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SYNTHESIS OF CYATHIFORMINES A-C: UNUSUAL FUNGAL METABOLITES DERIVED FROM CHORISMIC ACID

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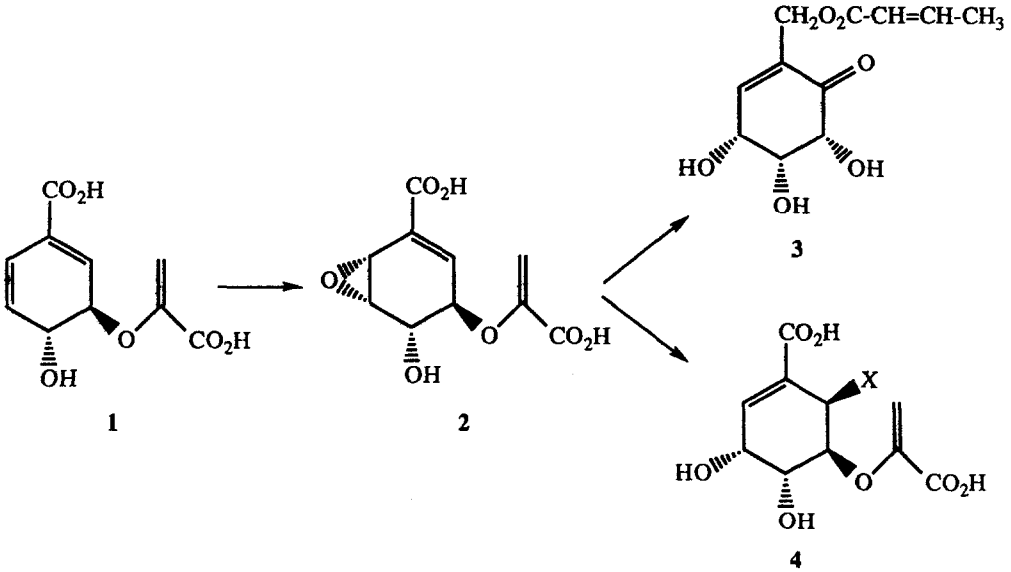
Abstract: *Expeditious syntheses of the title compounds are reported which lend credence to the apparent biogenetic relationship between the cyathiformines and (-)-chorismic acid.*

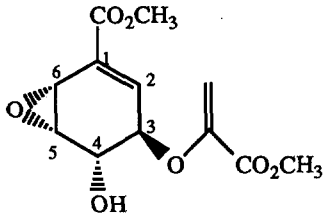
INTRODUCTION

During studies on the shikimic acid metabolic pathway, which is used by plants and microorganisms to biosynthesize aromatic compounds,[1] we recently had occasion to investigate the oxidation of chorismic acid **1** to its disubstituted epoxide **2**. This substance was of interest in connection with total syntheses of the potent glyoxalase inhibitor[2] COTC **3**, as well as compounds of general structure **4** for use as probes in studying the enzyme chorismate synthase.[3,4] In fact, epoxide **2** turns out to be closely related to a new family of chorismate-derived metabolites whose structures were recently reported,[5] thus prompting this independent disclosure of their synthesis.



DISCUSSION AND RESULTS

Screening of the genus *Clitocybe cyathiformis* for new fungal-derived natural products led Arnone *et al.*[5] to isolate and identify an unexpected series of alicyclic metabolites representing the first examples of chorismate-derived substances detected in higher fungi. These fungal-derived natural products include cyathiformines A-D **5-8**. Lactone **9**, the corresponding δ-lactone derived from **7**, was earlier reported to be the first chlorine-containing shikimate-related metabolite from fungi.[6] The structures of **5-9** suggest a biosynthetic pathway from chorismate that would appear to proceed by an *in vivo* epoxidation of **1**. Prompted by those reports, and by the antibacterial and antifungal activity of cyathiformine A,[5] we herein disclose short syntheses of cyathiformines A-C (**5-7**) which were developed independently during studies on selective hydrolysis of epoxide **2**.



