Communications to the Editor

[Chem. Pharm. Bull. 31(10)3781—3784(1983)]

ISOLATION AND STRUCTURE OF MACOMMELINS, NOVEL METABOLITES OF MACROPHOMA COMMELINAE

Sakae Shimizu, Ikuo Sakurai and Yuzuru Yamamoto*
Fuculty of Pharmaceutical Sciences, Kanazawa University,
Takara-machi 13-1, Kanazawa 920, Japan

The novel metabolites, named macommelin, macommelin-9-ol, macommelin-8-ol and macommelin-8,9-diol, were isolated from the culture broth of Macrophoma commelinae IFO 9570. The structures were determined to be 5-ethyl-, 5-(2'-hydroxyethyl)-, 5-(1'S-hydroxyethyl)- and 5-(1'S,2'-dihydroxyethyl)-4-methoxy-6-methyl-2H-pyran-2-one, respectively. This fungus also produced rosellisin, an antibiotic α-pyrone.

KEYWORDS——Macrophoma commelinae IFO 9570; Coelomycetes; fungi; metabolic product; macommelin; macommelin-9-ol; macommelin-8-ol; macommelin-8,9-diol; rosellisin; α-pyrone

Coelomycetes, Fungi Imperfecti, include many phytotoxic fungi. One of them, Macrophoma causes a fruit rot disease of apple[1a] and some other plants.[1b] In this paper, we deal with the isolation and structure of the metabolites of Macrophoma commelinae IFO 9570.

The fungus was cultivated stationarily on a malt extract medium at 27°C for three weeks. The culture filtrate was concentrated *in vacuo* and extracted with AcOEt The AcOEt extract was chromatographed on a silica gel column using $CHCl_3$-MeOH and three new metabolites (1, 2 and 3) and rosellisin[2] (5) were isolated. The aqueous layer was adsorbed on a charcaol column and eluted with acetone. From this fraction, metabolite 4 was isolated. Of metabolite 1, a main product, 1.6 g was obtained from 6 l of the culture broth, and the yields of the minor metabolites 2, 3, 4 and 5 were about 20 mg, 10 mg, 200 mg and 50 mg, respectively.

Metabolite 5[3] was identified as rosellisin (islandic acid-II methyl ester) by comparing it with an authentic sample.

Metabolite 1, mp 115-117°C,[4] was obtained as colorless needles by recrystallization from C_6H_6. Its molecular formula, $C_9H_{12}O_4$, was determined by elemental and MS analyses.[5] The UV absorption maximum appeared at 285 nm (logε; 3.82) and IR absorption appeared at 3462 (-OH), 1700 (>C=O), 1637 (>C=C<) and 1558 (>C=C<) cm^{-1}. In the ^{1}H-NMR spectrum, protons of methyl (δ2.23, s), hydroxy (δ2.32, s, exchanged with D_2O), methoxy (δ3.75, s), olefinic (δ5.33, s) and two methylenes coupling to each other (δ2.56 and 3.64, each t, J=6.0 Hz) were observed. In 1-acetate, mp 88-89°C, one of methylene protons shifted to a lower field (δ4.06 from 3.64) which suggested the presence of a hydroxyethyl group as a side chain in 1. The presence of a methylketone or a ketone group was excluded by iodoform or 2,4-dinitrophenylhydrazine (2,4-DNP) tests. The UV and IR spectra suggest that 1 may have a 4-methoxy-α-pyrone ring.[6]

By alkaline treatment of 1, the obtained 1,3-diketone derivative (6, colorless oil) was converted to a pyrazole derivative (7, mp 118-121°C) with semicarbazide. 7 was synthesized as shown in Chart and identified by the spectral data and mixed mp. From the above results, 1 was determined to be 5-(2'-hydroxyethyl)-4-methoxy-6-methyl-α-pyrone.

Metabolite 2,[7] colorless needles, mp 127-130°C (from CCl_4), had the same molecular formula $C_9H_{12}O_4$ as 1, and the UV and IR spectra were also similar. In the ^{1}H-NMR spectrum, methyl (δ1.46) and methine (δ4.82) protons coupling to each other were observed instead of two methylene signals as in 1. The chemical shifts of other signals were almost like those in 1. 2 was positive to the iodoform test. These data suggest that 2 is an isomer of 1, and has a 1'-hydroxyethyl group instead of the 2'-hydroxyethyl group of 1. This was confirmed by synthesis as follows.

