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A PHENOLIC AMIDE FROM ROOTS OF *CHENOPODIUM ALBUM*

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Key Word Index—*Chenopodium album*; Chenopodiaceae; pigweed; roots; *Aphanomyces cochlioides*; zoospore; attracting activity; *N-trans*-feruloyl-4-*O*-methyldopamine.

Abstract—A new phenolic amide has been isolated from the roots of *Chenopodium album*. Its structure was determined as *N-trans*-feruloyl-4-*O*-methyldopamine by spectroscopic evidence and chemical synthesis. It showed attracting activity toward the zoospores of *Aphanomyces cochlioides*, a pathogenic fungus against some plants of Chenopodiaceae.

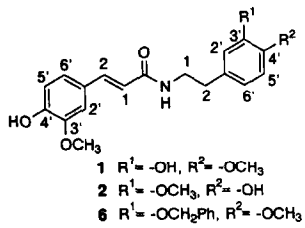
INTRODUCTION

Aphanomyces cochlioides (Saprolegniaceae), one of the plant pathogenic fungi, infects some species of Chenopodiaceae. It has been known that motile zoospores of *Aphanomyces* spp. show positive chemotaxis toward root exudates of their hosts [1–3]. In the course of our investigation of attractants of zoospores of *A. cochlioides*, we have isolated a new phenolic amide (**1**) from methanol extracts of pigweed (*Chenopodium album* L.).

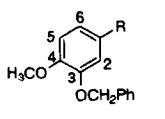
RESULTS AND DISCUSSION

The molecular formula of **1** was determined to be $C_{19}H_{21}NO_5$ by HR-mass spectrometry. In the ^{1}H NMR spectrum of **1**, signals of two methylene groups, two methoxyl groups, six aromatic protons, and one *trans*-olefin group were observed. Most of the signals were very similar to those of *N-trans*-feruloyl-3-*O*-methyldopamine (**2**) isolated from *Spinacia oleracea* [4], except for the signals of H-2′ (δ6.74, *d*, J=2.1 Hz) and H-5′ (δ6.85, *d*, J=8.1 Hz) in the phenethylamine unit. This indicates that **1** consists of feruloyl and 4-*O*-methyldopamine moieties. The structure was also supported by the evidence that a NOE interaction was observed between methoxyl protons (δ3.81, *s*) and H-5′ (δ6.85, *d*, J=8.1 Hz) in the phenethylamine unit. Thus, the structure of **1** was considered to be *N-trans*-feruloyl-4-*O*-methyldopamine.

This structure was further confirmed by the chemical synthesis as follows. 3-*O*-Benzylisovanillin (**3**) was transformed into 3-benzyloxy-4-methoxy-β-nitrostylene (**4**) by condensation with nitromethane. Reduction of **4** with $LiAlH_4$ provided 3-*O*-benzyl-4-*O*-methyldopamine (**5**). Acylation of **5** with ferulic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) afforded *N-trans*-feruloyl-3-*O*-benzyl-4-*O*-methyldopamine (**6**). Finally, **1** was acquired by deprotection of **6**. The physicochemical properties of synthesized **1** were in good agreement with those of the isolated amide. Thus, the structure of **1** was confirmed as *N-trans*-feruloyl-4-*O*-methyldopamine.



1 R¹= -OH, R²= -OCH₃
2 R¹= -OCH₃, R²= -OH
6 R¹= -OCH₂Ph, R²= -OCH₃



3 R= -CHO
4 R= $\sim\!\!\sim$NO₂
5 R= $\sim\!\!\sim$NH₂

The attracting activity of **1** toward the zoospores of *A. cochlioides* was tested. At the concentration of 1×10^{-8} M, zoospore aggregation was observed.

Compound **1** is the first naturally occurring amide which has 4-*O*-methyldopamine moiety, whereas 4-*O*-methyldopamine was only isolated from Cactaceae [5]. On the other hand, *N-trans*-feruloyl-3-*O*-methyldopamine (**2**) was previously isolated from Chenopodiaceae [4, 6] and Lauraceae [7].

EXPERIMENTAL

^{1}H NMR spectra were measured in Me_2CO-d_6 at 500 MHz using TMS as int. standard. UV spectra were measured in MeOH. Silica gel CC and reverse phase (RP) CC was performed on Wako-gel C-200 and cosmosil 75-C18-OPN, respectively. Bioassay method for the measurement of attracting activity conformed to the method of ref. [8].

