LITERATURE CITED

1. N. M. Rebachuk, V. A. Denisenko, and S. A. Fedoreev, Khim. Prir. Soedin., 793 (1987).
2. R. T. Luibrand, T. R. Erman, J. J. Vollmer, P. J. Scheuer, J. Finer and J. Clardy, Tetrahedron, 35, 609 (1979).
3. G. M. Sheldrick, SHELX 76: Program for Crystal Structure Determination, Cambridge University Press, Cambridge (1976), p. 42.
4. G. M. Sheldrick, Crystallographic Computing 3, Oxford University Press, Oxford (1985), p. 175.

SECONDARY METABOLITES OF Pyricularia oryzae

II. POLYKETIDE METABOLITES

S. I. Sviridov

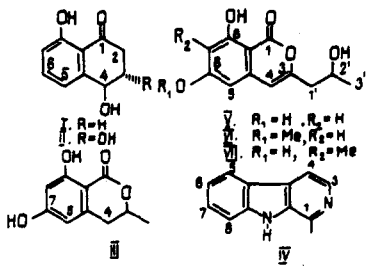
UDC 633.511:632.4:582.288

A number of exometabolites have been isolated from the deuteromycete Pyricularia oryzae Cav., of which harman and O-demethyldiaporthin have been isolated from this species for the first time. The latter is the first example of a hexaketide from fungi of the genus Pyricularia, for which only penta- and heptaketides have previously been described.

The isolation and identification of a number of secondary metabolites forming derivatives of o-nitrophenol from the neutral and acid fractions of an extract of the culture fluid of Pyricularia oryzae Cav. has been described previously. In this paper we consider the isolation and identification of five compounds from the neutral fraction of the extract.

Compounds (I)-(III) were identified by comparing their physicochemical properties with those of substances isolated previously from this fungus. They were metabolites of pentaketide origin and were identical with the isosclerone (4,8-dihydroxy-1(2H)-naphthalenone) (I), 3,4,8-trihydroxy-1(2H)-naphthalenone (II), and 6-hydroxymellein (III), that had been isolated previously from P. oryzae by Japanese workers [2, 7]. The PMR spectra that we obtained from these substances differed from those described previously by a considerably higher resolution and are given in the Experimental part.

Compound (IV), isolated from the neutral fraction, was identified on the basis of its physical properties as harman [3]. Harman is an indole alkaloid that is widely distributed in nature in higher plants. Of microorganisms it has been found in Streptomyces and Nocardia actinomycetes [3] as a result of the screening in the search for substances possessing growth inhibiting activity with respect to plants. In view of the fact that the scheme for isolating metabolites that we selected was designed for acidic and neutral compounds, while harman is a weak base, it may be assumed that its amount in the culture fluid was considerably higher than the amount that we had isolated.



All-Union Scientific-Research Institute of Phytopathology, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 471-475, July-August, 1991. Original article submitted July 23, 1990.

0009-3130/91/2704-0410$12.50

The structure of the fifth metabolite, which we have called O-demethyldiaporthin (V) was deduced from an analysis of its PMR and ^{13}C NMR spectra. The PMR spectrum taken at a frequency of 80 MHz contained signals at 11.25 ppm (1H, s), 9.63 ppm (1H, br. s), and 3.18 ppm (1H, br. s) undergoing exchange in D_2O (the proton giving a signal at 11.25 ppm was exchanged very slowly), which indicated the presence of three hydroxy groups. The hydroxyls with shifts of 11.25 and 9.63 ppm were phenolic, and the weaker of them participated in the formation of a hydrogen bond of the chelate type.