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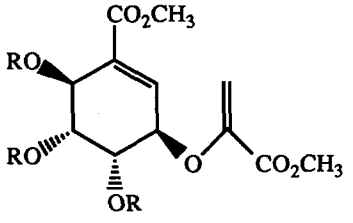
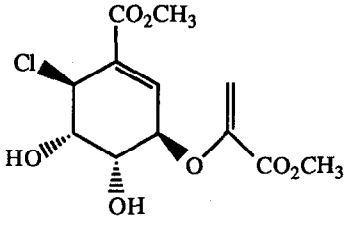


6 R= H
10 R= Ac



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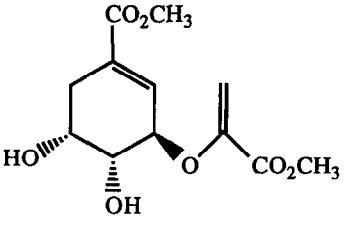

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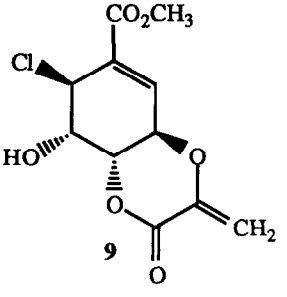

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Slow addition of ethereal diazomethane to a -78°C methanol solution of epoxychorismic acid **2**[7] produced the corresponding dimethyl ester **5** (49%) after flash chromatography to remove traces of pyrazoline resulting from the known cycloaddition of CH_2N_2 to the enoate moiety of **1**.[7] Diester **5** tended to decompose upon prolonged exposure to silica gel; however, when pure, its

melting point and specific rotation were both significantly higher than values reported for the natural material.[5] Since NMR data for synthetic and natural **5** were in good agreement, there seems little doubt of its structure. Moreover, the spectroscopic data and specific rotations of natural and synthetic cyathiformine C **7** (derived from synthetic **5**; vide infra) are in substantial agreement.

Initial studies on **5** were designed to investigate the formation of 1,2 and 1,4-diols in the acid-catalyzed hydrolysis of its epoxide ring. Somewhat to our surprise, we discovered that the enol pyruvate sidechain tended to lactonize when **5** was exposed to acids like CF_3CO_2H.[8] To prevent this undesired cyclization, epoxide **5** was subjected to the standard conditions of acetylation. However, in the presence of acetic anhydride-pyridine, **5** underwent acetolysis of the oxirane ring and subsequent acetylation to produce triacetate **10** as the major product (78%). The ^{1}H-NMR spectrum of compound **10** matched that of cyathiformine B triacetate, which was described in the initial characterization of this natural product.[5] Moreover, the ^{13}C-NMR of **10**, run under DEPT conditions, confirmed the presence of seven non-hydrogen-bearing (including five carbonyl), five methine, one methylene, and five methyl carbon atoms in this structure. Alcoholysis using K_2CO_3-CH_3OH (0°C, 1 h) smoothly transformed **10** to a sample of the naturally-occurring triol **6** which proved identical with cyathiformine B.

To synthesize cyathiformine C, an acetone solution of epoxydiester **5** was briefly exposed to concentrated hydrochloric acid. In this fashion, chlorohydrin **7** was produced in quantitative yield as a white foam. Spectroscopic and polarimetric data obtained on this sample were identical with published data for cyathiformine C, thus confirming the (3R,4R,5S,6S) absolute configuration for the natural product.

By establishing the absolute configurations of **5-7**, our work lends credence to the apparent biogenetic relationship between the cyathiformines and (-)-chorismic acid, thus linking these new fungal metabolites to the shikimic acid biosynthetic pathway. Although they have been proposed as likely defensive compounds or as potential sources of hydroxylated or aminated aromatic acids,[5] the true function(s) of the cyathiformines must await further study, now facilitated by the efficient total syntheses of **5-7** described here.

EXPERIMENTAL

Proton-NMR spectra were taken on Bruker WM-300 or Varian VXR-400S spectrometers. All chemical shifts were reported on the δ scale in parts per million downfield from Me_4Si (0.00 ppm). Carbon-13-NMR spectra were taken on a Varian VXR-400S or Bruker WM-300 (75 MHz) spectrometer. Infrared spectra were taken on a Mattson Galaxy Model infrared spectrometer. Mass spectra were acquired using a Finnigan 3300 mass spectrometer at Cornell or at the University of Illinois Mass Spectrometry Laboratory using a VG ZAB-SE or VG 70-VSE instrument. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter.

