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HYDROXYPHENYLAZOFORMAMIDE DERIVATIVES FROM *CALVATIA CRANIFORMIS*

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Key Word Index—*Calvatia craniformis*; higher fungi; Gastromycetes; craniformin; hydroxyphenylazoformamide derivatives.

Abstract—The new hydroxyphenylazoformamide derivatives, 4-hydroxyphenyl-1-azoformamide, 4-hydroxyphenyl-1-*ONN*-azoformamide and 2-methylsulfonyl-4-hydroxy-6-methylthiophenyl-1-azoformamide, which we named craniformin, as well as three known related compounds, 4-methoxyphenyl-1-azoformamide, 4-methoxyphenyl-1-*ONN*-azoformamide and 2-methylsulfinyl-4-hydroxy-6-methylthiophenyl-1-azoformamide (phenolic tautomer of rubroflavin), and three known steroids, ergosta-4,6,8(14), 22-tetraene-3-one, ergosta-7, 22-diene-3-ol and ergosterol peroxide were isolated from *Calvatia craniformis*. Structure of the compounds was established by chemical and spectroscopic means, including X-ray analysis.

INTRODUCTION

Calvatia craniformis is used in Japanese and Chinese folk medicine as a haematoic [1]. Gasteromycetes are unique higher fungi because they have gleba in the asexual peridium. We are interested in the constituents of the Gasteromycetes and have elucidated the structures of three new lanostane triterpenes having a δ-lactone in the side-chain from *Astraeus hygrometricus* [2] and a polyoxygenated steroid from *Lasiophaere nipponica* [3]. The constituents of other Gastromycetes are known, *viz.*, *Lycoperdon pyriforme* [4, 5], *L. perlatum* [6], *Pisolithus tinctoirum* [7], *Calvatia craniformis* [8] and *C. cyathiformis* [9–11]. We now report on the structural elucidation of the new hydroxphenylazoformamide derivatives **1** and **3**, craniformin (**6**) and two known derivatives, **2**, **4**, **5**, and the ergosterol derivatives **7**–**9** from *C. craniformis*.

RESULTS AND DISCUSSION

The methanol extract from fresh fruit bodies of *C. craniformis* was partitioned between water and ethyl acetate. The ethyl acetate extracts were separated using column chromatography to give compounds **1**–**9**.

Compound **1**, $C_7H_7N_3O_2$, showed absorptions at 3440 cm^{-1} (OH), 1703 and 1631 cm^{-1} (amide), 1580 and 864 cm^{-1} (benzene ring) in its IR spectrum. The UV spectrum showed absorption at 242 and 342 nm. The ^1H NMR spectrum of **1** in C_5D_5N showed only three signals at δ 7.17, 8.06 (each 2H, *d*, $J = 9.0$ Hz) and δ 8.74 (2H, *br s*). The ^{13}C NMR spectrum showed five signals at δ 117.2 (*d*), 126.7 (*d*), 145.0 (*s*), 164.3 (*s*) and 165.5 (*s*). These spectral data are similar to those of 4-methoxyphenyl-1-azoformamide (**2**) which has been isolated from a culture of *Lycoperdon pyriforme* [4], except for the presence of a methoxyl group in **2**. Methylation of **1** using diazomethane gave compound **1a**, which was identical to **2**. The isolation of **1** from a natural source is reported for the first time.

Compound **2**, was identified from its spectral data as 4-methoxyphenyl-1-azoformamide [4].