Extraction and fractionation. The fractionation was monitored by measuring the activity in attracting the zoospores of *A. cochlioides*. Fresh roots of *Chenopodium album* (1.2 kg), collected on the Hokkaido University campus, were extracted with MeOH. The MeOH extracts were partitioned between Et_2O and H_2O. The Et_2O layer was washed with 2 M HCl and then with 5% aq. $NaHCO_3$. The resulting neutral phenolic fr. (4.0 g) was chromatographed by silica gel CC and eluted with a $CHCl_3$–MeOH gradient. Active frs (0.2 g), eluted with

2–5% MeOH in $CHCl_3$, were applied to Sephadex LH-20 CC (75 ml). Elution with MeOH yielded an active fr. (retention vol. V_R 0–85 ml). The active fr. (0.14 g) was rechromatographed on RP CC (7 g) eluting with THF–H_2O (2:3) to give an active fr. (V_R 0–23 ml, 8.9 mg). Further purification was achieved by HPLC [Inertsil ODS 5 μm (250 × 4.6 mm), MeOH–H_2O (3:2), flow rate: 5 ml min^{-1} and UV detector 320 nm] to yield **1** (0.1 mg) as an oil.

N-trans-*Feruloyl*-4-O-*methyldopamine* (**1**). Oil. EIMS *m/z* (rel. int.): 343 $[M]^+$ (3), 193 (16), 177 (48), 150 (100). EIHRMS *m/z*: 343.1450 ($[M]^+$, calcd for $C_{19}H_{21}NO_5$: 343.1420). IR ν_{max}^{film} cm^{-1}: 3300, 1650, 1580, 1510. UV λ_{max}^{MeOH} nm: 219, 231, 290, 319. ^1H NMR δ(Me_2CO-d_6): 2.73 (2H, *t*, *J* = 7.3 Hz, amine H-2), 3.50 (2H, *q*, *J* = 7.3 Hz, amine H-1), 3.81 (3H, *s*, amine-OMe), 3.88 (3H, *s*, feruloyl-OMe), 6.50 (1H, *d*, *J* = 15.6 Hz, feruloyl H-1), 6.66 (1H, *dd*, *J* = 2.1, 8.1 Hz, amine H-6'), 6.74 (1H, *d*, *J* = 2.1 Hz, amine H-2'), 6.83 (1H, *d*, *J* = 8.2 Hz, feruloyl H-5'), 6.85 (1H, *d*, *J* = 8.1 Hz, amine H-5'), 7.04 (1H, *dd*, *J* = 1.9, 8.2 Hz, feruloyl H-6'), 7.16 (1H, *d*, *J* = 1.9 Hz, feruloyl H-2'), 7.44 (1H, *d*, *J* = 15.6 Hz, feruloyl H-2).

Synthesis of 3-*benzyloxy*-4-*methoxy*-β-*nitrostylene* (**4**) [9]. $MeNH_2 \cdot HCl$ (0.2 g) and Na_2CO_3 (0.28 g) were added to MeOH (2.8 ml), and the mixture filtered to give the filtrate. A soln of 3-*O*-benzylisovanillin (**3**, 10 g) in MeOH (30 ml), nitromethane (2.7 g) and the above filtrate were added, and the reaction mixture allowed to stand for 8 days at room temp. The ppt. was filtered, and recrystallized from MeOH to give yellow needles **4** (5.8 g, 48%). MS *m/z* (rel. int.): 285 $[M]^+$ (2), 253 (0.9), 162 (3), 91 (100). IR ν_{max}^{KBr} cm^{-1}: 1620, 1480, 1330. UV λ_{max}^{MeOH} nm: 245, 258, 363. ^1H NMR δ(Me_2CO-d_6): 3.92 (3H, *s*, -OMe), 5.21 (2H, *s*, -C$\underline{H}_2$-Ph), 7.11 (1H, *d*, *J* = 8.4 Hz, H-5), 7.32–7.54 (5H, *m*, $-CH_2-C_6\underline{H}_5$), 7.53 (1H, *dd*, *J* = 2.0, 8.4 Hz, H-6), 7.59 (1H, *d*, *J* = 2.0 Hz, H-2), 7.92 (1H, *d*, *J* = 13.6 Hz, $-CH=C\underline{H}-NO_2$), 8.02 (1H, *d*, *J* = 13.6 Hz, $-C\underline{H}=CH-NO_2$).

