tains a nontemplate metal cation. The conclusion reminds us of the correlation lying between the metal template effect in the synthesis of and the solvent extraction with crown compounds. We believe that the methodology for metal recognition with calix[n]arenes can be exploited not only on the basis of the change in the ring size but also on the basis of the conformational change.

Acknowledgment. The work was supported at the University of Maryland by equipment grants from the National Science Foundation (CHE-85-02155) for a diffractometer–computer system and the National Institutes of Health (RR-03354) for a computer graphics system.

Supplementary Material Available: Tables A–C containing anisotropic temperature factors, bond lengths, and bond angles of a single crystal of partial-cone-3 (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Pyridoacridine Alkaloids from Deep-Water Marine Sponges of the Family Pachastrellidae: Structure Revision of Dercitin and Related Compounds and Correlation with the Kuanoniamines

Geewananda P. Gunawardana,*[†,1] Frank E. Koehn,[†] Angela Y. Lee,[‡] Jon Clardy,*[‡] Hai-yin He,[§] and D. John Faulkner*[§]

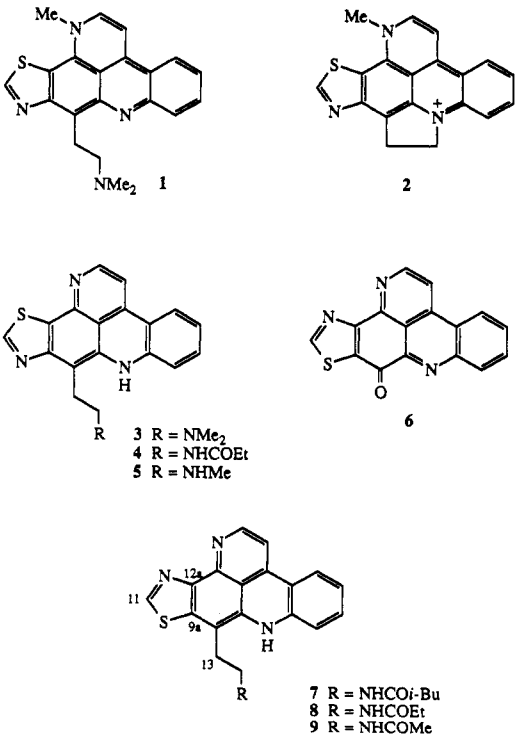
Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Fort Pierce, Florida 34946, Department of Chemistry—Baker Laboratory, Cornell University, Ithaca, New York 14853-1301, and Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0212

Received October 3, 1991

The single-crystal X-ray diffraction of a novel alkaloid, stellettamine (**15**), together with long-range ^{1}H–^{13}C coupling constants obtained by inverse detection methods, and metal chelation studies have shown that the previously reported regiochemistry of the thiazole moiety of dercitin (**1**) and four other related alkaloids **2–5** has to be revised. The corrected structures **10–14** are related to, and in one case identical to, those of the kuanoniamines **6–9**.

The structure of dercitin (**1**), a DNA interacting alkaloid from a deep-water marine sponge *Dercitus* sp. was deduced by a combination of long-range ^{1}H–^{13}C (COLOC) and ^{13}C–^{13}C (2D INADEQUATE) correlation information on the parent compound and its tetrahydro derivative.[2] Although the carbon skeleton of dercitin was established unambiguously from these data, the regiochemistry of the thiazole moiety was incorrectly assigned by comparison of the ^{13}C NMR chemical shifts of C-9a and C-12a with those of the respective carbons in simple thiazoles. Several attempts were made to establish the regiochemistry of the thiazole ring by desulfuration, but the various metal catalysts used for this reaction caused hydrogenation of the aromatic rings to give complex mixtures of products. Subsequently, cyclodercitin (**2**), which is a minor metabolite of the sponge *Dercitus* sp., and nordercitin (**3**), dercitamide (**4**), and dercitamine (**5**), which are metabolites of another deep-water sponge, *Stelletta* sp., were isolated and their structures proposed based on long-range ^{1}H–^{13}C (HMBC) correlation information and spectral comparison to dercitin (**1**).[3] The regiochemistry of the thiazole ring in these compounds was assumed to be the same as that of dercitin.

