The structure of the compound was determined by X-ray diffraction. The unsolvated form[15] crystallizes in space group $P2_1/c$ with $a = 23.912$ (4) Å, $b = 27.466$ (6) Å, and $c = 17.173$ (6) Å, $\beta = 90.86$ (2)°, and $Z = 4$. There are two independent anions, both lying on inversion centers, and four independent cations in the asymmetric unit. The configuration of the anion, shown in Figure 1, consists of a cube of cobalt atoms within bonding distance (mean Co···Co distance in the two anions, 2.66 Å) circumscribed by a concentric octahedron of nonbonding sulfur atoms. This arrangement generates a rhombic dodecahedral $[Co_8(\mu_4\text{-}S)_6]^{4+}$ core unit (idealized O_h symmetry) with nearly planar Co_2S_2 faces.[16] Each cobalt atom occupies a site of local idealized C_{3v} symmetry with coordination completed by a terminal benzenethiolate ligand whose Co–S bond is an extension of the body diagonal of the Co_8 cube. Ranges and mean values of structural parameters of the two anions are collected in Table I. In terms of metal and core sulfur atom positions, $[Co_8S_6(SPh)_8]^{4-}$ is the inverse of $[Fe_6(\mu_3\text{-}S)_8(PEt_3)_6]^{2+}$,[10] in which an S_8 cube encompasses an Fe_6 octahedron.

The following properties of $[Co_8S_6(SPh)_8]^{4-}$ have been observed in acetonitrile solution: (i) a red-purple color with intense S → core charge-transfer bands at λ_{max} (ϵ_M) 343 (62900) and 518 (30800) nm obscuring tetrahedral Co(II) $^4A_2 \rightarrow {}^4T_1$ (ν_2), $^4T_1$(P) (ν_3) ligand field bands (if present); (ii) EPR-silent (7 K); (iii) $\mu_{Co} = 1.03\text{-}1.11\ \mu_B$ (233-323 K); (iv) equivalent phenyl groups with ^{1}H isotropic shifts of +2.79 (*o*-H), -1.21 (*m*-H), +4.48 (*p*-H) ppm (297 K); (v) chemically reversible ($i_{p,c} \simeq i_{p,a}$, 100 mV/s) one-electron oxidation and reduction reactions at $E_{1/2} = -0.54$ and -1.18 V vs. SCE, respectively. Mononuclear $[Co(SPh)_4]^{2-}$,[14,17] and adamantane-like $[Co_4(SPh)_{10}]^{2-}$[14] (mean Co···Co distance 3.87 (1) Å) display larger μ_{Co} and isotropic shift values, all LMCT bands at ≲440 nm, and well-developed ν_2 and ν_3 features and do not sustain reversible oxidation and reduction reactions. The properties of $[Co_8S_6(SPh)_8]^{4-}$ and their differences with those of the foregoing species afford the view of an intact cluster in solution containing essentially equivalent and electronically coupled Co(II) sites.

Discrete species containing a cubic arrangement of directly interacting metal atoms are most uncommon. Indeed, the only previous example is $Ni_8(\mu_4\text{-}PPh)_6(CO)_8$, which is described as containing a completely bonding metal cube.[18] A much closer precedent of the core unit of $[Co_8S_6(SPh)_8]^{4-}$ is found in the extended lattice of the natural[19] and synthetic[20,21] mineral pentlandite (Co_9S_8). Here occur recognizable Co_8S_6 clusters that are linked by coordination to octahedral cobalt and tetrahedral sulfide sites. The comparison in the table demonstrates the structural similarity; metrical deviations between the soluble and mineral clusters may be due partly to the lower mean oxidation state of cobalt in the latter.

The synthesis and characterization of $[Co_8S_6(SPh)_8]^{4-}$ provides a new structural type for clusters constructed of weak field ligands and raises several attractive possibilities. One is the synthesis of other chalcogenide (X) clusters, perhaps solubilized as thiolate derivatives, that are known only in the solid state (e.g., "$Fe_4Ni_4S_6$" in one form of natural pentlandite,[19] "isolated" Mo_6X_8 and Mo_9X_{11} clusters in certain Mo–X[22] and M–Mo–X[23] phases). A second is the occurrence of a M_8S_6 framework in the Fe–Mo–S cluster that is the FeMo cofactor of nitrogenase. The species $[L_3MoFe_7S_6(SR)_7]^{z-}$,[24] containing the $MoFe_7S_6$ core, offers reasonable consistency with current analytical and spectroscopic data of the cofactor. Such complexes and $[Co_8S_6(SPh)_8]^{3-,5-}$ are current objects of synthesis in this laboratory.

