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GLIOTOXIN: UNCOMMON ^{1}H COUPLINGS AND REVISED ^{1}H- AND ^{13}C-NMR ASSIGNMENTS

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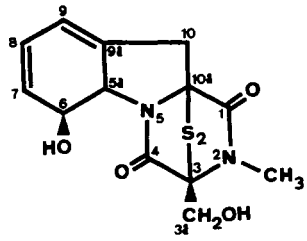
ABSTRACT.—Gliotoxin, isolated from the fungus *Dichotomomyces cejpii*, is shown to exhibit uncommon couplings that are temperature- and solvent-dependent. Furthermore, previously reported ^{1}H- and ^{13}C-nmr assignments are corrected.

During investigations of fungal metabolites with potential biological activity, we isolated a component identified as gliotoxin [**1**] from *Dichotomomyces cejpii* (Mil'ko) Scott (CMPG 893). We report unexpected ^{1}H couplings exhibited by the hydroxy group attached to the methylene and corrections to previously reported ^{1}H- and ^{13}C-nmr assignments (1).



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The isolated compound, $C_{13}H_{14}N_2O_4S_{21}$, $[M]^+$ *m/z* 326, was at first considered to be an isomer of gliotoxin as a consequence of the presence of three coupled signals in the ^{1}H-nmr spectrum ($CDCl_3$) at δ 4.43 (dd, J = 12.7, 6.1 Hz), δ 4.26 (dd, J = 12.7, 9.5 Hz), and δ 3.55 (dd, J = 9.5, 6.1 Hz), deduced to be representative of a $>$CH-CH_2OH chain. These findings were supported by the published ^{1}H nmr of gliotoxin ($CDCl_3$), in which the CH_2OH was recorded as two sharp doublets (J = 13 Hz) at δ 4.44 and 4.18 (Table 1). It should be mentioned that Cole and Cox (1) had as-

TABLE 1. ^{1}H-nmr Data of Gliotoxin [**1**] in $CDCl_3$.

Proton	Compound	
	1[a]	**1**
Me-2	3.22	3.21, s
H-3a$_A$	4.80	4.43, dd, J = 12.7 and 6.1 Hz
H-3a$_B$	4.80	4.26, dd, J = 12.7 and 9.5 Hz
3a-OH		3.55, dd, J = 9.5 and 6.1 Hz
H-5a	2.90, J = 18 Hz	4.82, s
H-6	3.72, J = 18 Hz	4.82, s
6-OH		5.81, s
H-7	5.90	5.99, br d, J = 5.1 Hz
H-8	5.90	5.95, br d, J = 10.1 and 5.1 Hz
H-9	5.90	5.79, br d, J = 10.1 Hz
H-10$_A$	4.44, J = 13 Hz	3.76, br d, J = 18 Hz
H-10$_B$	4.18, J = 13 Hz	2.96, br d, J = 18 Hz

[a]Data taken from Cole and Cox (1).

signed the broad singlet integrating for two protons at δ 4.80 to this methylene, as well as the doublets (J = 18 Hz) at δ 2.90 and 3.72 to H-5a and H-6, respectively, and the doublets (J = 13 Hz) at δ 4.44 and 4.18 to the endocyclic methylene CH_2-10. In contrast to the assignments given in Cole and Cox (1), when the shielding and coupling constants observed for the endocyclic geminal methylene protons are taken into account, the doublets at δ 2.90 and 3.72 have to be assigned to CH_2-10, similar to those reported for rotenone-like components, which also exhibit a methylene in a heteropentacycle (2,3). Finally, the remaining two protons, broad singlet at δ 4.80, must be attributed to both H-5a and H-6 in gliotoxin. As the ^{1}H-nmr spectra of the isolated metabolite and gliotoxin were different, the disulfide bridge was deduced to bridge either C-5a and C-10a or C-6 and C-10a instead of attachment to C-3 and C-10a as in gliotoxin. The first hypothesis was ruled out, based on the presence of H-5a observed as a singlet at δ 4.82. This signal showed a marked cross peak in the H,H-COSY spectrum, arising from a long range coupling with the doublet at δ 2.96 from the methylene H-10_B. In order to confirm this result, the carbon-hydrogen shift correlated spectrum was examined. Surprisingly, no carbon connectivity to the proton double doublet centered at δ 3.55 was observed, clearly indicating that this proton is attached to a heteroatom and thus permitting assignment as the hydroxy proton. Consequently, the isolated compound must be gliotoxin, and the above-mentioned ^{1}H-nmr assignments as well as ^{13}C-nmr assignments were, of course, confirmed (see Experimental). Comparison with an authentic sample of gliotoxin (tlc, hplc, uv, ^{1}H- and ^{13}C-nmr) verified this conclusion.

Because the signals in the ^{1}H-nmr spectrum are temperature-dependent and the nmr data acquisition parameters were not given by Cole and Cox (1), it may be that these authors did not record the ^{1}H-nmr spectrum at room temperature as in this study. To our knowledge, this is the first report of a proton showing such large coupling values (J = 9.5 and 6.1 Hz) with a methylene group through an oxygen atom. In C_6D_6, these couplings decreased too much to be seen: the involved hydroxy group appeared as a singlet resonating at δ 3.66, and the methylene gave rise to two sharp doublets (J = 6.5 Hz) at δ 3.99 and 3.64. Furthermore, H-5a and H-6 were recorded as two broad doublets (J = 13 Hz) at δ 4.84 and 4.31 instead of a two-proton broad singlet at δ 4.82 in $CDCl_3$. To the best of our knowledge, such vari-

TABLE 2. ^{13}C-nmr Chemical Shifts of Gliotoxin [1] in $CDCl_3$.

