

## Communications to the Editor

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ISOLATION AND STRUCTURE OF MACOMMELINS,  
NOVEL METABOLITES OF MACROPHOMA COMMELINAE

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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'-di-hydroxyethyl)-4-methoxy-6-methyl-2H-pyran-2-one, respectively. This fungus also produced rosellisin, an antibiotic  $\alpha$ -pyrone.

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

Coelomycetes, Fungi Imperfecti, include many phytotoxic fungi. One of them, Macrophoma causes a fruit rot disease of apple<sup>1a)</sup> and some other plants.<sup>1b)</sup> 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  $\text{CHCl}_3$ -MeOH and three new metabolites (1, 2 and 3) and rosellisin<sup>2)</sup> (5) were isolated. The aqueous layer was adsorbed on a charcoal 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<sup>3)</sup> was identified as rosellisin (islandic acid-II methyl ester) by comparing it with an authentic sample.

Metabolite 1, mp 115–117°C,<sup>4)</sup> was obtained as colorless needles by recrystallization from  $\text{C}_6\text{H}_6$ . Its molecular formula,  $\text{C}_9\text{H}_{12}\text{O}_4$ , was determined by elemental and MS analyses.<sup>5)</sup> The UV absorption maximum appeared at 285 nm ( $\log \epsilon$ ; 3.82) and IR absorption appeared at 3462 ( $-\text{OH}$ ), 1700 ( $>\text{C}=\text{O}$ ), 1637 ( $>\text{C}=\text{C}<$ ) and 1558 ( $>\text{C}=\text{C}<$ )  $\text{cm}^{-1}$ . In the  $^1\text{H}$ -NMR spectrum, protons of methyl ( $\delta$ 2.23, s), hydroxy ( $\delta$ 2.32, s, exchanged with  $\text{D}_2\text{O}$ ), methoxy ( $\delta$ 3.75, s), olefinic ( $\delta$ 5.33, s) and two methylenes coupling to each other ( $\delta$ 2.56 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 ( $\delta$ 4.06 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- $\alpha$ -pyrone ring.<sup>6)</sup>

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- $\alpha$ -pyrone.

Metabolite 2,<sup>7)</sup> colorless needles, mp 127-130°C (from CCl<sub>4</sub>), had the same molecular formula C<sub>9</sub>H<sub>12</sub>O<sub>4</sub> as 1, and the UV and IR spectra were also similar. In the <sup>1</sup>H-NMR spectrum, methyl ( $\delta$ 1.46) and methine ( $\delta$ 4.82) 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,<sup>8)</sup> was methylated with CH<sub>2</sub>N<sub>2</sub>. 5-Acetyl-4-methoxy-6-methyl- $\alpha$ -pyrone (12, mp 102-104°C) was produced together with its  $\gamma$ -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<sub>4</sub> in EtOH to yield the racemic 2, mp 133-135°C, and its IR spectrum in CHCl<sub>3</sub> and the <sup>1</sup>H-NMR spectrum were identical to that of the optically active 2.