Acknowledgment. This research was supported by NSF Grant CHE 81-06017. X-ray and NMR equipment used in this research were funded by NSF Grants CHE 80-00670 and CHE 80-08891.

Registry No. $(n\text{-}Pr_4N)_4[Co_3S_6(SPh)_8]$, 80753-71-5; $(n\text{-}Pr_4N)_2[Co_4(SPh)_{10}]$, 80720-97-4.

(15) The compound has been obtained in solvated and unsolvated forms in different recrystallizations from acetonitrile/ether; the latter afforded superior crystals. From 7830 unique data ($I > 2.5\sigma(I)$) collected in the range $3.5 \le 2\theta$ (Mo Kα radiation) $\le 43°$ with a Nicolet R3M four-circle automated diffractometer, the structure was solved by use of direct methods (MULTAN) and subsequent difference Fourier maps. Anisotropic refinement of all nonhydrogen atoms with phenyl groups as rigid bodies gave $R = 5.9\%$ and the anion parameters in the table; hydrogen atoms have not been included in the refinement. A solvated form crystallizes in space group $P2_1/n$. Current refinement to $R = 9.9\%$ confirms the presence of $[Co_8S_6(SPh)_8]^{4-}$, whose metrical features are in satisfactory agreement with those in the table.

(16) Displacements from unweighted least-squares planes: Co, -(0.04-0.05) Å; S, 0.04-0.05 Å.

(17) Holah, D. G.; Coucouvanis, D. *J. Am. Chem. Soc.* **1975**, *97*, 6917. Swenson, D.; Baenziger, N. C.; Coucouvanis, D. *Ibid.* **1978**, *100*, 1932.

(18) Lower, L. D.; Dahl, L. F. *J. Am. Chem. Soc.* **1976**, *98*, 5046.

(19) Rajamani, V.; Prewitt, C. T. *Can. Mineral.* **1973**, *12*, 178.

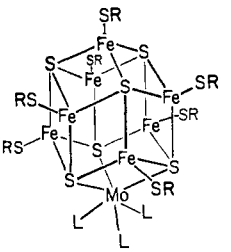
(20) Geller, S. *Acta Crystallogr.* **1962**, *15*, 1195.

(21) Rajamani, V.; Prewitt, C. T. *Can. Mineral.* **1975**, *13*, 75.

(22) Chevrel, R.; Sergent, M.; Prigent, J. *Mat. Res. Bull.* **1974**, *9*, 1487.

(23) Guillevic, J.; Bars, O.; Grandjean, D. *Acta Crystallogr. Sect. B*, **1976**, *B32*, 1338. Grüttner, A.; Yvon, K.; Chevrel, R.; Potel, M.; Sergent, M.; Seeber, B. *Ibid.* **1979**, *B35*, 285. Potel, M.; Chevrel, R.; Sergent, M. *Ibid.* **1981**, *B37*, 1007.

(24) The proposed structure, obtained by replacing cobalt atoms with seven iron atoms and a molybdenum atom in a trigonal octahedral site, is given below. Thiolate ligands, shown for the sake of definiteness, are absent in the cofactor but not necessarily in the native enzyme. The core composition falls within the range of analytical atom ratios of the cofactor: (7-8)Fe/(4-6)S/Mo.[25] The $MoFe_3S_3$ core fragment is geometrically similar to one Mo site configuration deduced from EXAFS analysis of enzyme and cofactor.[26]



(25) Shah, V. K.; Brill, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3249. Burgess, B. K.; Jacobs, D. B.; Stiefel, E. I. *Biochim. Biophys. Acta* **1980**, *614*, 196. Smith, B. E. In "Molybdenum Chemistry of Biological Significance"; Newton, W. E., Otsuka, S., Ed., Plenum Press: New York, 1980; pp 179-190.

(26) Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398. Cramer, S. P.; Gillum, W. O.; Hodgson, K. O.; Mortenson, L. E.; Stiefel, E. I.; Chisnell, J. R.; Brill, W. J.; Shah, V. K. *Ibid.* **1978**, *100*, 3814.

