The results of the electrochemical studies of compounds **2** and **4** are summarized in Table II. Typical differential pulse and cyclic voltammograms are shown in Figure 2. Electrochemical oxidation of the head-to-tail platinum(II) dimer (**4**) takes place reversibly at scan rates (v) slower than 50 mV s^{-1} with only one wave observed at E_p = +0.63 V in the range examined, from +1.0 to −0.1 V, with CV. At scan rates faster than 50 mV s^{-1} both ΔE_{pp} and the anodic to cathodic current ratio (i_a/i_c) increase, and $i_a v^{-1/2}$ decreases as v increases. These observations are consistent with a chemical reaction coupled with oxidation of the Pt(II) complex.[12] This reaction may involve coordination of axial ligands (NO_3^- or H_2O) to the resulting Pt(III) dimer. Exhaustive electrolysis of **4** at +0.85 V produced a colorless to orange color change in the solution and a net loss of 0.95 electrons per platinum atom. CV experiments conducted after the electrolysis or on authentic samples of **3** gave results identical with those obtained on **4** prior to electrolysis. From the CV data obtained on **3** and **4** at slow scan rates the difference in the reduction potentials, $\Delta E_{1/2} = E^1_{1/2} - E^2_{1/2}$, was estimated to be −30 mV.[5c,13] This value is in reasonable agreement with the value of −20 mV obtained for $\Delta E_{1/2}$

$$Pt^{III}Pt^{III} + e^- \overset{E^1_{1/2}}{\rightleftharpoons} Pt^{II}Pt^{III} + e^- \overset{E^2_{1/2}}{\rightleftharpoons} Pt^{II}Pt^{II}$$

from the DPV experiment (Table II). The negative value obtained for $\Delta E_{1/2}$ indicates that, in the oxidation of **4**, the removal of the second electron is less difficult than the first. This result appears to be one of the few reported such cases in inorganic systems.[5]

The electrochemical behavior of the head-to-head Pt(III) dimer **2** is similar in some respects to that observed for the head-to-tail isomer (Table II). A single wave was observed at E_p = +0.63 V in both CV and DPV studies, and the redox process also appeared to involve a coupled chemical reaction following an overall two-electron transfer. The major difference found was in the kinetics of the coupled chemical reaction. Although this system approaches quasi-reversible behavior at slow scan rates, ΔE_p, i_a/i_c, and $i_p v^{-1/2}$ all vary as a function of v down to the slowest measured scan rate (5 mV s^{-1}). Controlled potential electrolysis of **2** showed that an additional chemical reaction occurs after the electron transfer. As the reductive electrolysis proceeded to completion, the solution changed color from orange to green to blue and finally became colorless. The blue color produced during this reversible process is almost certainly due to the formation of the mixed-valence PPB, **1**. A blue color does not form in the controlled potential electrolysis of **4** presumably because the head-to-tail isomer cannot associate into a tetranuclear species, a result of steric effects which prevent the close approach of two dimeric units. If one assumes that the CV and DPV data for **2** approach reversible behavior in the slow scan rate limit, approximate values of +30 to +50 mV can be obtained for $\Delta E_{1/2}$ (Table II). This result implies that, for the head-to-head isomer, the second electron is harder to remove than the first. The mixed valence Pt(II)–Pt(III) dimer is therefore a possible intermediate in the electrochemical formation of PPB. In particular, reaction of the Pt(II)–Pt(III) intermediate with a Pt(II)–Pt(II) species, formed by disproportionation of the mixed-valence dimer, would generate the tetranuclear blue complex, PPB, having the formal oxidation state of +2.25. Further chemical and electrochemical studies of this system are in progress.

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Supplementary Material Available: Atomic positional and thermal parameters for compound **2** (2 pages). Ordering information is given on any current masthead page.

(12) (a) Nicholson, R. S.; Shain, I. *Anal. Chem.* **1964**, *36*, 706. (b) Nicholson, R. S. *Ibid.* **1965**, *37*, 1351.

