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Chemical and Chemotaxonomical Studies of Filices. XLVII.[1] Chemical Studies on the Constituents of *Arachniodes nigrospinosa* (CHING) CHING, *A. festina* (HANCE) CHING and *A. mutica* OHWI

NOBUTOSHI TANAKA,[a] NAOMI YAMAZAKI,[a] KAZUYUKI HORI,[a] TAKAO MURAKAMI,*,[a] YASUHISA SAIKI[b] and CHIU-MING CHEN[c]

Faculty of Pharmaceutical Sciences, Science University of Tokyo,[a] Funakawara-machi, Shinjuku-ku, Tokyo 162, Japan, Department of Pharmaceutical Sciences, Kobe Gakuin University,[b] Arise, Igawatani-machi, Tarumi-ku, Kobe 673, Japan and Department of Chemistry, National Tsing Hua University,[c] Kuang Fu Road, Hsinchu, Taiwan, China

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Two new compounds, II and III, were isolated, together with 4-hydroxynicotinamide, from the fronds of both *Arachniodes nigrospinosa* and *A. festina*. The structures of II and III were elucidated as (*E*)-1-(2,3,4,6-tetramethoxyphenyl)but-2-en-1-one and (*E*)-1-(2,3,4,6-tetramethoxyphenyl)pent-2-en-1-one, respectively. Ryomenin (I) was isolated from the fronds of *A. mutica*.

Keywords——*Arachniodes nigrospinosa*; *Arachniodes festina*; *Arachniodes mutica*; Aspidiaceae; (*E*)-1-(2,3,4,6-tetramethoxyphenyl)but-2-en-1-one; (*E*)-1-(2,3,4,6-tetramethoxyphenyl)-pent-2-en-1-one; ryomenin; 4-hydroxynicotinamide; phloroglucinol; chemotaxonomy

In the previous paper,[2] we reported the structural elucidation of six new compounds isolated from *Arachniodes standishii* OHWI (Aspidiaceae). As a continuation of our studies on ferns of this genus, the constituents of *A. nigrospinosa* (CHING) CHING (Japanese name: Inu-ryomenshida), *A. festina* (HANCE) CHING (Japanese name: Taiwan-ryomenshida) and *A. mutica* OHWI (Japanese name: Shinobukaguma) were investigated.

From *A. mutica*, a novel sesquiterpene, ryomenin (I), was isolated. From both *A. nigrospinosa* and *A. festina*, two new compounds, II and III, were isolated, together with 4-hydroxynicotinamide (IV). The structures of II and III were elucidated as described below.

Compound II, $C_{14}H_{18}O_5$, colorless needles, mp 61—62 °C, showed ultraviolet (UV) absorption maxima at 225 ($\log\varepsilon$ 4.20) and 284 nm ($\log\varepsilon$ 3.14) in a methanol solution. The infrared (IR) spectrum of II indicated the presence of a benzene ring ($\nu_{max}^{CHCl_3}$ cm^{-1}: 1603, 1497), a conjugated carbonyl group (1660 cm^{-1}) and a *trans*-disubstituted double bond (970 cm^{-1}). In the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum (in $CDCl_3$), compound II showed an aromatic proton signal at δ 6.29 (1H, s) and four aromatic methoxyl signals at δ 3.73 (3H, s), 3.79 (3H, s), 3.81 (3H, s) and 3.88 (3H, s), indicating that the benzene ring is substituted unsymmetrically by four methoxy groups. Compound II also showed a methyl signal at δ 1.90 (3H, dd, J=7 and 1 Hz) and two olefinic proton signals at δ 6.28 (1H, dd, J=15 and 1 Hz) and 6.61 (1H, dq, J=15 and 7 Hz), indicating the presence of a *trans*-1-propenyl group.