Fungal Xanthone Biosynthesis. Distribution of Acetate-Derived Oxygens in Ravenelin

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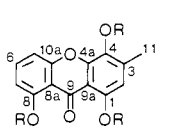
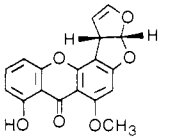
Received December 3, 1981

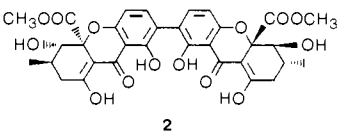
Ravenelin (**1**) is a xanthone pigment isolated from the mycelium



1 R = H
7 R = Ac



3



2

of the phytopathogenic fungi *Helminthosporium ravenelii* and *H. turcicum*.[1,2] Although its biological function is undetermined,

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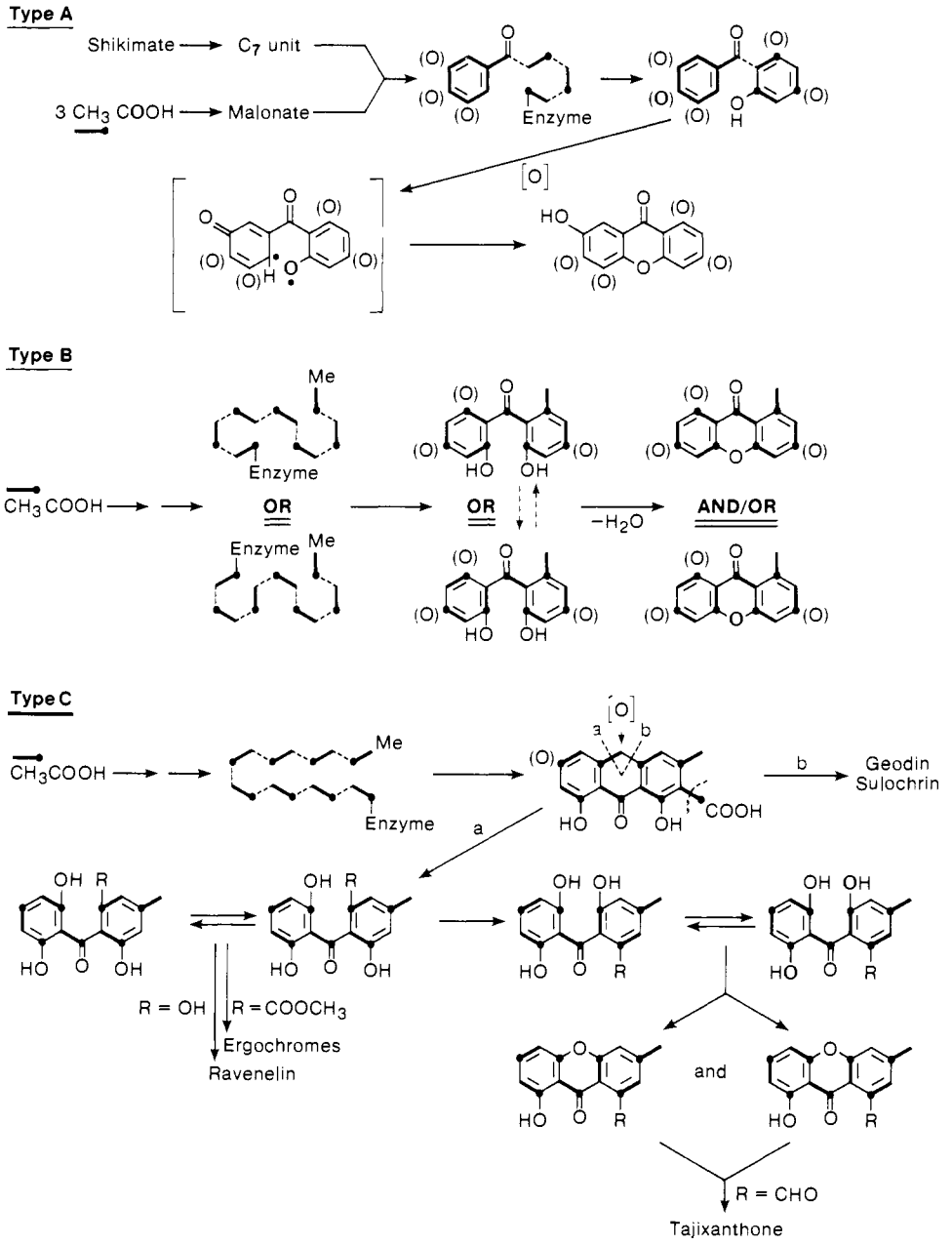
Scheme I



the biosynthesis of its carbon skeleton from acetate[3] has interested chemists because of a structural relationship to various polyketides including mycotoxic food contaminants such as ergochrome AA (secalonic acid A) (**2**)[4] and sterigmatocystin (**3**), an aflatoxin precursor.[5] Despite knowledge of the arrangement of precursor carbons in a host of such secondary metabolites, the sequence and mechanistic details of intermediate transformations usually remain speculative. This is mainly because of difficulty in obtaining and reincorporating compounds along the biogenetic path, but is also partly due to lack of knowledge about the fate of precursor atoms other than carbon. However, recent development of methodology for detection of oxygen-18 by chemical shift changes (isotope shifts) induced in ^{13}C NMR spectra[6] has facilitated mechanistic[7] and biosynthetic[8] studies using oxygen labeling and promises

(1) Raistrick, H.; Robinson, R.; White, D. E. *Biochem. J.* **1936**, *30*, 1303–1314.

