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THE ANTIBIOTIC BOSTRYCIN FROM *ALTERNARIA EICHHORNIAE*

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Key Word Index—*Alternaria eichhorniae*; fungus; *Eichhornia crassipes*; water hyacinth; bostrycin; anthraquinone; antibiotic.

INTRODUCTION

Many attempts have been made to find suitable and effective biological controls for the pernicious weed, water hyacinth, which is represented by seven genera and 30 species having a world-wide distribution. Nag Raj and Ponnappa [1] have investigated the phytotoxic effect of several fungi on water hyacinth and found *Alternaria eichhorniae* to hold some promise in the control of *Eichhornia crassipes* (Mart.) Solms., a species of water hyacinth prevalent in Pakistan. The present investigation is directed towards the isolation and characterization of the phototoxic substances produced by the *Alternaria* fungi

RESULTS AND DISCUSSION

Chloroform extraction of the broth on which *A. eichhorniae* had been grown gave, after crystallization from warm acetic acid, a bright red compound (81% of extract), mp 236–237° (sealed capillary, corrected), having λ_{max}^{EtOH} 226 (25,200), 300 (6260), 470 (4830), 501 (5460), 540 (3690) nm. Its IR spectrum (KBr) showed strong absorption bands at 3525, 3490, and 3380 cm^{-1} (—OH) as well as a strong carbonyl absorption at 1595 cm^{-1}. Mass spectral analysis gave a strong parent ion at *m/e* 336 ($C_{16}H_{16}O_8$).

The identity of this compound with the antibiotic, bostrycin (**1**) isolated by Noda [2, 3] from *Bostrychonema alpestre* Cesati was confirmed by its ^1H NMR spectrum in DMSO-d_6 which was virtually identical to that published by Noda. However, the ^1H NMR spectrum in pyridine-d_5 was more revealing and gave: δ 1.79 (3H, *s*, C_2 -Me), 3.07 (1H, *d*, J = 18 Hz, C_1 -H), 3.44 (1H, *d*, J = 18 Hz, C_1 -H), 3.69 (3H, *s*, —OMe), 4.36 (1H, *d*, J = 4 Hz, C_3 -H), 5.68 (1H, *d*, J = 4 Hz, C_4 -H), 6.37 (1H, *s*, C_6 -H). The low coupling constant between the C_3 and C_4 protons as well as the chemical shifts confirm the identity of this mold metabolite as bostrycin. Other reduced anthraquinones have been isolated from other *Alternaria* spp., e.g. altersolanol A (**2**) and altersolanol B (**3**) [4–6] have been isolated from *Alternaria solani*.

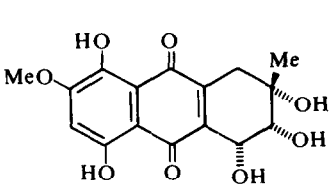
In our present studies, the broth concentrate, from which the mold mycelium has been removed, showed activity against water hyacinth by producing necrosis in the leaves and also the crude red metabolic product; the chloroform extract of the broth gave the same results. Ponnappa [7] has reported that the metabolic pigment produced by *Alternaria eichhorniae* appears to have a herbicidal value against water hyacinth which can be used as a biologically produced 'weed killer' in a concentrated form. This confirms our findings, but the major metabolic constituent (81%) 'bostrycin' of *Alternaria eichhorniae*, which is an antibiotic against gram-positive bacteria reported by Noda [3], has shown no effect as a 'weed killer'. These observations indicate that the total crude metabolic product has some pathogenic effect, but the major component showed no activity. There must therefore be some other constituents present in the crude metabolic product which might be biologically active in producing diseases on the host plant, although their concentrations in the crude extract may be very low. This assumption is very much supported by Ponnappa's [1] findings that the activity of cultures of *Alternaria eichhorniae* against water hyacinth is correlated with the degree of colouration of the culture medium.

EXPERIMENTAL

Mps are corr.; ^1H NMR spectra were obtained at 90 MHz.

Culture of A. eichhorniae. A strain of *Alternaria eichhorniae* Nag Raj and Ponnappa sp. nov. IMI no. 121518 were obtained from the International Mycological Institute, U.K. and cultured in potato-dextrose broth (24 g/l.) having an initial pH of 5.1. The mold was allowed to grow for 12 days with temps. ranging from 24 to 30° and being irradiated with daylight and/or incandescent light. Filtration of the broth (pH 4.6) gave a bright red soln which was phytotoxic to water hyacinth.