From these spectral data and the molecular formula, $C_{14}H_{18}O_5$, the structure of compound II was determined as (*E*)-1-tetramethoxyphenylbut-2-en-1-one. The possible substitution patterns of methoxy groups on the benzene ring were 2,3,4,5- or 2,3,4,6-. It has been reported that the chalcone having the former substitution pattern (V) shows an aromatic

proton signal at δ 7.03[3] and that having the latter pattern (VI) shows a signal at δ 6.40.[4] In the case of compound II, the aromatic proton signal appeared at δ 6.29, suggesting that the substitution pattern is the latter. This was confirmed by alkaline hydrolysis of compound II to yield 2,3,4,6-tetramethoxyacetophenone.[5]

On the basis of the above evidence, the structure of compound II was established as (*E*)-1-(2,3,4,6-tetramethoxyphenyl)but-2-en-1-one.

Compound III, $C_{15}H_{20}O_5$, colorless oil, showed UV [λ_{max}^{MeOH} nm (log ε): 225 (4.19), 284 (3.14)] and IR [$\nu_{max}^{CHCl_3}$ cm^{-1}: 1650, 1605, 1495, 975] spectra similar to those of compound II. The ^{1}H-NMR spectrum showed signals due to a *trans*-1-butenyl group at δ 1.05 (3H, t, J= 7 Hz), 2.25 (2H, d of quintets, J=7 and 1 Hz), 6.24 (1H, dt, J=15 and 1 Hz) and 6.63 (1H, dt, J=15 and 7 Hz) together with signals due to a 2,3,4,6-tetramethoxyphenyl group at δ 3.73 (3H, s), 3.78 (3H, s), 3.81 (3H, s), 3.87 (3H, s) and 6.31 (1H, s). Thus, the structure of compound III was determined as (*E*)-1-(2,3,4,6-tetramethoxyphenyl)pent-2-en-1-one.

The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra (see Table I) of compounds II and III were consistent with the proposed structures.

A. nigrospinosa and *A. festina* are both morphologically uniform except for their scales. Their methanol extracts also gave the same thin layer chromatogram on silica gel. Further, their constituents, II and III, are analogous to compounds isolated from *A. standishii, e.g.*, compound VII.[2] *A. standishii* also contains ryomenin (I), which was isolated from *A. mutica* as mentioned above. These findings support the close relationship of these four ferns. Further studies on the constituents of other ferns of this genus are in progress.

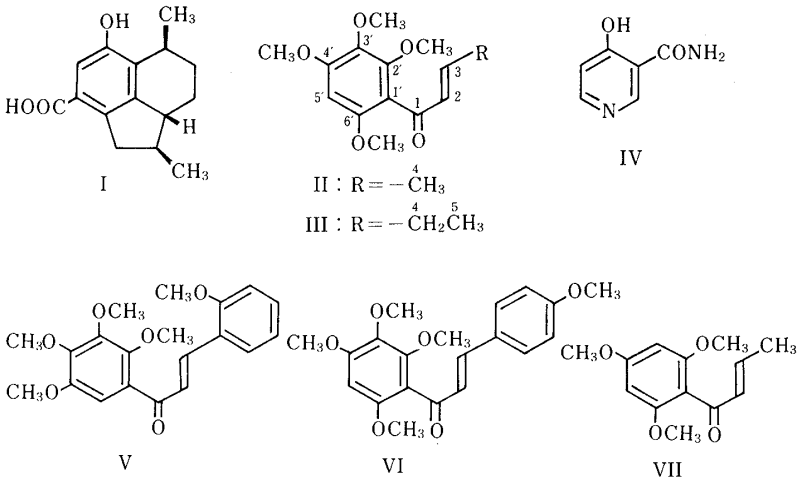

Fig. 1

TABLE I. ^{13}C-NMR Data for Compounds II and III (in $CDCl_3$)

Carbon	II	III	Carbon	II	III
C-1	193.9	194.0	C-1′	116.4	116.5
C-2	134.2	131.8	C-2′	152.8[b]	152.9[b]
C-3	145.7	151.5	C-3′	136.0	136.1
C-4	18.2	25.5	C-4′	154.6[b]	154.8[b]
C-5	—	12.2	C-5′	92.6	92.8
2′-OMe	61.6[a]	61.6[a]	C-6′	151.3[b]	151.5[b]
3′-OMe	60.9[a]	60.9[a]			
4′-OMe	56.1	56.1			
6′-OMe	56.1	56.1			

Assignments with the same superscript for each compound may be interchanged.

