Pentaketide Metabolites of *Verticillium dahliae*. 3.[1] Identification of (−)-3,4-Dihydro-3,8-dihydroxy-1(2*H*)-naphthalenone [(−)-Vermelone] as a Precursor to Melanin

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(−)-Vermelone [3,4-dihydro-3,8-dihydroxy-1(2*H*)-naphthalenone] was isolated and identified from culture filtrates of the melanin-deficient *brm-1* mutant of *Verticillium dahliae* fed 1,3,8-trihydroxynaphthalene. (−)-Vermelone was readily dehydrated both chemically and biologically to 1,8-dihydroxynapthalene (1,8-DHN). Since 1,8-DHN oxidizes to a black pigment (melanin), (−)-vermelone is probably the terminal ketone in the biosynthetic pathway from (+)-scytalone to melanin. Thus, the melanin in *V. dahliae* is apparently a polymer composed of oxidized 1,8-DHN subunits.

Recently Bell et al.[2] isolated a series of melanin-deficient mutants from the fungus *Verticillium dahliae*. The mutant *brm-1* (brown microsclerotia) accumulated (+)-scytalone (**1**)[3] that served as a substrate for natural melanin synthesis in *alm* (albino microsclerotia) mutants.[2,3] When fed to a different brown mutant (*brm-2*), (+)-scytalone (**1**) was enzymatically dehydrated to 1,3,8-trihydroxynaphthalene (1,3,8-THN, **2**) but was not converted to normal black melanin.

We fed 1,3,8-THN (**2**) to the *brm-1* mutant to determine if **2** would be converted to melanin. However, a new compound **4** was produced in quantity and accumulated without melanin synthesis. Compound **4** was converted to normal black melanin by either *brm-2* or *alm* mutants.[4] Therefore, **4** also appears to be in the biosynthetic pathway leading from (+)-scytalone to melanin. We propose the trivial name (−)-vermelone (derived from *Verticillium* melanin ketone) for **4**.

The high-resolution mass spectrum of (−)-vermelone (**4**) indicated the formula $C_{10}H_{10}O_3$ (*m/e* 178, 98%). Thus **4** was a dihydro derivative of 1,3,8-THN (**2**). The ir, NMR, and mass spectra of (−)-vermelone (**4**) and (+)-scytalone (**1**) were very similar. The ir spectra of both showed hydrogen-bonded carbonyl groups (1635 cm^{-1} for **1** and 1642 cm^{-1} for **4**). The NMR spectra confirmed the hydrogen bonded phenolic groups (δ 12.45 for **1** and δ 12.42 for **4**). Three aromatic protons appeared in the NMR spectrum of (−)-vermelone (**4**). Two formed complex doublets at δ 6.75 (J = 7.3 Hz) and were coupled to a third aromatic proton (δ 7.45, t, J = 7.3 Hz). The methine proton (CHOH) in (+)-scytalone (**1**) appeared at δ 4.20 whereas that in (−)-vermelone (**4**) gave a multiplet at δ 4.32. In each compound the methine proton was coupled to four methylene protons; these appeared as complex multiplets at δ 2.75–2.95 in **1** and δ 3.00–3.20 in **4**. Thus, the NMR spectrum indicated that vermelone is 3,4-dihydro-3,8-dihydroxy-1(2*H*)-naphthalenone (**4**).

Fragment ions of (−)-vermelone observed in the high-resolution mass spectrum also agreed with structure **4**. (−)-Vermelone (**4**) was found to readily lose a molecule of water (*m/e* 160, $C_{10}H_8O_2$, 100%) and to undergo a reverse Diels–Alder fragmentation giving $CH_2{=}CHOH$ and the fragment *m/e* 134 ($C_8H_2O_2$, 98%).

To confirm its structure, we oxidized (−)-vermelone (**4**) to 2-hydroxyjuglone; **4** also was dehydrated in alkali to give 1,8-dihydroxynaphthalene (1,8-DHN).

The diketone tautomer **3** probably is an intermediate in the conversion of 1,3,8-THN (**2**) to (−)-vermelone (**4**). Free-energy calculations[5] for the tautomers **2** and **3** indicated similar thermodynamic stabilities. The ^1H NMR spectrum showed only the **2** tautomer; however, on the surface of an enzyme the predominant tautomer might have been the diketone **3**. An alcohol dehydrogenase then could reduce **3** to (−)-vermelone (**4**).