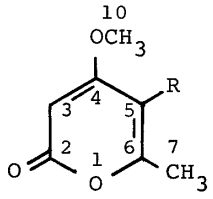
11, was synthesized by the condensation of acetylacetone (8) and malonyl chloride (10), according to the literature,[8] was methylated with CH_2N_2. 5-Acetyl-4-methoxy-6-methyl-α-pyrone (12, mp 102-104°C) was produced together with its γ-pyrone derivative (mp 120-121°C) in a ratio of 3 : 1. These were separated by silica gel column chromatography. 12 was hydrogenated with $NaBH_4$ in EtOH to yield the racemic 2, mp 133-135°C, and its IR spectrum in $CHCl_3$ and the ^{1}H-NMR spectrum were identical to that of the optically active 2.

Metabolite 3,[9] colorless prisms, mp 85-87°C (from cyclohexane), had the molecular formula $C_9H_{12}O_3$ indicating one oxgen atom less than 1. The IR spectrum indicated the absence of an -OH group and in the ^{1}H-NMR spectrum an ethyl group appeared. The structure of 3 was confirmed by chemical conversion from 1 as follows: 5-(2'-chloroethyl)-4-methoxy-6-methyl-α-pyrone (13, mp 107-109°C), synthesized by the treatment of 1 with $SOCl_2$, was reductively dechloronated with $NaBH_4$ in DMSO. The resulting compound was identical to 3, so 3 was determined to be 5-ethyl-4-methoxy-6-methyl-α-pyrone.

Metabolite 4,[10] colorless plates, mp 185-186.5°C (from $CHCl_3$-MeOH), had the molecular formula $C_9H_{12}O_5$ which has one more oxygen atom than 1. The UV and IR spectra were similar to 1. 4 showed ABX type protons (δ4.03 and 4.10, AB part; δ5.36, X part) and two hydroxy protons (δ4.9 and 6.4, each br) together with protons of methyl, methoxy and olefinic as in 1 and 2. These data suggest that 4 has a 1,2-dihydroxyethyl group at the C-5 position of the 4-methoxy-6-methyl-α-pyrone ring. The 1,2-diol moiety was confirmed by oxidation with $NaIO_4$. Formaldehyde (14, 2,4-DNP derivative, 15, mp 165-167°C) and 5-formyl-4-methoxy-6-methyl-α-pyrone (16, mp 130-133°C) were obtained. 16 showed an aldehyde proton at δ10.16 in the ^{1}H-NMR spectrum and evolved into the semicarbazone (17, mp 230-233°C (dec.)). 16 was intramolecularly rearranged to 18 (mp 109-110°C) by treatment with 90 % H_2SO_4 or $AgNO_3$. From these results, 4 was determined to be 5-(1',2'-dihydroxyethyl)-4-methoxy-6-methyl-α-pyrone.

The stereochemistry at C-8 in metabolites 2 and 4 was examined. The methylketone derivative (12) was stereoselectively hydrogenated by a chiral borane complex.[11] By comparing the yielded (1'R)- and (1'S)-alcohols (2) [(1'R)-, $[\alpha]_D$ +8.41°; (1'S)-, $[\alpha]_D$ -5.71°: chemical and optical yields of about 25 %] with the natural product ($[\alpha]_D$ -32.6°), 2 was determined to be the (1'S)-hydroxy derivative. This was also confirmed by the result of the shift reagent-induced CD method.[12a] When copper (II) hexafluoroacetylacetonate (10^{-4}M) was added to ca. a 10^{-3}M solution (CCl_4) of 2,

a CD extreme appeared at 333 nm and the sign was negative ($\Delta\varepsilon$ -0.15). Similary, when nickel(II) acetylacetonate (4×10^{-5}M) was added to ca. a 10^{-4}M solution (1M t-BuOH in CCl_4) of 4, a negative Cotton effect ($\Delta\varepsilon$ -0.68) at 309 nm and a positive Cotton effect ($\Delta\varepsilon$ +0.21) at 284 nm were observed in the differential CD curve.[12b] And when praseodimium(III) dipivaloylmethanate (4.5×10^{-5}M) was added to ca. a 5.0×10^{-5}M