The aromatic protons gave a signal in the form of a complex narrow multiplet at about 6.4 ppm; however, at 250 MHz the multiplet was split into three one-proton signals, at 6.37 ppm (d, J = 2.2 Hz), 6.40 ppm (dd, J = 2.2 and 0.4 Hz), and 6.42 ppm (ddd, J = 0.7, 0.5, and 0.4 Hz). The signals at 6.37 and 6.40 ppm belonged to two protons in a benzene ring with the m-arrangement which was substituted in the manner of a 4-acyl-5-alkenylresorcinol. The signal at 6.42 ppm, having three long-range SSCCs was due to the 5-alkenyl substituent. The long-range constant of 0.4 Hz indicated spin-spin coupling with one of the aromatic protons, and the other two were coupled with aliphatic protons giving a signal at 2.60 ppm. This signal, having the form of a "doublet" with an SSCC of 6.6 Hz at 80 MHz formed at 250 Mz the AB part of very degenerate ABX → A_2X system with a geminal SSCC of 17 Hz. The high-field part of the spectrum contained, in addition to this signal, the signal of a methyl group at 1.25 ppm (d, J = 62 Hz) and a one-proton broadened sextet at 4.18 ppm (J = 6.4 Hz). The appearance of these signals indicated the presence of the 2-hydroxypropyl group of O-methyldiaporthin such groups being found frequently in polyketides.

The most intense fragments in the mass-spectroscopic breakdown of O-demethyldiaporthin were the molecular ion with m/z 236 (23%) and an ion corresponding to M − CH_3CHO, m/z 192 (100%). An M− CH_3 ion had a low intensity (2%).

The ^{13}C NMR spectrum contained seven signals from a 4-acylresorcinol nucleus, three signals of a 2-hydroxypropyl residue, and signals at 156.38 and 140.95 ppm belong the 5-alkenyl substituent of resorcinol. The signal at 156.38 ppm indicated a substitution of the double bond by oxygen.

The combination of these facts enabled O-demethyldiaporthin to be ascribed the structure of 6,8-dihydroxy-3-(2-hydroxypropyl)isocoumarin (V). Two related metabolites have been isolated from natural sources: diaporthin (VI) from Endotia parasitica (Murr.) And [4] and lunatinin (VII) from Cochliobolus lunata [5]. They are O- and C-methyl derivatives of O-dimethyldiaporthin, respectively. The treatment of O-demethyldiaporthin with CH_2N_2 in Et_2O/MeCN (O-demethyldiaporthin is practically insoluble in ether) gave a monomethyl ether as the main product, which, by its physicochemical properties, including the sign and magnitude of its optical rotation, was identical with diaporthin, and this served as an additional proof ot its structure.

When this paper was being prepared for publication, a report [6] appeared on the isolation of O-demethyldiaporthin, identical with that which we have isolated, from Drechslera siccans. The physicochemical and spectral characteristics of this compound practically coincide with those that we have obtained, with the exception of the fact that Hallock et al. were unable to obtain the substance in the crystalline form, which, in all probability, was due either to an insufficient amount of material or to an enantiomeric composition different from ours.

EXPERIMENTAL

The PMR spectra were obtained on Bruker AC80 and WM250 spectrometers, and ^{13}C NMR spectra on the Bruker AC80 spectrometer. The other conditions for performing the investigation were identical with those described in [1].

Isolation. Harman (I) in an amount of 10 mg was obtained when fraction 2 of the neutral part was chromatographed on silica gel L 5/40 μ (Chemapol) in ethyl acetate with subsequent purification on Al_2O_3 (Woelm for TLC, basic) in $CHCl_3$ and crystallization from aqueous MeCN (with the addition of a few drops of 25% NH_3). The isolation of isosclerone (I) has been described previously [1]. Compounds (II), (III), and (V) were eluted after the o-nitrophenol derivatives [1] when fractions 4 and 5 of the neutral part were separated.

Subsequent purification by PTLC on Kieselgel 60 (0.2 mm, C_6H_6–ethyl acetate (8:2)) and recrystallization from acetone gave 15 mg of (II). The fractions containing (III) were

purified successively on silica gel L 5/40 μ in the $CHCl_3$–acetone (95:5) system, on polyamide in hexane–$CHCl_3$–acetone (95:95:10), and on Sephadex LH-20 in MeOH. Recrystallization from aqueous methanol gave 30 mg of (III). O-demethyldiaporthin was purified on a mixture of polyamide and silica gel (1:3) in $CHCl_3$–acetone (9:1 → 9.15). Recrystallization from acetone gave 80 mg of pure substance.