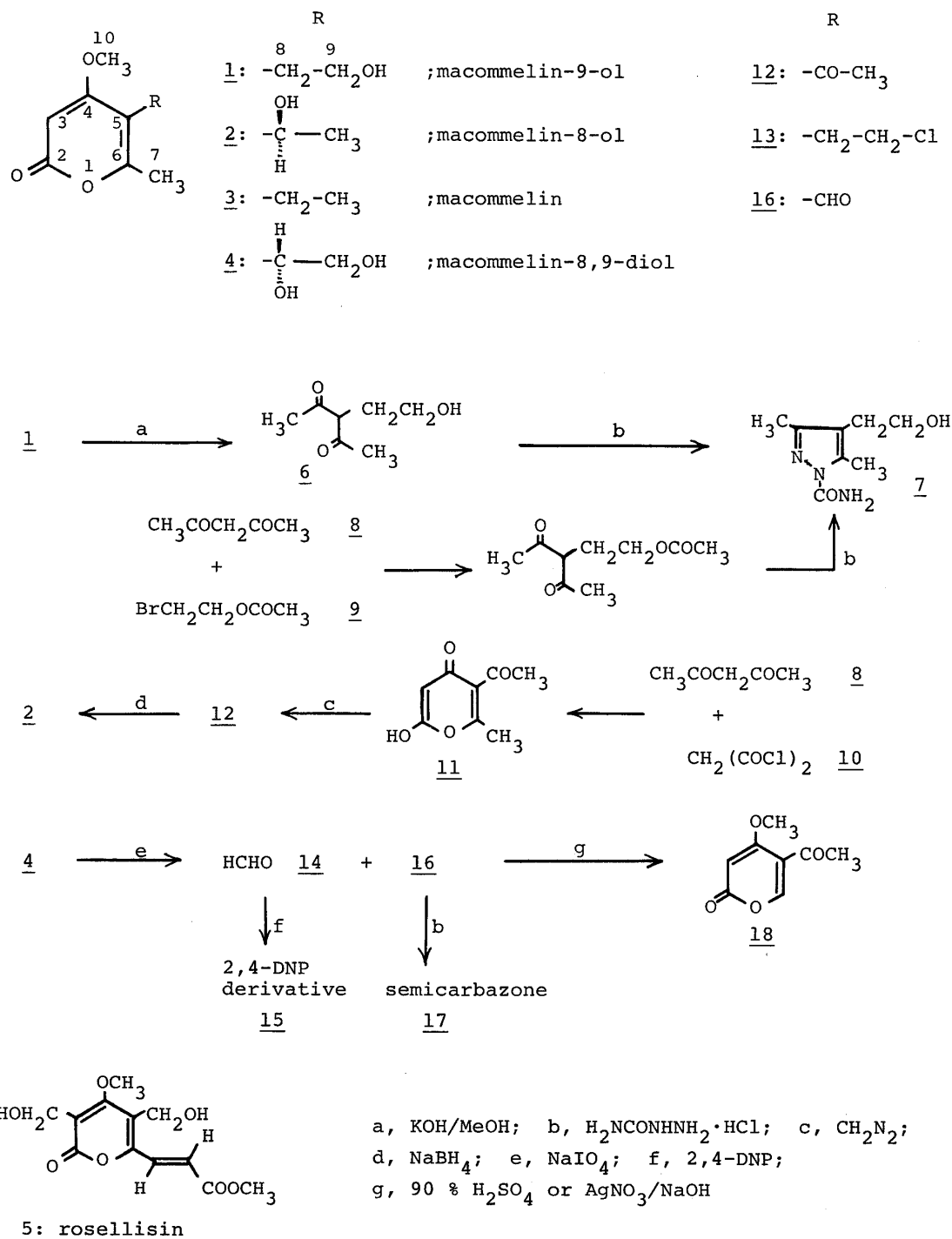
Metabolite 3,<sup>9)</sup> colorless prisms, mp 85-87°C (from cyclohexane), had the molecular formula C<sub>9</sub>H<sub>12</sub>O<sub>3</sub> indicating one oxygen atom less than 1. The IR spectrum indicated the absence of an -OH group and in the <sup>1</sup>H-NMR spectrum an ethyl group appeared. The structure of 3 was confirmed by chemical conversion from 1 as follows: 5-(2'-chloroethyl)-4-methoxy-6-methyl- $\alpha$ -pyrone (13, mp 107-109°C), synthesized by the treatment of 1 with SOCl<sub>2</sub>, was reductively dechlorinated with NaBH<sub>4</sub> in DMSO. The resulting compound was identical to 3, so 3 was determined to be 5-ethyl-4-methoxy-6-methyl- $\alpha$ -pyrone.

Metabolite 4,<sup>10)</sup> colorless plates, mp 185-186.5°C (from CHCl<sub>3</sub>-MeOH), had the molecular formula C<sub>9</sub>H<sub>12</sub>O<sub>5</sub> which has one more oxygen atom than 1. The UV and IR spectra were similar to 1. 4 showed ABX type protons ( $\delta$ 4.03 and 4.10, AB part;  $\delta$ 5.36, X part) and two hydroxy protons ( $\delta$ 4.9 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- $\alpha$ -pyrone ring. The 1,2-diol moiety was confirmed by oxidation with NaIO<sub>4</sub>. Formaldehyde (14, 2,4-DNP derivative, 15, mp 165-167°C) and 5-formyl-4-methoxy-6-methyl- $\alpha$ -pyrone (16, mp 130-133°C) were obtained. 16 showed an aldehyde proton at  $\delta$ 10.16 in the <sup>1</sup>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<sub>2</sub>SO<sub>4</sub> or AgNO<sub>3</sub>. From these results, 4 was determined to be 5-(1',2'-dihydroxyethyl)-4-methoxy-6-methyl- $\alpha$ -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.