Experimental

The instruments, materials and experimental conditions were the same as described in Part XXXVII[6] of this series.

Isolation Procedure

1) ***Arachniodes nigrospinosa*** **(CHING) CHING**——The air-dried fronds (490 g) of *A. nigrospinosa*, collected at Tashulinshan, Ping Tung county, Taiwan, China, in December, were extracted 3 times with 3 l of methanol under reflux for 6 h. The extracts and then a further 10 l of methanol were passed over activated charcoal (60 g) packed in a column of 6 cm diameter. The resulting solution was concentrated to a syrup under reduced pressure. The syrup was mixed with silica gel (80 g) and applied to a silica gel column (70 g, 7 cm diameter). The column was eluted successively with $CHCl_3$ (800 ml), 10% MeOH in $CHCl_3$ (600 ml, frac. 1) and 20% MeOH in $CHCl_3$ (600 ml, frac. 2). Frac. 1 was rechromatographed on alumina using $CHCl_3$ as an eluent, followed by preparative layer chromatography (PLC) (solvent system, *n*-hexane : ethyl acetate = 2 : 1) to yield compound II (80 mg) and compound III (95 mg). Frac. 2 was concentrated to a syrup and distributed between the upper and lower layers of a mixture of $CHCl_3$ (20 ml), MeOH (20 ml) and water (15 ml). The upper layer was evaporated under reduced pressure. The residue was rechromatographed on silica gel using 10% MeOH in $CHCl_3$ as an eluent followed by PLC (solvent system, ethyl acetate : MeOH = 10 : 3) to yield 4-hydroxynicotinamide (IV, 12 mg).

2) ***Arachniodes festina*** **(HANCE) CHING**——The air-dried fronds (740 g) of *A. festina*, collected at Sileng, Tao Yuan county, Taiwan, China, were extracted 3 times with 4 l of methanol under reflux for 6 h. The extracts and then 10 l of methanol were passed over activated charcoal (60 g) packed in a column of 7 cm diameter. The resulting solution was worked up in the same manner as described for *A. nigrospinosa* to yield compounds II (10 mg), III (45 mg) and IV (40 mg).

3) ***Arachniodes mutica*** **OHWI**——The air-dried fronds (1100 g) of *A. mutica*, collected in August at Mt. Nyugasa, Nagano prefecture, were extracted 3 times with 4 l of methanol under reflux for 6 h. The extracts and then 15 l of methanol were passed over activated charcoal (110 g) packed in a column of 7 cm diameter. The resulting solution was concentrated under reduced pressure. The resulting syrup was mixed with silica gel (80 g) and applied to a silica gel column (150 g, 7 cm diameter). The column was eluted successively with $CHCl_3$ (600 ml), 10% MeOH in $CHCl_3$ (400 ml), 20% MeOH in $CHCl_3$ (400 ml) and 30% MeOH in $CHCl_3$ (400 ml, frac. 1). Frac. 1 was distributed between the upper and lower layers of a mixture of $CHCl_3$ (20 ml), MeOH (20 ml) and water (15 ml). The lower layer was evaporated under reduced pressure and rechromatographed on silica gel using $CHCl_3$ and ether as eluents, followed by PLC (solvent system, $CHCl_3$: acetone = 4 : 1) to yield ryomenin (I, 15 mg).

Ryomenin (I)——Colorless needles from a mixture of benzene and *n*-hexane, mp 206—209 °C, $[\alpha]_D^{20}$ −119° (c = 0.3, MeOH). UV λ_{max}^{MeOH} nm (log ε): 211 (4.51), 252 (3.98), 310 (3.58). IR ν_{max}^{KBr} cm^{-1}: 3500, 2925, 2865, 1675, 1610, 1427, 1195, 1040, 725. ^{1}H-NMR (100 MHz, in Pyr.-d_5) δ: 1.22 (3H, d, J = 7 Hz), 1.68 (3H, d, J = 7 Hz), 7.98 (1H, s), 10.73 (1H, br s). MS m/z: 246, 231, 213, 208. This compound was identical with an authentic sample on direct comparison (TLC, IR, ^{1}H-NMR, MS and mixed fusion).

