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SHORT COMMUNICATION

A NAPHTHO [1,2-b] FURAN DERIVATIVE FROM THE FUNGUS *ROESLERIA PALLIDA*

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Abstract—The strongly blue fluorescent compound present in the dark green extracts of the fungus *Roesleria pallida* (Pers.) Sacc. was obtained in a pure state and determined as 4,7-dihydroxy-2,3,3,9-tetramethyl-2,3-dihydronaphtho [1,2-b] furan-5,6-dicarboxylic anhydride.

THE BLUE fluorescent compound, obtained in small amount from *Roesleria pallida* was shown to be 4,7-dihydroxy-2,3,3,9-tetramethyl-2,3-dihydronaphtho [1,2-b] furan-5,6-dicarboxylic anhydride (I). The molecular formula $C_{18}H_{16}O_6$ was shown by mass spectrometry. Table 1 shows the interpretation of the metastable ions. Other interesting transitions in the spectrum were $295^+ \rightarrow 267^+$ (18·3) + 28 and $285^+ \rightarrow 267^+ + 18$.

TABLE 1. METASTABLE TRANSITIONS IN THE MASS SPECTRUM OF THE ISOLATED COMPOUND

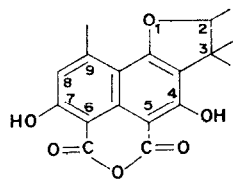
Metastable ion M*	Transition† $M_1^+ \rightarrow M_2^+$ + neutral fragment
298·7	328^+ (80,8) $\rightarrow 313^+$ (100) + 15 (CH_3)
278·0	313^+ (100) $\rightarrow 295^+$ (31·1) + 18 (H_2O)
259·5	313^+ (100) $\rightarrow 285^+$ (40·0) + 28 (CO)
231·2	313^+ (100) $\rightarrow 269^+$ (51·6) + 44 (CO_2)

†Abundances are given in parentheses.

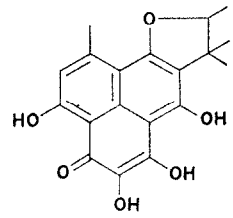
Owing to the small quantity of compound, it was not possible to measure a NMR spectrum in the usual way. We obtained a fairly good spectrum after time averaging of 36 scans. Signals expressed as δ units (ppm) were found at 1·34 and 1·40 (singlets, 2 CH_3 groups), 1·59 (doublet, $J = 7$ c/s, 1 CH_3 group), 2·90 (singlet, assigned to 1 ϕ-CH_3), 4·74 (quartet, $J = 7$ c/s, 1 H) and 6·87 (singlet, 1 aromatic H). The spectrum was scanned up to 10 ppm.

A search of the literature revealed a compound with the same melting point and molecular formula as our product and with similar UV and IR spectra.[1] The data of the mass and NMR spectra described above fitted in with the structure reported. The substance was a degradation product of atrovenetin (II) and of herqueinone (III) and the product I was synthesized from these compounds, isolated from *Penicillium herquei* CBS 336·48. The product obtained was compared with the isolated compound from *R. pallida* and showed complete agreement in all respects (m.p., UV and IR spectra, TLC). Since X-ray study has

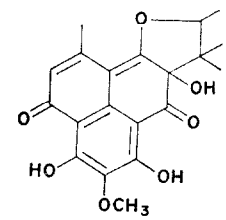
[1] D. H. R. BARTON, P. DE MAYO, G. A. MORRISON and H. RAISTRICK, *Tetrahedron* **6**, 48 (1959).



(I)



(II)



(III)

shown the final structure of atrovenetin[2] as II, the structure of I is thus also proved. Recent studies,[3] concerning the constitution of herqueinone have confirmed this finding.

I had previously been isolated only from *Penicillium herquei*,[4] and its presence in *R. pallida* is remarkable since there is no taxonomic relationship between the two genera. *R. pallida* also produced a dark green pigment. It is interesting that dark green pigments have also been reported in *P. herquei* and *P. atrovenetum*.[5,6] The pigments were not isolated in a pure state, but the pigments of both fungi appeared to be the same. It is possible that a close relationship exists between the green pigments indicated above and that from *R. pallida*. Preliminary experiments showed that the UV and IR spectra of the *R. pallida* pigment are very similar to those reported for the *Penicillium* pigment.[4] Finally, it may be noted, that there is evidence for a relationship between the blue fluorescent compound (I) and these dark green *Penicillium* pigments.