<sup>11)</sup> By comparing the yielded (1'R)- and (1'S)-alcohols (2) [(1'R)-,  $[\alpha]_D +8.41^\circ$ ; (1'S)-,  $[\alpha]_D -5.71^\circ$ ; chemical and optical yields of about 25 %] with the natural product ( $[\alpha]_D -32.6^\circ$ ), 2 was determined to be the (1'S)-hydroxy derivative. This was also confirmed by the result of the shift reagent-induced CD method.<sup>12a)</sup> When copper (II) hexafluoroacetylacetonate (10<sup>-4</sup>M) was added to ca. a 10<sup>-3</sup>M solution (CCl<sub>4</sub>) of 2,

a CD extreme appeared at 333 nm and the sign was negative ( $\Delta\epsilon -0.15$ ). Similarly, when nickel(II) acetylacetonate ( $4 \times 10^{-5} \text{ M}$ ) was added to ca. a  $10^{-4} \text{ M}$  solution (1M t-BuOH in  $\text{CCl}_4$ ) of 4, a negative Cotton effect ( $\Delta\epsilon -0.68$ ) at 309 nm and a positive Cotton effect ( $\Delta\epsilon +0.21$ ) at 284 nm were observed in the differential CD curve.<sup>12b)</sup> And when praseodimium(III) dipivaloylmethanate ( $4.5 \times 10^{-5} \text{ M}$ ) was added to ca. a  $5.0 \times 10^{-5} \text{ M}$



Chart

solution ( $\text{CCl}_4$ ) of 4, CD extremes  $\Delta\epsilon$  -0.14 at 305 nm and  $\Delta\epsilon$  +0.61 at 288 nm were observed in the induced curve.<sup>12b)</sup> From these results, a positive chirality of the  $\alpha$ -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.

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- 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 characterized by elemental analyses, UV, IR, MS,  $^1\text{H}$ - and  $^{13}\text{C}$ -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  $\text{C}_9\text{H}_{12}\text{O}_4$ : C, 58.69; H, 6.57. Found: C, 58.59; H, 6.58. MS  $m/z$ : 184 ( $\text{M}^+$ ).
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- 7) 2: Anal. Calcd for  $\text{C}_9\text{H}_{12}\text{O}_4$ : C, 58.69; H, 6.57. Found: C, 58.66; H, 6.64. MS  $m/z$ : 184 ( $\text{M}^+$ ).  $\text{UV}\lambda_{\text{max}}^{\text{EtOH}}$  nm (log $\epsilon$ ): 284 (3.82).  $[\alpha]_{\text{D}}^{21}$  -32.6° (c=1, EtOH).  $\text{IR}\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3440, 1704, 1633, 1556.  $^1\text{H}$ -NMR (in  $\text{CDCl}_3$ )  $\delta$ : 1.46(3H, d,  $J=6.4$  Hz,  $-\text{CH}-\text{CH}_3$ ), 2.28(3H, s,  $-\text{CH}_3$ ), 2.61(1H, s, exchanged with  $\text{D}_2\text{O}$ ), 3.81(3H, s,  $-\text{OCH}_3$ ), 4.82(1H, q,  $J=6.4$  Hz,  $-\text{O}-\text{CH}-\text{CH}_3$ ), 5.39(1H, s).
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- 9) 3: Anal. Calcd for  $\text{C}_9\text{H}_{12}\text{O}_3$ : C, 64.27; H, 7.19. Found: C, 63.95; H, 7.18. MS  $m/z$ : 168 ( $\text{M}^+$ ).  $\text{UV}\lambda_{\text{max}}^{\text{EtOH}}$  nm (log $\epsilon$ ): 223 (sh), 286 (3.82).  $\text{IR}\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 1705, 1643, 1564.  $^1\text{H}$ -NMR (in  $\text{CDCl}_3$ )  $\delta$ : 1.02(3H, t,  $J=7.2$  Hz,  $-\text{CH}_2-\text{CH}_3$ ), 2.18(3H, s,  $-\text{CH}_3$ ), 2.32(2H, q,  $J=7.2$  Hz,  $-\text{CH}_2-\text{CH}_3$ ), 3.76(3H, s,  $-\text{OCH}_3$ ), 5.34(1H, s).
- 10) 4: Anal. Calcd for  $\text{C}_9\text{H}_{12}\text{O}_5$ : C, 53.99; H, 6.04. Found: C, 53.70; H, 6.01. MS  $m/z$ : 200 ( $\text{M}^+$ ).  $\text{UV}\lambda_{\text{max}}^{\text{EtOH}}$  nm (log $\epsilon$ ): 284 (3.81).  $[\alpha]_{\text{D}}^{21}$  -7.6° (c=0.5, EtOH).  $\text{IR}\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3350, 1689, 1633, 1558.  $^1\text{H}$ -NMR (in  $\text{C}_5\text{D}_5\text{N}$ )  $\delta$ : 2.48(3H, s,  $-\text{CH}_3$ ), 3.52(3H, s,  $-\text{OCH}_3$ ), 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  $\text{D}_2\text{O}$ ), 5.36(1H, dd,  $J=6.6, 5.4$  Hz), 5.56(1H, s), 6.4(1H, br, exchanged with  $\text{D}_2\text{O}$ ).
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