$^{13}C,^{2}H_2$]malonate in this process, an experiment patterned after the earlier work of Abell and Staunton with mono- and trideuterated acetic acid as polyketide precursors.[4a] Stereospecific proton removal will result in nearly equal degrees of deuterium loss from a CHD and a CD_2 methylene group in the polyketide, because there is no intramolecular competition between H and D. However, if the proton removal is nonstereospecific, a substantially higher deuterium retention from the CHD than from the CD_2 methylene group is expected because of the high primary kinetic deuterium isotope effect ($k_H/k_D \sim 4-7$)[2a] associated with enolization reactions. [2-$^{2}H_1$]Malonate (87% D_1)[10] and [2-$^{13}C,^{2}H_2$]malonate (99% ^{13}C, 98% D_2) were converted into orsellinic acid in the same coupled enzyme system used with the chiral malonate samples. GC-MS analysis of the products showed 51% deuterium retention per labeled site from the monodeuterated precursor and 26% from the dideuterated precursor.[11]

The results of the above experiments strongly suggest that the proton abstractions from C-3 and C-5 of the polyketide precursor in the formation of orsellinic acid are not stereospecific and therefore not enzyme-mediated (Scheme I), although they may occur while the molecule still resides in the chiral environment of the protein. This contrasts with the biosynthesis of 6-methylsalicylic acid and of rubrofusarin, for which Staunton and co-workers[4] proposed stereospecific proton removal based on comparisons of the incorporation of [2-$^{2}H_1$]- and [2-$^{2}H_3$]acetic acid.

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(10) Prepared by generating the trianion of malonic acid with sodium hydride and quenching with D_2O.

(11) Theoretical values, in the absence of any exchange and isotope discrimination, in both cases are 50%. The data indicate extensive (~50%) exchange and a sizeable primary kinetic isotope effect ($k_H/k_D > 2$).

Ohioensin-A: A Novel Benzonaphthoxanthenone from *Polytrichum ohioense*

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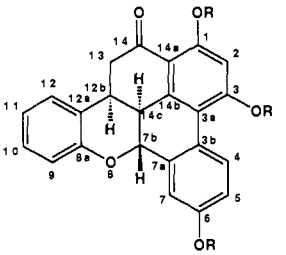
The National Cancer Institute has conducted an initial screening program to discover novel biologically active compounds from the Bryophyta.[1] As part of this program to isolate antineoplastic agents from mosses, *Polytrichum ohioense* (*Polytrichaceae*) has been studied. Although a number of mosses have been examined phytochemically, there were no published examinations of this species prior to our investigations. In this communication we report the structure of ohioensin-A (**1**) which contains a novel polycyclic skeleton and exhibits cytotoxicity against PS and MCF-7 tumor cells in culture at ED_{50} 1.0 and 9.0 μg/mL, respectively.[2]



