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STEMPHYLTOXIN III FROM *ALTERNARIA ALTERNATA*

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ABSTRACT.—Stemphyltoxin III, (6a*R**,6b*S**,7*R**,8*R**)-3,6a,10-trihydroxy-4,9-dioxo-4,6a,6b,7,8,9-hexahydro-7,8-epoxyperylene, a known metabolite of *Stemphylium botryosum* var. *lactucum*, has been identified as a mutagenic metabolite of *Alternaria alternata* by spectroscopic studies. The ^{13}C-nmr spectral data, which were not reported previously, are presented.

Species of *Alternaria* are plant pathogens and common decay organisms of mature fruits and vegetables. A large number of secondary metabolites have been reported for these molds (1,2). Recently we reported the isolation and characterization of three mutagenic perylenequinone metabolites, altertoxins I, II, and III (3). Other perylenequinone metabolites, stemphyltoxins I, II, III, IV, and stemphyperylenol, have recently been isolated and characterized from another mold and plant pathogen, *Stemphylium botryosum* var. *lactucum* (4). We report here the isolation, from *Alternaria alternata* (Fries) Keissler, of a compound with physical and spectral properties essentially identical to those of stemphyltoxin III [(6a*R**,6b*S**,7*R**,8*R**)-3,6a,10-trihydroxy-4,9-dioxo-4,6a,6b,7,8,9-hexahydro-7,8-epoxyperylene; note the different numbering scheme employed here as compared to Arnone *et al.* (4)]. The ^{13}C-nmr spectral data, which were not reported by Arnone *et al.* (4), are given here. Because no temperature was given for the reported (4) optical rotation measurement for stemphyltoxin III and no sample was available for comparison, the quantitative difference from our value cannot be reconciled. Preliminary studies using Ames *Salmonella typhimurium* TA98, TA100, and TA1537 assays indicate that stemphyltoxin III is also a potent mutagen.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting point was determined on a Kofler apparatus and is uncorrected. The uv spectrum was recorded on a Beckman DU-7 spectrophotometer and the ir spectrum on a Nicolet 740 instrument. The mass spectrum was obtained on a VG-ZAB 2F mass spectrometer at 70 eV. ^{1}H- and ^{13}C-nmr spectra were determined at 400 and 100 MHz, respectively, on a Varian VXR-400 nmr spectrometer. The optical rotation was measured on a Perkin-Elmer 241 MC polarimeter.

ORGANISM.—*A. alternata*, isolated from cherries and designated strain No. 42, is deposited with the Division of Microbiology, Food and Drug Administration, Washington, D.C.

CULTURE CONDITIONS AND ISOLATION PROCEDURE.—*A. alternata* was cultured on rice and H_2O and extracted with $CHCl_3$ as in Stack and Prival (5). Stemphyltoxin III occurs as a minor metabolite that is midway in polarity between altertoxin II and altertoxin I in tlc and hplc separations. It was isolated by Si gel cc as described by Stack and Prival (5), and it elutes from the column with $CHCl_3$-MeOH (98:2). Crystallization from $CHCl_3$/hexane yielded 154 mg brown microcrystalline solid, mp 190–300° (dec) $[\alpha]^{20}D$ +891° (c=0.12, MeOH) [lit. (4) mp >300° (dec), $[\alpha]D$ +688.5° (c=0.13, MeOH)]; uv λ max (MeOH) 215 (29,000), 269 (24,600), 287 sh (20,000), 300 sh (16,500), 374 nm (4700); ir ν max (KBr) 3439, 3047, 3028, 1658, 1644, 1607, 1457, 1335, 1228, 1172, 843, 827 cm^{-1}; ms *m/z* $[M]^+$ 348.0636 (calcd for $C_{20}H_{12}O_6$, 348.0634), 330, 319, 314, 302; ^{1}H nmr (Me_2CO-d_6) 3.77 (dd, J=3.6, 0.7, 1H, H-8), 3.9 (bs, 1H, H-6b), 4.60 (d, J=3.6, 1H, H-7), 5.15 (s, 1H, OH-6a), 6.55 (d, J=10.4, 1H, H-5), 7.02 (dd, J=8.8, 0.8, 1H, H-11), 7.09 (d, J=8.8, 1H, H-2), 7.86 (d, J=10.4, 1H, H-6), 8.14 (d, J=8.8, 2H, H-1 and H-12), 12.2 (s, 1H, OH-3), 12.4 (s, 1H, OH-10); ^{13}C nmr (Me_2CO-d_6) 43.4 (d, C-6b), 53.5 (d, C-8), 57.2 (d, C-7), 66.7 (C-6a), 113.7 (C-3a/9a), 115.2 (C-9a/3a), 117.4 (d, C-11/2), 118.8 (d, C-2/11), 125.0 (C-12a/12b), 125.8 (C-12b/12a), 129.3 (d, C-5), 132.7 (d, C-1/12), 133.7 (d, C-12/1), 136.5 (C-12c), 140.6 (C-9b), 147.8 (d, C-6), 161.8 (C-3/10), 163.6 (C-10/3), 191.0 (C-4), 198.4 (C-9).

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