(2) *Helminthosporium ravenelii* is a synonym for *Drechslera ravenelii* CBS 200.29 (Centraalbureau voor Schimmelcultures, Baarn, Netherlands).

(3) (a) Birch, A. J.; Simpson, T. J.; Westerman, P. W. *Tetrahedron Lett.* **1975**, 4173–4177. (b) Birch, A. J.; Baldas, J.; Hlubucek, J. R.; Simpson, T. J.; Westerman, P. W. *J. Chem. Soc., Perkin Trans. 1* **1976**, 898–904.

(4) (a) Franck, B. "The Biosynthesis of Mycotoxins, a Study in Secondary Metabolism"; Steyn, P., Ed.; Academic Press: New York, 1980; pp 157–191 and references therein. (b) Turner, W. B. "Fungal Metabolites"; Academic Press: New York, 1971; pp 167–173. (c) Kurobane, I.; Vining, L. C.; McInnes, A. G.; Walter, J. A.; Wright, J. L. C. *Tetrahedron Lett.* **1978**, 1379–1382.

(5) (a) Steyn, P. S.; Vleggaar, R.; Wessels, P. L. In ref 4a, pp 105–155. (b) Zamir, L. O.; Hufford, K. D. *Appl. Environ. Microbiol.* **1981**, *42*, 168–173. (c) Pachler, K. G. R.; Steyn, P. S.; Vleggaar, R.; Wessels, P. L.; Scott, D. B. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1182–1189.

(6) (a) Theoretical prediction: Jameson, C. J. *J. Chem. Phys.* **1977**, *66*, 4983–4988. (b) Hackney, D. D.; Sleep, J. A.; Rosen, G.; Hutton, R. L.; Boyer, P. D. "NMR and Biochemistry. A Symposium Honoring Mildred Cohn"; Opella, S. J., Lu, P., Eds.; Marcel Dekker: New York, 1979; pp 299–300. (c) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252–253. (d) Vederas, J. C. *Ibid.* **1980**, *102*, 374–376. (e) Risley, J. M.; Van Etten, R. L. *Ibid.* **1980**, *102*, 4609–4614, 6699–6702. (f) Diakur, J.; Nakashima, T. T.; Vederas, J. C., *Can. J. Chem.* **1980**, *58*, 1311–1315. (g) Moore, R. N.; Diakur, J.; Nakashima, T. T.; McLaren, S. L.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1981**, 501–502.

(7) (a) Darensbourg, D. J. *J. Organomet. Chem.* **1979**, *174*, C70–C76. (b) Darensbourg, D. J.; Baldwin, B. J. *J. Am. Chem. Soc.* **1979**, *101*, 6447–6449. (c) Darensbourg, D. J.; Baldwin, B. J.; Froelich, J. A. *Ibid.* **1980**, *102*, 4688–4694. (d) Brandänge, S.; Dahlman, O.; Mörch, L. *J. Chem. Soc., Chem. Commun.* **1980**, 555–556. (e) Benner, S. A.; Maggio, J. E.; Simmons, H. E., III *J. Am. Chem. Soc.* **1981**, *103*, 1581–1582. (f) Risley, J. M.; Van Etten, R. L. *Ibid.* **1981**, *103*, 4389–4392.