Cyathiformine A **5** -- A solution of epoxychorismic acid (308 mg, 1.27 mmol) in absolute CH_3OH (45 mL) was cooled under Ar to -78°C and stirred while ethereal diazomethane (prepared from Diazald) was added via gas-tight syringe and Teflon™ needle. The reaction was followed by thin layer chromatography until no more starting material remained. The colorless solution was concentrated under reduced pressure to afford a pale oil (490 mg). Purification by flash chromatography on SiO_2 (1:4 CH_3CN:CH_2Cl_2) afforded 164 mg (49%) of the desired epoxide: mp 101.5-103.5°C, lit[5] 88-90°C; $[\alpha]_D$ -162° (c=0.76, $CHCl_3$), -159° (c= 0.14, CH_3OH), lit[5] -104° (c= 0.1, $CHCl_3$); R_f 0.2 (1:4 CH_3CN:CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) 6.93 (dd, 1 H, J= 2.0, 2.0 Hz), 5.60, 4.85 (ABq, 2 H, J= 3.0, 3.0 Hz), 4.58 (dd, 1 H, J= 8.2, 1.6 Hz), 4.24 (dd, 1 H, J= 8.2, 1.0 Hz), 4.10 (dd, 1 H, J= 4.3, 2.3 Hz), 3.83, 3.82 (2s, each 3 H), 3.71 (dd, 1 H, J= 4.2, 0.8 Hz); ^{13}C-NMR ($CDCl_3$) 164.8, 163.8, 149.5, 139.3, 129.7, 99.1, 77.4, 70.5, 53.7, 52.8, 52.4, 48.5; IR (film) 3440, 2975, 1720, 1620, 1445 cm^{-1}; CIMS (NH_3) m/z 288 ($M + NH_4^+$, 100%), 271 ($M + H^+$, 56%).

Acetylation of **5** to form triacetate **10** -- To a solution of epoxydiester **5** (21.5 mg, 0.080 mmol) in anhydrous pyridine (400 μL) at 5°C was added freshly distilled acetic anhydride (23 μL, 3.0 equiv) and *ca.* 1 mg of 4-dimethylaminopyridine. The pale yellow solution was stirred at rt for 1 h, at which time tlc indicated starting material was absent. The reaction mixture was diluted with ethyl acetate (3 mL) and washed with aqueous $NaHCO_3$ (2 x 3 mL) then with saturated NaCl (3 mL). The combined aqueous layers were back-extracted once with ethyl acetate (3 mL), then the

combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum to afford 34.5 mg of an oil. Purification by flash chromatography on SiO_2 (3:97 CH_3CN:CH_2Cl_2) afforded **10** (26 mg, 78%) as an oil: $[\alpha]_D$ = -50° (c =1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) 7.14 (d, 1 H, J= 2.5 Hz), 5.76 (d, 1 H, J= 3.5 Hz), 5.62 (d, 1 H, J= 2.9 Hz), 5.44 (m, 1 H), 5.42 (dd, 1 H, J= 2.5, 8.0 Hz), 4.95 (d, 1 H, J= 2.9 Hz), 4.89 (dd, 1 H, J= 2.4, 7.8 Hz), 3.82, 3.78 (2s, each 3 H), 2.10 (s, 6 H), 2.03 (s, 3 H); ^{13}C-NMR ($CDCl_3$, DEPT; (0) = quarternary, (1) = methine, (2) = methylene, (3) = methyl carbon) 169.8(0), 169.13(0), 169.10(0), 164.4(0), 163.0(0), 149.9(0), 138.3(1), 128.9(0), 99.0(2), 72.5(1), 69.4(1), 68.9(1), 65.8(1), 52.6(3), 52.4(3), 20.8(3), 20.78(3), 20.70(3); IR (film) 2975, 1750, 1625, 1440, 1370, 1240, 1210, 1165, 1030 cm^{-1}; CIMS (NH_3) m/z 432 (M+ NH_4^+, 29%).