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Biosynthesis of Dibromotyrosine-Derived Antimicrobial Compounds by the Marine Sponge *Aplysina fistularis* (*Verongia aurea*)[1,2]

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Marine natural products have been a focus of recent interest as potential pharmaceuticals, with sponges proving to be particularly rich in bioactive compounds. The brominated phenols and quinones isolated from *Aplysina* (*Verongia*) species include active antibacterial agents which are proposed to be derived from mono- or dibromotyrosines.[3] Attempts by other workers to demonstrate such a conversion in vivo have been unsuccessful.[4,5] However, we have now reinvestigated the biosynthesis of brominated phenols and bromoquinones in *Aplysina fistularis* by using liposome-enclosed precursors and have demonstrated the conversion of phenylalanine (Phe) and tyrosine (Tyr) to the dienone **3** as well as to the rearranged product dibromohomogentisamide (**4**). This work supports the biosynthetic pathway in Scheme I.

Samples of *Aplysina fistularis*, with their natural rock substrates intact, were collected by scuba operations at several coves offshore from the Catalina Marine Science Center, Santa Catalina Island, CA. An antibiotic dip of benzylpenicillin, streptomycin sulfate, and chloramphenicol in sea water was used to prevent the bacterial infection normally observed after the organisms are handled.[8] Sponges were incubated in 3 L of aerated sea water in polyethylene containers maintained at environmental temperatures by a fresh running sea water bath. Algal growth was inhibited by limiting the light incident on the aquaria. These precautions allowed sponge survival in the laboratory for as long as 2 weeks.[9]

Conventionally, radiolabeled precursors have been administered to sponges by simple solution in sea water.[4,6,7] Since sponges are known to filter feed on particulates such as dead bacteria,[10] we presumed that liposome encapsulation would improve incorporation of precursors by sponges. Multilamellar lipid vesicles of bacterial dimensions were prepared by agitating an aqueous solution of the precursor in a flask coated with a lipid film.[11] Phosphatidylcholine

(1) Presented at the Marine Chemistry Symposium, 64th Conference, Chemical Institute of Canada, Halifax, Nova Scotia, June 4, 1981.

(2) Dr. Robert Given, Catalina Marine Science Center, originally identified the bright yellow sponge as *Verongia aurea sensu* de Laubenfels, 1948. This sponge has been reclassified as *Aplysina fistularis* (Pallas) *sensu* Wiedenmayer, 1977 (Dr. G. J. Bakus, University of Southern California, personal communication).

(3) (a) Minale, L.; Cimino, G.; DeStefano, S.; Sodano, G. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 1–72. (b) Goo, Y. M.; Rinehart, K. L.; Jr. In "Drugs and Food from the Sea", Kaul, P. N.; Sindermann, C. J., Eds.; The University of Oklahoma Press: Norman, OK, 1978; pp 107–115.

(4) DeRosa, M.; Minale, L.; Sodano, G. *Comp. Biochem. Physiol. B* **1973**, *45B*, 883–893.

(5) The biosynthetic origins of fatty acids,[4] sterols,[6] and terpenes[7] in sponges have been studied with greater success.

(6) (a) DeRosa, M.; Minale, L.; Sodano, G. *Experientia* **1975**, *31*, 408–410. (b) DeRosa, M., Minale, L.; Sodano, G. *Ibid.* **1975**, *31*, 758–759. (c) DeRosa, M.; Minale, L.; Sodano, G. *Ibid.* **1976**, *32*, 1112–1113. (d) Minale, L.; Riccio, R.; Scalona, O.; Sodano, G.; Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C. *Ibid.* **1977**, *33*, 1550–1552. (e) Minale, L.; Persico, D.; Sodano, G. *Ibid.* **1979**, *35*, 296–297.

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(8) McMammon, H. M. In "Culture of Marine Invertebrate Animals"; Smith, W. L.; Chanley, M. H., Eds.; Plenum Press: New York, 1975; p 15.

