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ISOLATION AND STRUCTURE IDENTIFICATION OF TWO NEW DERIVATIVES OF THE MYCOTOXIN FUSAROCHROMENONE PRODUCED BY *FUSARIUM EQUISETI*

WEIPING XIE, CHESTER J. MIROCHA,

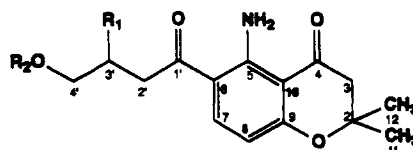
Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108

and YECHUN WEN

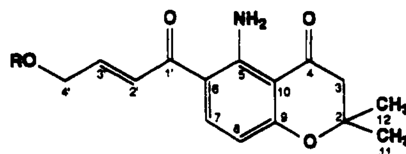
Shanghai Research Centre of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, People's Republic of China

ABSTRACT.—Two new chromone derivatives, 2,2-dimethyl-5-amino-6-(4′-hydroxylbutyryl)-4-chromone [**2**] and 2,2-dimethyl-5-amino-6-(2′*E*-ene-4′-hydroxylbutyryl)-4-chromone [**4**], were isolated from rice cultures of *Fusarium equiseti*. Their structures were deduced from chemical and spectral data.

Fusarochromanone [**1**] is a mycotoxin produced by an isolate of *Fusarium equiseti* (Corda) Sacc. Alaska 2-2 (originally identified as *F. graminearum*) (1–5). This toxin causes tibial dyschondroplasia, a common bone disease in chicks, and reduced hatchability of fertile chicken eggs under experimental conditions (2). Fusarochromanone [**1**] has also been detected in chicken feed samples associated with tibial dyschondroplasia (1). It is a 4-chromone derivative with some unusual substituents. Several fusarochromanone derivatives have been found in cultures of *F. equiseti* (3, 6–8). Two minor metabolites, **2** and **4**, were also isolated as yellow powders from a rice culture of the fungal isolate. Compound **2** is light yellow and exhibited a blue fluorescence under uv light. Compound **4** is bright yellow but did not fluoresce under uv.



1 R_1=NH_2, R_2=H
2 R_1=R_2=H
3 R_1=H, R_2=Ac



4 R=H
5 R=Ac

Eims of **2** resulted in the observation of a $[M]^+$ at *m/z* 277 and $[M-H_2O]^+$ ion at *m/z* 259 indicating the mol wt to be 277 daltons with the presence of a hydroxy group. This conclusion was supported by fabms which exhibited a $[M+H]^+$ ion at *m/z* 278 and an $[M+H-H_2O]^+$ ion at *m/z* 260; and by pcims which yielded a $[M+H]^+$ ion at *m/z* 278, a $[M+C_2H_5]^+$ ion at *m/z* 306 and a $[M+H-H_2O]^+$ ion at *m/z* 260; as well as ncims which yielded a $[M]^-$ ion at *m/z* 277. Hreims of **2** gave the $[M]^+$ at *m/z* 277.1309 (calculated as 277.1314 for $C_{15}H_{19}NO_4$, with seven double bond equivalents). Acetylation of **2** yielded a monoacetate [**3**] with a $[M]^+$ ion at *m/z* 319 by eims, also supporting the mol wt of **2** as 277 daltons and the presence of a hydroxy substituent. The very similar eims spectra of **2** and **1** (2) suggested that their structures were closely related. The fragment ion at *m/z* 218, which resulted from an α-cleavage between C-1′ and C-2′, were base peaks in the eims spectra of both **1** (2) and **2** suggesting that **2** had the same 4-chromone basic structure and similar substitutents to **1**. A fragment ion $[M-CH_2CHOH]^+$ at *m/z* 233.1017

