**Table I.** Mössbauer Effect Spectral Parameters[a]

| compd | site | $\delta_{Fe}$ | $\Delta E_Q$ | $\Gamma$ | $A$, % | $X^2$ | $\Delta\delta$ | $\sum\delta$ | Fe–CO[b] | Fe–$\mu$-CO | $n_e$[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fe$_3$(CO)$_{12}$ | Fe(1,2) | 0.115 (4) | 1.119 (6) | 0.259 (2) | 61.4 | 1.29 | 0 | 0.287 (7) | 3 | 2 | 0 |
| | Fe(3) | 0.057 (4) | 0[d] | 0.316 (3) | 38.6 | | 0 | | 4 | 0 | 0 |
| [PPN][Fe$_3$(CO)$_{10}$CH] | Fe(1,2) | 0.002 (3) | 1.145 (5) | 0.251 (2) | 66.7[d] | 1.27 | 0.113 (5) | 0.005 (6) | 3 | 1 | $^1/_2$ |
| | Fe(3) | 0.001 (4) | 1.540 (7) | 0.334 (4) | 33.3[d] | | 0.056 (6) | | 3 | 0 | 0 |
| [PPN][Fe$_2$Co(CO)$_9$CCO] | Fe(1) | −0.019 (4) | 0.905 (4) | 0.236 (3) | 50.0[d] | 0.64 | 0.134 (6) | −0.017 (6) | 3 | 1 | $^1/_2$ |
| | Fe(3) | 0.002 (5) | 0.746 (5) | 0.276 (6) | 50.0[d] | | 0.055 (6) | | 3 | 0 | 0 |
| [PPN]$_2$[Fe$_3$(CO)$_9$CCO] | Fe(1,2) | −0.041 (4) | 0.494 (2) | 0.267 (4) | 66.7[d] | 0.85 | 0.156 (6) | −0.101 (7) | 3 | 1 | 1 |
| | Fe(3) | −0.019 (4) | 0.224 (2) | 0.253 (6) | 33.3[d] | | 0.076 (6) | | 3 | 0 | 0 |

[a] All data in mm/s obtained at 78 K relative to natural abundance $\alpha$-iron foil. [b] The number of indicated bonds at each iron site. [c] The approximate localization of the electron added to the cluster upon reduction. [d] Component variable constrained to the given value.
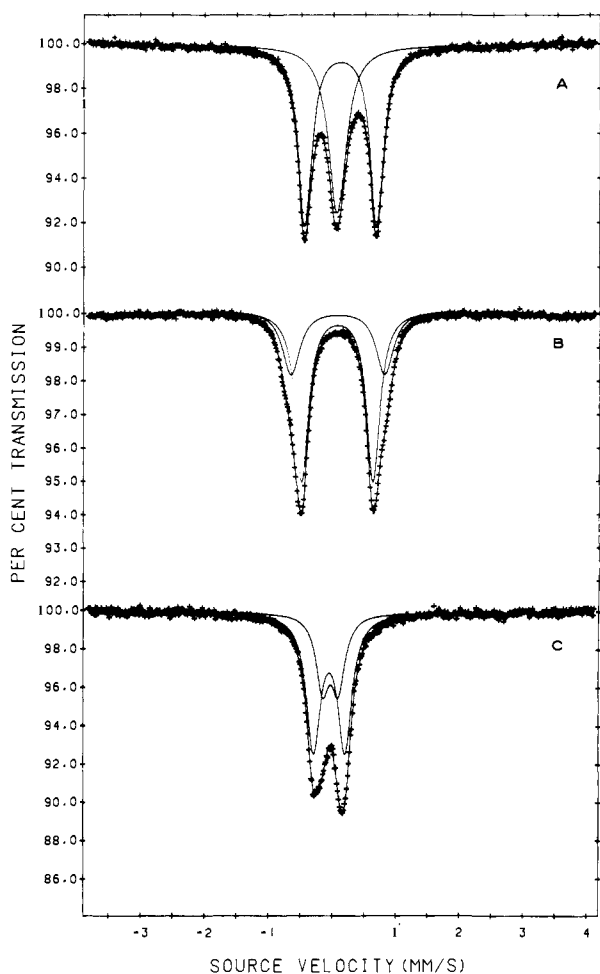


