

A NEW ANTIFUNGAL SUBSTANCE,
BROMOMONILICIN, AND ITS
PRECURSOR PRODUCED BY
MONILINIA FRUCTICOLA

Sir:

In a previous paper¹⁾ we reported the structural elucidation of a new antifungal metabolite, chloromonilicin (**1**), isolated as a growth self-inhibitor of phytopathogenic fungi *Monilinia fructicola*. It contained a novel seven-membered lactone ring presumably formed by oxidative cleavage of a benzene nucleus in a xanthone system. Subsequently we have isolated a new chlorine containing metabolite, chloropinselin (**2**), a probable precursor of **1**. Also, bromomonilicin (**3**) and bromopinselin (**4**) were prepared for activity and biosynthetic studies. Biosynthetic incorporation of **4** into **3** was accomplished using *M. fructicola* culture.

During the course of isolation of **1**, a new product $C_{16}H_{11}O_6Cl$ was isolated from the culture filtrate as yellow needles (**2**: mp 246~247.5°C; IR ν_{\max}^{KBr} cm^{-1} 3315, 1715, 1650, 1621, 1582, 1500). Its 1H NMR (δ , acetone- d_6) spectrum showed the presence of a *C*-methyl group (2.43 (br s) ppm), an *O*-methyl group (3.90 (s) ppm), a pair of aromatic *meta*-coupling protons (6.66 (m) and 6.91 (m) ppm, $J=ca.$ 2 Hz), an isolated aromatic proton (7.61 (1H, s) ppm) and two chelated phenolic hydroxyl groups (8~9 (br) and 12.0 (s) ppm). The UV spectrum of **2** (λ_{\max}^{MeOH} nm (ϵ) 237 (37,000), 263 (45,000), 294 (13,000), 324 (sh, 5,700), and 386 (8,700)) was superimposable on that of sydowinin B²⁾ (methyl 2,8-dihydroxy-6-hydroxymethylxanthone-1-carboxylate), suggesting the presence of a 2,8-dihydroxyxanthone-1-carboxylate structure in **2**. By catalytic hydrogenation over palladium on

charcoal in a 10% methanolic potassium hydroxide solution, **2** gave a dechlorinated product: mp 222.5~223.0°C; MS m/z 300 (M^+), whose 1H NMR spectrum (360 MHz, $CDCl_3$) showed characteristic signals of aromatic *ortho*-coupling protons at δ 7.36 and 7.48 (ABq, $J=9$ Hz) ppm. The product was identified with pinselin (**5**) by comparison of UV and IR spectra³⁻⁵⁾ and melting point⁶⁾. The position of chlorine atom in **2** was determined by the nuclear Overhauser effect (NOE) experiment on the dimethyl ether of **2**. Irradiation of the methoxyl signal at δ 3.88 ppm increased the intensity of the signal of an aromatic one-proton singlet at δ 7.36 ppm (increment: 21%). Thus, the structure of **2** was assigned to 4-chloropinselin. **2** showed no antifungal and antibacterial activities.

A new compound considered promising as an antifungal substance, bromomonilicin (**3**), was prepared by fermentation and was identified, as in the following. The spore suspension of *M. fructicola* was inoculated into 100 ml culture medium containing sucrose (commercial) 2%, malt extract 2% and sodium bromide 0.1% in 500-ml Sakaguchi flask and the flasks were incubated at 24°C for 12 days on a reciprocal shaker. Combined ethyl acetate extracts of the culture filtrate were chromatographed over Silica gel BW-820 with benzene - EtOAc to give fractions 2 and 4. Rechromatography of the fraction 2 over Silica gel CQ-3 and its purification by HPLC (SIL-CN, *n*-hexane - THF - AcOH (5:1:0.03)) and preparative TLC (Kieselgel, benzene - EtOAc (20:1)) afforded bromomonilicin as yellow needles: mp 183~183.5°C; $[\alpha]_D^{25} +170^\circ$ ($CHCl_3$); MS m/z 394 and 396 (1:1, M^+); IR ν_{\max}^{KBr} cm^{-1} 3430, 3052, 1739, 1725, 1650, 1612, 1586; UV λ_{\max}^{MeOH} nm (ϵ) 279 (39,000); 1H NMR ($CDCl_3$) δ 12.08 (1H, br s,

Fig. 1. Structures of halomonilicins (**1** and **3**), halopinselins (**2**, **4** and **6**), pinselin (**5**) and the keto-form of 4-bromopinselin (**7**).