Compound **3**, yellow needles, $C_7H_7N_3O_3$, showed IR absorptions at 3392 cm^{-1} (hydroxyl), 1709 and 1625 cm^{-1} (amide), and 1594 and 850 cm^{-1} (benzene ring). The ^1H NMR and ^{13}C NMR spectral data were similar to those of **1**, except for the carbon signals at δ 145.0 (*s*) for **2** and δ 138.8 (*s*) for **1**. The mass spectrum of **3** showed a $[M]^+$ at *m/z* 181, which is 16 mu higher than that of **1**. From these data, the structure of compound **3** was deduced to be 4-hydroxyphenyl-1-*ONN*-azoxyformamide. Methylation of **3** using diazomethane gave compound **3a**, which was identified as 4-methoxyphenyl-1-*ONN*-azoxy-

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Table 1. ^{13}C NMR spectral data of compounds **1–6** (C_5D_5N: **1**, **3**, **5a**, DMSO-d_6; **2**, **4**, **5**, **6**, TMS as int. standard)

Carbon	1	2	3	4	5	5a	6
1	145.0	145.1	138.8	138.9	135.9	136.0	137.0
2	126.7	125.0	124.8	123.9	147.9	147.7	147.4
3	117.2	114.5	116.0	114.2	107.8	105.5	114.8
4	164.3	163.1	161.4	159.6	163.0	161.4*	163.8
5	117.2	114.5	116.0	114.2	113.3	112.3	117.0
6	126.7	125.9	124.8	123.9	146.4	144.1	141.4
$-CONH_2$	165.6	164.6	163.3	162.7	163.2	161.5*	165.3
$-OCH_3$	—	55.8	—	55.8	—	54.1	—
$-SCH_3$	—	—	—	—	15.2	14.8	16.9
$-SOCH_3$	—	—	—	—	44.1	42.8	—
$-SO_2CH_3$	—	—	—	—	—	—	45.4

* Values interchangeable within same column.

formamide (**4**) from its spectral data [4]. The isolation of compound **3** from a natural source is also reported for the first time.

Compound **4** was identified from its spectral data as 4-methoxyphenyl-1-*ONN*-azoxyformamide [4].

Compound **5**, red needles, showed IR absorptions at 3444 cm^{-1} (hydroxyl), 1703 and 1596 cm^{-1} (amide). The ^1H NMR spectrum in C_5D_5N showed two singlet methyl signals at δ 2.37 and 2.90, two aromatic proton signals at δ 7.13 and 8.02 (each 1H, *d*, J = 2.4 Hz) and amide proton signals at δ 8.60 and 9.02. The ^{13}C NMR spectral data (Table 1) of **5** in DMSO-d_6 showed two methyl signals at δ 15.2 and 44.1, two methine signals at δ 107.8 and 113.3, and five quaternary carbon signals at δ 135.9, 146.4, 147.9, 163.0 and 163.2. The UV spectrum of **5** was almost the same as that of **1**, indicating the presence of a *p*-hydroxyphenylazo moiety in the compound. The FAB mass spectrum of **5** showed a $[M+H]^+$ ion peak at *m/z* 274 (positive mode) and a $[M-H]^-$ ion peak at *m/z* 272 (negative mode). From the HR mass spectrum and elementary analysis, the molecular formula of **5** was suggested to be $C_9H_{11}N_3O_3S_2$. By comparing the ^{13}C NMR spectrum of **1** with that of **5**, the quaternary carbon signals at δ 163.0 and 163.2 were assigned to the benzene carbon attached to oxygen function and amide carbonyl carbon signals, respectively. The remaining carbon signals at δ 107.8, 113.3, 135.9, 146.4 and 147.9 were assigned to benzene ring carbons. In the ^1H NMR spectrum of **5** in C_5D_5N, the coupling constant of the proton signals at δ 7.13 and 8.02 was 2.4 Hz, indicating that these protons are in a *meta*-position. The methyl signals at δ 2.37 and 2.90 were assigned to $-SCH_3$ and $-SOCH_3$ from the molecular formula and chemical shifts (^1H and ^{13}C NMR). Methylation of **5** with diazomethane gave a monomethylate derivative **5a**, whose ^1H NMR spectrum showed a methoxyl signal at δ 4.03. From these data, the structure of **5** was suggested to be a benzene substituted with –OH, $-SCH_3$, $-SOCH_3$ and $-N{=}N-CONH_2$. To confirm the substitution pattern, we measured the NOE spectra of **5a** in $CDCl_3$ and the ^{13}C-^1H long-range correlation spectrum of **5** in DMSO-d_6 (Fig. 1). Thus, the structure of compound **5** was determined as shown in the formula. This structure is in agreement with the phenolic tautomer of rubroflavin previously isolated from the North America puff ball, *C. rubroflava*, by Gill and Steglich [12]; equilibrium between the tautomers (phenol–benzoquinone) was strongly dependent on the solvents and we used the same solvents for NMR experiments to prove the identity of both compounds. They above authors reported ^1H and ^{13}C NMR spectral data of rubroflavin in DMSO-d_6 and CD_3OD, respectively [12]. However, the ^1H and ^{13}C NMR spectral data of both compounds were different using the same solvents for NMR experiments. It is very difficult to explain this difference; we suggest that compound **5** exists as the phenolic tautomer in the CD_3OD, DMSO-d_6 and C_5D_5N. Compound **5** is also chiral and exhibits an unusually high optical rotation, the same as rubroflavin [**5**: $[\alpha]_D$ −1740°, *c* 0.40, pyridine]. The