**Figure 1.** Mössbauer effect spectra obtained at 78 K for (A) Fe$_3$(CO)$_{12}$ (I), (B) (PPN)[Fe$_3$(CO)$_{10}$CH] (II), and (C) (PPN)$_2$[Fe$_3$(CO)$_9$CCO] (III).

increase in site electronic symmetry is largely expected as the loss of the $\mu$-CO group yields a site with roughly 6-fold octahedral coordination. The even larger reduction of $\Delta E_Q$ for the Fe(3) site in III and its resultant high electronic symmetry is no doublt a result of the interaction[16] between the Fe(3) atom and the ketenylidene group as indicated by the broken bond in the schematic of III. A similar increase in the electronic symmetry at Fe(3) in IV is observed if one compares the decreased quadrupole interaction at Fe(3) with that found in II. In fact, this interaction to increase the electronic symmetry at the Fe(3) in III and IV may be the driving force behind the tilting of the CCO toward the metal plane as expected in III and observed[16] in IV. No similar interaction would be needed in H$_2$Os$_3$(CO)$_9$(CCO), which is observed[17] to have the CCO group normal to the Os$_3$ metal plane.

Our results help to expain the nucleophilic reactivity of III toward methylating agents and acids.[16] The selective partial localization of the added electrons on the Fe(1,2) sites in the reduced clusters yields a stronger nucleophilic potential at the bridging carbon. The smaller added electronic density at Fe(3) upon reduction permits a significant interaction between Fe(3) and the ketenylidene group facilitating the breaking of the carbon–carbon bond and the formation of the resulting $\mu$-CO group from the ketenylidene ligand.

## Isolation and Structure of Tunichrome B-1, a Reducing Blood Pigment from the Tunicate *Ascidia nigra* L.

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The hematology of tunicates, a class of common marine organisms that selectively accumulate metals such as V, Fe, Mo, Nb, has puzzled scientists for over 70 years.[1] The black tunicate *Ascidia nigra* (Linnaeus) sequesters vanadium as the pentavalent vanadate, concentrates it by 10$^6$-fold,[2] and stores the metal in its reduced V(III) or V(IV) states[3,4] at physiological pH (ca. 7.2),[5] possibly as a hexaaquo or other complex.[6] The apparent contradiction that V(III) is unstable in aqueous media at pH above 2.5 can be overcome by assuming the presence of a strong reductant that also serves as a good complexing agent. We believe that this role is fulfilled by the tunichromes,[7] the bright yellow

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blood pigments[8] of *A. nigra*, and various other tunicates.[9] Tunichromes, especially in the crude state, are extremely labile, consist of a complex mixture of closely related compounds, and have eluded all isolation attempts. We report the isolation and characterization of tunichrome B-1 (TB-1), the first member of a group of new biological reducing agents. All following operations were carried out under dry, $O_2$-free Ar and in degassed solvents.
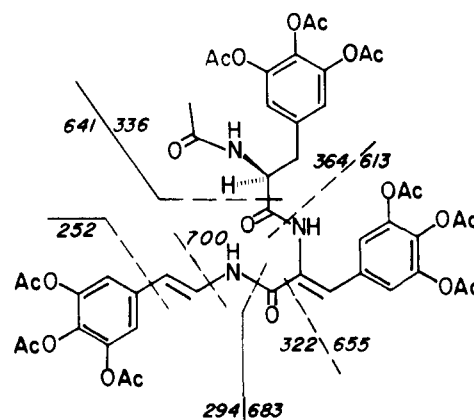
Blood was taken from the hearts of adult *A. nigra* (collected off Key Biscayne, FL) and centrifuged. The pellets were immersed in liquid $N_2$, lyophilized, ground with $Na_2SO_4$, and placed on a Sephadex LH-20 column. After stepwise gradient elution with 1:1 $CH_2Cl_2$/*i*-PrOH, *i*-PrOH, *i*-PrOH/EtOH (90–10%), and EtOH (all containing 0.2% 3-*tert*-4-hydroxy-5-methylphenyl sulfide[10]), the bright yellow tunichromes began to move with EtOH/MeOH (4:1). When the first yellow band reached the column end, the gel was extruded into a glovebag and sliced into three portions according to color intensities: TA (least polar)/ TB/TC. The major segment containing TB was extracted with MeOH/*tert*-butyl sulfide (*t*-BS) (99:1), concentrated, and repeatedly precipitated to give TB as a yellow powder. At this stage 1000 tunicates yielded 120 mg of TA, 1300 mg of TB, and 300 mg of TC amounting to ca. 1.7 mg of tunichrome/animal.[11]