Scheme II

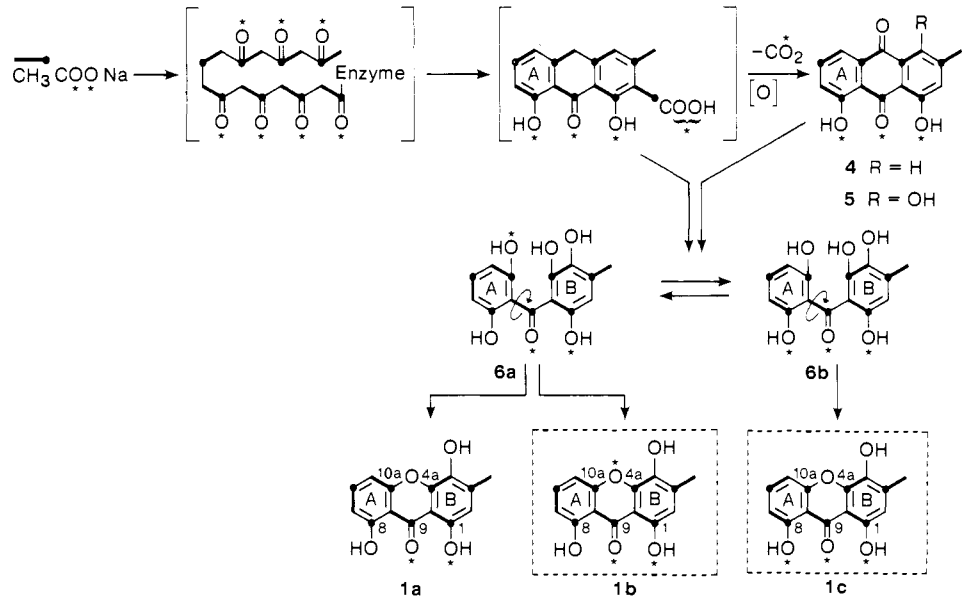


Table I. ^{13}C NMR Data of Ravenelin (1) and Triacetate 7 Derived from Sodium [1-^{13}C,$^{18}O_2$]Acetate[a]

carbon	δ(1) (ppm)	Δδ(1)[b] (ppm × 10²)	^{16}O:^{18}O (1)[c]	δ(7) (ppm)	Δδ(7)[b] (ppm × 10²)	^{16}O:^{18}O (7)[c]
1	151.8	1.0	53:47	146.8	2.1	53:47
8	160.5	1.0	58:42	156.2	2.2	63:37
9	185.2	2.7	43:57	173.9	3.7	46:54
10a	155.7	1.9	62:38	150.2	2.1	61:39

[a] Fourier transform spectra were measured on a Bruker WH 400 spectrometer at 100.6 MHz with ca. 0.1 M solutions of 1 in Me_2SO-d_6 or 7 in $CDCl_3$ with Me_4Si internal standard. Signals for carbons bearing oxygens were first expanded by using 32K data block/2000 Hz, 500–1000 scans, 16.4-s acquisition time and 45° pulse angle. Spin-echo expansions (vide infra) employed similar conditions except for 5.4-s acquisition time and pulse sequence. [b] ^{18}O upfield-shift values are relative to ^{13}C–^{16}O signal as internal reference and are ±0.1 (ppm × 100). [c] Approximate values obtained from relative peak heights of ^{13}C–^{16}O and ^{13}C–^{18}O signals in normal (non-spin-echo) spectra.

additional insights. Application of the technique to ravenelin (**1**) in the present work locates the acetate-derived oxygens, provides a means for determining the direction of ring closure in xanthone biosynthesis, and supports a suggested oxidative sequence[3] for its formation.

Hydroxylated benzophenones are generally accepted as immediate precursors of most xanthones, but their biogenesis in higher plants usually differs from that in fungi and lichens.[9] In the former, benzophenones partly derived from shikimate appear to generate the central xanthone ring by oxidative coupling (Scheme I, type A),[9b,c] whereas in fungi and lichens the substitution pattern of totally acetate-derived benzophenones presumably encourages ring closure by an addition–elimination process (types B, C).[9a,10] A "looped" folding (type B) of the fungal polyketide chain analogous to that observed with bikaverin[11] leads directly to precursors of griseoxanthones and the related spiro derivative griseofulvin.[8g,10,12] The "circular" folding (type C) which is proposed for ravenelin (**1**),[3] ergochrome AA (**2**),[4] and a number of other fungal metabolites (e.g., tajixanthone, geodin, sulochrin)[13] requires oxidative cleavage of an intermediate anthrone or anthraquinone. After fission of the central ring by a still unknown mechanism,[14] the benzophenone often possesses an axis of symmetry in one conformationally labile ring which affords a mixture of two labeling patterns in the xanthone.[15] The direction of addition–elimination cyclization[10,12a] of *o,o'*-dihydroxybenzophenones to xanthones is unknown. During the suggested biosynthesis of ravenelin (**1**) (Scheme II) each ortho hydroxyl group in either rotamer **6a** or **6b** is in principle capable of nucleophilic attack on the adjacent ring, thereby yielding three different carbon–oxygen labeling patterns (**1a**, **1b**, **1c**). In order to distinguish between these possibilities and to exclude participation of reduced (deoxygenated) intermediates, we incorporated[16] doubly