In the biosynthetic pathway to melanin, (−)-vermelone (**4**) apparently dehydrates to 1,8-DHN. Allport and Bu'Lock previously showed that 1,8-DHN can be chemically oxidized to a black polymer. When fed to the *alm-1* mutant, (−)-vermelone (**4**) was dehydrated to a naphthol with chromatographic and spectral properties identical with those of 1,8-DHN. Also, the 1,8-DHN like (−)-vermelone (**4**) was rapidly converted to a black pigment (melanin) by *alm* or *brm-2* cultures.

The *brm-1* mutant lacks the enzyme activity necessary to convert either of the 3-hydroxytetralones (**1** or **4**) to their corresponding naphthols. This suggests that the same enzyme catalyzes both dehydratase reactions in the conversion of (+)-scytalone to 1,8-DHN.

Experimental Section[10]

Melting points were determined on a Kofler hot stage and are uncorrected. Low-resolution spectra were recorded at 70 eV on a Varian CH-7 mass spectrometer, with a probe temperature of 20 °C and a source temperature of 200 °C. High-resolution mass measurements were made with a CEC 21-110 spectrometer. Peaks above *m/e* 50 and 10% are reported. NMR spectra were recorded on a JEOL MH-100 in $(CD_3)_2CO$ with Me_4Si as an internal standard. Phenolic protons were determined by D_2O exchange. Coupling between protons was determined by spin decoupling techniques. Uv spectra were determined in 95% EtOH or 95% EtOH containing 0.03 M NaOH (EtONa) with a Beckman ACTA-MVI.

Biological Conversion of 1,3,8-Trihydroxynaphthalene (2) to (−)-Vermelone (4) and of 4 to 1,8-Dihydroxynaphthalenone. The mutants *brm-1* and *alm-1* of *Verticillium dahliae* and their growth on potato–carrot–dextrose–agar (PCDA) have been described.[2] Conidia were washed from the outer 1 cm of fungal colonies with sterile distilled water and adjusted to 10^6 conidia/ml. Aliquots (0.2 ml) of the conidial suspension were spread on fresh PCDA in culture dishes (9 cm diameter) and incubated in the dark at 24 °C for 6 days.

Compound **2** was prepared from (+)-scytalone (**1**) by acid dehydration.[3] Excess **2** was stirred under N_2 for 1 h in 0.01 M potassium phosphate buffer (pH 6) containing 1% sucrose. The saturated solution was filtered and added to the 6-day-old cultures of *brm-1* (10 ml/dish), and the cultures were incubated for another 18 h. Solutions were then decanted and each culture dish was rinsed twice with 12 ml of hot (90 °C) water. All aqueous fractions were combined and filtered. Crude metabolites were then prepared as described previously.[3]

Similarly, (−)-vermelone (**4**) was dissolved in the sucrose–buffer solution at 1 mg/ml, and was added to the *alm-1* mutant for conversion to 1,8-DHN.

Purification of (−)-Vermelone [3,4-Dihydro-3,8-dihydroxy-1(2*H*)-naphthalenone, 4]. (−)-Vermelone (**4**) was purified by sequential TLC on polyamide developed with 9:1 chloroform–acetone (R_f 0.77), on Silicar TLC-7GF (Mallinkrodt Chemical Co., St. Louis, Mo.) with 50:50:1 ethyl ether–naphtha solvent–formic acid (R_f 0.28), and on Silicar with 9:1 chloroform–acetone (R_f 0.34). Silica gels with zinc silicate phosphor were avoided because they gave poorer resolution. Compound **4** was located on TLC layers as a quenching spot under 254-nm uv light or a yellow fluorescing spot under 365-nm uv

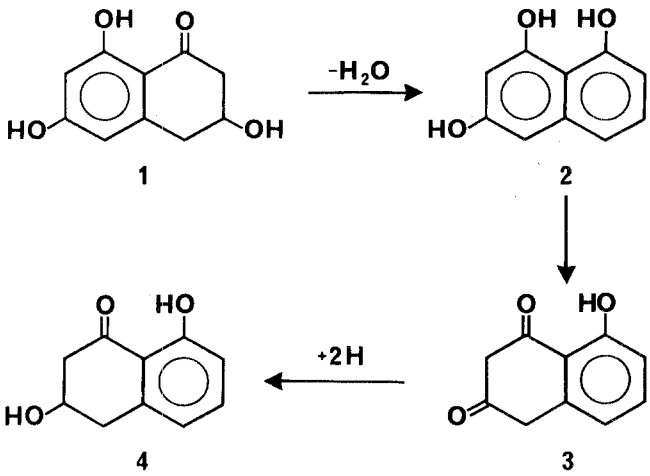

Figure 1. Probable biosynthetic pathway for the conversion of (+)-scytalone (1) to (−)-vermelone (4).

light. Compound 4 also formed a red-brown chelate when chromatograms were sprayed with 1% $FeCl_3$ and a gray spot with DMB reagent (equal volumes of 1% 2,4-dimethoxybenzaldehyde in ethanol and concentrated HCl, freshly mixed). Bands of silica gel containing 4 were scraped from TLC plates, packed in 1-cm chromatography columns, and eluted with ethyl ether.