1 R = H

2 R = Ac

Ohioensin-A (**1**, 1.5 × 10^{-4}% yield) was isolated from the ethanol extract of *P. ohioense* following solvent partitioning and silica gel ($CHCl_3$–MeOH, 97:3) chromatography. Ohioensin-A (**1**) ($C_{23}H_{16}O_5$), yellow needles from $CHCl_3$–MeOH (1:1), mp 274–275 °C dec, $[\alpha]_D^{27}$ +37° (*c* 0.1, MeOH), had IR bands at 3500–2500 (OH, br), 1620 (C=O), 1600, and 1570 (aromatic ring) cm^{-1}, indicating the presence of intramolecularly hydrogen bonded hydroxyl and conjugated carbonyl functions. The UV bands at 272.5 and 361.0 nm, which exhibited a bathochromic shift upon adding $AlCl_3$, suggested the presence of a phenolic hydroxyl group in the vicinity of a keto function. EIMS showed a significant fragment at *m/z* 354 by a loss of water from the stable molecular ion. This ion may originate by a rearrangement involving the transfer of H-13 to the C-14 carbonyl oxygen to give the enol form.[3] On the basis of this, the carbonyl carbon was linked to an aliphatic carbon with at least one hydrogen attached. Analysis of the ^{13}C NMR spectrum established the presence of one carbonyl, one methylene, 11 methine (eight aromatic), and ten aromatic quaternary carbons, which supported the presence of a polycyclic skeleton with highly aromatic character. The ^{1}H NMR spectrum of **1** indicated three exchangeable singlets at δ 7.43, 8.81, and 12.13 assigned to three phenolic hydroxyls, one of which was hydrogen bonded. This was confirmed by the formation of a triacetate (**2**). One uncoupled proton in the high field aromatic region (6.53, 1 H, s) could be assigned to H-2. This signal showed NOE enhancements of 9% on irradiation of the hydroxy group at δ 12.13 of **1** and of 16% on irradiation of the C-3 acetoxyl methyl protons at δ 2.36 of **2**. Upon acetylation, the H-2 signal exhibited an unusual downfield shift of δ 0.63 which confirmed its location between two phenolic hydroxyl groups. The ^{1}H NMR spectrum of **1** also indicated two groups of aromatic proton signals based on homonuclear ^{1}H–^{1}H decoupling experiments. The downfield shifts of H-7($\Delta\delta$ 0.32) and H-5($\Delta\delta$ 0.47) in the ^{1}H NMR of **2** established that a hydroxyl group was substituted at the C-6 position. The remaining interrelated aliphatic proton signals were also unambiguously assigned by selective decoupling experiments.

A computer-generated perspective drawing of the final X-ray model of **1** is given in the Supplementary Material.[4] The X-ray analysis did not define the absolute but only the relative stereochemistry; the enantiomer shown was arbitrarily chosen. The asymmetric unit of crystalline **1** consists of two independent molecules with identical structures and only minor conformational differences. The sp^3 centers at C7b, C14c, C12b, and C13 give the essentially planar molecule some slight three-dimensionality. For example, the twist about the C3a–C3b biphenyl bond is −17°. With the stereochemistry being relative, **1** was named

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(4) Two molecules of composition $C_{23}H_{16}O_5 \cdot CH_3OH$ formed the asymmetric unit. The structure was solved and refined with the SHELXTL PLUS package of programs. The final model using anisotropic heavy atoms and fixed isotropic hydrogens has refined in a full-matrix least-squares to a conventional *R*-factor of 0.041 for the observed reflections. Both molecules comprising the asymmetric unit had identical stereostructures. See the paragraph entitled Supplementary Material Available for additional crystallographic details.

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(7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,3,6-trihydroxy-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthen-14-one.

The biogenetic pathway to **1** in *P. ohioense* apparently involves *O*-hydroxycinnamate and hydroxylated bibenzyls as intermediates. These compounds have been detected as natural products in a few mosses.[5,6]

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Supplementary Material Available: Details of the X-ray study including space group, experimental conditions, tables of atomic coordinates, thermal parameters, interatomic distances, and interatomic angles for **1**, and spectral data (UV, IR, MS, ^{13}C NMR, and ^{1}H NMR) for **1** and **2** (6 pages). Ordering information is given on any current masthead page.

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A Binuclear Mixed-Valence Ferromagnetic Iron System with an $S = 9/2$ Ground State and Valence Trapped and Detrapped States

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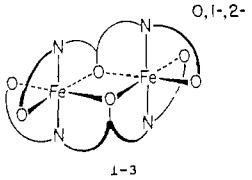
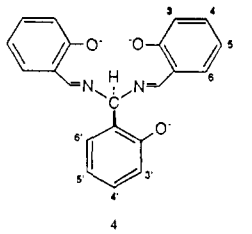
We have recently described the synthesis and certain physicochemical properties of a series of binuclear iron complexes $[Fe_2(salmp)_2]^{0,1-,2-}$ (**1–3**), derived from trianionic binucleating



1–3

ligand salmp (**4**).[1,2] These complexes are part of a set whose members contain the bridge unit $Fe_2(\mu\text{-}OR)_2$ and, in turn, are



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members of a much larger set including some ten types of oxygen-bridged binuclear complexes.[1] There is substantial interest in binuclear Fe complexes because of the presence of binuclear, magnetically coupled units in proteins.[3] Subset **1–3** is unique because each oxidation level has been structurally defined and each, from magnetic susceptibility behavior, is *ferromagnetic*.[1] The current EPR and Mössbauer studies confirm this property and provide data elucidating the temperature dependence of the electron distribution in mixed-valence complex **2**.