(8) (a) Vederas, J. C.; Nakashima, T. T. *J. Chem. Soc., Chem. Commun.* **1980**, 183–185. (b) Vederas, J. C.; Nakashima, T. T.; Diakur, J. *Planta Med.* **1980**, *39*, 201–202. (c) Sankawa, U.; Ebizuka, Y.; Noguchi, H.; Ishikawa, Y.; Kitagawa, S.; Kobayashi, T.; Seto, H. *Heterocycles* **1981**, *16*, 1115–1118. (d) Hutchinson, C. R.; Sherman, M. M.; Vederas, J. C.; Nakashima, T. T., *J. Am. Chem. Soc.* **1981**, *103*, 5953–5956. (e) Cane, D. E. Hasler, H.; Liang, T. C. *Ibid.* **1981**, *103*, 5960–5962. (f) Cane, D. E.; Liang, T. C.; Hasler, H. *Ibid.* **1981**, *103*, 5962–5965. (g) Lane, M. P.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1982**, *104*, 913–915.

(9) (a) Manitto, P. "Biosynthesis of Natural Products"; Wiley: New York, 1981; pp 200–202. (b) Gupta, P.; Lewis, J. R. *J. Chem. Soc. C.* **1971**, 629–631. (c) Sultanbawa, M. U. S. *Tetrahedron* **1980**, *36*, 1465–1506. (d) Afzal, M.; Al-Hassan, J. M. *Heterocycles* **1980**, *14*, 1173–1205.

(10) (a) Sandifer, R. M.; Bhattacharya, A. K.; Harris, T. M. *J. Org. Chem.* **1981**, *46*, 2260–2267 and references therein. (b) Sundholm, E. G. *Tetrahedron* **1978**, *34*, 577–586.

(11) McInnes, A. G.; Smith, D. G.; Walter, J. A.; Vining, L. C.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1975**, 66–68.

(12) (a) Harris, C. M.; Roberson, J. S.; Harris, T. M. *J. Am. Chem. Soc.* **1976**, *98*, 5380–5386. (b) Simpson, T. J.; Holker, J. S. E. *Phytochemistry* **1977**, *16*, 229–233. (c) Sato, Y.; Oda, T.; Miyata, E.; Saito, H. *FEBS Lett.* **1979**, *98*, 271–275.

(13) (a) Bardshiri, E.; Simpson, T. J. *J. Chem. Soc., Chem. Commun.* **1981**, 195–196. (b) Fujimoto, H.; Flasch, H.; Franck, B. *Chem. Ber.* **1975**, *108*, 1224–1228. (c) Gatenbeck, S.; Malmström, L. *Acta Chem. Scand.* **1969**, *23*, 3493–3497.

(14) Probable mechanisms[4a] include biological Baeyer–Villiger oxidation (Schwab, J. M. *J. Am. Chem. Soc.* **1981**, *103*, 1876–1878) of the anthraquinones or cleavage via hydroperoxide adducts (Franck, B.; Berger-Lohr, B. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 818–819. Money, T. *Nature (London)* **1963**, *199*, 592–593).

(15) Biosynthesis of sterigmatocystin (**3**) involves a slightly different "circular" folding of the polyketide.[5c] Cleavage of the anthraquinone precursor and xanthone formation does not proceed via a symmetrical intermediate since a single discrete distribution of acetate units is observed.

(16) Fourteen-day-old potato-dextrose agar slants of *D. ravenelii* were used to innoculate three 500-mL Erlenmeyer flasks each containing 100 mL of sterile medium (per liter: 50 g of D-fructose, 2 g of peptone, 2 g of $NaNO_3$, 1 g of KH_2PO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4$), which were incubated in the dark at 25 °C on a rotary shaker (180 rpm). Each flask was injected with 1.0 mL of aqueous labeled sodium acetate solution (1.02 g/9.0 mL, pH 10) every 24 h from day 4 (96 h) to day 6 of the fermentation. After a further 48 h, the mycelium was filtered, dried in vacuo, and extracted with hot $CHCl_3$. Chromatographic separation of the extract (70–230 mesh Merck silica gel 60, toluene/methanol) and recrystallization (toluene) yielded 35 mg of pure ravenelin with the expected[1,3b] spectral (IR, ^1H NMR, ^{13}C NMR, UV, MS) and physical properties.

labeled sodium [1-^{13}C,$^{18}O_2$]acetate (isotopic purity: 90% ^{13}C, 18% $^{18}O_1$, 81% $^{18}O_2$)[8g] into ravenelin (**1**) with growing cultures of *Drechslera ravenelii* CBS 200.29.[2]