Isosclerone (I). Colorless needles from a mixture of hexane and ethyl acetate with mp 69-70°C (according to the literature [7] 74-76°C). UV spectrum: λ_{max}^{MeOH}: 214, 259, 331 nm (lg ε: 4.25; 3.98; 3.61). UV spectrum: ν_{max} (paraffin oil), cm^{-1}: 3320 s, 3220 s, 3030 w, 1630 br. s, 1595 sh., 1570, 1450 s, 1404 w, 1365, 1335 s, 1325, 1307, 1257 s, 1218, 1195 s, 1157 s, 1095, 1075, 1065, 1048, 1030 w, 983 s, 891 s, 841 s, 820 s, 805 s, 771 w, 746 s, 705 w, 650 w, 621, 580 w.

PMR spectrum ($CDCl_3$, ppm): 1.98 (1H, br. s, HO-4); 2.18 (1H, dddd, J = 18.0; 8.2; 7.3; 4.9, H-3ax); 2.31 (1H, dddd, J = 18.0; 8.2; 4.9; 3.7, H-3eq); 2.62 (1H, ddd, J = 13.4; 8.2; 4.9, H-2eq); 2.98 (1H, ddd, J = 13.4; 8.2; 4.9, H-2ax); 4.89 (1H, br. dd, J = 7.3; 3.7, H-4ax); 6.90 (1H, dd, J = 8.2; 1.2; H-7) 7.00 (1H, ddd, J = 7.6; 1.2; 0.6, H-5); 7.47 (1H, dd, J = 8.2; 7.6, H-6); 12.40 (1H, br. s, HO-8).

Mass spectrum, m/z (%): 179 (M^+ + 1.10), 178 (M^+, 100), 177(6), 161(5), 160(19), 150(31), 149(12), 136(11), 133(8), 132(21), 122(41), 121(96), 105(4), 104(11), 103(9), 94(13), 93(17), 92(7), 91(7), 78(7), 77(17), 75(6), 66(9), 65(26), 64(7), 63(12).

R_f 0.57 (system 2).

trans-3,4,8-Trihydroxy-1(2H)-naphthalenone (II). Colorless needles from acetone with mp 189-192°C (according to the literature [2], 191-192°C from acetone) and $[\alpha]_\lambda^{20}$ (nm: −31.7° (589; Na D-line), −33.3° (578), −40.0° (546), −91.0°(435), −129° (406) (c 0.96; MeOH) (lit. $[\alpha]_D$ −36.0° (MeOH) [2]). UV spectrum, λ_{max}^{MeOH}: 212, 214 sh., 258, 333 nm (lg ε: 4.05; 4.05; 3.96; 3.65); $\lambda_{max}^{0.1N.KOH/MeOH}$: 226, 231, 258, 365 nm (lg ε: 4.03; 4.01; 3.79; 3.60). IR-spectrum, ν_{max} (paraffin oil), cm^{-1}: 3430 s, 3330 s, 3250 sh. s, 1630 s, 1615 sh. s, 1570 w, 1445 s, 1370, 1330 s, 1290, 1240 s, 1210, 1160, 1120, 1090 w, 1075 s, 1040, 1020, 985 w, 960, 860, 820, 800 s, 785 s, 745, 720, 650 w, 630 br. w, 470 br. w.

PMR spectrum (250 MHz, acetone-d_6, ppm): 2.11 (2H, br. s, HO-3,4); 2.73 (1H, dd, J = 17.1; 8.5 Hz, H-2ax); 3.05 (1H, dd, J = 17.1; 4.2 Hz, H-2eq); 4.11 (1H, ddd, J = 8.5; 4.2; 7.2 Hz, H-3): 4.67 (1H, br. dt, J = 7.2; 1.5 Hz, H-4); 6.83 (1H, ddd, J = 8.6; 1.0; 0.7 Hz, H-7); 7.16 (1H, ddd, J = 7.6; 1.0; 2.2 Hz, H-5); 7.54 (1H, dd, J = 8.6; 7.6 Hz, H-6); 12.33 (1H, s, HO-8).

Mass spectrum, m/z (%): 194 (M^+, 92), 176(20), 151(6), 150(77), 149(6), 148(6), 147(20), 123(11), 122(65), 121(100), 120(6), 104(4), 94(6), 93(13), 91(5), 77(3), 66(3), 65(11).

R_f 0.53 (system 2).