Compound II [=(*E*)-1-(2,3,4,6-Tetramethoxyphenyl)-but-2-en-1-one]——Colorless needles from methanol, mp 61—62 °C. UV λ_{max}^{MeOH} nm (log ε): 225 (4.20), 284 (3.14). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3010, 2950, 2845, 1660, 1603, 1497, 1470, 1407, 1290, 1120, 1025, 970. ^{1}H-NMR (100 MHz, in $CDCl_3$) δ: 1.90 (3H, dd, J = 7 and 1 Hz), 3.73 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.88 (3H, s), 6.28 (1H, dq, J = 15 and 1 Hz), 6.29 (1H, s), 6.61 (1H, dq, J = 15 and 7 Hz). MS m/z: 266, 251, 225, 167. Calcd for $C_{14}H_{18}O_5$: 266.115 (M), Found: 266.115 (M^+).

Compound III [=(*E*)-1-(2,3,4,6-Tetramethoxyphenyl)-pent-2-en-1-one]——Colorless oil. UV λ_{max}^{MeOH} nm (log ε): 225 (4.19), 284 (3.14). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3010, 2950, 2850, 1650, 1605, 1495, 1470, 1405, 1280, 1120, 975. ^{1}H-NMR (100 MHz, in $CDCl_3$) δ: 1.05 (3H, t, J = 7 Hz), 2.25 (2H, dq, J = 7 and 1 Hz), 3.73 (3H, s), 3.78 (3H, s), 3.81 (3H, s), 3.87 (3H, s), 6.24 (1H, dt, J = 15 and 1 Hz), 6.31 (1H, s), 6.63 (1H, dt, J = 15 and 7 Hz). MS m/z: 280, 265, 251, 225, 167. Calcd for $C_{15}H_{20}O_5$: 280.131 (M), Found: 280.129 (M^+).

Compound IV (=4-Hydroxynicotinamide)——Colorless needles from MeOH, mp 263—265 °C. UV λ_{max}^{MeOH} (log ε): 251 (3.94), 282 (2.60). IR ν_{max}^{KBr} cm^{-1}: 3280, 2930, 1680, 1640, 1535, 1350, 1240, 1165, 1045, 880, 775. ^{1}H-NMR (100 MHz, in CD_3OD) δ: 6.53 (1H, d, J = 7 Hz), 7.72 (1H, dd, J = 7 and 1.5 Hz), 8.50 (1H, d, J = 1.5 Hz). MS m/z: 138, 122, 121. Calcd. for $C_6H_6N_2O_2$: 138.044 (M), Found: 138.043 (M^+). The properties and spectral data of this compound were in good agreement with those reported.[7]

Alkaline Degradation of Compound II——A solution of compound II (20 mg) in 15% KOH–70% EtOH (10 ml) was heated under reflux for 7 h. The reaction mixture was poured into ice-cold water and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to PLC (solvent system, $CHCl_3$: ether = 5 : 1) to yield 4 mg of 2,3,4,6-tetramethoxyacetophenone. This product was identical with an authentic sample derived from 2-hydroxy-3,4,6-trimethoxyacetophenone on direct comparison (GLC, IR, ^{1}H-NMR and MS).

2,3,4,6-Tetramethoxyacetophenone from 2-Hydroxy-3,4,6-trimethoxyacetophenone——A mixture of 2-hydroxy-3,4,6-trimethoxyacetophenone (4 mg), CH_3I (7 ml), anhydrous K_2CO_3 (1 g) and anhydrous acetone (7 ml) was heated under reflux for 10 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The extract

was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was subjected to PLC (solvent system, $CHCl_3$: ether = 5 : 1) to yield 3 mg of 2,3,4,6-tetramethoxyacetophenone. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700, 1605, 1500, 1475, 1410, 1275, 1115, 1030. ^{1}H-NMR (60 MHz, in $CDCl_3$) δ 2.46 (3H, s), 3.80 (6H, s), 3.87 (6H, s), 6.25 (1H, s). MS *m/z*: 240, 225, 210, 195, 167, 165.

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