Purpurone, an Inhibitor of ATP-Citrate Lyase: A Novel Alkaloid from the Marine Sponge *Iotrochota* sp.

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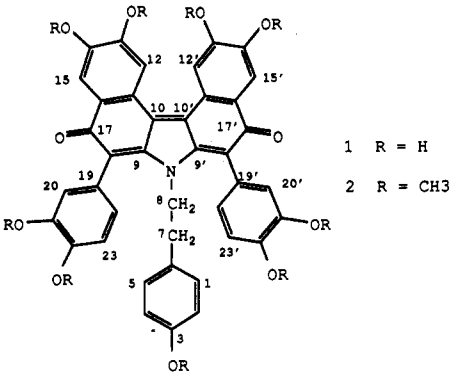
A novel compound, purpurone (1), with ATP-citrate lyase inhibitory activity, was isolated from the marine sponge *Iotrochota* sp. Its structure was established mainly on the basis of NMR spectroscopic data. Purpurone represents the first example of a new class of marine products.

With low-density lipoproteins (LDL) playing a key role in hypercholesterolemia, agents that can stimulate LDL catabolism[1,2] or inhibit LDL synthesis[3,4] are of important interest in the treatment of the disease. Since the very-low-density lipoproteins (VLDL) as metabolic precursors of LDL are produced from fatty acids and cholesterol, the intervention of VLDL synthesis at the acetyl coA and the citrate lyase level has provided a strategic target for hypercholesterolemia therapy. It is known that an inhibitor of HMG-CoA reductase in the liver can reduce cholesterogenesis by interfering the conversion of acetyl coA to mevalonic acid. An inhibitor of the enzyme ATP-citrate lyase (ACL), on the other hand, is anticipated to reduce the production of acetyl CoA and can affect both lipogenesis and cholesterogenesis.[5] During the course of screening natural products for ATP-citrate lyase activity, we selected an extract of the marine sponge *Iotrochota* sp., from which purpurone (1) was isolated. In this paper, we report the purification, structure elucidation, and biological activity of purpurone.

The aqueous ethanol extract of *Iotrochota* sp. obtained from Suntory[6] showed good ATP-citrate lyase inhibitory activity (IC_{50} = 25 μg/mL). Initial bioassay-guided fractionation[7] of the extract on reversed-phase supports yielded several purple aqueous fractions with enhanced activity. However, no pure active components could be isolated until the extract had been subjected to mild acid hydrolysis using 2 N methanolic HCl. Chromatography of the hydrolysate by repeated RP18 HPLC afforded a single active compound, purpurone (1), in low yield. Our speculation is that the more lipophilic compound 1 came from precursors that were either sugar or protein conjugates. These conjugated components, present in small amounts, were extremely polar, showing no affinity to reversed-phase supports, and were inseparable from each other. Purpurone, as indicated by its name, is a purple colored compound. Its unusual spectral characteristics (high mass versus few NMR signals) eliminated the known purple marine products[8,9] from structural considerations.



Purpurone was obtained as a purple, noncrystalline glassy solid. Its HRFAB mass spectrum ($[M + H]^+$, m/z = 698.1673) established a molecular formula of $C_{40}H_{27}NO_{11}$. Since only 22 carbon signals and nine proton signals were observed for the compound (Table I), purpurone must be a highly symmetrical molecule in which the nitrogen atom, one oxygen atom, and four carbon atoms lie on a plane of symmetry. The IR spectrum contained a strong hydroxyl band at 3600-3100 cm^{-1} and a carbonyl band at 1680 cm^{-1} which was assigned to a highly conjugated

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(6) The *Iotrochota* sp. obtained from Suntory was of an Indopacific origin. Specimens of *Iotrochota* species from the Caribbean were devoid of the purpurone-type compounds, and their extracts were inactive in the ATP-citrate lyase assay.
(7) Bioassay of ATP-citrate lyase (ACL): Function of ACL was measured in a coupled enzyme assay in which the oxaloacetate produced from citrate and coenzyme A is reduced by malate dehydrogenase to malate using NADH as the source of reducing potential. Substrate concentrations were adjusted so that the amount of ACL was limiting and progress of reaction was measured by following absorbance at 340 nm. Briefly, in a final volume of 250 μL of 50 mM Tris·HCl, pH 8.0, containing 1 mM citrate, 5 mM ATP, 0.2 mM coenzyme A, 10 mM MCl_2, 10 mM KCl, 10 mM dithiothreitol, 10 units of malate dehydrogenase (EC 1.1.1.37 from porcine heart), 0.2 mM NADH, and 1μg/mL of ACL was incubated 20 min at 25 °C while A_{340} was measured using a UVmax kinetic spectrophotometer. Crude extract samples were dissolved in DMSO and added to the reaction mixture so that the final concentration of DMSO was 2.5% (v/v). All assays were done in flat-bottom, 96-well microtiter plates with all controls (without enzyme or without inhibitor), as well as a standard curve ranging from 0.067 to 0.2 μg/mL ACL. The amount of ACL activity remaining in the presence of a putative inhibitor was calculated by interpreting the slope of ΔA_{340} in the test sample with the curve-fit data from standard samples in the same microtiter plate.
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Table I. ^1H and ^{13}C NMR Data of Purpurone (1)[a]