(9) Survival of the sponges was clearly dependent on the sea water temperature and dropped off rapidly above 17 °C.

(10) (a) Reiswig, H. M. *Biol. Bull.* **1971**, *141*, 568–591. (b) *Can. J. Zool.* **1975**, *53*, 582–589.

(11) Poste, G.; Papahadjapoulos, D.; Vail, W. J. In "Methods in Cell Biology"; Prescott, D. M., Ed.; Academic Press: New York, 1976; Vol. 14, pp 33–71.

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Scheme I

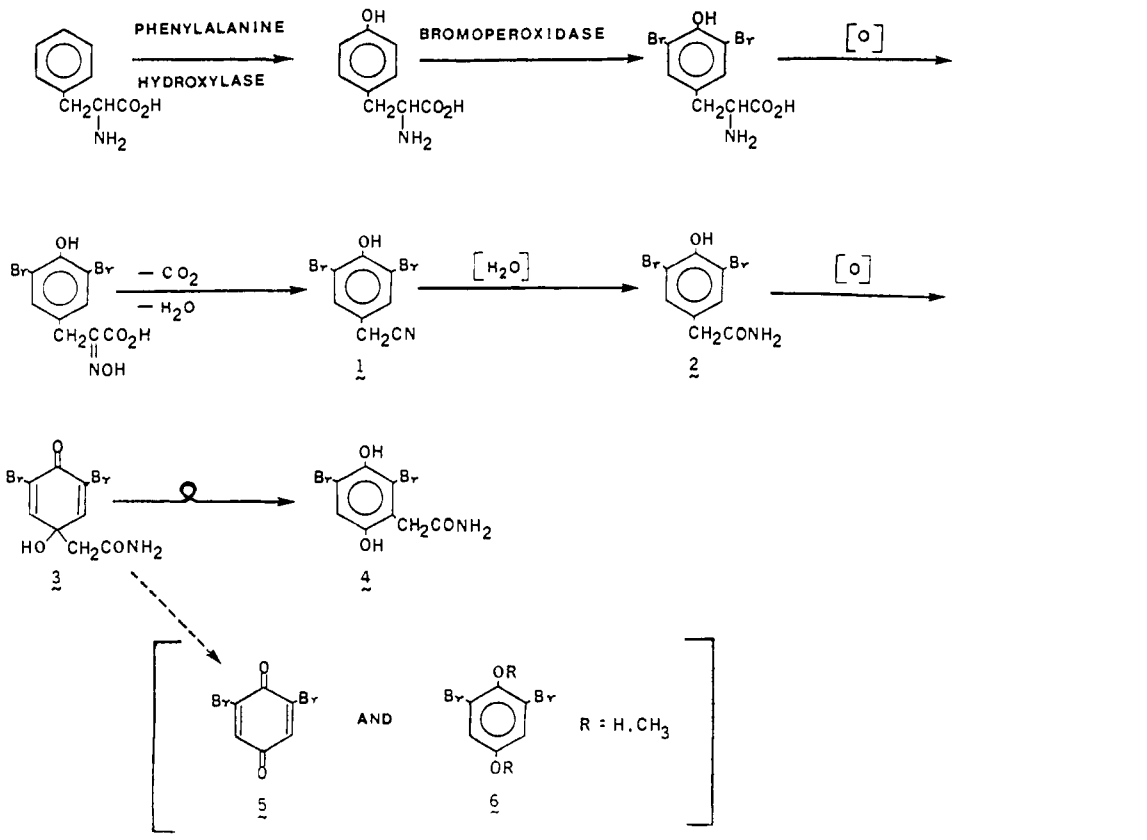


Table I. Incorporation of Labeled Precursors

	NaOAc[a] [1-^{14}C]	L-Met[a] [$^{14}CH_3$]	D,L-Phe[a] [U-^{14}C]	D,L-Phe[a] [U-^{14}C,^{15}N]	D,L-Tyr[a] [U-^{14}C]	D,L-Tyr[b] [U-^{14}C]
precursor						
DPM × 10^7	25.0	10.0	5.0	52.0	12.0	16.0
DPM/mmol × 10^9	1.3	1.32	1.22	0.092	1.34	1.34
mg ^{15}N[c]				80.2		
mg ^{15}N/mmol[c]				14.2		
hexane extract						
% incorporation	0.13	1.76	0.28	0.26	0.17	0.21
methanol extract						
% incorporation	0.05	0.92	1.13	0.50	2.03	1.34
dienone **3**						
DPM × 10^3	1.9	2.9	15.2	65.4	38.5	12.3
DPM/mmol × 10^3	8.1	9.0	93.7	14.0	74.0	54.0
% incorporation, × 10^{-2} [d]	0.08	0.29	3.04	1.26	3.21	0.77
dilution of ^{14}C, × 10^3 [e]	160	150	13	6.6	18	25
mg ^{15}N × 10^{-3} [c]				3.73		
mg ^{15}N/mmol × 10^{-4} [c]				7.98		
% ^{15}N incorporation, × 10^{-3}				4.65		
dilution of ^{15}N, × 10^3 [e]				17.8		
^{15}N dilution/^{14}C dilution				2.7		
homogentisamide **4**						
DPM/mmol × 10^3				25.9		

[a] Using liposomes. [b] Without liposomes. [c] Excess ^{15}N above natural abundance (synthetic Phe and sponge-derived dienone naturally contain 0.3722 and 0.3744 atom % ^{15}N, respectively). [d] See footnote 14. [e] Ratio of ^{14}C specific activities or ^{15}N enrichments (precursor/product).

and cholesterol were chosen for the vesicle membranes since they occur in sponge tissues[12] and form vesicles with desirable properties.[13]