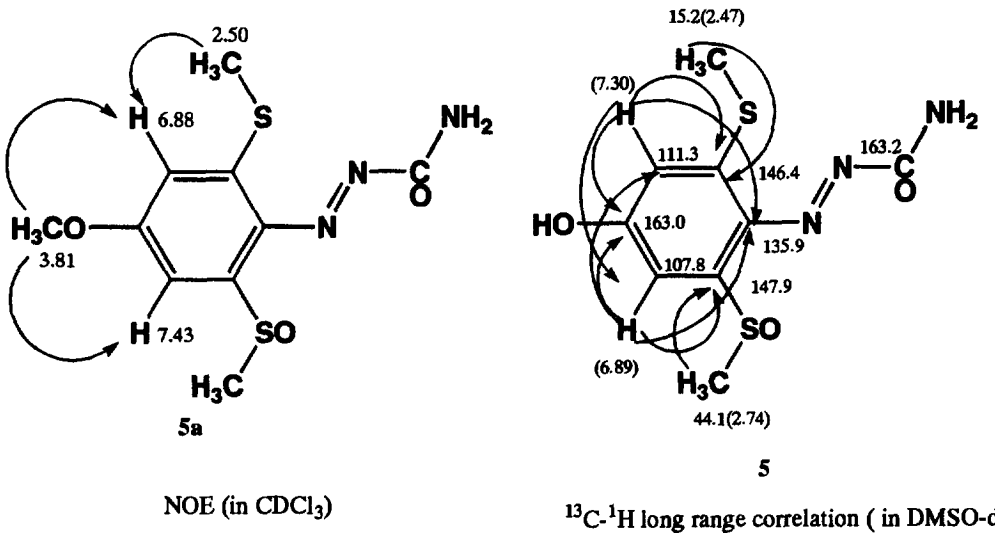


Fig. 1. NOE and ^{13}C–^1H long-range correlation of compounds **5** and **5a**.

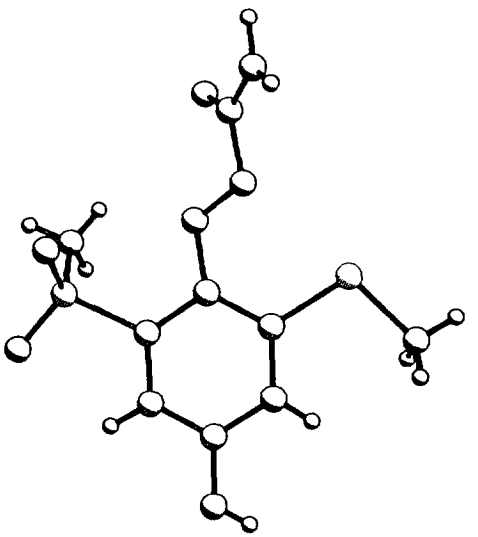


Fig. 2. ORTEP drawing of craniformin (**6**).

configuration of **5** was deduced to be (*S*), the same as ruboflavin.