Expansion of the 100.6-MHz ^{13}C NMR spectra of **1** showed that isotopically shifted signals were present at C-1, C-8, C-9, and C-10a (Table I), but the peaks were disappointingly broad and poorly resolved. Since previous experience had demonstrated that attachment of electron-withdrawing groups on hydroxyl oxygens increases ^{18}O isotope shift magnitude,[6d,8d] the [^{13}C, ^{18}O] ravenelin (**1**) was converted to its triacetate, **7**.[1] The ^{13}C NMR spectra of **7** confirmed[17] the presence of ^{18}O at the four sites, but the signals were still complicated due to long-range carbon–carbon couplings. The cause of this common problem, which often obscures the small ^{18}O isotope shifts, is excess incorporation of [^{13}C, ^{18}O] precursor.[8e-g] This results in multiple ^{13}C labels within a single molecule. Fortunately this difficulty can be overcome by inverting only the uncoupled ^{13}C–^{16}O and ^{13}C–^{18}O singlets with a spin-echo Fourier transform (SEFT) NMR sequence: $(90°-\tau-180°-\tau-\text{acquisition}-T)_N$, where $\tau = 1/(2J_{CC})$ and J_{CC} is the estimated long-range coupling constant.[8g,18] Application of SEFT ^{13}C NMR spectrometry to **7** eliminated interference from coupling and permitted exact measurement of isotope shift magnitudes. Although the isotope ratios in ^{13}C-coupled spectra and in SEFT experiments must be viewed with caution,[8g] comparison of all results showed that the $^{16}O/^{18}O$ ratios in Table I are approximately correct (±5–10%).

The low natural abundance of ^{13}C (1.1%) and the relatively limited chance of incorporating two neighboring ^{13}C labels into the same molecule enable detection of intact carbon–oxygen bonds by ^{13}C NMR spectrometry through use of doubly labeled [^{13}C,^{18}O] precursor. In contrast, such information is not easily accessible through mass spectrometry. Appearance of a significant isotope shift at C-10a, but not at C-4a, which is also derived from the carboxyl of acetate, demonstrates that ravenelin **1b** (Scheme II) is present. Since the two carbon labeling patterns in ring A of **1** occur in about 1:1 ratio,[3b] the total amount of ^{18}O at C-10a and C-8 (Table I) indicates that the hydroxyl group in ring A of possible intermediates[19] **4** or **5** must have had a ^{16}O:^{18}O ratio close to 30:70. Comparing this value to the amounts of ^{18}O present in ravenelin (**1**) at C-1 and C-9 (which represent maximal oxygen labeling except for exchange during biosynthesis[20]) suggests xanthone ring closure proceeds by nucleophilic attack of the ring A hydroxyl group of **6a** or **6b** on the ortho position of ring B. The eliminated ring B ortho substituent need not be hydroxyl, but **6** is a chemically reasonable[10] hypothetical intermediate. Its potential precursors, chrysophanol (**4**) and islandicin (**5**), have not yet been proven to be on the direct biogenetic pathway; however, carbon[21] and oxygen[22] labeling studies on **5** agree with their possible involvement.[19]

Although our results do not exclude a small proportion of cyclization in the opposite sense, they do demonstrate that **1b** and **1c** represent the major acetate-derived carbon–oxygen labeling patterns of ravenelin. Possible participation of deoxygenated intermediates that undergo aerobic aromatic hydroxylation (except at C-4 of **1**) is also eliminated. This methodology is applicable not only to investigations of xanthone biosynthesis but, more generally, to studies on cyclization mechanisms involving attack by one oxygen-bearing carbon on another.

Acknowledgment. We thank the Stable Isotopes Resource of Los Alamos National Laboratory for generous gifts of [^{18}O]water and the Natural Sciences and Engineering Research Council of Canada for financial support (Grant A0845 and NSERC summer studentship to J.G.H.). We are very grateful to Professor M. A. Pickard (University of Alberta) for advice on culturing *D. ravenelii*.

Registry No. 1, 479-44-7; triacetate **7**, 80754-76-3.

(17) The ^{13}C NMR spectra of **7** was assigned by comparison to **1** by using substituent effects in xanthones and by examination of ^1H-coupled spectra of unlabeled **7** (Westerman, P. W.; Gunasekera, S. P.; Uvais, M.; Sultanbawa, S.; Kazlauskas, R. *Org. Magn. Reson.* **1977**, *9*, 631–636. Sundholm, E. G. *Acta Chem. Scand., Ser. B* **1978**, *B32*, 177–181). The assignments ($CDCl_3$, ppm relative to Me_4Si) are 173.9 (C-9), 169.6 (Ac), 169.5 (Ac), 168.0 (Ac), 156.2 (C-8), 150.2 (C-10a), 148.6 (C-4a), 146.8 (C-1), 138.2 (C-3), 135.3 (C-4), 134.4 (C-6), 119.8 (C-2), 118.9 (C-7), 115.9 (C-5), 115.4 (C-8a), 114.3 (C-9a), 21.1 (2 × Ac), 20.2 (Ac), 16.5 (C-11).