(calculated as 233.1051 for $C_{13}H_{15}NO_3$) in the hreims of **2** was suggestive of a 4′-hydroxylbutyryl unit in the molecule. The ^{1}H-nmr spectrum of **2** showed signals for C-2[$(CH_3)_2$], C-3 (CH_2), C-7 (H), C-8 (H), and C-5 (NH_2), which agreed with the ^{1}H-nmr spectrum of **1** (2) and confirmed that they had the same chromone skeleton. Proton resonances at 1.95–2.18 ppm (2H, m), 2.99 ppm (2H, t, J=7.0 Hz) and 3.71 ppm (2H, t, J=6.0 Hz) confirmed the presence of a 6-(4′-hydroxylbutyryl) unit in **2**. The ^{1}H-nmr spectrum of **3** showed a singlet at 2.04 ppm (3H), confirming the acetylation and presence of one -OH group in **2**. A downfield shift of the singlet from 3.71 ppm (C-4′ protons) in the ^{1}H-nmr spectrum of **2** to 4.14 ppm (C-4′ protons) in the ^{1}H-nmr spectrum of **3** confirmed the OH group at C-4. Therefore, the structure of **2** was established as 2,2-dimethyl-5-amino-6-(4′-hydroxylbutyryl)-4-chromone.

The eims of **4** showed a $[M]^+$ ion at *m/z* 275 and a $[M-H_2O]^+$ ion at *m/z* 257, indicating the mol wt of **4** to be 275 daltons, again with the presence of an -OH group. Supporting evidence came from the fabms which exhibited a $[M+H]^+$ ion at *m/z* 276 and a $[M+H-H_2O]^+$ ion at *m/z* 258 as well as the ncims which showed a $[M]^-$ ion at *m/z* 275. Acetylation of **4** yielded a monoacetate [**5**], identified by eims, and which confirmed the presence of a hydroxy substituent in **4**. Most of the fragment ions, including that at *m/z* 218 in the eims spectrum of **4**, were also found for **2**, indicating that they were analogous except for a structural difference in the 6-butyryl group. The eims of **4** had a $[M]^+$ ion at *m/z* 275 as the base peak, suggesting a more stable structure compared with **2**. This increased stability was consistent with increased unsaturation in the butyryl group. The eims of **4** gave an intense fragment ion at *m/z* 188 (40) resulting from cleavage between C-6 and C-1′ and a weaker ion at *m/z* 218 (30), that may be postulated as being due to cleavage between C-1′ and C-2′. This fragmentation pattern differed from that of **2**, where *m/z* 218 was the base peak while *m/z* 188 was very weak. This difference in their ms fragmentation patterns agreed with the proposed structures of **2** and **4**. In the eims of **1** and **2**, cleavage between C-6 and C-1′ was reduced due to conjugation between the carbonyl at C-1′ and the ring, while cleavage between C-1′ and C-2′ was very much favored. In the eims of **4**, cleavage between C-1′ and C-2′ was reduced due to conjugation between the carbonyl at C-1′ and the C-2′, C-3′ double bond, while cleavage between C-6 and C-1′ increased because of reduced competition. Accurate mass measurement of **4** by hreims, which gave the $[M]^+$ at *m/z* 275.1086 (calculated as 275.1015 for $C_{15}H_{17}NO_4$, with 8 double bond equivalents), supported the presence of one more double bond in **4** compared with **2**. Comparison of the ^{1}H- and ^{13}C-nmr spectra of **4** with published data of **1** (2) showed good agreement and supported the 4-chromone skeleton of **4**. The ^{1}H-nmr spectrum of **4** showed signals at 4.43–4.45 ppm (2H, m), 6.97 ppm (1H, dt, J=15.2, 3.7, and 3.7 Hz) and 7.17 ppm (1H, d, J=15.2 Hz), and supported a partial structure of -COCH=CHCH$_2$OH. The C-2′, C-3′ double bond was in the *E* form since $J_{2'3'}$=15.2 Hz was observed. In the ^{1}H-nmr spectrum of **5**, a signal at 4.79–4.81 ppm (H_2-4′, m), which showed a downfield shift compared with the analogous signal in the ^{1}H-nmr spectrum of **4**, indicated that the -OH substituent was at C-4′. The ^{13}C-nmr and DEPT nmr spectra of **4** showed signals at 104.01 ppm (s) and 124.59 ppm (s), which indicated the presence of a C-2′, C-3′ double bond. The uv spectrum of **4**, which exhibited absorbances at 260 and 390 nm in comparison with values 245 and 278 nm in the spectrum of **2**, was also indicative of increased conjugation in **4**. The structural relationship of **2** and **4** was