Cyathiformine B **6** -- A saturated solution (cloudy) of anhydrous K_2CO_3 in absolute CH_3OH (500 µL) was added to a solution of cyathiformine B triacetate **10** (11.8 mg) in CH_3OH (285 µL) at 0°C under Ar. After 1 h, the reaction was terminated by adding saturated aqueous NH_4Cl (4 mL). After removing CH_3OH on the rotary evaporator, the aqueous residue was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to an oil. Purification by flash chromatography on SiO_2 (5:95 CH_3OH:CH_2Cl_2) afforded **6** as a white foam (8.5 mg) in quantitative yield: $[\alpha]_D$ -111° (c= 0.76, $CHCl_3$) lit[5] -98° (c= 0.1, $CHCl_3$); ^{1}H-NMR (acetone-d_6) 6.77 (d, 1 H, J= 2.2 Hz), 5.44 (d, 1 H, J= 2.7 Hz), 4.97 (d, 1 H, J= 2.7 Hz), 4.84 (dd, 1 H, J= 7.7, 2.0 Hz), 4.56 (d, 1 H, J= 3.1 Hz), 4.50 (d, 1 H, J= 5.3 Hz), 4.48 (d, 1 H, J= 5.5 Hz), 4.35 (d, 1 H, J= 3.5 Hz), 4.14 (dd after D_2O, 1 H, J= 7.8, 2.3 Hz), 4.05 (dd after D_2O, 1 H, J= 3.2, 2.3 Hz), 3.76, 3.73 (2s, each 3 H); ^{13}C-NMR ($CDCl_3$) 166.6, 163.9, 149.4, 135.3, 131.8, 99.0, 76.1, 72.7, 68.9, 68.2, 52.8, 52.3; IR (film) 3425, 2975, 1725, 1625, 1445, 1220, 1175, 1100, 1050 cm^{-1}; CIMS (NH_3) m/z 306 (M+NH_4^+, 100%), 289 (M+H^+, 100%).

Cyathiformine C **7** -- A solution of epoxydiester **5** (22.6 mg, 0.08 mmol) in acetone (1 mL) was cooled to 0°C and conc. HCl (9 µL) was added. Starting ester disappeared and a slightly more polar spot was observed after 5 minutes. After 20 min, solvents were removed *in vacuo* to yield 28 mg of a colorless oil. Purification by flash chromatography on SiO_2 (1:4 CH_3CN:CH_2Cl_2) afforded **7** as a white foam (26 mg, 100%): $[\alpha]_D$ -84° (c= 1.2, $CHCl_3$) lit[5] -76.5° (c= 0.12, $CHCl_3$);

^{1}H-NMR ($CDCl_3$) 6.95 (d, 1 H, J= 2.2 Hz), 5.66 (d, 1 H, J= 2.8 Hz), 5.05 (d, 1 H, J= 2.7 Hz), 4.90 (dd, 1 H, J= 2.7, 0.8 Hz), 4.76 (dd, 1 H, J= 8.1, 1.6 Hz), 4.45 (dd, 1 H, J= 8.3, 2.2 Hz), 4.41 (m, 1 H), 3.92 (br. s., 1 H), 3.85, 3.82 (2s, each 3 H), 2.91 (br. s, 1 H); ^{13}C-NMR ($CDCl_3$) 164.6, 164.0, 149.4, 135.9, 130.3, 101.4, 77.3, 73.9, 68.0, 53.0, 52.9, 52.4; ^{13}C-NMR (acetone-d_6, DEPT) 165.4(0), 163.9(0), 151.3(0), 137.7(1), 131.1(0), 97.4(2), 77.0(1), 75.1(1), 68.6(1), 55.5(1), 52.6(3), 52.5(3); IR (film) 3440, 3000, 2950, 1725, 1620, 1440, 1250, 1210, 1175, 1100 cm^{-1}; CIMS (isobutane) m/z 307, 309 (M + 1, ^{35}Cl, ^{37}Cl).

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8. The facility of this cyclization may be pertinent to the biosynthetic origin of **9** (Ref. 6).

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