(−)-Vermelone formed crystals from cyclohexane: mp 91–94 °C; $[\alpha]^{25}D$ −18° (*c* 0.36, EtOH); MS *m/e* (%) 178.062345 (98, M^+; $C_{10}H_{10}O_3$ requires 178.062980), 161 (20), 160 (100, M − H_2O), 135 (25), 134.037171 (98, M − CH_2=CHOH; $C_8H_6O_2$ requires 134.036770), 132 (44), 131 (20), 107 (13), 106 (62), 105 (32), 104 (28), 103 (16), 78 (54), 77 (44), 63 (13), 52 (19), 51 (31); uv-visible λ_{max} (EtOH) (ε) 333.5 nm (4000), 259 (10 600); λ_{max} (EtONa) (ε) 374 (5500), 346 (5200), 333 (sh), 266 (sh).

Chemical Conversions of (−)-Vermelone (4). Compound 4 was oxidized with Jones reagent[7] to give a single orange quinone. The R_f values and uv-visible spectra of this quinone agreed with those[1] of synthetic 2-hydroxyjuglone.[8] (−)-Vermelone (4) was dehydrated with 50% aqueous KOH by the methods described for (+)-scytalone.[3] The uv-visible and mass spectra of the phenol obtained from 4 agreed with those of 1,8-DHN synthesized from 8-hydroxy-1-naphthalenesulfonic acid (sodium salt) according to Tanaka et al.[9]

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Maytansinoids. Synthesis of a Fragment of Known Absolute Configuration Involving Chiral Centers C-6 and C-7

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An efficient, stereocontrolled synthesis is described for compound **2**, which represents a fragment corresponding to carbons 5–12 of the maytansinoid ring skeleton. The total yield from (*Z*)-2-butene-1,4-diol is 78%. The dioxepane **6** has been resolved via the α-phenethylurethane and the absolute configuration of the enantiomers determined by the Horeau method. The specific rotations of all intermediates are reported.

The maytansinoids[1–4] are a group of structurally related ansa macrolides isolated from *Maytenus* and *Colubrina* species, which are of great current interest because of their high antileukemic potency and cytotoxicity. They are characterized by structure **1**, in which R may be CH_3 (maytansine),[1] C_2H_5 (maytanprine),[2] $CH(CH_3)_2$ (maytanbutine),[2] or $CH_2CH(CH_3)_2$ (maytanvaline).[3] 15ξ-Hydroxymaytanbutine (colubrinol)[4] and its acetate have also been described. More recently,[3] maytansine, the most thoroughly investigated representative of this class of compounds, has also been found to possess significant activity against solid murine tumor systems, and it is presently undergoing clinical trials.

Sparked by our interest in the unusual biological properties of these structurally interesting natural products, whose isolated yields from their respective plant sources are in the order of 10^{-4}% or less, we have initiated a synthetic program aimed at the natural products themselves as well as at structurally related substances, which might retain the biological properties of the former. Two groups, Meyers et al.[5–7] and Corey and Bock,[8] have recently reported on their approaches to this problem. In this paper we wish to describe the synthesis of compound **2**, which represents a fragment corresponding to carbon atoms 5–12 of the maytansinoid ring skeleton. The final intermediate **3** has also been prepared in optically active form of known absolute configuration. The elaboration of this intermediate which contains the chiral centers corresponding to C-6 and C-7 in maytansine in the correct relative configuration seemed to us an appropriate point of departure since such a precursor could in turn direct the development of stereochemistry of all the remaining chiral centers, C-3, C-4, C-5, and C-10. The synthesis of **3**, which follows a plan similar to that of Corey and Bock,[8] and is identical with the latter up to compound **7**, had been completed when that paper appeared. Interestingly, the further utilization of that intermediate proceeds along quite different lines. It was envisioned that the relative stereochemistry at C-6 and C-7 could be created by a methyllithium opening of a suitably protected (*Z*)-2,3-epoxybutane-1,4-diol. Thereafter the vicinal hydroxyl