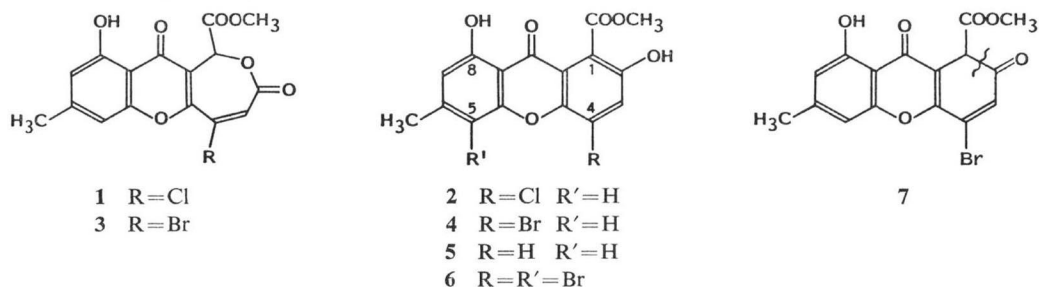


Table 1. Antimicrobial spectrum of bromomonilicin (3).

Test organism	MIC ($\mu\text{g/ml}$)
<i>Staphylococcus aureus</i> 209P	> 50
<i>Escherichia coli</i> NIHJ	> 50
<i>Shigella flexneri</i> IID642	> 50
<i>Pseudomonas aeruginosa</i> 1001	> 50
<i>Candida albicans</i> YU 1200	25
<i>Trichophyton asteroides</i>	6.2
<i>T. interdigitale</i>	6.2
<i>T. rubrum</i> IFO 5467	6.2

Agar dilution method on glucose nutrient agar.

OH), 7.13 (1H, s), 6.83 (1H, m), 6.73 (1H, m), 6.41 (1H, br s), 3.79 (3H, s), 2.42 (3H, br s). Its structure (3) was confirmed by alkaline methanolysis. The methanolysis product (mp 195.5~197.5°C) was identical in all respects (UV, IR and MS) with that derived from chloromonilicin¹⁾. Bromomonilicin (3) showed expected antifungal activity against *Candida* and *Trichophyton* sp. (Table 1), but the activity was about one-fourth of that of 1.

The fraction 4 was rechromatographed over Silica gel CQ-3 to give crude yellow crystals of 4-bromopinselin, which were crystallized from acetonitrile and were purified by HPLC (ODS, H₂O - acetonitrile (1:1)). 4-Bromopinselin showed: mp 223.5~224.0°C; MS m/z 378 and 380 (1:1, M⁺); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ) 238 (26,000), 263 (31,500), 292 (11,000), 324 (sh, 5,400), 385 (6,400); ¹H NMR (acetone-*d*₆) δ 12.3 (1H, s, OH), 7.79 (1H, s), 6.90 (1H, m), 6.68 (1H, m), 3.92 (3H, s), 2.43 (1H, br s). From these spectral data, its structure was assigned to 4-bromopinselin (4). The position of bromine atom was confirmed by the NOE experiment on the dimethyl ether of 4; a NOE was observed between a methoxyl signal at δ 3.85 ppm and an aromatic one-proton singlet at δ 7.52 ppm. In addition, a very minor metabolite 6: mp ca. 340°C, MS m/z 456, 458, 460 (1:2:1, M⁺), was isolated from the 4-bromopinselin fraction by preparative TLC (Silica gel GF₂₅₄, benzene - EtOAc (5:1), multiple developments (7 times)), and was identified to be 4,5-dibromopinselin on the basis of spectral data of its dimethyl ether: mp 243~245°C; MS m/z 484, 486, 488 (1:2:1, M⁺); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 1730, 1661; UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ) 243 (36,400), 254 (sh, 35,900), 293 (7,800), 319 (6,300), 373 (9,200); ¹H NMR (CDCl₃) δ 7.58 (3-H), 6.73 (7-H), 4.04 (COOCH₃), 3.97 (2-OCH₃), 3.89

(8-OCH₃), 2.57 (6-CH₃). (A NOE was observed between 2-OCH₃ and 3-H, and 8-OCH₃ and 7-H).

The incorporation of 4-bromopinselin (4), as biosynthetic precursor, into bromomonilicin (3) was performed as described below. *M. fructicola* was cultured in a 500-ml Sakaguchi flask containing 100 ml of a medium consisting of malt extract 1% and sucrose (commercial) 2%. 4 (1.5 mg in 0.06% sodium carbonate solution (0.2 ml)) was added to the fermentation broth (100 ml) 10 days after inoculation. After an additional 4 days the broth (pH 4.4) was filtered and extracted with EtOAc. The chloro- and bromomonilicin fraction prepared from the extract by preparative TLC was subjected to mass spectrometric analysis. The molecular ions m/z 394 and 396, and fragment ions m/z 307 and 309 (M⁺ - COOCH₃ - CO) formed from 3 were all clearly detected in the mass spectrum of chloromonilicin, suggesting that bromomonilicin is generated by selective oxidative cleavage of one of the benzene nuclei in 4-bromopinselin. Addition of 6 as a control experiment did not give substantially the molecular and fragment ions of 3 in the mass spectral analysis. HPLC analysis showed that 3 was incorporated to the extent of 4%. To the best of our knowledge, this is the first instance of Baeyer-Villiger type cleavage of phenolic metabolites⁷⁾, which are oxidized *via* the corresponding keto-form 7.

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