While the present work was in progress, kuanoiamines A–D (**6–9**) were obtained from an unidentified tunicate and its mollusc predator *Chelynotus semperi* by Carroll and Scheuer.[4] The carbon skeleton of the kuanoniamines was established by interpretation of spectral data, and the regiochemistry of the thiazole moiety was correctly assigned by interpretation of the HMBC experiment, which shows the large difference in the three-bond ^{1}H–^{13}C coupling constants across the thiazole ring caused by the difference in electron delocalization through C–N and C–S bonds. The value of $^{3}J_{H\text{-}C\text{-}N\text{-}C}$ is 12–16 Hz while $^{3}J_{H\text{-}C\text{-}S\text{-}C}$



† Harbor Branch Oceanographic Institution.
‡ Cornell University.
§ Scripps Institution of Oceanography.

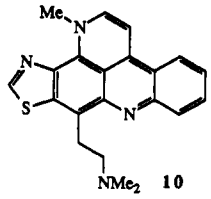
(1) Present Address: Abbott Laboratories, One Abbott Parkway, Abbott Park, IL 60046.

(2) (a) Gunawardana, G. P.; Kohmoto, S.; Gunasekara, S. P.; McConnell, O. J.; Koehn, F. E. *J. Am. Chem. Soc.* **1988**, *110*, 4856–4858. (b) Burres, N. S.; Sazesh, S.; Gunawardana, G. P.; Clement, J. J. *Cancer Res.* **1989**, *49*, 5267–5274.

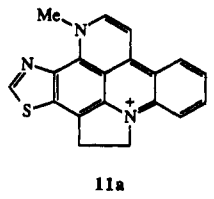
0022-3263/92/1957-1523$03.00/0

is less than 5 Hz.[4,5] The three-bond correlation from H-13 to C-9a allowed the unambiguous assignment of the C-9a signal in kuanoniamines B–D (**7–9**). Unfortunately, Carroll and Scheuer did not consider kuanoniamine C and dercitamide to be identical molecules.

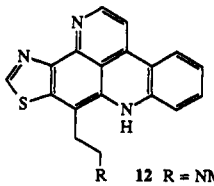
The spectral data of dercitin (**1**), cyclodercitin (**2**), and the *Stelletta* metabolites **3–5** have been reexamined using NMR experiments that were not available at the time of the original work. Both the HMBC spectrum and a fully coupled ^{13}C NMR spectrum of dercitin recorded in TFA showed $^{3}J_{H\text{-}11,C\text{-}12a} = 16$ Hz while $^{3}J_{H\text{-}11,C\text{-}9a} \cong 0.8$ Hz. The new evidence, together with the connectivity data established previously, confirmed that the regiochemistry for the thiazole ring in the *Dercitus* and *Stelletta* metabolites must be reversed and that the assignment based on chemical shift comparisons is erroneous. The structures of metabolites **1–5** must be revised to **10–14**. In addition,



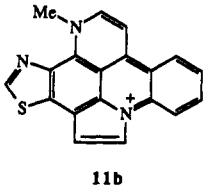
10



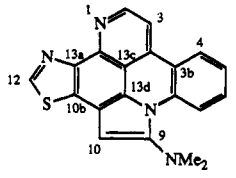
11a



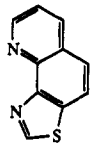
12 R = NMe_2
13 R = NHCOEt
14 R = NHMe



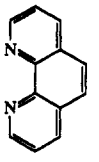
11b



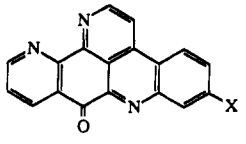
15



16



17



18 X = H
19 X = Br

we independently examined a purple tunicate, tentatively identified as a species of *Cystodytes*,[6] from Pohnpei and isolated samples of kuanoniamine C (**8**) and kuanoniamine D (**9**). We immediately recognized that kuanoniamine C had spectral data identical to those reported for dercitamide.[3] Gated decoupling and selective heteronuclear proton decoupling experiments were used to measure $^{3}J_{H\text{-}11,C\text{-}12a} = 12$ Hz and $^{3}J_{H\text{-}11,C\text{-}9a} < 3$ Hz. Thus, there is no doubt that kuanoniamine C (**8**) and dercitamide (**13**) are identical.