Craniformin (**6**), $C_9H_{11}N_3O_4S_2$, showed IR absorptions at 3437 cm^{-1} (hydroxyl), 1719 and 1588 cm^{-1} (amide). The ^1H NMR spectrum showed two singlet methyl signals at δ 2.20 and 3.73, *meta*-coupled aromatic proton signals at δ 7.16 and 8.02 (each 1H, *d*, $J = 2.4$ Hz) and two amide proton signals at δ 8.97 and 9.23 (each 1H, *br s*). The ^{13}C NMR spectrum of **6** showed two methyl carbon signals at δ 16.9 and 45.4, and aromatic carbon signals and amide carbon signals at δ 114.8 (*d*), 117.0 (*d*), 137.0 (*s*), 141.4 (*s*), 147.4 (*s*), 163.8 (*s*) and 165.3 (*s*). The ^1H NMR spectra data were similar to those of compound **5**, except for the methyl signal at δ 3.73 in **6** and δ 2.90 in **5**. The chemical shifts and multiplicity of the ^{13}C NMR spectral data of compound **6** were also similar to those of compound **5** (Table 1). These data, including the molecular formula, suggested that in **6**, the sulphone group is replaced by sulphoxide in **5**. In order to confirm the structures of compounds **5** and **6**, an X-ray analysis of **6** was attempted (Fig. 2). The structure of craniformin (**6**) was confirmed and from a comparison between the spectral data of compounds **6** and **5**, the structure of **5** was also confirmed.

Compounds **7**–**9** were identified from their spectral data as ergosta-4,6,8(14), 22-tetraene-3-one [2], ergosta-7.22-diene-3-ol [13] and ergosterol peroxide [14], respectively.

Related to our compounds, there are some reports concerning antitumour activity. Calvatic acid (*p*-carboxyphenylazocyanide) showed a carcinostatic effect, inhibiting Yoshida sarcoma cells growth and increasing survival of mice with leukemia [8]. Also, calvatic acid and some analogues induced a strong decrease of ornithine decarboxylase (ODC) activity in AH-130 hematoma cells at low concentrations [15]. We measure the carcinostatic activity of compounds **5** and **6** against K562 leukemia cells; compound **5** and **6** showed activities of 38.7 $\mu g\ ml^{-1}$.

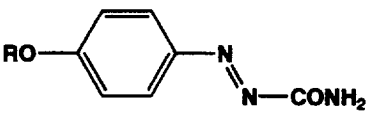
EXPERIMENTAL

Mps: uncorr. ^1H NMR: 400 MHz with TMS as int. stand. ^{13}C NMR: 100 MHz. CC: silica gel 60 (Merck), Sephadex LH-20 (Pharmacia) and Toyo Pearl HW-40 (Tosho).

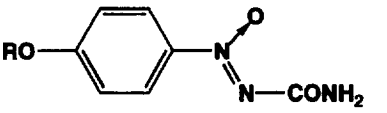
Material. Fresh fruit bodies of *C. craniformis* (Schw.) Fr. were collected as follows: (I) 2.32 kg from Kamiyama-Chyo, Tokushima, Japan in October 1984; (II) 0.4 kg from Hiwasa-Chyo, Tokushima, Japan in September 1985; (III) 3.6 kg Itano-Chyo, Tokushima, Japan in September 1990.