proved by generation of **2** through the hydrogenation of **4**. The hydrogenation product was identified by eims. Therefore, the structure of **4** was established as 2,2-dimethyl-5-amino-6-(2′*E*-ene-4′-hydroxylbutyryl)-4-chromone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were measured on a VG 7070EQ instrument. Eims spectra were obtained at 70 eV. Fabms spectra were obtained in thioglycerol as matrix. Methane was used as reactant gas in cims. ^{1}H-, ^{13}C-, and DEPT nmr spectra were measured on a Bruker AC-200 instrument at 200 MHz, using TMS as internal standard. Uv spectra were recorded on a Beckman DB-GT spectrophotometer in MeOH.

FUNGAL MATERIAL.—The fungal isolate was *F. equiseti* (Alaska 2-2).

EXTRACTION AND ISOLATION.—Procedures for culture growth, extraction, and column fractionation have been described in a previous report (8). The fractions containing **2** and **4**, as detected by tlc, were purified by repeated Si gel prep. tlc, with a fluorescence indicator, in $CHCl_3$-MeOH (19:1). The tlc spots and bands of **2** were visualized by long-wavelength uv. The spots and bands of **4** were visualized by short-wavelength uv. Compounds **2** and **4** had R_f values of 0.74 and 0.47, respectively, on Si gel tlc in $CHCl_3$-MeOH (19:1). Five mg of **2** and 20 mg of **4** were obtained as yellow powders.

2,2-Dimethyl-5-amino-6-(4′-hydroxylbutyryl)-4-chromone [**2**].—Ir ν max (film) 3397, 3285, 2934, 2872, 1740, 1655, 1597, 1566, 1458, 1373, 1315, 1559, 1107, 995, 895 cm^{-1}; uv λ max (MeOH) 276, 246, 213 nm; ms *m/z* [M]$^+$ ($C_{15}H_{19}NO_4$) 277 (56), 259 (19), 244 (19), 233 (67), 218 (100), 204 (16), 190 (14), 176 (17), 162 (80), 106 (16); ^{1}H nmr ($CDCl_3$, 50 MHz) δ 1.45 (6H, s, H_3-11 and H_3-12), 1.95–2.18 (1H, m, H-3′), 2.69 (2H, s, H_2-3), 2.99 (1H, t, J=7.0 Hz, H-2′), 3.71 (2H, t, J=6.0 Hz, H_2-4′), 6.06 (1H, d, J=8.9 Hz, H-8), 7.87 (1H, d, J=8.9 Hz, H-7), 9.37 (1H, br s, ArNHa), 9.58 (1H, br s, ArNHb).