Additional evidence for the proposed structural revision came from an X-ray crystallographic study. Further purification of a selected chromatographic fraction from *Stelletta* sp. by HPLC led to the isolation of a yellow pigment (mp 280–282 °C from $CHCl_3$) that was named stellettamine (**15**). The molecular formula, $C_{20}H_{14}N_4S$ (MH^+, $m/z = 343.1039$), was determined by high-resolution FAB mass spectroscopy. Examination of the ^1H and ^{13}C NMR spectra revealed its close structural resemblance to dehydrocyclodercitin (**11b**), which is an oxidation product of cyclodercitin (**11a**) formed under acidic conditions. As in the case of cyclodercitin, the analysis of long-range ^1H–^{13}C correlations allowed the assignment of a structure for this compound, but the regiochemistry of the thiazole moiety could not be deduced by NMR spectroscopy alone. The carbons attached to nitrogen and sulfur in the thiazole ring could be identified by the magnitudes of the three-bond coupling constants to H-12 ($^{3}J_{C\text{-}13a,H\text{-}12} = 16$ Hz, $^{3}J_{C\text{-}10b,H\text{-}12} = 3$ Hz) that were observed in the HMBC spectrum. However, in several HMBC experiments optimized for coupling constants varying from 10 to 3 Hz, no long-range correlations were observed from H-10 to the quaternary carbons in the thiazole ring. Since the orientation of the thiazole moiety cannot be elucidated without this information, the structure of stellettamine (**15**) was determined by a single-crystal X-ray diffraction experiment.

A computer-generated perspective drawing of the final X-ray model of stellettamine (**15**) is given in Figure 1. The entire molecule, with the exception of the two *N*-methyl groups, is planar within experimental error, and bond distances suggest a highly delocalized system of π-bonds. The *N,N*-dimethylamino group is rotated to minimize the steric interactions of the methyl groups with the C-7 and C-10 hydrogens, and this orientation results in the nitrogen lone pairs being oriented well out of conjugation with the ring. The molecules pack as closely stacked (3.6-Å interplanar spacing) aromatic pairs around an inversion center. There is a chloroform of crystallization located so that the chloroform hydrogen makes close contacts with both N-1 (2.30 Å) and N-13 (2.76 Å).

Stellettamine constitutes another addition to the rapidly growing class of marine alkaloids bearing the pyridoacridine nucleus.[7] This is the first report of the X-ray crystallographic analysis of this hexacyclic system. Unlike most other compounds of this class, stellettamine was found to be devoid of any activity in antifungal and cytotoxicity screens.

The substructure **16**, which is similar in geometry to 1,10-phenanthroline (**17**), was expected to bind metals in the same manner as 1,10-phenanthroline.[8] The metal binding properties of kuanoniamine D (**9**) were therefore investigated using both ^1H NMR and fluorescence spectroscopy. These studies provided evidence for the formation of 2:1 complexes between the alkaloid **9** and divalent metal ions such as Co^{2+}, Cu^{2+}, and Zn^{2+}. The first evidence for metal binding was obtained by observing the ^1H NMR spectrum of kuanoniamine D (**9**) in the presence of added zinc chloride. The signals due to H-2, H-3, and H-11 were substantially broadened upon addition of small quantities of metal ion but sharpened and remained unchanged once a 2:1 ratio of alkaloid to metal had been attained: in contrast, all other signals remained virtually unchanged. As a free base, kuanoniamine D (**9**) is a strongly fluorescent compound, and when irradiated at 350

(3) Gunawardana, G. P.; Kohmoto, S.; Burres, N. S. *Tetrahedron Lett.* **1989**, *30*, 4359–4362.
(4) Carroll, A. R.; Scheuer, P. J. *J. Org. Chem.* **1990**, *55*, 4426–4431.
(5) Faure, R.; Galy, J.-P.; Elguero, J.; Vincent, E. *Can. J. Chem.* **1978**, *56*, 46–55.
(6) The voucher speciment was too poorly preserved for classical taxonomic identification.
(7) For a listing of all marine alkaloids bearing the pyridoacridine nucleus see: Schmitz, F. J.; De Guzman, F. S.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1991**, *56*, 804–808.
(8) For a review of the copper complex of 1,10-phenanthroline and its role in the oxidative cleavage of DNA, see: Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180–186.