EXPERIMENTAL

Cultural conditions. The fungus *Roesleria pallida* (Pers.) Sacc., CBS 407·51 was grown on X-agar medium for 30–35 days at 15° in the dark. The X medium was 1 l. cherry agar (*med. a*), 1 l. peptone-glucose-sucrose agar (*med. b*) and 1 l. oatmeal agar (med. c) followed by autoclaving at 104° for 8 min.

Preparation of *med. a.* Cherries (200 g) were extracted with 200 ml hot H_2O for several hours. After filtration through glass cloth (± 85 mesh/cm²), the solution was autoclaved at 110° for 30 min, mixed with 800 ml H_2O containing 20 g agar. Finally the solution was autoclaved again at 102° for 5 min.

Composition of *med. b.* Peptone 10 g, glucose 20 g, sucrose 10 g, K_2HPO_4 1 g, $NaNO_3$ 1 g, $MgSO_4 \cdot 7H_2O$ 0,5 g, agar 15 g, tap H_2O 1 l. Sterilization was carried out at 110° for 30 min. *Med. c.* was prepared by extracting 30 g oatmeal in 1 l. H_2O at 80° for 2 hr. The extract was filtered through glass cloth and subsequently sterilized at 120° for 20 min.

Isolation procedures. At the end of the incubation period, a dark green velvety mycelial mat had grown over the whole plate. Mycelium and agar were extracted 4× with peroxide free Et_2O and 4× with EtOH. The extracts were evaporated *in vacuo* and dried (P_2O_5). Chromatography was carried out using a column (4 × 48 cm) of silica (Mallinckrodt, 100 mesh) and kieselguhr (Merck) in a ratio of 2:1 with light petroleum b.p. 60–80°/$CHCl_3$ = 1:1 (v/v) as the eluent. The first colorless fractions contained the fluorescent compound. Further purification was accomplished by means of preparative TLC. Merck 1 mm silicagel G layers were used with hexane saturated with H_2O/EtOH = 100:2 (v/v) as the developing solvent.

Finally the fluorescent compound was sublimed at 200° at normal pressure. Cream-coloured needles were obtained with m.p. 259–261°.

Spectrometry. UV spectra were taken with a Perkin–Elmer 402 UV-visible spectrophotometer. Spectrum in MeOH, λ_{max}: 215, 255, 295, 362, 375 nm; in MeOH/KOH, λ_{max}: 227, 257, 308, 344, 390 nm. IR spectra (KBr disc) were measured on a Grubb–Parsons Spectromaster. Absorptions: 1706 and 1667 cm^{-1} (carbonyl stretching), 1618 and 1610 cm^{-1} (C=C stretching), bands between 1000–1300 cm^{-1} (C—O stretching) and bands in the region of 2850–3000 cm^{-1} (alkane C—H stretching).

[2] I. C. PAUL, G. A. SIM and G. A. MORRISON, *Proc. Chem. Soc.* 352 (1962).
[3] J. S. BROOKS and G. A. MORRISON, *Tetrahedron Letters* 963 (1970).
[4] N. NARASIMHACHARI and L. C. VINING, *Can. J. Chem.* **41**, 641 (1962).
[5] J. A. GALARRAGA, K. G. NEILL and H. RAISTRICK, *Biochem. J.* **61**, 456 (1955).
[6] K. G. NEILL and H. RAISTRICK, *Biochem. J.* **65**, 166 (1957)

The NMR spectrum was obtained after time averaging 36 successive scans with a Varian C 1024 CAT coupled to a Varian HA 100 spectrometer. The spectrum was recorded in $CDCl_3$ using trimethylsilane as the internal standard. The mass spectrum was taken on an A.E.I. MS-9 instrument.

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