Each experiment was terminated prior to any visible sponge deterioration[14] by extracting the tissue to yield a methanol extract rich in brominated metabolites.[3b] Sponge tissue was cut, washed with distilled water, and continuously extracted with diethyl ether for 2 days in a Soxhlet apparatus. The ether extract was then

(12) (a) Litchfield, C.; Morales, R. W. In "Aspects of Sponge Biology"; Harrison, F. W.; Cowden, R. C., Eds.; Academic Press: New York, 1976; p 183. (b) DeRosa, M.; Minale, L.; Sodano, G. *Comp. Biochem. Physiol. B* **1973**, *46B*, 823–837.

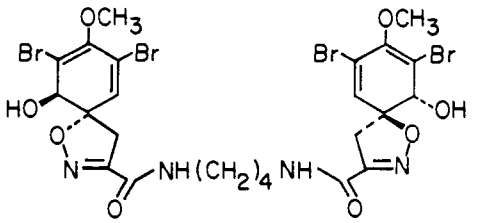
(13) Pagano, R. E.; Weinstein, J. N. *Annu. Rev. Biophys. Bioeng.* **1978**, *7*, 435–468.

(14) Concurrent tyrosine feeding experiments (with and without the use of liposomes, Table I) were conducted for 3 days. Other incubation times varied from 2 to 6 days.

dried and triturated sequentially to provide hexane- and methanol-soluble portions. The hexane extract was more heavily labeled than the methanol extract by sodium acetate and methionine (presumably in sterols), but the reverse was true for Tyr and Phe which gave the brominated metabolites (Table I). Approximately 0.5% of the sponges' wet weight was recovered in the methanolic extract and, of this, one-third[15] corresponded to the antimicrobial[16] metabolite **3**. The dienone **3** was easily isolated as the solid, recrystallized from methanol and characterized.[17] The structures of other brominated metabolites (**1**, **2**, **4**–**6**) found in the methanol extract were assigned by comparison of TLC, HPLC, MS, and GC/MS parameters of the mixture relative to those of synthetically prepared compounds.[3b,18] The methanol extract was fractionated chromatographically (silica TLC followed by reversed-phase C_{18} HPLC) to afford small amounts of radiochemically pure **4**.