6-Hydroxymellein (III). Colorless plates from aqueous methanol with mp 209-211°C (according to the literature [2], 204-205°C) and $[\alpha]_\lambda^{20}$ (nm): +50.9° (589), +53.5° (578), +62.9° (546), +118° (435), +142° (406), +195° (365) (c 0.37; MeOH). UV spectrum, λ_{max}^{MeOH}: 214, 268, 301 nm (lg ε: 4.31; 4.11; 3.74); $\lambda_{max}^{0.1KOH/MeOH}$ 218, 245, 309 nm (lg ε: 4.00; 3.98, 4.37). IR spectrum, ν_{max} (paraffin oil), cm^{-1}: 3175, 3100 sh, 1620 s, 1580 w, 1495 w, 1470, 1410 w, 1375 s, 1320, 1285, 1250 s, 1220, 1190, 1160 s, 1115, 1060, 1020 w, 990 w, 945 w, 850, 790, 725 s, 705 s, 610, 585, 520.

PMR spectrum (250 MHz, acetone-d_6, ppm): 1.45 (1H, d, J = 6.4 Hz, Me-3); 2.83 (1H, dddd, J = 16.5; 10.7; 1.2; 0.7 Hz, H-4a); 2.96 (1H, dddd, J = 16.5; 3.8; 0.7; 0.4 Hz, H-4b); 4.70 (1H, ddd, J = 10.7; 3.8; 6.4 Hz, H-3); 6.27 (1H, ddd, J = 2.3; 0.7; 0.4 Hz, H-7); 6.30 (1H, ddd, J = 2.3; 1.2; 0.7 Hz, H-5); 9.35 (1H, br. s, HO-6); 11.28 (1H, s, HO-8).

Mass spectrum, m/z (%): 194(M^+, 94), 179(9), 176(24), 165(14), 151(24), 150(100), 148(12), 147(6), 122(11), 121(7), 120(5), 91(11), 81(6), 79(6), 77(8), 69(31), 66(9), 65(12), 63(6), 55(7), 53(10), 51(13).

R_f 0.50 (system 2).

Harman(IV). Colorless needles from hexane with mp 225-227°C (according to the literature [3], 230-232°C from acetone). UV spectrum, λ_{max}^{MeOH}: 212, 235, 247 sh, 281 sh, 287, 335, 348 nm (lg ε: 4.38; 4.62; 4.45; 4.11; 4.22; 3.81; 3.82); $\lambda_{max}^{MeOH+HCl}$ 204, 248, 301, 367 nm (lg ε: 4.37; 4.53; 4.28; 3.76).

PMR spectrum (250 MHz, acetone-d_6, ppm): 2.77 (3H, d, J = 0.7 Hz, Me-1); 7.23 (1H, ddd, J = 7.8; 6.8; 1.2 Hz, H-6); 7.51 (1H, ddd, J = 8.3; 6.8; 1.2 Hz, H-7); 7.60 (1H, ddd, J = 8.3; 1.2; 0.7 Hz, H-8); 7.89 (1H, br. s, J = 5.4 Hz, H-4); 8.18 (1H, dddd, J = 7.8; 1.2; 0.7; 0.7 Hz, H-5); 8.25 (1H, br. d, J = 5.4 Hz, H-3); 10.63 (1H, br, NH).

Mass spectrum: m/z (%): 183 (M^+ + 1.14), 182 (M^+, 100), 181(21), 168(3), 167(2), 155(6), 154(16). 149(5), 140(4), 128(2), 127(4).

R_f 0.44 (system 1); 0.11 (system 4).

O-Demethyldiaporthin (V). Colorless needles from acetone with mp 173-174°C and $[\alpha]_\lambda^{20}$ (nm): +64.4° (589), +68.0° (578), +78.3° (546), +163° (435), +197° (406), +424° (365) (c 0.82; MeOH). UV spectrum, λ_{max}^{MeOH}: 243, 255 sh, 277, 286 sh, 327 nm (lg ε: 4.64; 4.09; 3.83; 3.66; 3.83); $\lambda_{max}^{0.1N.KOH/MeOH}$ 246 sh, 253, 307, 328 sh, nm (lg ε: 4.55; 4.62; 4.10; 3.89). IR spectrum, ν_{max} (paraffin oil), cm^{-1}: 3400, 3050, 1670 s, 1625 s, 1575, 1480, 1390, 1350, 1295, 1275 sh, 1260, 1225, 1170 s, 1145, 1110 w, 1090 w, 1060, 1040, 1000 w, 970 w, 930 w, 835 s, 790, 750.