Carbon	Compound	
	1[a]	1
C-1	165.9[b]	166.0[b]
C-3	77.3	75.6[c]
C-3a	60.5	60.7
C-4	165.1[b]	165.3[b]
C-5a	69.7	69.7
C-6	73.1	73.1
C-7	129.8	130.1
C-8	120.1	123.3
C-9	123.3	120.2
C-9a	130.7	130.8
C-10	36.6	36.6
C-10a	77.3	under $CDCl_3$ peak[c]
2-Me	27.5	27.5

[a]Data taken from Cole and Cox (1).
[b,c]Assignments with the same superscript in each column may be reversed.

TABLE 3. Antifungal Bioassay Relative to the cc Fractions (diameter of inhibition in mm).

Fraction	Strain	
	Candida albicans	*Candida tropicalis* R_2
I	0	0
II	0	9
III	11	24
IV	40	40
V	0	9

ations of scalar coupling constants as a result of solvent dependency have never been previously reported.

EXPERIMENTAL

BIOLOGICAL MATERIAL.—A strain belonging to the mycological collection of the Laboratory of Cryptogamy (CMPG: Collection Mycologie Pharmacie Grenoble) was used in this study: *D. cejpii* (CMPG 893) was isolated from marine sediments (Barcelona, Spain). The strain was preserved at 4° on a gelose medium with malt extract (1.5%). The fungus was grown on the same medium at 24° for 8 days and used for the inoculum. Cultivation was performed at 22° in 10-liter fermentor (Biolafitte) containing 8 liters of potato dextrose agar medium (Difco) under shaking (100 rpm) with aeration (1 liter/min) after sterilization by autoclaving for 20 min at 121°. The pH value was 5.55.

GENERAL EXPERIMENTAL PROCEDURES.—Analytical hplc was performed with μ-Bondapak C_{18} (10 μm) column (3.9 × 300 mm) (Waters) on Waters Model 510 apparatus and M 480 Lambda-Max™ spectrophotometer (254 nm) using H_2O-MeOH (55:45) as eluent at 1.0 ml/min. Semipreparative hplc was performed with Spherisorb ODS 2 (5 μm) column (7.5 × 300 mm) (Société Française de Chromatographie) using the same conditions. The uv spectrum was measured in MeOH on an Acta C II spectrophotometer. ^{1}H- and ^{13}C-nmr spectra ($CDCl_3$, δ ppm/TMS) were run on AC200 and AM300 Bruker nmr spectrometers at room temperature. The ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra were recorded with Bruker standard software (4,5). The ir spectrum was taken on an Unicam SP1100 spectrometer. Ei and fab$^+$ mass spectra were obtained with a Nermag 10-10C apparatus (80 eV).

ISOLATION PROCEDURE.—After 5 days of cultivation, the culture medium was filtered off and the aqueous phase (pH 6.00) extracted with EtOAc. Combined extracts were deposited on a Bio-Sil A (200–400 mesh) column (18 × 280 mm) and eluted with a nonlinear gradient of *n*-hexane/$CHCl_3$ leading to five fractions of 1 liter each: I (*n*-hexane), II [*n*-hexane–$CHCl_3$ (55:45)], III [*n*-hexane–$CHCl_3$ (50:50)], IV [*n*-hexane–$CHCl_3$ (45:55)], and V [*n*-hexane–$CHCl_3$ (40:60)]. Antifungal activity was detected by the agar-well diffusion method (6,7) using *Candida albicans* (CMPG 684) and *Candida tropicalis* R_2 IP (Polyene-resistant, Institut Pasteur). After chromatography on Bio-Sil A column, antifungal activity was detected in fractions III and IV (Table 3). After drying the active fractions were crystallized in MeOH. Tlc was carried out in the following solvent systems; C_6H_6-EtOAc (60:40) (R_f = 0.47) and C_6H_6-dioxane-HOAc (90:25:4) (R_f = 0.64); silica plates (Kieselgel 60 F 254, Merck).

GLIOTOXIN.—A white crystalline compound: mp 221°; hplc retention time 19 min; uv λ max (MeOH) 213, 235 sh, 272 nm; ir ν KBr 3500, 3420, 3090, 2950, 2880, 2820, 1670, 1450, 1425, 1360, 1330, 1255, 1240, 1200, 1090, 1070, 1020, 820, 720, 710, 650 cm^{-1}; eims (80 eV) *m/z* (rel. int.) $[M]^+$ 326 (10), $[M-S_2]^+$ 262 (100), $[M-S_2-H_2O]^+$ 244 (14), $[M-S_2-{}^{\cdot}CHO]^+$ 233 (26), 227 (7), 217 (22), 203 (4), 188 (2), 175 (2), 160 (3), 153 (5), 136 (5), 116 (5), 107 (15), 101 (19), 94 (6), 89 (15), 77 (41), 73 (47), 55 (48), 42 (84); hrms *m/z* $[M]^+$ 326.0390 ($C_{13}H_{14}N_2O_4S_2$ requires 326.0395), $[M-S_2]^+$ 262.09460 ($C_{13}H_{14}N_2O_4$ requires 262.09536), $[M-S_2-{}^{\cdot}CHO]^+$ 233.09280 ($C_{12}H_{13}N_2O_3$ requires 233.09285), 107.0461 ($C_2H_7N_2O_3$ requires 107.0461), 73.0528 (C_3H_7NO requires 73.0528); fab$^+$ ms *m/z* (rel. int.) $[MH]^+$ 327 (39), $[MH-S_2]^+$ 263 (100), 262 (98), 245 (41), 233 (16), 227 (18), 165 (47); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

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