Both Phe and Tyr were shown to be biosynthetic precursors of **3** (Table I), implying the ready conversion of Phe to Tyr[19] by the sponge. Improved incorporation of Tyr in the liposome feeding experiment (relative to that without liposomes) argues that this technique should prove valuable in future marine biosynthetic studies, particularly for the introduction of less polar precursors. The presence of comparable radioactivity in **3** and **4** agrees with our previous hypothesis[20] that **4** is formed from **3** via a skeletal rearrangement parallel to that observed in the mammalian biosynthesis of homogentisic acid. Although the enzymatic mechanism of the side-chain migration to form homogentisic acid is still unclear,[21] the conversion of 4-hydroxy-2,5-cyclohexadienone-4-acetic acid to homogentisic acid in aqueous alkali has been demonstrated.[22]

Crystalline **3**, isolated from sponges incubated with a mixture of [^{15}N]Phe and [U-^{14}C]Phe, was digested by the Kjeldahl procedure, and the resulting ammonium chloride was oxidized to nitrogen gas for ^{15}N quantitation by isotope ratio mass spectrometry.[23] Table I summarizes the incorporation results. Although partial transamination of the amino acid (ca. $^2/_3$) is apparent, the observed retention of ^{15}N relative to ^{14}C indicates that the sponge can convert the alanine to the acetamide side chain without deamination. The biosynthetic pathway in Scheme I is consistent with our labeling studies as well as the known occurrence of bromophenol nitriles and oximes in *Verongia* species.[24] The isolation of (*p*-hydroxyphenyl)pyruvic acid oxime[25] from *Hymeniacidon sanguinea* and aerothionin (**7**)[26] from *Aplysina fistularis* supports the proposed mechanism. The intermediacy of compound **3** is currently being investigated with purified material from the ^{15}N/^{14}C labeling experiment.[27,28]



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Acknowledgment. Field work carried out at the Santa Catalina Marine Science Center was supported in part by the Institute of Marine and Coastal Studies and the Marine and Freshwater Biomedical Center, both at the University of Southern California. Chemical studies at the University of Illinois were supported in part by grants from the National Institute of Allergy and Infectious Diseases (AI 04769, AI 01278) and the National Institute of General Medical Sciences (GM 27029). We thank Dr. W. O. McClure, University of Southern California, and Dr. C. J. Pearce, University of Illinois, for liquid scintillation counting and gas chromatography of sponge extracts, J. C. Cook, Jr., University of Illinois, and Dr. L. Dunkerton, University of Southern California, for mass spectra, Dr. T. Kurtz, University of Illinois, for isotope ratio measurements, and Dr. R. Given, Catalina Marine Science Center, for assistance with sponge collection and identification.

(15) The amount of **3** in each methanolic extract was determined by TLC purification (silica, 4:1 $CHCl_3$–MeOH) to give a mixture of **3** and **4**, followed by UV quantitation of both compounds in methanol [**3**: λ_{max} 255 nm (6500). **4**: λ_{max} 222 nm (5400)].

(16) The crude extract (1:3 toluene/methanol) of the sponge was tested for antimicrobial activity by the disk method (100 μL/disk): *Bacillus subtilis* (18 mm); *Escherichia coli* (13 mm). Purified **3** was tested at 100 μg/disk: *B. subtilis* (35 mm); *E. coli* (33 mm).

(17) Physical data obtained for **3** [mp 190–191 °C; IR (Nujol) 3420, 3125, 2700, 2660, 1650 cm^{-1}; MS (EI), *m/z* 323, 306, 278, 264, 244, 227, 199, 185; ^{1}H NMR (CD_3COCD_3) 7.59 (2 H), 2.97 (3 H), 2.75 (2 H) ppm; ^{13}C NMR (CD_3OD) 174.5, 173, 153.2 (2 C), 121.5 (2 C), 72.8, 46.0 ppm] and its acetate [mp 168–171 °C; ^{1}H NMR (CD_3COCD_3) 7.75 (2 H), 2.9 (4 H), 2.08 (3 H) ppm] were in agreement with values published for a synthetic sample (Sharma, G. M.; Burkholder, P. R. *Tetrahedron Lett.* **1967**, 4147–4150).