The TB fraction separated on TLC[12] into three orange fluorescent bands. However, HPLC of TB employing a UV diode array detector[13] showed that each TLC spot consisted of two compounds with closely related UV spectra. The six components TB-1 (major) to TB-6 could not be separated on a scale larger than analytical due to extensive decomposition during adsorption chromatography. However, the new centrifugal counter-current chromatography (CCCC) technique[14] was ideally suited for TB separation due to its rapid nondestructive nature, the possibility of medium-scale operation and the ability to switch from "normal" to "reversed phase"[15] during separation. The following unusually complex two-phase system was found to be optimal: *i*-AmOH/*n*-BuOH/*n*-PrOH/$H_2O$/HCOOH/*t*-BS (8:12:10:30:0.25:1), upper phase = mobile phase, 2.4 mL/min, 1000 rpm. After elution of the two less polar components TB-5/6 and TB-3/4, the phases were switched (lower phase = mobile phase) allowing the most polar pair TB-1/2 to elute as a sharp peak after 7 h (30 mg of TB-1/2 from 200 mg of crude TB mixture). Semipreparative reversed-phase HPLC of the TB-1/2 fraction employing two columns in series[16] finally yielded pure TB-1, however, with considerable loss, i.e., 500 μg of pure TB-1 from 5 mg TB-1/2.[17] TB-1 (**1**) has the following spectral characteristics: SIMS,[18] *m/z*
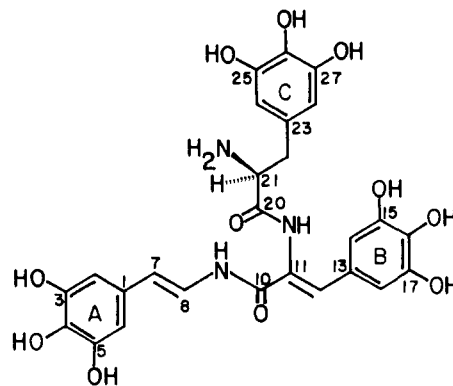


556 (M + H); UV (MeOH) 210 nm (ε 68 000), 245 (sh), 285 (sh), 340 (19,600); CD (MeOH) 243 nm (Δε −2.5), 281 (+1.2), 340 (−3.3); IR (KBr) 3700–2400 cm⁻¹ (br), 1648; ¹H NMR (250 MHz, pyr-*d*₅) δ 10.69 (d, *J* = 10.5 Hz, N⁹H; other NH and OH peaks coalesce as broad band), 8.06 (dd, *J* = 10.5, 14.6 Hz, 8-H), 6.45 (d, *J* = 14.6 Hz, 7-H), 7.63 (s, 1 H, 12-H), 7.37 (s, 2 H Ar), 7.02 (s, 2 H, Ar), 6.85 (s, 2 H, Ar), 4.38 (dd, 1 H, *J* = 4, 10 Hz)/3.75 (dd, 1 H, *J* = 4, 14 Hz)/3.12 (dd, 1 H, *J* = 10, 14 Hz) for NCHCH₂. The instability of TB-1 hindered further spectral measurements; the permethyl ether (TB-1-Me) (CH₂N₂/MeOH, <20% yield), also unstable, showed the presence of nine methyls: DCI-MS,[19] *m/z* 682 (M + H).