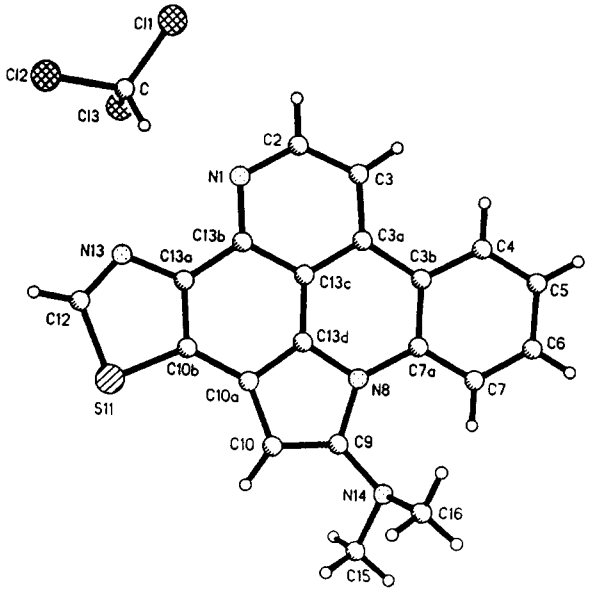


Figure 1. Computer-generated perspective drawing of the final X-ray model of stellettamine (**15**). All hydrogens are shown as is the chloroform of crystallization.

nm, an emission peak at 524 nm was observed. The emission peak could be greatly decreased in intensity and shifted in wavelength upon addition of excess of the metal ions Zn^{2+}, Fe^{2+}, and Cu^{2+}, and was completely quenched by adding $CoCl_2$ (Figure 2). The quenching of the fluorescence was therefore used to study the binding ratios of **9** to metal ions and to determine the stability constants of the complexes.

In order to determine the binding ratios and stability constants, it is essential that the metal–ligand complex does not fluoresce at the wavelength observed. This is true of the cobalt complex but for the copper complex it was necessary to change the observation wavelength from 524 to 508 nm. The binding ratios to Co^{2+} and Cu^{2+} were measured using the method of continuous variation.[9] By using a total concentration of ligand and metal ion of 10^{-5} M, binding ratios of 1.86 and 1.94 were obtained for $Cu(OAc)_2$ and $CoCl_2$, respectively. Both values can be rounded to 2, which is the expected ratio considering that the coordination number of these ions is four and that there are two binding sites, N-1 and N-12, per molecule. In order to estimate the stability constants of the complexes, kuanoniamine D (**9**) was titrated against $CoCl_2$ and $Cu(OAc)_2$ in 20% aqueous methanol solutions. The relative intensity of the fluorescence was plotted against the ratio of the total concentration of metal ion to the total concentration of the ligand. The calculated stability constants[10,11] were 2.5×10^{10} M^{-2} for $CoCl_2$ and 1.3×10^{10} M^{-2} for $Cu(OAc)_2$. It is interesting to note that the fluorescence spectrum of **9** was substantially quenched and the wavelength of emission was changed to 593 nm by the addition of calf thymus DNA. This observation provides support for a mechanism of action that involves complexation with DNA: similar quenching experiments may be used to detect complexation between DNA and molecules with a suitable fluorescence spectrum.