Isolation of compounds **1**–**9**. Fresh fruit bodies (II), (0.4 kg) were cut and extracted with hot MeOH until the MeOH extract were colourless. The MeOH soln was evapd to dryness (21 g), dissolved in H_2O and extracted with EtOAc. The EtOAc extract was evapd under red. pres. to give a residue (1.73 g), which was chromatographed on a silica gel column and eluted successively with $CHCl_3$–MeOH (19:1) and MeOH to afford 6 frs (I-1–3). Fr. 3 (70 mg) was recrystallized from MeOH to give 30 mg of **5**. Fresh fruit bodies (I), (2.32 kg) were treated in the same manner as described above to give an EtOAc residue (14.2 g), which was partitioned between EtOAc and 5% Na_2CO_3. The EtOAc layer was washed with satd aq. NaCl, dried ($MgSO_4$) and evapd to give a neutral EtOAc residue (10 g). This was sepd by silica gel CC, eluting successively with hexane–EtOAc (4:1), EtOAc and MeOH to give 10 frs (II-1–II-10). Fr. II-8 was recrystallized from MeOH to give 43 mg of **4**. Fr. II-10 was separated by silica gel CC using $CHCl_3$–Me_2CO (9:1), prep. TLC with $CHCl_3$–MeOH (9:1) and recrystallized from MeOH to give 14 mg of **3**. Frs II.2, II.4 and II.6 were recrystallized from MeOH to give 38 mg of **7**, 11 mg of **8**, 102 mg of **9**, respectively. Fresh fruit bodies (III), (3.60 kg) were also treated in the same manner as described above to give an EtOAc residue (15 g), which was chromatographed on a silica gel column eluting successively with $CHCl_3$–MeOH (19:1) and MeOH to give 8 frs (III-1–III-8). Fr. III-1 (10.1 g) was separated by silica gel CC with $CHCl_3$–MeOH (19:1) and on Sephadex LH-20 with $CHCl_3$–MeOH (1:95) to give 31.3 mg of **4**. Fr. III-2 (0.72 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (19:1) and on Sephadex LH-20 with MeOH to give 209.5 mg of **1**. Fr. III-3. (0.218 g) was chromatographed on Sephadex LH-20 with $CHCl_3$–MeOH (1:9) and recrystallized from MeOH to give 160.3 mg of **5**. Fr. III-4 (0.117 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1) and recrystallized from MeOH to give 22 mg of **6**.

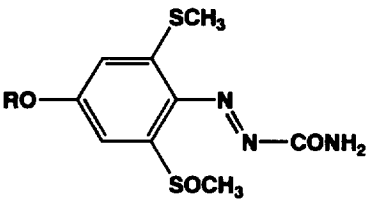
Compound **1**. Yellow needles, mp 177–179°. IR ν_{max}^{KBr} cm^{-1}: 3440, 1703, 1631, 1580, 864. UV λ_{max}^{MeOH} nm (log ε): 242 (4.12), 342 (4.27). ^1H NMR (C_5D_5N): δ 7.17 (2H, *d*, $J = 9.0$ Hz), 8.06 (2H, *d*, $J = 9.3$ Hz), 8.74 (2H, *br s*). ^{13}C NMR (C_5D_5N): Table 1. EI-MS



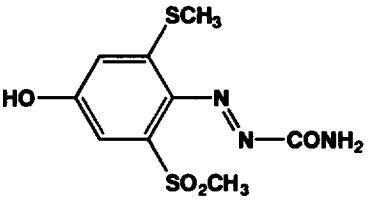
1: R=H
1a=2: R=Me



3: R=H
3a=4: R=Me



5: R=H
5a: R=Me



6

m/z (rel. int.): 164 $[M-H]^+$ (0.3), 122 (40), 96 (66), 66 (55), 49 (100), 44 (41). HR-MS *m/z* 166.0593 $[M+H]^+$ $C_7H_8N_3O_2$ required 166.0617.

Methylation of compound **1**. A CH_2N_2 soln (2 ml) in Et_2O was added to a soln of compound **1** (5 mg) in MeOH (2 ml), followed by usual work-up to give **1a** (3 mg). Compound **1a** was identified as **2** by comparison of spectral data and TLC.