Extraction of metabolites. After culture, the broth was adjusted to pH 3.5 with conc HCl. To this soln was added an equal vol. of $CHCl_3$ and allowed to stand for 1 day with occasional shaking. Separation and distillation of the $CHCl_3$ under red. pres. at *ca* 40° gave a gummy residue which was washed with Et_2O to



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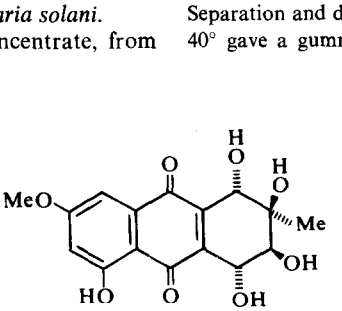

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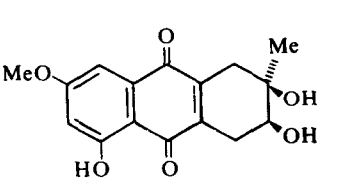

3

remove waxy matter; thus producing, a red, free-flowing solid (*ca* 10 mg/l. broth).

Isolation of bostrycin (**1**). Bostrycin was isolated by dissolving the above extract (250 mg) in warm HOAc, filtering to remove insoluble material, then allowing the soln to cool. Dark red crystals deposited (203 mg), mp 236–237°, $[\alpha]_D^{25}$ −81° (*c*, 1.19 in DMSO), which were identical to bostrycin (*vide supra*).

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COUMARINS FROM *APIUM GRAVEOLENS* SEEDS

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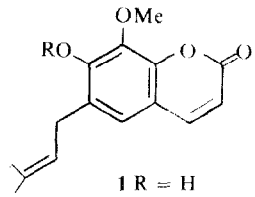
Key Word Index—*Apium graveolens*; Umbelliferae; seeds; seselin; bergapten; isoimperatorin; isopimpinellin; 7-hydroxy-8-methoxy-6-(3-methyl-2-butenyl)2H-1-benzopyran-2-one; apigravin; osthenol; structural determination.

Continuing our investigation [1–3] of *Apium graveolens* seeds, we now wish to report the isolation of 6 coumarins from the petrol extract of the seeds. Three of the coumarins not previously reported from *A. graveolens* seeds are seselin, isoimperatorin and osthenol, the other two, bergapten and isopimpinellin, are known [4, 5]. The sixth, a novel coumarin named apigravin, has been assigned the structure 7-hydroxy-8-methoxy-6-(3-methyl-2-butenyl)2H-1-benzopyran-2-one, **1**, based on the following experimental evidence.

Spectral data of **1** and its Me ether revealed the presence of a coumarin skeleton with a OH, a OMe and a C-prenyl group in it. Its ^{1}H NMR spectrum indicated that the 3-, 4- and 5-positions in the coumarin are unsubstituted. The similarity in the UV spectrum of **1** (λ_{max}^{MeOH} nm: 260, 325) and umbelliferone indicated oxygenation at the 7-position. **1** readily formed a cyclized product (HCO_2H), **2**, suggesting that the OH and prenyl groups are *ortho* to each other, and that the prenyl group occupies the 6- or 8-position. The IR and UV absorptions of **1** were different from those reported for 7-hydroxy-6-methoxy-8-*C*-prenyl coumarin [6] indicating the placement of the prenyl group at the 6-position. The negative Gibb's reaction [7] of **1** and a positive Gibb's test of its demethylated derivative (**4**), obtained by treating **1** with pyridinium hydrobromide [8], suggested the presence of the OMe group at the 5- or 8-position. The possibility of **1** having a 5,6,7-substitution pattern was ruled out by direct comparison of its Me ether (**3**) with an authentic sample of 5,7-dimethoxy-6-C-prenyl coumarin (toddaculin) [9].



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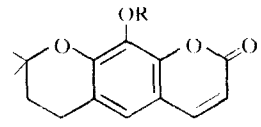
3 R = Me



2 R = Me
4 R = H

EXPERIMENTAL

Isolation. Dried *A. graveolens* seeds (4 kg) were extracted with petrol. The petrol extract was concd and chromatographed on Si gel (1 kg) using petrol with increasing amounts of C_6H_6 as eluent. The 6 main fractions obtained were crystallized to yield compounds A, B, C, D, E and F, respectively.

Identification. Compounds A, B, C, D and F were identified as seselin, bergapten, isoimperatorin, isopimpinellin and osthenol,