(18) Goo, Y. M. Ph.D. Thesis, University of Illinois, Urbana, IL, 1980.

(19) Weiss, U.; Edwards, J. M. "The Biosynthesis of Aromatic Compounds"; Wiley: New York, 1980; p 144.

(20) Krejcarek, G. E.; White, R. H.; Hager, L. P.; McClure, W. O.; Johnson, R. D.; Rinehart, K. L., Jr.; McMillan, J. A.; Paul, I. C.; Shaw, P. D.; Brusca, R. C. *Tetrahedron Lett.* **1975**, 507–510.

(21) Nozaki, M. *Top. Curr. Chem.* **1979**, *78*, 145–186.

(22) Saito, I.; Chujo, Y.; Shimazu, H.; Yamane, M.; Matsuura, T.; Cahnmann, H. J. *J. Am. Chem. Soc.* **1975**, *97*, 5272–5277.

(23) Caprioli, R. M. In "Biochemical Applications of Mass Spectrometry"; Waller, G. R., Ed.; Wiley: New York, 1972; pp 746–748.

(24) Minale, L. *Pure Appl. Chem.* **1976**, *48*, 7–23.

(25) Cimino, G.; DeStefano, S.; Minale, L. *Experientia* **1975**, *31*, 756–757.

(26) McMillan, J. A.; Paul, I. C.; Goo, Y. M.; Rinehart, K. L., Jr.; Krueger, W. C.; Pschigoda, L. M. *Tetrahedron Lett.* **1981**, 39–42.

(27) We presume **3** (or the related acid or nitrile) is also the precursor for the brominated quinones and hydroquinones **5** and **6**, as shown in Scheme I, though that has not yet been demonstrated.

(28) The enzymes involved may be rather nonspecific and complementary pathways may also exist, involving, e.g., bromination of (*p*-hydroxyphenyl)-acetonitrile, oxidation of Tyr to its oxime, or oxidation of the nitrile analogue of **2** to the corresponding dienone.

Stereospecific Conversion of Penicillin to Thienamycin

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The control of absolute stereochemistry is a critical problem in the synthesis of chiral molecules. A convenient access to chirality is the stereocontrolled modification of suitable natural products. In this paper, we wish to report a stereospecific synthesis of the potent antibiotic thienamycin[1] (**9**) from readily available 6-aminopenicillanic acid (**1**).

The strategy employed to transform the stereochemistry of penicillin, 5*R*, 6*R*, to that of thienamycin, 5*R*, 6*S*, 8*R*, takes advantage of the strong preference of β-lactam systems for trans substitution. Thus, replacement of the penicillin 6-nitrogen with acetyl (**1** → **2**) and reduction of the chelated *trans*-ketone from the β side (**2** → **3**) set the stereochemistry at C-8 and C-6 as *R* and *S*. Degradation of the thiazolidine ring to **5d** followed by trans substitution of the corresponding immonium intermediate established the remaining center C-5 as *R*. Noteworthy features of this synthesis are the stereospecific amine–borane reduction of ketone **2** and use of a novel β-carbene-anion equivalent for annelation.

(1) For previous synthesis, see: (a) Melillo, D. G.; Shinkai, I.; Liu, T.; Ryan, K.; Sletzinger, M. *Tetrahedron Lett.* **1980**, 2783. (b) Saltzman, T. N.; Ratcliffe, R.; Christensen, B. G.; Bouffard, F. A. *J. Am. Chem. Soc.* **1980**, *102*, 6161. (c) Johnston, D. B. R.; Schmitt, S. M.; Bouffard, F. A.; Christensen, B. G. *Ibid.* **1978**, *100*, 313. Kametami, T.; Huang, S.-P.; Suzuki, J.; Yokohama, S.; Shara, M. *Heterocycles* **1979**, *12*, 1301.

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