atom	δ (^{13}C) (m[b])	δ (^1H) (integ, m, J, Hz)	long-range coupled ^{13}C[c]
1(5)	130.9 (d)	6.43 (2 H, d, 8.5)	C3, C7
2(4)	115.7 (d)	6.38 (2 H, d, 8.5)	C6
3	156.7 (s)		
6	129.8 (s)		
7	34.5 (t)	2.20 (2 H, t, 7.0)	C1(5), C6, C8
8	49.6 (t)	2.95 (2 H, t, 7.0)	C6, C7, C9(9′)
9(9′)	156.1 (s)		
10(10′)	126.5 (s)[d]		
11(11′)	125.0 (s)		
12(12′)	114.1 (d)	7.80 (2 H, s)	C10(10′), C13(13′), C14(14′), C16(16′)
13(13′)	150.1 (s)[e]		
14(14′)	149.0 (s)[e]		
15(15′)	115.1 (d)	7.45 (2 H, s)	C11(11′), C13(13′), C14(14′), C17(17′)
16(16′)	132.1 (s)[d]		
17(17′)	185.4 (s)		
18(18′)	118.9 (s)		
19(19′)	126.1 (s)		
20(20′)	119.5 (s)	6.85 (2 H, d, 1.8)	C18(18′), C19(19′), C22(22′), C24(24′)
21(21′)	146.2 (s)		
22(22′)	146.6 (s)		
23(23′)	116.2 (d)	6.78 (2 H, d, 8.0)	C19(19′), C21(21′)
24(24′)	124.1 (d)	6.67 (2 H, dd, 8.0, 1.8)	C20(20′), C22(22′)

[a] Recorded in methanol-d. [b] Multiplicity deduced from GASPE and INEPT. [c] Observed from HMBC. [d,e] Assignments interchangeable.

carbonyl group; a signal at δ 185.4 in the ^{13}C NMR spectrum supported that assignment. The UV absorptions at 296 and 500 nm were shifted to 315 and 511 nm upon addition of base, indicating the presence of phenolic groups. No shifts were observed, however, upon the addition of acid, suggesting that the nitrogen was not basic. Permethylation of 1 with diazomethane produced an unstable nonamethyl derivative **2** (*m/z* 824.3082) which showed five methyl signals in a ratio of 2:2:2:2:1 in the ^1H NMR spectrum. Thus, purpurone contains nine phenolic groups, one of which lies on the plane of symmetry. Each of the symmetric units consequently has one carbonyl and four phenolic groups.

The presence of a tyramine unit in the plane of symmetry is evident from the NMR spectra. The proton signals at δ 2.20 (t, J = 7 Hz, 2 H) and 2.95 (t, J = 7 Hz, 2 H) correlating to carbon signals at δ 34.5 (t) and 49.6 (t), respectively, clearly arise from methylene groups. Each of the other seven proton signals in the ^1H NMR spectrum therefore represents two hydrogens by integration. The signals at δ 6.38 (d, J = 8.5 Hz, 2 H) and 6.43 (d, J = 8.5 Hz, 2 H) were assigned to the protons on the *p*-hydroxyphenyl ring. The HETCOR and HMBC experiments identified the carbon and the proton signals due to the tyramine unit, giving chemical shift assignments as listed in Table I. The HMBC correlation from the proton signal at δ 2.95 to a carbon signal signal at δ 156.1 suggested that the tyramine nitrogen was attached to two symmetrical units, each $C_{16}H_9O_5$, through an aromatic carbon atom. This aromati carbon must be conjugated to a carbonyl atom to account for a significant downfield shift.

The ^1H NMR spectrum also contained an isolated spin system with signals at δ 6.67 (dd, J = 8, 1.8 Hz, 2 H), 6.78 (d, J = 8 Hz, 2 H), and 6.85 (d, J = 1.8 Hz, 2 H). The HMBC correlations indicated that the signals could be assigned to two symmetrically located 1,2-dihydroxyphenyl rings that were substituted at the 4-position. Irradiation of both proton signals at δ 2.95 and 2.20 caused nuclear Overhauser enhancements of the signals at δ 6.67 and 6.85. These enhancements suggested that the two 4-substituted 1,2-dihydroxyphenyl rings were flanking the tyramine unit. The HMBC spectrum showed that each ring was attached to an aromatic carbon at δ 118.8 (s), which is an appropriate chemical shift for C-18.