Most structural studies were performed by using the decaacetate (TB-1-Ac) (excess Ac₂O/pyr; prep TLC 5% *i*-PrOH/CH₂Cl₂; then HPLC, YMC gel SI-5μ, 4% *i*-PrOH/CH₂Cl₂): C₄₆H₄₅N₃O₂₁ FAB-HRMS,[20] *m/z* 976.2625, calcd 976.2624 (M + H) (see **2**).



¹³C and ¹H NMR spectra clarified the nature of all carbons and hydrogens.[21] Ozonolysis of TB-1-Ac gave triacetylgallaldehyde (2 equiv by UV), whereas hydrolysis of TB-1-Me (4N HCl/reflux overnight) yielded (3,4,5-trimethoxyphenyl)alanine (TPA, compared with synthetic *dl* sample). The most plausible structure **1** derived from these data contains hydroxylated phenyl enamide and dehydro-5-hydroxy-DOPA moieties such as found in clinoamide[22] and the celenamides.[23] Structure **1** was then established by ¹H NMR of tetrahydro-TB-1-Ac[24] (H₂/5% Pd–C) and NOE measurements on TB-1-Ac.[21] The methoxydiphenylfuranone (MDPF) derivative[25] of the trimethoxyphenylalanine from the

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(11) Amount in animals collected in Sept 1984. Values season dependent; an isolation carried out in Feb 1984, for example, gave only half the amount.

(12) Whatman LHP-KF silica plates, toluene/methyl ethyl ketone/ethyl acetate/water/formic acid (6:24:50:10:10), sprayed with 1% diphenylboric acid–ethanolamine complex, 366-nm detection.

(13) Column: YMC-gel ODS II, 3 μm, 4.6 × 200 mm (distributed by YMC, Inc., P.O. Box 492, Mt. Freedom, NJ 07970); 0.001 M HCOONH₄/CH₃CN (88.5:11.5; adjusted to pH 2.8), containing 0.02% *t*-BS; LKB 2140 rapid spectral detector, spectral width 200–370 nm, scanning interval 0.4 s.

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(15) In normal-phase mode, the organic layer of a biphasic solvent system is used as mobile phase; in reversed-phase mode the mobile phase is the aqueous layer.

(16) YMC gel ODS II, 15 and 5 μm, both 25 × 250 mm, 0.03 M HCOONH₄/CH₃CN (91:9; adjusted to pH 3.0), containing 0.5% 2-methoxyethanol and 0.02% *t*-BS.

(17) A detailed description of the isolation of tunichromes will be given elsewhere.

(18) Hitachi EPI-G2 spectrometer, secondary ion MS, glycerol matrix. We thank Dr. H. Naoki, Suntory Institute for Bioorganic Research, for measurements.

(19) Ribermag R-10-10 spectrometer, desorption/chemical ionization mode (CH₄ or NH₃ as reactant gas). We thank Slavica Sporer and Vinka Parmakovich for these MS measurements.

(20) Kratos M-50 spectrometer, high-resolution fast atom bombardment mode (Xe ionizing gas; thioglycerol matrix). We thank Drs. Michael L. Gross and Kenneth Tomer, University of Nebraska at Lincoln, for measurements.

(21) See supplementary material.

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(24) COSY (Bruker WM 300) measurements clarified couplings within pertinent spin systems: δ 2.74 (2 H, m, 7-H)/3.24 and 3.56 (1 H each, both m, 8-H's)/6.78 (dd, N⁹-H), 3.00 (m, 12-H's)/4.59 (m, 11-H)/6.68 (d, N¹⁹-H).

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hydrolysate of TB-1-Me exhibited a Cotton effect of $\Delta\epsilon$ +2.3 at 380 nm, thus showing the C-21 configuration to be $S$.

The tunichrome B-1 structure is derived from three (3,4,5-trihydroxyphenyl)alanine units; the reducing nature is due to the pyrogallol moiety which is known to reduce V(V) to V(IV) and form complexes.[26] Elucidation of the TB-1 structure will now enable one to clarify the long-standing controversy regarding the biological/biochemical function of tunichromes and vanadium and the mechanism of V(V) to V(III) reduction in ascidians, as well as to contribute to the understanding of the biochemical role of vanadium in animals.[27]

**Supplementary Material Available:** ^{1}H and ^{13}C NMR, mass spectra, NOEs, Cosy, and other spectroscopic data of 1 and derivatives (8 pages). Ordering information is given on any current masthead page.

