CHLOROMONILICIN, A NEW ANTIFUNGAL METABOLITE PRODUCED BY *MONILINIA FRUCTICOLA*

Sir:

In the course of an attempt to isolate a growth self-inhibitor produced by benomyl-resistant strains of cherry rot fungus *Monilinia fructicola*, we have recently obtained an active fraction (Frn. 2) from the culture filtrate of the benomyl-resistant strains[1] and, later, from that of the benomyl-sensitive strains. We wish to report the chemical and physical properties and the structural elucidation of the active principle, which was named chloromonilicin (**1**), which contains a novel seven-membered lactone ring presumably formed by oxidative cleavage of a benzene nucleus in a xanthone system.

The Frn. 2 obtained from the ethyl acetate extract of the culture filtrate[1] was recrystallized from benzene - *n*-hexane to give chloromonilicin as yellow needles. Its physico-chemical properties are listed in Table 1. The molecular formula was determined to be $C_{16}H_{11}O_7Cl$ by high resolution mass spectrometry. Its IR spectrum and ^1H NMR spectrum, together with a positive ferric chloride test (violet in ethanol), indicated the presence of a chelated phenolic hydroxyl group. The NMR spectrum revealed also the presence of a pair of *meta*-coupling protons (δ 6.85 and 6.75 ppm, J=1.5 Hz) on a benzene nucleus and a methyl group (δ 2.45 ppm) allylically coupled to each of the *meta*-coupling protons (J=*ca.* 0.5 Hz). In addition, a long-range coupling was observed between the one-proton singlet at δ 6.44 ppm (half-band width 1.3 Hz) and a methoxyl signal at δ 3.82 ppm; the latter was assignable to that of methoxycarbonyl group because of the presence of a fragment ion at m/z 291 ($M^+ - COOCH_3$) in the mass spectrum.

By catalytic hydrogenation over palladium charcoal, chloromonilicin gave the hydrogenolysis

Fig. 1. Structures of chloromonilicin (**1**) and its reaction products.

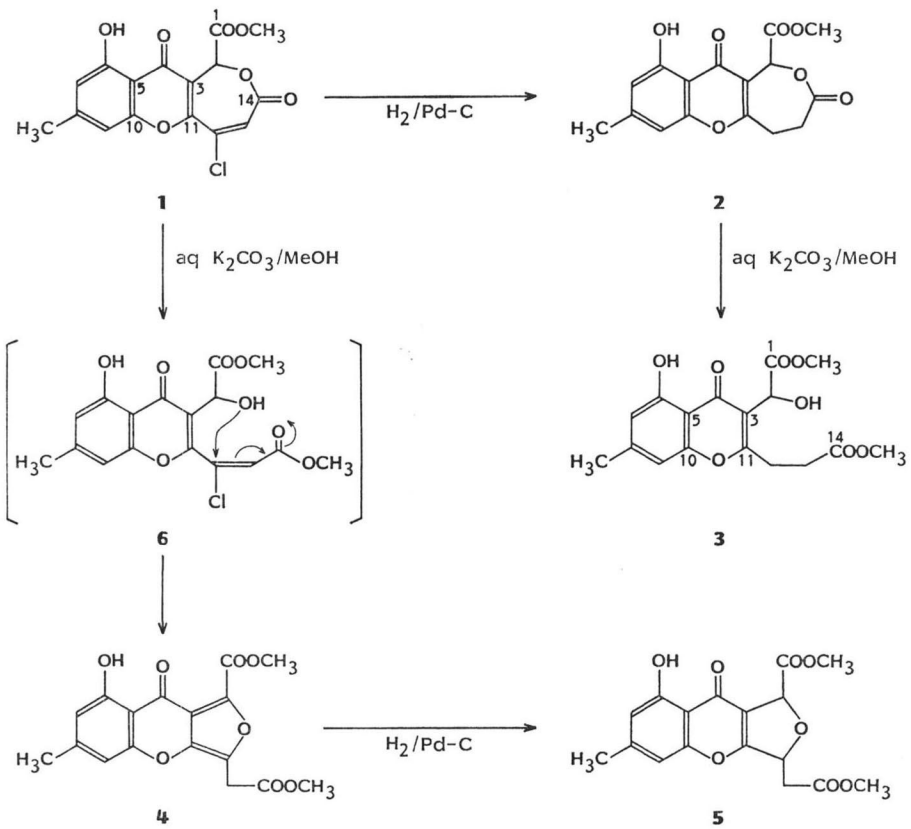

Table 1. Physico-chemical properties of chloromonilicin (**1**).

MP (°C)	169.5~170.5
$[\alpha]_D$ (*c* 0.4, $CHCl_3$)	+212°
High MS	350.01973
(Calcd for $C_{16}H_{11}O_7Cl$)	(350.01969)
UV λ_{max}^{MeOH} nm (ε)	276 (20,000)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	3040, 1755, 1733, 1655, 1620, 1602
^1H NMR δ^{CDCl_3} ppm	11.68 (1H, s, OH), 6.89 (1H, s), 6.85 (1H, m), 6.75 (1H, m), 6.44 (1H, br s), 3.82 (3H, s), 2.45 (3H, br s)

Table 2. ^{13}C NMR chemical shifts for chloromonilicin (**1**) and **3**.