Compound **2**. Orange needles, mp 156–158°. IR ν_{max}^{KBr} cm^{-1}: 3350, 3160, 1700, 1670. UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 238 (4.13), 334 (4.24). ^1H NMR (C_3D_3OD): δ 3.78 (3H, *s*), 7.12 (2H, *d*, J = 9.3 Hz), 7.73 (2H, *br d*), 8.10 (2H, *d*, J = 9.3 Hz). ^{13}C NMR. (DMSO-d_6): Table 1. EI-MS *m/z* (rel. int.): 179 $[M]^+$ (10), 163 (3), 135 (100), 107 (87).

Compound **3**. Yellow needles, mp 156–158°. IR ν_{max}^{KBr} cm^{-1}: 3392, 1709, 1625, 1594, 885, 850. UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 227 (4.19), 302 (4.25). ^1H NMR: (C_5D_5N): δ 7.06 (2H, *d*, J = 9.3 Hz), 8.23 (2H, *d*, J = 9.3 Hz), 9.12 (2H, *br s*). ^{13}C NMR (C_5D_5N): Table 1. EI-MS *m/z* (rel. int.): 181 $[M]^+$(5), 152 (15), 121 (12), 110 (44), 93 $[M-ONNCONH_2]^+$ (53), 65 (64), 43 (100). HR-MS *m/z* 182.0548 $[M+H]^+$ $C_7H_8N_3O_3$ required 182.0566.

Methylation of compound **3**. Methylation of compound **3** (5 mg) with CH_2N_2 in MeOH (2 ml), followed by usual work-up gave **3a** (3 mg). Compound **3a** was identified as **4** by comparison of spectral data and TLC.

Compound **4**. Yellow needles, mp 196–198°. IR ν_{max}^{KBr} 3350, 3160, 1700, 1670. UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 227 (4.27), 298 (4.33). ^1H NMR (C_5D_5N): δ 3.65 (3H, *s*) 6.91 (2H, *d*, J = 9.3 Hz), 8.21 (2H, *d*, J = 9.3 Hz), 9.15 (2H, *br s*). ^{13}C NMR (DMSO-d_6): Table 1. EI-MS *m/z* (rel. int.): 195 $[M]^+$ (7), 165 (16), 107 (42), 43 (100). HR-MS *m/z* 195.0639 $[M]^+$ $C_8H_9N_3O_3$ required 195.0644.

Compound **5**. Red needles, mp 192–194°. $[\alpha]_D^{25}$ −1740° (pyridine *c* 0.40). IR ν_{max}^{KBr} cm^{-1}: 3444, 3323, 1703, 1596, 1267, 1219, 1014, 836. UV λ_{max}^{MeOH} ($\log\varepsilon$): 248 (4.27), 285 (4.28), 355 (4.10). ^1H NMR (C_5D_5N): δ 2.37 (3H, *s*, SCH_3), 2.90 (3H, *s*, $-SOCH_3$), 7.13, 8.02 (each 1H, *d*, J = 2.4 Hz), 8.60, 9.02 (each 1H, *br s*). ^1H NMR (DMSO-d_6): δ 2.47 (3H, *s*, SCH_3), 2.74 (3H, *s*, $-SOCH_3$), 6.89, 7.31 (each 1H, *d*, J = 2.4 Hz), 7.71, 7.75 (each 1H, *br s*). ^1H NMR: δ (CD_3OD): 2.49 (3H, *s*, SCH_3), 2.85 (3H, *s* $-SOCH_3$), 6.89, 7.31 (each 1H, *d*, J = 2.4 Hz). ^{13}C NMR (DMSO-d_6): Table 1. ^{13}C NMR (CD_3OD): δ 15.4 (*q*), 44.5 (*q*), 109.5 (*d*), 114.4 (*d*), 136.8 (*s*), 148.7 (*s*), 149.4 (*s*), 164.9 (*s*), 166.6 (*s*). EI-MS *m/z* (rel. int.): 272 $[M-H]^+$ (2), 186 $[M-NCONH_2-CH_3]+$(4), 163 (73), 110 (100). FAB-MS (pos.): 274 $[M+H]^+$. FAB-MS (Neg.): 272 $[M-H]$. HR-MS *m/z* 272.0236 $[M-H]^+$ $C_9H_{10}N_3O_3S_2$ required 272.0242. (Found: C 39.72; H 4.01; N 15.31; S 23.20 $C_9H_{11}N_3O_3S_2$ required: C; 39.55, H; 4.06, N; 15.37, S; 23.46).