PMR spectrum (acetone-d_6): in the text.

^{13}C NMR spectrum (20 MHz, acetone-d_6, ppm): 23.63(C3'), 43.90(C1'), 65.68(C2'), 99.99 (C5 or C7), 102.34(C7 or C5), 103.38(C4a or C8a), 106.40(C8a or C4a), 140.95(C4), 156.38(C3), 164.61(C8 or C6), 166.32(C6 or C8), 167.12(C1).

Mass spectrum, m/z (%): 236 (M^+, 23), 221 (M^+–Me, 2), 193(12), 192 (M^+–MeCHO, 100), 177(4), 164(11), 163(8), 150(4), 146(13), 121.5, 118(3).

R_f 0.49 (system 2).

Diaporthin (VI). A solution of 20 mg of O-demethyldiaporthin in 1 ml of MeCN was treated with an excess of CH_2N_2 in ether. After being left for an hour, the reaction mixture was evaporated to dryness and the residue was chromatographed on LH-20 in the C_6H_6–$CHCl_3$–MeOH (6:4:1) system. The fractions containing diaporthin were combined and recrystallized from a mixture of hexane and acetone, which gave 18 mg of diaporthin in the form of colorless plates with mp 88-90°C (according to the literature [4], 91.5-92.5°C, sublimed, or 90-92°C, from benzene–ether), and $[\alpha]_\lambda^{20}$ (nm): ±56.5° (589), +61.2° (578), +71.5° (546), +153° (435), +210° (406), +417° (365) (c 1.15; MeOH) (lit.: $[\alpha]_D$ +54° (c 0.87; $CHCl_3$) [4].

IR spectrum, ν_{max} (paraffin oil, cm^{-1}): 3370, 3320, 1670 s, 1615 s, 1590 w, 1565, 1520, 1450, 1440, 1410, 1370, 1355, 1345, 1325, 1290, 1235 s, 1190 s, 1160 s, 1110, 1090, 1070, 1050, 1025 w, 975, 935, 885 w, 860, 835, 810 w, 795 w, 720 s, 615 w, 575 w.

PMR spectrum (80 MHz, $CDCl_3$ ppm): 1.30 (3H, d, J = 6.2 Hz); 2.22 (1H, br. s); 2.60 (2H, br. d, J = 6.4 Hz); 6.26 (1H, br. s); 6.29 (1H, d, J = 2.3 Hz); 6.42 (1H, d, J = 2.3 Hz; 10.97 (1H, s).

Mass spectrum, m/z (%): 250 (M^+, 26), 235 (M^+–Me, 2), 206 (M^+–MeCHO, 100), 191 (M^+–MeCHO–Me, 3), 188(2), 178(4), 177(6), 164(3), 163(6), 160(11), 149(3), 135(4).

R_f 0.47 (system 2).

LITERATURE CITED

1. S. I. Sviridov and B. S. Ermolinskii, Khim. Prir. Soedin., No. 6, 811 (1990).
2. S. Iwasaki, S. Nozoe, Z. Sato, and T. Kozaka, Tetrahedron Lett., 3977 (1969); S. Iwasaki, H. Muro, S. Mozoe, S. Okuda, and Z. Sato, Tetrahedron Lett., 13 (1972).
3. K. Yomosa, A. Hirota, H. Sakai, and A. Hogai, Agric. Biol. Chem., 51, 921 (1987).
4. A. Boller, E. Gauman, E. Hardegger, F. Kugler, S. Naef-Roth, and M. Rosher, Helv. Chim. Acta, 40, 875 (1957); E. Hardegger, W. Rieder, A. Walser, and F. Kugler, Helv. Chim. Acta, 40, 1283 (1966).
5. M. Nikina, T. Sassa, and S. Marumo, Tennen Yuki Kagobutsu, Toronkai Koen Yoshishu, 21, 144 (1978) (Chem. Abstr., 90, 99737).
6. Y. F. Hallock, J. Clardy, D. S. Kenfield, and G. Strobel, Phytochemistry, 27, 3123 (1988).
7. T. Morita and H. Aoki, Agric. Biol. Chem., 38, 1501 (1974).