*2,2-Dimethyl-5-amino-6-(2′*E*-ene-4′-hydroxylbutyryl)-4-chromone* [**4**].—Ir ν max (film) 3669, 3389, 3277, 2957, 2892, 1660, 1651, 1599, 1566, 1556, 1466, 1373, 1275, 1174, 1159, 1107 and 895 cm^{-1}; uv λ max (MeOH) 390, 260, and 210 nm; ms *m/z* [M]$^+$ ($C_{15}H_{17}NO_4$) 275 (100), 260 (10), 257 (23), 244 (49), 242 (28), 220 (19), 218 (30), 201 (13), 188 (40), 162 (17), 106 (17); ^{1}H nmr ($CDCl_3$, 200 MHz) δ 1.45 (6H, s, H_3-11 and H_3-12), 2.69 (2H, s, H_2-3), 4.43–4.45 (2H, m, H_2-4′), 6.07 (1H, d, J=9.0 Hz, H-8), 6.97 (1H, dt, J=15.2, 3.7, and 3.7 Hz, H-3′), 7.17 (1H, d, J=15.2 Hz, H-2′), 7.93 (1H, d, J=9.0 Hz, H-7), 9.42 (1H, br s, ArNHa), 9.73 (1H, br s, ArNHb); ^{13}C nmr ($CDCl_3$, 50 MHz) δ 26.58 (q), 49.02 (t), 65.28 (t), 104.01 (d), 124.59 (d), 140.46 (d), 144.14 (d), 79.41 (s), 104.56 (s), 112.16 (s), 155.46 (s), 166.04 (s), 189.76 (s) and 193.82 (s).

ACETYLATION OF COMPOUND **2**.—Compound **2** (5 mg) was acetylated with $(Ac)_2O$ (1 ml) in pyridine (1 ml) at room temperature for 18 h. The fluorescent product [**3**] was isolated by Si gel prep. tlc in $CHCl_3$-MeOH (97:3). Compound **3** exhibited: eims (70 eV) *m/z* [M]$^+$ 319 (54), 259 (11), 244 (20), 233 (30), 218 (100), 204 (10), 202 (6), 190 (8), 177 (8), 162 (59), 106 (13); ^{1}H nmr ($CDCl_3$, 200 MHz) δ 1.45 (6H, s, H_3-11 and H_3-12), 1.96–2.16 (2H, m, H_2-3′), 2.04 (3H, s, 4′-$OCOCH_3$), 2.69 (2H, s, H_2-3), 2.92 (2H, t, J=7.3 Hz, H_2-2′), 4.14 (2H, t, J=6.4 Hz, H_2-4′), 6.06 (1H, d, J=9.0 Hz, H-8), 7.84 (1H, d, J=9.0 Hz, H-7), 9.36 (1H, br s ArNHa), 9.55 (1H, br s, ArNHb).

ACETYLATION OF COMPOUND **4**.—Compound **4** (4 mg) was acetylated with *N*-acetylimidazole (3 mg) in $CHCl_3$ (1 ml) at room temperature for 18 h. The reaction product [**5**] that absorbed short-wavelength uv light, was purified by Si gel prep. tlc in $CHCl_3$-MeOH (19:1). Compound **5** exhibited: eims (70 eV) *m/z* [M]$^+$ 317 (100), 262 (12), 258 (37), 257 (84), 244 (42), 242 (66), 228 (14), 218 (51), 214 (25), 202 (37), 201 (18), 191 (15), 188 (36), 174 (28), 162 (45), 144 (11), 136 (13), 135 (13), 118 (12), 106 (17); ^{1}H nmr ($CDCl_3$, 200 MHz) δ 1.46 (6H, s, H_3-11 and H_3-12), 2.14 (3H, s, 4′-$OCOCH_3$), 2.70 (2H, s, H_2-3), 4.79–4.81 (2H, m, H_2-4′), 6.09 (1H, d, J=9.0 Hz, H-8), 6.85 (1H, dt, J=15.3, 4.7, and 4.7 Hz, H-3′), 7.07 (1H, d, J=15.3 Hz, H-2′), 7.86 (1H, d, J=9.0 Hz, H-7), 9.45 (1H, br s, ArNHa), 9.67 (1H, br s, ArNHb).

HYDROGENATION OF **4**.—A solution of **4** (1 mg) in MeOH (10 ml) with a catalytic amount of Pd/C (5%) was stirred for 2 h under H_2. The crude product was chromatographed by prep. tlc (Si gel) to give pure **2**.

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