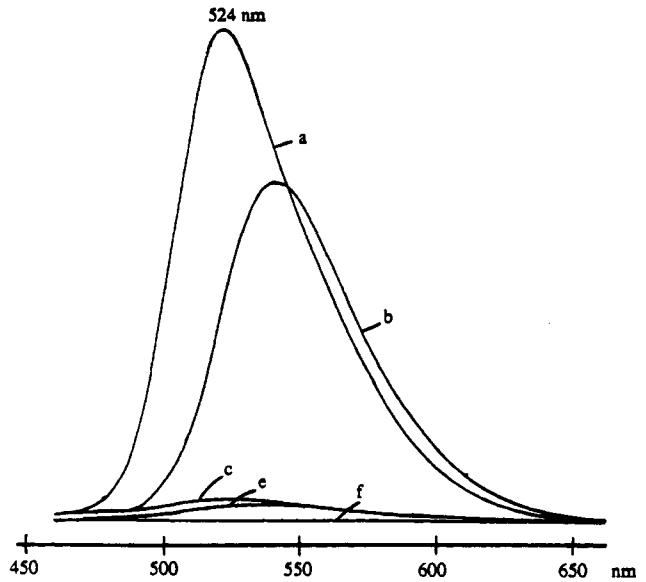


Figure 2. Fluorescence spectra of kuanoniamine B (**7**, 10^{-5} M) alone and in the presence of metal ions: (a) **7**, (b) **7** + $ZnCl_2$, (c) **7** + $FeSO_4$, (d) **7** + $Cu(OAc)_2$, (e) **7** + $CoCl_2$. The excitation wavelength = 350 nm; solvent, 4:1 MeOH–H_2O; pH = 8.5; T_M/T_L = 100.

Our observation that alkaloids in this series can bind metals in 2:1 complexes is in contrast with the observations of Kobayashi et al.[12] and Bloor and Schmitz,[13] who have independently reported that ascididemin (**18**) and 2-bromoleptoclinidone (**19**) did not form red complexes with ferrous salts, as had been expected by analogy with 1,10-phenanthroline (**17**). On the basis of our results, we would expect that complexes were formed but that they could not be detected visually, possibly because of interference from the extended chromophore already present in these alkaloids. We recommend use of fluoresence spectroscopy for detection of metal complexion as a method of identifying compounds possessing substructures **16** and **17**. The combination of the HMBC experiments, the X-ray experiment on stellettamine (**15**), and establishing that dercitamide (**13**) and kuanoniamine C (**8**) were identical clearly requires that structures **1–5** be revised to **10–14**.

Experimental Section

Isolation of Stellettamine (15). The extraction of the sponge material and the preliminary fractionation of the extracts by preparative counter-current chromatography (PCCC) has been described previously.[3] The stellettamine containing PCCC fraction was further purified by HPLC on a Lichrosorb RT NH_2 7-μm column using 96:4 CH_2Cl_2/MeOH as the solvent system to obtain stellettamine (**15**).

Stellettamine (15): dark brown roughly cubic crystals from chloroform; mp 280–282 °C; UV (MeOH) 205 nm (log ε 4.197), 260 (3.973), 309 (4.032) 338 (3.570), 364 (3.575), 391 (3.534), 460 (3.136); IR ($CHCl_3$) 1690, 1658, 1629, 1590, 1535, 1450, 1428, 1345, 1260, 1220 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 9.18 (1 H, dd, J = 8.6, 1.4 Hz, H7), 9.16 (1 H, d, J = 5.3 Hz, H2), 9.06 (1 H, s, H12), 8.55 (1 H, dd, J = 8, 1.9 Hz, H4), 8.10 (1 H, d, J = 5.3 Hz, H3), 7.67 (1 H, ddd, J = 8.6, 7.9, 1.8 Hz, H6), 7.51 (1 H, ddd, J = 8, 7.9, 1.4 Hz, H5), 6.86 (1 H, s, H10) 2.95 (6 H, s, NMe_2); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 149.4 (C9), 149.1 (C12), 147.5 (C2), 145.0

(9) Beck, M. T. In *Chemistry of Complex Equilibria*; Van Nostrand Reinhold: London, 1970; pp 86–89.
(10) Connors, K. A. In Binding Constants. The Measurement of Molecular Complex Stability; Wiley: New York, 1987; Chapter 2.
(11) The stability constants (β_2) are calculated by eq 1, which is derived from eqs 2–4 and uses the result that kuanoniamine D (**9**) forms of 2:1 complexes with $CoCl_2$ and $Cu(OAc)_2$.

$$\beta_2 = [ML_2]/[M][L]^2 = T_L - [L]/2T_M[L]^2 - (T_L - [L])[L]^2 \quad (1)$$

$$[ML_2] = \beta_2[M][L]^2 \quad (2) \qquad [M] = T_M - [ML_2] \quad (3) \qquad [L] = T_L - 2[ML_2] \quad (4)$$

(12) Kobayashi, J.; Cheng, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177–1180.
(13) Bloor, S. J.; Schmitz, F. J. *J. Am. Chem. Soc.* **1987**, *109*, 6134–6137. De Guzman, F.; Schmitz, F. J. *Tetrahedron Lett.* **1989**, *30*, 1069–1070.