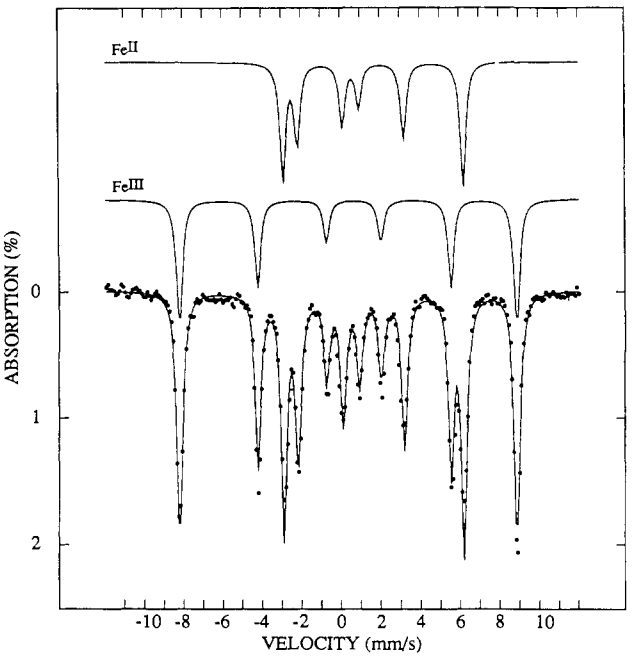

Figure 1. Mössbauer spectrum of polycrystalline $(Et_4N)[Fe_2(salmp)_2]$ at 1.5 K. Solid lines are simulations using an $S = 9/2$ spin Hamiltonian with the parameters quoted in the text. For the electronic system at hand, the values of the asymmetry parameter η and the angle β are not unique.[5] We used $\eta = +2$, $\beta = 30°$ for the Fe^{II} site and $\eta = -2$ and $\beta = 25°$ for the Fe^{III} site. Spectral decompositions into these two sites are shown above the data.

The X-band EPR spectrum of **2** (not shown) exhibits broad resonances centered at $g \approx 6.4$ and 11. From variable-temperature studies, we tentatively conclude that these resonances originate from the first ($g_y \approx 11$) and second excited Kramers doublet of an $S = 9/2$ system with $D \gtrsim 1.5$ cm^{-1} and $E/D \approx 0.15$–0.33, where D and E are zero-field splitting parameters. For this range of E/D, an $S = 9/2$ system has a ground-state doublet with uniaxial magnetic properties ($g_x \approx g_z \approx 0$, $g_y \approx 17.5$). Such doublets produce Mössbauer spectra that exhibit a six-line pattern for each Fe site. The exceptionally well-resolved spectrum of **2** in Figure 1 is consistent with this expectation.[6] Spectral simulations confirm the $S = 9/2$ spin state and yield the following information. (i) The two Fe sites occur in a 1:1 occupation ratio; there is no evidence for additional Fe species. (ii) The quadrupole splitting $\Delta E_Q = -0.92$ mm/s, the isomer shift[7] $\delta = 0.55$ mm/s, and the magnetic hyperfine coupling constant $\mathbf{a} = 29.0$ MHz are typical of high-spin Fe(III), whereas the parameters of the other site ($\Delta E_Q = +2.35$ mm/s, $\delta = 1.12$ mm/s, $\mathbf{a}_y = 18.1$ MHz) are characteristic of high-spin Fe(II).[8-10] The value $\mathbf{a} = 29.0$ MHz

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