(18) (a) Rabenstein, D. L.; Nakashima, T. T. *Anal. Chem.* **1979**, *51*, 1465A–1474A and references therein. (b) Brown, D. W.; Nakashima, T. T.; Rabenstein, D. L. *J. Magn. Reson.* **1981**, *45*, 302–314.

(19) Dr. Thomas J. Simpson (Chemistry Department, University of Edinburgh) has isolated trace amounts of islandicin (**5**) along with ravenelin (**1**) (personal communication). After submission of this manuscript chrysophanol (**4**) was shown to co-occur with ravenelin (**1**) (Van Eijk, G. W.; Roeymans, H. J. *Exp. Mycol.* **1981**, *5*, 373–375).

(20) Normalization of ^{13}C NMR spectra of [^{13}C,^{18}O]ravenelin (**1**) to unlabeled **1** shows about 10-fold total ^{13}C enhanced at each labeled carbon. The ^{16}O:^{18}O isotope ratios indicate a little less than 50% loss of ^{18}O to the medium after accounting for precursor isotopic purity and removal of one of the acetate oxygens in the biogenetic process.

(21) Casey, M. L.; Paulick, R. C.; Whitlock, H. W. *J. Org. Chem.* **1978**, *43*, 1627–1634.

(22) Gatenbeck, S. *Acta Chem. Scand.* **1960**, *14*, 296–302.

Nocardicin A Biosynthesis: Stereochemical Course of Monocyclic β-Lactam Formation

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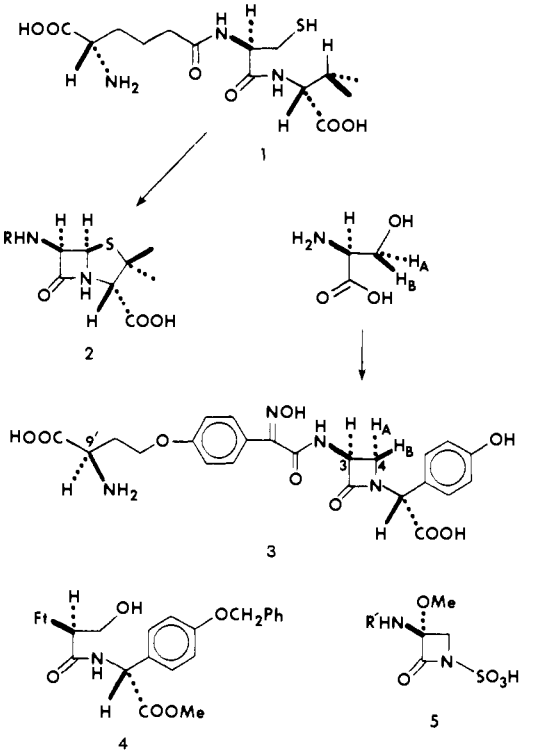
Received November 19, 1981

Unlike the complex and poorly understood sequence of oxidative reactions that occur in vivo to convert the Arnstein tripeptide **1**



to isopenicillin N (**2**) [R = δ-(L-α-aminoadipyl)],[1] biosynthetic results obtained in these laboratories[2] have clearly shown that the amino acid precursors of nocardicin A (**3**), the L isomers of methionine, (*p*-hydroxyphenyl)glycine, and serine, are assembled

(1) O'Sullivan, J.; Bleaney, R. C.; Huddleston, J. A.; Abraham, E. P. *Biochem. J.* **1979**, *184*, 421–26. Konomi, T.; Herchen, S.; Baldwin, J. E.; Yoshida, M.; Hunt, N. A.; Demain, A. L. *Ibid.* **1979**, *184*, 427–30. For a review see: O'Sullivan, J.; Abraham, E. P. In "Antibiotics: Biosynthesis"; Corcoran, J. W., Ed.; Springer Verlag: Berlin, 1981; Vol. 4, pp 101–22.

(2) Townsend, C. A.; Brown, A. M. *J. Am. Chem. Soc.* **1981**, *103*, 2873–74. See also: Hosoda, J.; Tani, N.; Konomi, T.; Ohsawa, S.; Aoki, H.; Imanaka, H. *Agric. Biol. Chem.* **1977**, *41*, 2007–12.

0002-7863/82/1504-1748$01.25/0