(C13a), 140.8 (C13b), 136.4 (C7a), 133.0 (C3a), 130.8 (C6), 129.7 (C10b), 125.2 (C4), 124.3 (C5), 122.0 (C13d), 121.4 (C3b), 118.4 (C7), 113.3 (C13c), 110.5 (C3), 110.6 (C10a), 94.2 (C10), 45.3 (NMe_2); HRFABMS obsd m/z = 365.0839 ($C_{20}H_{14}N_4S$ + Na requires 365.0837), 343.1039 ($C_{20}H_{15}N_4S$ requires 343.1019).

Single-Crystal X-ray Diffraction Analysis of Stellettamine (15). Preliminary diffraction photographs of stellettamine (15) displayed monoclinic symmetry, and accurate cell constants of a = 13.812 (3) Å, b = 12.638 (4) Å, c = 11.987 (3) Å, and β = 106.57 (2)° were determined from 20 diffractometer measured 2θ values. Systematic extinctions and density considerations were uniquely consistent with space group $P2_1/c$ with one molecule of composition $C_{20}H_{14}N_4S{\cdot}CHCl_3$ in the asymmetric unit. A total of 2879 reflections with $2\theta \leq 116°$ were collected on a computer controlled four-circle diffractometer using CuKα radiation and θ:2θ scans at approximately -15 °C. After Lorentz, polarization, background, and analytical absorption corrections, 2736 (95%) were judged observed ($|F_o| \geq 3\sigma|F_o|$). The structure was solved and refined routinely using the SHELXS system of programs. In the final model the non-hydrogen atoms are anisotropic, the hydrogens are isotropic and riding the appropriate heavy atom, and the final discrepancy index is R = 0.056. Additional X-ray parameters are available and are described in the supplementary material. A drawing of the final X-ray model of stellettamine (15) is given in Figure 1.

Isolation of Kuanoniamine D (9). A dark purple encrusting tunicate, tentatively identified as a species of *Cystodyes*, was collected from patch reefs (-2 m) near the airport at Pohnpei in April 1989. The freeze-dried tunicate (20 g) was extracted successively with hexane (200 mL) and 2:1 $MeOH/CH_2Cl_2$ (3 × 200 mL). The purple extract was chromatographed on a Sephadex LH-20 column using MeOH as eluant to yield two red fractions. The first fraction was concentrated to a gum, washed with $CHCl_3$ (2 × 5 mL), and redissolved in MeOH (2 mL) at 45 °C. After the solution had been allowed to stand at room temperature for 1 h, a purple solid precipitated. The solid (36 mg) was dissolved in water, and the solution was basified with 1% NaOH solution to pH 9. The yellow precipitate was extracted with $CHCl_3$ (2 × 20 mL) and chromatographed on reversed-phase HPLC (20:1 $MeOH/H_2O$) to yield kuanoniamine D (9, 14.4 mg, 0.07% dry wt) and dercitamide (13 ≡ 8, kuanoniamine C, 7.9 mg, 0.04% dry wt), both of which possess spectral data that are identical to literature values.[3,4] The second red fraction was dissolved in warm MeOH (2 mL) and the solution was cooled to obtain debromoshermilamine (17 mg, 0.085% dry wt) as a purple precipitate.[14]