Methylation of compound **5**. A CH_2N_2 soln (4 ml) in Et_2O was added to a soln of compound **5** (10 mg) in $CHCl_3$–MeOH (1:1, 5 ml), followed by usual work-up and CC on silica gel with $CHCl_3$–MeOH (9:1) to give **5a** (9 mg). **5a**. IR ν_{max}^{KBr} cm^{-1}: 3441, 1719, 1579, 1250, 1217. ^1H NMR ($CDCl_3$): δ 2.50 (3H, *s*, $-SCH_3$), 2.86 (3H, *s*, $-SOCH_3$), 4.03 (3H, *s*, $-OCH_3$), 6.88 (1H, *s*, J = 2.4 Hz), 7.60 (1H, *s*, J = 2.4 Hz), 6.60, 7.43 (each 1H, *br s*, $-CONH_2$). ^1H NMR (C_5D_5N): δ 2.42 (3H, *s*), 2.93 (3H, *s*), 3.81 (3H, *s*), 6.93, 7.81 (each 1H, *d*, J = 2.4 Hz). ^{13}C NMR (C_5D_5N): Table 1. EI-MS *m/z* (rel. int.): 287 $[M]^+$ (4), 272 $[M-CH_3]^+$ (12), 256 $[M-OCH_3]^+$ (4), 243 $[M-CONH_2]^+$ (15), 216 $[M-N{=}NCONH_2+H]^+$ (85), 201 $[M-N{=}NCONH_2-CH_3+H]^+$ (100), 184 (27), 170 (25), 153 (28), 138 (21), 121 (49). FD-MS *m/z*: 287 $[M]^+$.

Compound **6**. Red plates, mp 192–194°. IR ν_{max}^{KBr} cm^{-1}: 3437, 3287, 1719, 1588, 1492, 1421, 1342, 1301, 1207, 1143, 982, 964, 938, 845, 752. ^1H NMR (C_5D_5N): δ 2.20 (3H, *s*, $-SCH_3$), 3.73 (3H, *s*, $-SO_2CH_3$), 7.16, 8.02 (each 1H, *d*, J = 2.4 Hz), 8.97, 9.23 (each 1H, *br*

s). ^{13}C NMR (DMSO-d_6): Table 1. FAB-MS *m/z*: 312 $[M+Na]^+$, 328 $[M+K]^+$.

