mean square shifts of all atoms of His-69, His-196, and Glu-72 from the Zn^{2+} enzyme to the Ni^{2+} enzyme are 0.15 Å and from the Zn^{2+} enzyme to the Co^{2+} enzyme are 0.10 Å. Only the Ni^{2+} and its bound H_2O have moved about 0.5 Å, to make an octahedral-minus-one geometry; there is no sixth ligand about Ni^{2+} within the limits of accuracy of some 5-10% in occupancy. Temperature factors of all atoms in the metal and its coordination sphere are similar in all three metalloenzymes, except that the metal-bound water has temperature factors (and related occupancies) of 15 (0.7), 8 (1.0), and 12 (1.0) Å^2 for the Zn^{2+}, Ni^{2+}, and Co^{2+} enzymes, respectively.

Table I. Metal Bonds for Zn-, Ni-, and CoCPA[a]

	His-69 ND1	His-196 ND1	Glu-72 OE2	Glu-72 OE1	H_2O
Zn	2.17	2.03	2.30	2.19	2.19
	(3.0)	(3.0)	(6.9)	(5.3)	(15.4)
Ni	2.15	2.08	2.49	2.11	2.07
	(3.0)	(3.8)	(3.7)	(3.0)	(8.4)
Co	2.13	2.06	2.26	2.24	2.00
	(3.0)	(3.0)	(3.0)	(3.0)	(12.0)

[a] Numbers in parentheses are temperature factors.

For the Co^{2+} enzyme, spectral and magnetic properties have led to an ambiguous assignment of between 4 and 5 ligands.[6] This result is consistent with our results for the Co^{2+} enzyme, which has a geometry about the metal essentially identical with that of the Zn^{2+} enzyme and differs only slightly from the Zn^{2+} enzyme in the occupancy and/or the range of slight disorder of the metal-bound water molecule.

These results call for a reevaluation of the magnetic susceptibility and spectroscopic results of the Ni^{2+} enzyme.[2] Also the essentially identical positions of atoms of the protein structures, including ligands to the metal site, among these three derivatives make it unlikely that the differences in peptidase and esterase activities can be explained in terms of the entatic state hypothesis.[7]

The activity of carboxypeptidase A in this crystal form[3] has been established previously.[8] While it would be desirable to test the activity of the Co^{2+} and Ni^{2+} enzymes in the crystalline state, the requirement of a substantial quantity of good single crystals has delayed this test to a future study.

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Registry No. Carboxypeptidase A, 11075-17-5; L-histidine, 71-00-1; L-glutamic acid, 56-86-0.

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Solubility Properties in Polymers and Biological Media. 4. Correlation of Octanol/Water Partition Coefficients with Solvatochromic Parameters

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Hansch and Leo[1] have established that hydrogen-bonding forces and solute molecular volumes are the major properties that in-

