Note

Isolation and Identification
of Dactylariol from the
Culture Liquid of
Alternaria porri (Ellis)
Ciferri†

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In the course of investigation on the metabolic pigments produced by *Alternaria porri* (Ellis) Ciferri, we reported that seven pigments, (I)~(VII),¹⁻⁶ were isolated and identified, of which altersolanol A (VII) and altersolanol B (VI) are tetrahydroanthraquinone derivatives. The present paper deals with the isolation and identification of another tetrahydroanthraquinone, dactylariol (VIII), 7-methoxy-2-methyl-(1*R*, 2*R*, 3*R*)-1,2,3,4-tetrahydro-1,2,3,5-tetrahydroxyanthraquinone, from the culture liquid, when the fungi was cultured on Brian's medium *T*, which was used in the case of isolation of altersolanol A.⁶

In the previous study,⁶ it was reported that coloring matter obtained from an ethyl acetate extract of the culture liquid was separated into 5% sodium bicarbonate soluble and non-soluble parts, and from the former part altersolanol A was isolated. In this experiment, the ethyl acetate part mentioned above was further separated into 5% sodium carbonate soluble and non-soluble ethyl acetate parts, and isolation and identification of the minor components of pigments in both parts were attempted.

From the 5% sodium bicarbonate soluble part obtained from the culture liquid (54 liters), altersolanol A (VII, 144 mg) was also identified as shown in the previous paper.⁶ While the non-soluble ethyl acetate part was further extracted with 5% sodium carbonate solution (3 × 300 ml) and after evaporation of ethyl acetate the extract that remained was subjected to repeated silicic acid column chromatography (Merck, Kieselgel 60) with a solvent system of methanol-chloroform-formic acid (5:95:2) to give 2-methylxanthopurpurin-7-methylether (I, 10 mg). The sodium carbonate solution was acidified with hydrochloric acid and extracted with ethyl acetate (3 × 80 ml). After removing the solvent, the extract was

- I: $R_1 = R_2 = R_3 = H$
 II: $R_1 = R_3 = H, R_2 = OH$
 III: $R_1 = R_2 = H, R_3 = OH$
 IV: $R_1 = H, R_2 = R_3 = OH$
 V: $R_2 = H, R_1 = R_3 = OH$

- VI: $R_1 = R_2 = H$
 VII: $R_1 = R_2 = OH$
 VIII: $R_1 = OH, R_2 = H$

subjected to silicic acid column chromatography under the same conditions mentioned above, and separated into four parts in order of increasing *R_f* values. After repeated column chromatography of these parts, it was revealed that the first to third pigments were identical with 3,4,5-trihydroxy-7-methoxy-2-methylanthraquinone (IV, 5 mg), macrosporin (II, 81 mg) and altersolanol B (VI, 72 mg), respectively. Pigments I,¹ II,¹ IV,³ VI⁵ and VII⁶ have already been reported as metabolic pigments of *Alternaria porri* in the course of our investigation. The identification of pigments was carried out by comparison of their *R_f* values on TLC and IR spectra with those of authentic samples. The fourth pigment, dark-red needles (20 mg), sublimed at near 220°C into needles and finally melted at 300~304°C. The mass spectrum gave a molecular ion at *m/z* 320, corresponding to $C_{16}H_{16}O_7$ (320.09), and peaks at 322 (*M* + 2*H*), 302 (*M* - *H*₂O), 284 (*M* - 2*H*₂O), 268, 259, 247 (*M* - *C*₃*H*₅O₂, *C*₁₃*H*₁₁O₅), 231 (259 - CO), 219 (247 - CO), 151 and 149. UV λ_{max}^{EtOH} nm (ϵ): 268 (12.5 × 10³), 286 (infl., 8 × 10³) and 422 (4.1 × 10³). IR ν_{max}^{KBr} cm⁻¹: 3200~3600 (broad OH), 1650 (non-chelated C=O), 1630 (chelated C=O) and 1200 (phenolic OH). [α]_D²⁰ -58° (*c* 0.043 in ethanol) [lit.⁷ records [α]_D²⁶ -33 ± 10° (*c* 0.032 in ethanol)]. The NMR spectrum revealed the presence of an aromatic methyl ether at 3.91 ppm (3H, s), two meta coupled aromatic protons at 6.71 ppm (1H, d, *J* = 2 Hz) and 7.17 ppm (1H, d, *J* = 2 Hz), and tertiary methyl protons at 1.50 ppm (3H, s). Moreover, peaks at 4.76 ppm (1H, s, *C*₁-H), 4.15 ppm (dd, *J* = 6 and 9 Hz, *C*₃-H), 3.13 ppm (dd, *J* = 6 and 20 Hz, *C*₄-H) and 2.59 ppm

† Studies of the Metabolic Products of *Alternaria porri* (Ellis) Ciferri. Part XI. Parts I to IV, see ref. 1.

(dd, $J=9$ and 20 Hz, C_4 -H) were shown.

Comparing the physical properties and spectral data of the pigment with those of dactylariol previously reported,⁷⁾ this compound was presumed to be dactylariol (VIII). And the presumption was proved to be correct by comparison of its NMR spectrum with that of an authentic sample.

When dactylariol (VIII, 10 mg) was heated at 120°C/0.5 torr, it sublimed into other pigments. On monitoring the TLC plate of silica gel (Merck Kieselgel 60G) using the solvent system of methanol-chloroform-formic acid (5:95:2), macrosporin (II) and other minor unidentified pigments were detected. By the preparative TLC 3 mg of macrosporin (II) was obtained. Consequently, it was confirmed that macrosporin (II) is formed from dactylariol (VIII) by dehydration. Stoessl *et al.*⁸⁾ have proved that altersolanol A (VII) is metabolized to altersolanol B (VI), macrosporin (II) and dactylariol (VIII) by the incorporation of (1,2-¹³C₂) acetate. (He used the term of altersolanol C for dactylariol.) In our experiment, however, dactylariol (VIII) was proved to be a minor metabolic component of *Alternaria porri* (Ellis) Ciferri.

Altersolanol A (VII), altersolanol B (VI), dactylariol (VIII), and bostrycin (IX)⁹⁾ have been reported as 1,2,3,4-tetrahydroanthraquinones, of which dactylariol (VII) has been known as a metabolite produced by *Dactylaria lutea*, Routien and *Alternaria solani*.⁸⁾ The occurrence of dactylariol (VIII) in both the genera *Dactylaria* and *Alternaria* is of taxonomic interest, because they are not regarded as related genera. However, it might be suggested from the

chemotaxonomical point of view that dactylariol (VIII) will be detected as a metabolite of other fungi of the *Alternaria* genus in the future.

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