1: -CH_2-CH_2OH ;macommelin-9-ol

2: -CH(OH)-CH_3 ;macommelin-8-ol

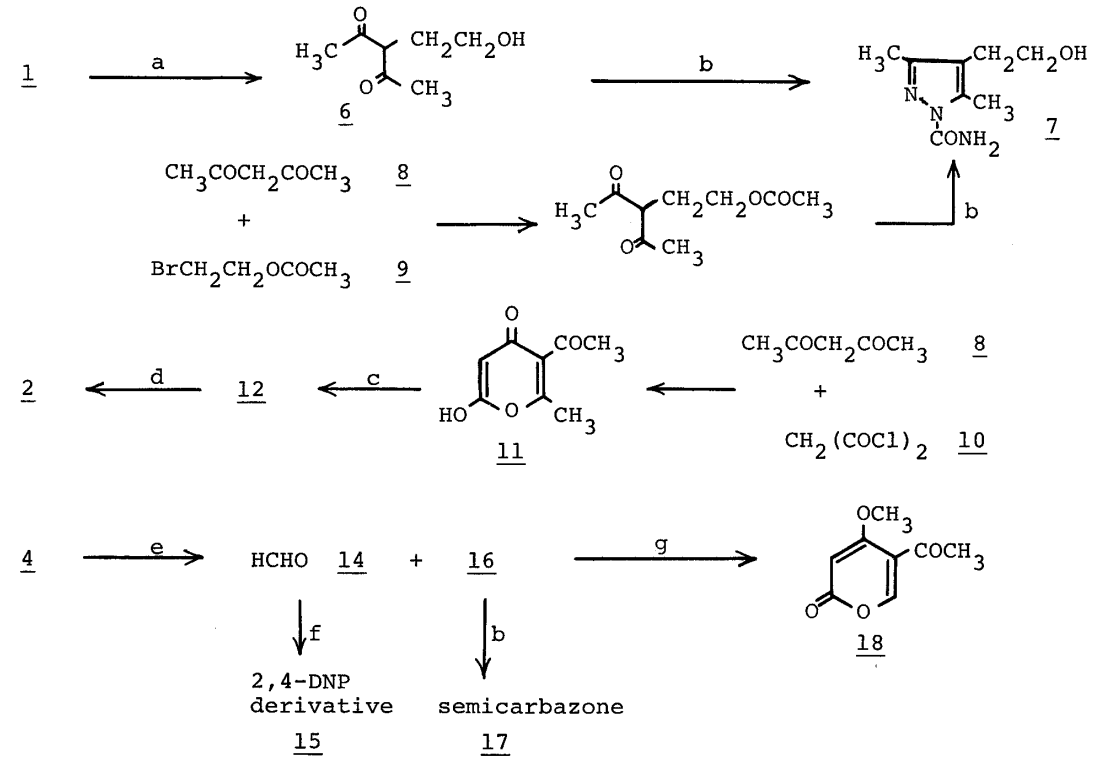
3: -CH_2-CH_3 ;macommelin

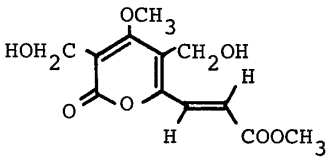
4: -CH(OH)-CH_2OH ;macommelin-8,9-diol

12: -CO-CH_3

13: -CH_2-CH_2-Cl

16: -CHO





5: rosellisin

a, KOH/MeOH; b, $H_2NCONHNH_2 \cdot HCl$; c, CH_2N_2; d, $NaBH_4$; e, $NaIO_4$; f, 2,4-DNP; g, 90 % H_2SO_4 or $AgNO_3$/NaOH

Chart

solution (CCl_4) of 4, CD extremes $\Delta\varepsilon$ -0.14 at 305 nm and $\Delta\varepsilon$ +0.61 at 288 nm were observed in the induced curve.[12b] From these results, a positive chirality of the α-glycol moiety in 4 was postulated and the absolute configuration at C-8 was determined to be (1'S).

Metabolite 3, the basal structure among the metabolites, is named macommelin and the other metabolites 1, 2 and 4 are named macommelin-9-ol, macommelin-8-ol and macommelin-8,9-diol, respectively.

These metabolites of Macrophoma commelinae IFO 9570 may be closely related to each other in the biosynthesis. The studies of biosynthesis and bioactivity of the macommelin group are in progress.