X-ray crystallographic analysis of compound **6**. Crystal $0.70 \times 0.50 \times 0.30$ mm, monoclinic, space group C2/c, $a = 18.488$ (2) Å, $b = 9.721$ (2) Å, $c = 15.569$ (2) Å, $\beta = 116.755$ (8)° $V = 2498.6$ (8) Å^3, $Z = 8$, $D_{calc} = 1.538$ g cm^{-3}. All measurements were made on a Rigaku AFC5S diffractometer with graphite monochromated MoKα radiation and a 50 kV 30 mA sealed tube X-ray generator. Data were collected using the ω–2θ scan technique to a maximum 2θ value of 50°; a total of 2348 independent reflections was collected The linear absorption coefficient for MoKα is 4.2 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction. Data were corrected for Lorenz and polarization effects and the structure solved by a direct method [16]. The resulting *E* map revealed the positions of all non-H atoms. All H atom positions were found on a difference Fourier map. The refinement of atomic parameters was carried out by full-matrix least squarement. Termal parameters were refined anisotropically for all non-H atoms and isotropically for the H atoms. The final refinement was based on 1063 observed reflections ($I > 3.00\sigma(I)$) and 207 variable parameters and converged (largest parameter shift was 0.03 times its esd) with unweighted and weighted agreement factors of $R = 0.032$ and $R_w = 0.0396$. The standard devision of an observation of unit weight was 1.34. The weighting scheme was based on counting statistics and included a factor ($p = 0.03$) to downweight the intense reflections. Plots of $\Sigma(|F_o|-|F_c|)^2$ vs $|F_o|$ reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices, showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded with 0.19 and -0.23 e$^-$/Å^3, respectively. Neutral atom scattering factors were taken from Cromer and Waber [17]. All calculations were performed using the TEXSAN [18] crystallographic software package of the Molecular Structure Corporation.

REFERENCES

1. Jianzhe, Y., Xiaolan, M., Qiming, M., Yichen, Z. and Huaan, W., *Iconos of Medicinal Fungi from China*. Science Press, Benjiing, 1987.
2. Takaishi, Y., Murakami, Y., Nakano, K., Mutakami, K. and Tomimatsu, T., *Phytochemistry*, 1987, **26**, 2341.
3. Takaishi, Y., Adachi, R., Murakami, Y., Ohashi, T., Nakano, K. and Tomimatsu, T., *Phytochemistry*, 1987, **31**, 243.
4. Okuda, T., Nakayama, N. and Fujiwara, A., *Transactions of the Mycological Society of Japan*, 1982, **23**, 225.
5. Hiramoto, M., Okada, K. and Nagai, S., *Tetrahedron Letters*, 1970, **13**, 1087.
6. Banga, N. R., Welter, A., Tadot, J. and Casimir, J., *Phytochemistry*, 1979, **18**, 482.
7. Lobo, A. M., Abreu, P. M., Prabhakar, S. and Godinho, L. C., *Tetrahedron Letters*, 1985, **26**, 2589.
8. Umezawa, H., Takeuchi, T., Iinuma, H., Ito, M., Ishizuka, M. and Kurahata, Y., *Journal of Antibiotics*, 1975, **28**, 87.
9. Kawahara, N., Sekita, S. and Satake, M., *Chemical and Pharmaceutical Bulletin*, 1993, **41**, 1318.
10. Kawahara, N., Sekita, S. and Satake, M., *Phytochemistry*, 1994, **37**, 213.
11. Kawahara, N., Sekita, S. and Satake, M., *Phytochemistry*, 1994, **38**, 947.
12. Gill, M. and Steglich, W., *Progress in the Chemistry of Organic Natural Products*, Vol. 51. Springer, New York, 1987, 242.
13. Takaishi, Y., Ohashi, T. and Tomomatsu, T., *Phytochemistry*, 1989, **28**, 945.
14. Takaishi, Y., Uda, M., Ohashi, T., Nakano, K., Murakami, K. and Tomomatsu, T., *Phytochemistry*, 1991, **30**, 4117.
15. Brossa, O., Gadoni, E., Olivero, A., Seccia, M., Miglietla, A., Garbriel, L. and Gravela, E., *Research Communications in Chemical Pathology and Pharmacology*, 1990, **70**, 143.
16. Gilmore, C., *Journal of Applied Crystallography*, 1984, **17**, 42.
17. Cromer, D. T. and Waber, J. T., *International Tables for X-ray Crystallography*, Vol. IV. The Kynoch Press, Birmingham, U.K., 1974.
18. TEXSAN-TEXRAY Structure Analysis Package, Molecular Structure Corporation, 1985.