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ANTHRAQUINONE PIGMENTS FROM THE PHYTOPATHOGEN *PHOMOPSIS JUNIPEROVORA* HAHN

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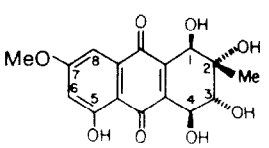
Key Word Index—*Phomopsis juniperovora*; *Alternaria solani*; fungi; *Juniperus virginiana*; Cupressaceae; 7-methoxy-2-methyl-1,2,3,4,5-pentahydroxy-1,2,3,4-tetrahydroanthraquinone; altersolanol A; macrosporin.

Phomopsis juniperovora Hahn causes the most damaging disease of eastern red cedar (*Juniperus virginiana* L.) in forest nurseries in the Great Plains of the U.S., and is also a destructive pathogen of many other Cupressaceae [1]. When the fungus is cultured on a variety of agar media a deep yellow or orange coloration is produced, usually accompanied by the appearance of bright orange–red crystals on the surface of the agar; the coloration has been reported as diagnostic for *P. juniperovora* [2]. No chemical work has been reported on the fungus.

We have extracted the orange–red material from the culture, and, using mainly spectroscopic methods, find that the major constituent is 7-methoxy-2-methyl-1,2,3,4,5-pentahydroxy-1,2,3,4-tetrahydroanthraquinone (**1**). The identity was confirmed by comparison with an authentic sample of (**1**), previously isolated by Stoessl from *Alternaria solani* and named by him altersolanol A [3a].

We also isolated small amounts of anthraquinones from the *Phomopsis* cultures. We identified macrosporin (**2**) definitely, by its UV and MS and by conversion to its diacetate [3b]. We have tentatively identified 6-methylxanthopurpurin-3-methyl ether (**3**) and macrosporin-3-methyl ether



(1)

from UV and MS. Compounds (**2**) and (**3**) were previously isolated from *A. solani* [3b] and *Alternaria bataticola* [4]. *Phomopsis* and *Alternaria* are not regarded as related fungi and so the isolation of similar anthraquinones from these fungi, particularly the same tetrahydroanthraquinone (one of a rare group of compounds [5,6]), is of interest.

EXPERIMENTAL

Unless otherwise specified UV spectra were taken in EtOH and IR spectra in CH_2Cl_2.

Culture of organisms. Isolates of *Phomopsis juniperovora* Hahn (ATCC 24902) were maintained on CMS (corn meal sucrose) agar. Cultures were grown on either PDA (potato dextrose agar) plates or CMS plates. Cultures were incubated at 24° until crystal development was extensive (usually 30 days).

Extraction of metabolites. Three methods of extraction were used. (1) *Isolation from a batch of 50 plates where orange needles appeared on surface.* The crystals were scraped from the agar surface, and extracted in a Soxhlet with acetone. Removal of the acetone gave 223 mg of fairly pure altersolanol A, m.p. 215–220° (darkening at 209°). The residual agar was broken up and extracted with acetone to yield a crude product, 226 mg. This material was chromatographed on a polyamide column and elution with C_6H_6–Me_2CO (4:1) gave a further 87 mg of altersolanol A, m.p. 217–220° (darkening at 212°).

(2) *Isolation from plates when there were few or no crystals on the surface of the agar.* The total agar mat was extracted with acetone or MeOH. The residue, 230 mg, after removal of the solvent was chromatographed on a polyamide column, which was eluted with MeOH to give 2 main fractions; evaporation of the combined early fractions gave mixture A, 21 mg, and of the later fractions mixture B, 269 mg. The latter was mainly altersolanol A (as shown by TLC), with some solvent and resin residues. Mixture A was rechromatographed on preparative silica plates, which on development with C_6H_6–EtOAc (4:1), gave 2 major bands, A1 (R_f 0·52, 22 mg) and A2 (R_f 0·82, 8 mg). A1 was macrosporin, m.p. 306–312° (lit. [3, 7] 308–316° and 300–302°), λ_{max} 380, 305, 285, 252 and 228 nm; MS: *m/e* (rel. intensity) 285 (18), 284 (100), 283 (11), 255 (14), 254 (11), 241 (6), 226 (10), 129 (25), 128 (18), 100 (41) and 85 (79). Macrosporin diacetate, (13·9 mg), on recrystallization from $CHCl_3$–petrol (b.p. 30–60°) had m.p. 215–219° (lit. [3b,7] 213–216° and 209–210°); the NMR spectrum ($CDCl_3$) was identical with that reported by Stoessl [3b].

The band A2 was rechromatographed on Kieselgel in C_6H_6–EtOAc (4:1). Three bands were obtained. The middle band, R_f 0·66, appears from its UV and MS to be a mixture of macrosporin monomethyl ether and 6-methylxanthopurpurin-3-methyl ether.

(3) In the third method of extraction, the agar mats from 50 plates were extracted in cold acetone; the residue after the removal of acetone was taken up in H_2O, and then the extraction procedure used by Stoessl for his culture filtrates was followed [3b]. When the aqueous phases were extracted with EtOAc and the dried EtOAc layers concentrated almost to dryness altersolanol A (254 mg) crystallized out. After two re-crystallizations from dioxan the altersolanol A (161 mg) had m.p. 218–220° (charring at 206°) undepressed by m.m.p. with an authentic sample; $[\alpha]_D^{25}$ − 154° (*ca* 7·1 mg in 10 ml, 100% EtOH); authentic sample $[\alpha]_D^{25}$ − 160° (*ca* 7·7 mg in 10 ml, 100% EtOH), IR, UV and NMR (DMSO-d_6) spectra were identical with those of an authentic sample; NMR (trifluoracetic acid) δ = 4·42, 4·11 (2 H pair *d*, *J* = 2 Hz, Ar-H), 3·19, 2·68 (2 H, AB *q*, *J* = 6 Hz; H_3 and H_4), 3·04 (1 H, *s*, H_1) 2·40 (3 H, *s*, $ArOCH_3$), 1·06 ppm. (3 H, *s*, CH_3); MS *m/e* (rel. intensity) 338 (4), 336 (4), 334 (4), 300 (36), 284 (71), 263 (51), 262 (60), 234 (100), 206 (31) and 128 (11). M* 209 ($M^*_{calc.}$ 262 → 234 = 209). Found: C, 56·74; H, 5·29; O, 37·79. Calc. for $C_{16}H_{16}O_8$: C, 57·14; H, 4·80; O, 38·06%. The mother liquors after the crystallization of (**1**) were evaporated to dryness and distributed between H_2O and $CHCl_3$. Extraction of the aqueous layers yielded altersolanol A (41 mg).

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