Metal Binding Studies. ^1H NMR spectra were recorded using a Bruker 200-MHz spectrometer at 25 °C, using 0.01 M solutions in 5:1 MeOH-d_4/$CDCl_3$. Fluorescence studies were performed on a Farrand MK2 spectrofluorometer at 25 °C and analytical- or spectral-grade solvents were used. In all fluorescence experiments to determine the binding ratios and stability constants, the pH was adjusted to 8.5, the excitation wavelength was set at 350 nm, and the observation wavelength was 524 nm for $CoCl_2$ and 508 nm for $Cu(OAc)_2$. The binding ratios to Co^{2+} and Cu^{2+} were measured using the method of continuous variation[8] with a total concentration of ligand and metal ion of 10^{-5} M. The x_{max} values, the fraction of the total ligand concentration that gives the maximum formation of complex, of 0.66 and 0.65 for the complexes of kuanoniamine D (9) with $CoCl_2$ and $Cu(OAc)_2$, respectively, were obtained by curve fitting, using the least squares method, and the binding ratios (n) were calculated according to the equation $n = x_{max}/(1 - x_{max})$.[9] The stability constants (β_2) were calculated by plotting the observed relative fluorescence intensity (F_{obs}) against the ratio of the total concentration of metal ion to the total concentration of ligand (T_M/T_L). The value of F_{obs} was arbitrarily set at 1000 for [L] = 10^{-5} M so that [L] = $100*F_{obs}$. The values of log β_2 were calculated as 10.1 ± 0.4 for $Cu(OAc)_2$ (24 data points) and 10.4 ± 0.3 for $CoCl_2$ (27 data points).[10,11]

Acknowledgment. We thank Dr. Jay S. Siegel for helpful discussions. The tunicate was tentatively identified by Dr. Chris Ireland. Research at Cornell was supported by a grant from the New York State Sea Grant Program and at Scripps by grants from the National Cancer Institute (CA 49084 and CA 50750).

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, and interatomic distances and angles (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(14) Rudi, A.; Kashman, Y. *J. Org. Chem.* **1989**, *54*, 5331-5337.

A *Pseudomonas* sp. Alcohol Dehydrogenase with Broad Substrate Specificity and Unusual Stereospecificity for Organic Synthesis

Curt W. Bradshaw, Hong Fu, Gwo-Jenn Shen, and Chi-Huey Wong*

Department of Chemistry, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, California 92037

Received September 17, 1991

A new alcohol dehydrogenase from *Pseudomonas* sp. strain PED has been isolated and characterized. The enzyme exhibits a broad substrate specificity, accepting aromatic, cyclic, and aliphatic compounds as substrates. The K_m values were determined as 525 μM for NAD and 75 μM for 2-propanol with a specific activity of 36 U/mg. The kinetic mechanism is ordered bi-bi with the cofactor binding first and releasing last. The enzyme transfers the *pro-R* hydride of NADH to the *si* face of carbonyl compounds to yield (*R*) alcohols. Synthetic-scale reductions of a number of representative compounds were carried out in high enantiomeric excess with in situ regeneration of NADH using 2-propanol as the hydride source and the same enzyme as catalyst.

Introduction

Alcohol dehydrogenases are now well-established catalysts in organic synthesis.[1] Enzymes from horse liver,[2] yeast,[2a] and *Thermoanaerobium brockii*[3] have been utilized extensively. Although the alcohol dehydrogenases isolated

(1) (a) Hummel, W.; Kula, M.-R. *Eur. J. Biochem.* **1989**, *184*, 1. (b) Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (c) Lemiere, G. L. *Enzymes as Catalysts in Organic Synthesis*; Schneider, M. P., Ed.; D. Reidel Publishing: Dordrecht, Holland, 1986; pp 19-34.

(2) (a) Jones, J. B.; Beck, J. F. In *Applications of Biochemical Systems in Organic Synthesis*; Jones, J. B., Sih, C. J., Perlman, D., Eds.; John Wiley and Sons: New York, 1976; pp 248-376. (b) Jones, J. B. *Mechanisms of Enzymatic reactions: Stereochemistry*; Frey, P. A., Ed.; Elsevier Science: Amsterdam, 1986; pp 3-14. (c) Jones, J. B. *Enzymes in Organic Synthesis*, Ciba Foundation Symposium III; Pitman: London, 1985; pp 3-14.

0022-3263/92/1957-1526$03.00/0