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## Electrochemical Permeability Control through a Bilayer-Immobilized Film Containing Redox Sites

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We report reversible permeability control through a viologen (4,4'-bipyridinium)-containing bilayer-film deposited on a platinum minigrid sheet by electrochemical redox reactions.

Developments of various types of permeability-controllable membranes have been prompted by the need to study the transport properties of biological membranes.[1] We have reported the signal-receptive, bilayer-coated capsule membranes, in which bilayers supported on the physically strong polymer membrane act as a valve of slow releases from the capsule responding to stimuli from the outside.[2] Kunitake, Kajiyama, and co-workers recently prepared various types of the bilayer-immobilized films[3,4]

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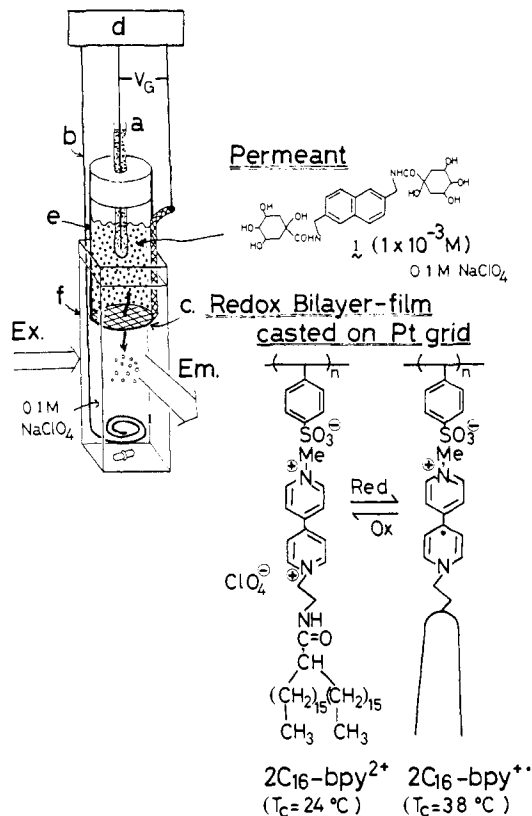


**Figure 1.** Experimental setup for the permeation of fluorescent probe 1 controlled by electrochemical redox reactions. (a) Reference electrode, Ag/AgCl in saturated KCl (−0.05 V vs. SCE); (b) Pt wire for counter electrode; (c) Pt minigrid sheet (100 mesh, 28 mm$^2$) embedded in 2C$_{16}$-bpy$^{2+}$-PSS$^-$ bilayer films; (d) potentiostat; (e) Polyethylene tube (diameter, 7 mm); (f) 1-cm quartz cell.
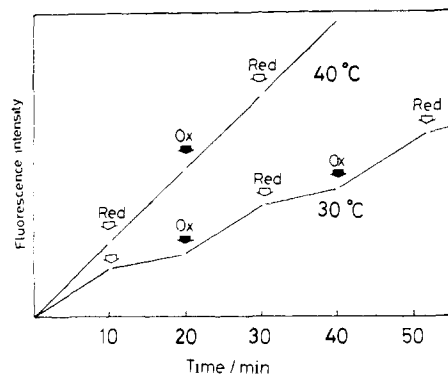


**Figure 2.** Permeation controls of the probe 1 across the viologen-containing film on a Pt grid by electrochemical redox reactions. The potential of −0.5 and 0 V vs. SCE was applied to Pt grid/film at Red and Ox, respectively.

and reported that the permeability across the bilayer- or liquid-crystal-immobilized film could be changed by phase transition phenomena[4] and photoirradiation.[5] We also observed the permeability of the bilayer film could be regulated by the electric transmembrane potential.[6] Burgmayer and Murray first reported that the permeability of anions through a polypyrrole film deposited on a Au grid could be changed by positive charge formation on the film by electrochemical redox reactions.[7]

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