ACKNOWLEDGEMENT We are grateful to the Insititute for Fermentation, Osaka, for supplying of the IFO strain, to Dr. T. Tatsuno for supplying islandic acid-II methyl ester (rosellisin). We are also grateful to Dr. H. Suda for guidance of the stereoselective hydrogenation. We thank Mr. Y. Itatani, Miss Y. Teranishi and Miss T. Ueda for the elemental analyses and spectral measurements.

REFERENCES AND NOTES

1) a) T. Sassa, Agric. Biol. Chem., 47, 1417 (1983); b) I. J. Kapoor and R. N. Tandon, Phytopathol. Z. 70, 137 (1971) [Chem. Abstr., 75, 59977a (1971)].
2) M. S. R. Nair, Phytochemistry, 15, 1090 (1976): Rosellisin is identical to the methyl ester of islandic acid-II reported by Y. Fujimoto, H. Tsunoda, J. Uzawa and T. Tatsuno, 23rd Symposium papers of the Chemistry of Natural Products, 1980, p. 640.
3) All compounds were characterrized by elemental analyses, UV, IR, MS, ^{1}H- and ^{13}C-NMR spectroscopies.
4) All melting points were determined on a micro melting point apparatus (Yanagimoto) and were uncorrected. The mixed melting point analysis was carried out with a capillary tube.
5) 1: Anal. Calcd for $C_9H_{12}O_4$: C, 58.69; H, 6.57. Found: C, 58.59; H, 6.58. MS m/z: 184 (M^+).
6) Y. Koyama, Y. Fukakusa, N. Kyomura and S. Yamagishi, Tetrahedron Lett., 1969, 355; K. Yamada, Bull Chem. Soc. Jpn., 35, 1323 (1962).
7) 2: Anal. Calcd for $C_9H_{12}O_4$: C, 58.69; H, 6.57. Found: C, 58.66; H, 6.64. MS m/z : 184 (M^+). $UV\lambda_{max}^{EtOH}$nm (logε): 284 (3.82). $[\alpha]_D^{21}$ -32.6° (c=1, EtOH). $IR\nu_{max}^{KBr}cm^{-1}$: 3440, 1704, 1633, 1556. ^{1}H-NMR(in $CDCl_3$) δ: 1.46(3H, d, J=6.4 Hz, -CH-CH_3), 2.28(3H, s, -CH_3), 2.61(1H, s, exchanged with D_2O), 3.81(3H, s, -OCH_3), 4.82(1H, q, J=6.4 Hz, -O-CH-CH_3), 5.39(1H, s).
8) M. Aslam Butt and J. A. Elvidge, J. Chem. Soc., 1963, 4483.
9) 3: Anal. Calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 63.95; H, 7.18. MS m/z : 168 (M^+). $UV\lambda_{max}^{EtOH}$nm (logε): 223 (sh), 286 (3.82). $IR\nu_{max}^{KBr}cm^{-1}$: 1705, 1643, 1564. ^{1}H-NMR(in $CDCl_3$) δ: 1.02(3H, t, J=7.2 Hz, -CH_2-CH_3), 2.18(3H, s, -CH_3), 2.32(2H, q, J=7.2 Hz, -CH_2-CH_3), 3.76(3H, s, -OCH_3), 5.34(1H, s).
10) 4: Anal. Calcd for $C_9H_{12}O_5$: C, 53.99; H, 6.04. Found: C, 53.70; H. 6.01. MS m/z : 200 (M^+). $UV\lambda_{max}^{EtOH}$nm (logε): 284 (3.81). $[\alpha]_D^{21}$ -7.6° (c=0.5, EtOH). $IR\nu_{max}^{KBr}cm^{-1}$: 3350, 1689, 1633, 1558. ^{1}H-NMR(in C_5D_5N) δ: 2.48(3H, s, -CH_3), 3.52(3H, s, -OCH_3), 4.03(1H, dd, J=11.2, 5.4 Hz), 4.10(1H, dd, J=11.2, 6.6 Hz), 4.9(1H, br, exchanged with D_2O), 5.36(1H, dd, J=6.6, 5.4 Hz), 5.56(1H, s), 6.4(1H, br, exchanged with D_2O).
11) H. Suda, S. Kanoh, N. Umeda, T. Nakajo and M. Motoi, Tetrahedron Lett., 1983, 1513.
12) a) J. Dillon and K. Nakanishi, J. Am. Chem. Soc., 96, 4055 (1974); b) idem, ibid., 96, 4057 and 4059 (1974).

(Received September 5, 1983)