Reduction of compound **4** to 3-O-*benzyl*-4-O-*methyldopamine* (**5**) [10]. To a refluxed mixture of $LiAlH_4$ (0.34 g) and THF (14 ml), a soln of **4** (0.5 g) in THF (5 ml) was added, and the reaction mixture refluxed for 10 hr. The reaction mixture was cooled to room temp., diluted with H_2O and filtered. The filtrate was extracted with Et_2O. The Et_2O layer was washed with aq. 2M NaOH and then with H_2O, dried over $MgSO_4$ and concd to give a yellow oil **5** (0.36 g, 78%). MS *m/z* (rel. int.): [257 $[M^+]$ (5), 228 (35), 167 (4), 137 (24), 91 (100). IR ν_{max}^{film} cm^{-1}: 3350, 1650, 1580, 1510, 1430. UV λ_{max}^{MeOH} nm: 230, 289, 319. ^1H NMR δ(Me_2CO-d_6): 2.77 (2H, *t*, *J* = 7.5 Hz, $C\underline{H}_2-CH_2-NH_2$), 3.37 (2H, *t*, *J* = 7.5 Hz, $CH_2-C\underline{H}_2-NH_2$), 3.78 (3H, *s*, -OMe), 5.08 (2H, *s*, -C$\underline{H}_2$-Ph), 6.78 (1H, *dd*, *J* = 2.0, 8.2 Hz, H-6), 6.87 (1H, *d*, *J* = 8.2 Hz, H-5), 6.96 (1H, *d*, *J* = 2.0 Hz, H-2), 7.29–7.50 (5H, *m*, $-CH_2-C_6\underline{H}_5$).

Synthesis of N-trans-*feruloyl*-3-O-*benzyl*-4-O-*methyldopamine* (**6**) [7]. To a mixture of **5** (2.5 g) and ferulic acid (2.1 g) in THF (100 ml), a soln of DCC (5.0 g) in THF (10 ml) was added, and the reaction mixture stirred for 13 hr at room temp. The reaction mixture was diluted with H_2O, and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried over $MgSO_4$ and evapd to dryness to give a brown oil. Purification by silica gel CC ($CHCl_3$–Et_2O, 4:1) afforded **6** as a yellow amorphous powder (2.75 g, 65%). MS *m/z* (rel. int.): 433 $[M]^+$ (4), 342 (0.6), 240 (55), 177 (38), 91 (100). IR ν_{max}^{KBr} cm^{-1}: 1620, 1480, 1330. UV λ_{max}^{MeOH} nm: 245, 258, 363. ^1H NMR δ(Me_2CO-d_6): 2.77 (2H, *t*, *J* = 7.0 Hz, amine H-2), 3.51 (2H, *q*, *J* = 7.0 Hz, amine H-1), 3.80 (3H, *s*, amine-OMe), 3.86 (3H, *s*, feruloyl-OMe), 5.09 (2H, *s*, $-C\underline{H}_2-Ph$), 6.50 (1H, *d*, *J* = 15.7 Hz, feruloyl H-1), 6.80 (1H, *dd*, *J* = 2.0, 8.1 Hz, amine H-6'), 6.83 (1H, *d*, *J* = 8.1 Hz, feruloyl H-5'), 6.90 (1H, *d*, *J* = 8.1 Hz, amine H-5'), 6.97 (1H, *d*, *J* = 2.0 Hz, amine H-2'), 7.05 (1H, *dd*, *J* = 1.9, 8.1 Hz, feruloyl H-6'), 7.16 (1H, *d*, *J* = 1.9 Hz, feruloyl H-2'), 7.29–7.48 (5H, *m*, $-CH_2-C_6\underline{H}_5$), 7.46 (1H, *d*, *J* = 15.7 Hz, feruloyl H-2).

Deprotection of compound **6** [11]. To a soln of **6** (0.22 g) in CH_2Cl_2 (1 ml), dimethyl sulphide (0.85 g) and BF_3–Et_2O (0.73 g) was added, and the reaction mixture stirred for 48 hr at room temp. The reaction mixture was diluted with H_2O, and extracted with EtOAc. The EtOAc layer was dried over $MgSO_4$ and evapd to give a brown oil. Purification by silica gel CC ($CHCl_3$–MeOH, 19:1) afforded **1** as an oil (27 mg, 16%).

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