The remaining signals in the ^1H NMR spectrum are at δ 7.45 (s, 2 H) and 7.80 (s, 2 H). The HETCOR spectrum indicated that the two proton signals at δ 7.45 and 7.80 were directly correlated to the carbon signals at δ 115.1 (d) and 114.1 (d), respectively. The HMBC spectrum indicated that both proton signals were correlated with the ^{13}C NMR signals at δ 149.0 (s) and 150.1 (s), which were assigned to phenolic carbons. These proton signals were therefore on two symmetrically located 1,2-disubstituted 4,5-dihydroxyphenyl rings. Since the HMBC spectrum showed a strong correlation between the ^1H NMR signal at δ 7.45 and the ^{13}C NMR signal at δ 185.4, the carbonyl groups must be adjacent to the 1,2-disubstituted 4,5-dihydroxyphenyl rings. In order to attain the correct degree of unsaturation, the structure of purpurone must be drawn as shown. Since the HMBC experiment did not show a two-bond correlation from the proton signal at δ 7.45 to either of the carbon signals at δ 126.5 and 132.1, the assignment of these two carbon signals is ambiguous. It is interesting to note that purpurone (1) cannot adopt a planar geometry due to the steric interaction between H-12 and H-12′. Although this could result in an optically active material, no optical rotation was observed for purpurone.

Purpurone inhibits ATP–citrate lyase in a dose-dependent manner and has an IC_{50} of 7μM. When tested for cytotoxicity, purpurone (1) was found to be nontoxic to Hep G2 cells, showing no reduction in cellular ATP levels at 100μg/mL. Preliminary data indicated the compound was able to reduce fatty acid but not cholesterol biosynthesis.

Experimental Section

General. Multiplicities of ^{13}C NMR resonances were obtained from GASPE[10] and INEPT. HETCOR and HMBC experiments were performed on instruments operating at 400 and 360 MHz for ^1H and 100 and 90 MHz for ^{13}C, respectively. The fast atom bombardment (FAB) mass spectra were measured on samples in a matrix of dithiothreitol and dithioerythritol. High-performance liquid chromatography (HPLC) was done using reversed-phase 10-μm columns and HPLC-grade solvents.

Collection and Isolation of Purpurone (1). The *Iotrochota* sponge was collected in September 1983 at the Koror island, Palau. The aqueous ethanolic extract of the sponge as a dark brown gum (900 mg) was heated in 12 mL of anhydrous 2 N methanolic HCl in a sealed tube at 70 °C for 12 h. The reaction mixture was evaporated to dryness. The residue was fractionated by repeated reversed-phase HPLC (ODS, MeCN–0.2% TFA (75:25)). Active purple fractions containing HPLC homogenous 1 were pooled and freeze-dried. Purpurone 1 was obtained in 8.0-mg yield.

Preparation of Permethyl Purpurone (2). A solution of compound 1 (2.0 mg, 3 μmol) in 2 mL of MeOH was treated with 0.4 mL of a 0.3 M ethereal solution of diazomethane. After being stirred at 25 °C for 4 h, the mixture was evaporated to dryness, and the residue was purified by silica TLC ($MeOH–CH_2Cl_2$ (6:94)) to give **2**.

Purpurone (1): purple glass; HRFABMS MH^+ 698.1673 (calcd for $C_{40}H_{28}NO_{11}$ Δ 1.1mmu); UV (MeOH) λ_{max} 216 (ε 28 100), 296 (15 100), and 500 nm (11 400); UV (MeOH/KOH) λ_{max} 215 (ε 30 400), 315 (16 400), and 511 nm (8500); IR (KBr) ν_{max} 3600–

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3100, 1680, 1631, 1596, 1558, 1515, 1455, 1384, 1296, 1251, 1205, 1119, 1060 and 722 cm^{-1}; ^1H and ^{13}C NMR, Table I.

Permethyl purpurone (2): Yellow-brown solid; HRFABMS MH^+ 824.3082 (calcd for $C_{49}H_{46}NO_{11}$ Δ 1.1 mmu); ^1H NMR (acetone-d_6) δ 7.85 (2 H, s), 7.50 (2 H, s), 7.02 (2 H, d, J = 1.6 Hz), 6.95 (2 H, d, J = 8.5 Hz), 6.90 (2 H, dd, J = 8.5, 1.6 Hz), 6.48 (2 H, d, J = 8.6 Hz), 6.34 (2 H, d, J = 8.6 Hz), 3.87 (6 H, s), 3.75 (6 H, s), 3.72 (6 H, s), 3.70 (6 H, s), 3.38 (3 H, s), 2.90 (2 H, t, J = 9.0 Hz), 2.20 (2 H, t, J = 9.0 Hz).

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