Carbon No.	Chemical shifts, δ ($CDCl_3$) (ppm)	
	1	**3**
1	166.9 s	172.7 s
2	66.5 d	65.7 d
3	118.3 s	118.0 s
4	178.5 s	181.2 s
5	108.0 s	107.7 s
6	160.2 s	159.9 s
7	108.0 d	106.8 d
8	149.6 s	147.4 s
9	113.7 d	111.9 d
10	155.2 s	155.7 s
11	155.2 s	166.9 s
12	135.1 s	26.6 t
13	129.3 d	30.6 t
14	161.5 s	171.8 s
C-CH_3	22.5 q	22.0 q
O-CH_3	53.8 q	52.6 q
		51.8 q

product dechloro-dihydro derivative **2**, mp 184~186°C, whose ^1H NMR spectrum displayed a four-proton multiplet at δ 3.3~2.7 ppm instead of the one-proton singlet at δ 6.89 ppm seen in that of chloromonilicin. Methanolysis of **2** afforded a methyl ester **3**; MS *m/z* 350 (M^+); UV λ_{max}^{MeOH} nm (ε) 322 (6,000), 260 (sh, 24,000), 238 (32,000), 230 (sh, 28,000); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3510, 1735, 1655; ^1H NMR δ ($CDCl_3$) ppm 11.90 (1H, OH), 6.60 (1H, br s), 6.52 (1H, br s), 5.16 (1H, s), 4.10 (1H, OH), 3.74 (3H, s), 3.67 (3H, s), 3.2~2.6 (4H, m), 2.33 (3H, s). Its UV spectrum was quite similar to that of a 2,3-dialkyl-5-hydroxychromone[2], suggesting the presence of a 5-hydroxy-7-methylchromone moiety ($C_{10}H_8O_3$) in chloromonilicin. This was supported by the chemical shifts of ^{13}C NMR spectra of chloromonilicin and **3** (Table 2)[3].

The ^1H NMR spectrum of **3** showed characteristic signals of a hydroxyl at δ 4.10 ppm and a methoxyl group at δ 3.67 ppm. The above mentioned signal for a proton on the carbon bearing a methoxycarbonyl group was shifted upfield relative to chloromonilicin and **2**, *i.e.* from δ 6.44 and 6.40 (**2**) ppm to δ 5.16 ppm. Furthermore, a typical A_2B_2 spectrum (symmetrical ten-line multiplet) was observed at δ 3.2~2.6 ppm. These results revealed that chloromonilicin and **2** contained a seven-membered lactone ring with a methoxycarbonyl group as the third ring in their molecule; in good accord with the carbonyl absorptions of chloromonilicin ($\nu_{max}^{CHCl_3}$: 1755 (α-acyloxy ester) and 1733 (β-halo-α,β-unsaturated ester) cm^{-1}). The ^{13}C-{^1H} long-range selective proton decoupling on chloromonilicin and **3** using an improved method of SPT differential spectrum[4] clearly indicated the heteronuclear long-range coupling between the methine proton and the chromone-carbonyl carbon. Therefore, the total structure of chloromonilicin, excluding the position of chlorine atom, was elucidated as that shown in **1**, which justified the ^{13}C NMR spectrum of chloromonilicin (Table 2). A lower field resonance of the methine proton (C2-H) in **1** seems to be due to the anisotropic effect of the C4-carbonyl group.

The presence of chlorine atom on C12 was confirmed by the following experiments. On treatment with alkaline methanol at 0°C, **1** gave the dechlorinated methyl ester **4**, mp 196~197.5°C, whose ^1H NMR showed a two-proton singlet at δ 3.99 ppm instead of two one-proton singlets at δ 6.89 and 6.44 ppm in that of **1**. The structure of **4** was elucidated by spectral analyses of its hydrogenation product **5**, mp 161~162°C. A partial structure ■–$CH_a(H_b)CH_c(O–)C=C–CH_d(O–)$–■ in **5** was revealed by ^1H NMR (360 MHz) and ^{13}C NMR spectrometry; H_a δ 3.00, H_b 3.11, H_c 5.61, H_d 5.62 ppm; J_{ab}=13, J_{ac}=4,

Table 3. Antimicrobial spectrum of chloromonilicin (**1**).

Test organism	MIC (μg/ml)
Staphylococcus aureus 209P	50
Escherichia coli NIHJ	>100
Shigella flexneri 2a	>100
Pseudomonas aeruginosa 1001	>100
Candida albicans YU 1200	6.2
Trichophyton asteroides	1.5
Trichophyton interdigitale	3.1
Trichophyton rubrum IFO 5467	1.5

Agar dilution method on glucose nutrient agar.

J_{bc}=8, J_{cd}=1.5 Hz; –$\overset{1}{C}$H(O–)–: δ 79.5 (d) and 78.3 (d) ppm. An unstable intermediate in the formation of **4** was isolated from the reaction mixtrue of **1** with methanol-d_4 under reflux and was characterized as the methanolysis product **6**, on the basis of the mass (**6**-d_3: m/z 385 and 387 (M^+, chlorine-containing ions)) and UV (λ_{max}^{MeOH} 243, 265 (sh) and 325 nm) spectra. When **1** in methanol-d_4 was treated with potassium carbonate in deuterium oxide, it gave a mixture of deuterated **4** having molecular ions at m/z 351 (**4**-d_4) and 352 (**4**-d_5) in the mass spectrum. Its ^{1}H NMR spectrum in $CDCl_3$ - C_6H_6 (1 : 1) showed a signal (0.7 H by integration) of the singlet of the methylene protons (C13) at δ 3.62 ppm, indicating the attachment of chlorine atom to C12.

As shown in Table 3, chloromonilicin (**1**) showed marked antifungal activity against *Candida* and *Trichophyton* sp. It possessed also hyphal growth-inhibiting activity against *M. fructicola* at 5 μg/disc.

Preparation and identification of bromomonilicin and biosynthetically monilicin-related metabolites are now in progress in our laboratory.

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