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EMEHETERONE, A PYRAZINONE DERIVATIVE FROM *EMERICELLA HETEROTHALLICA**

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Key Word Index—*Emericella hetrothallica*; Eurotiaceae; 2(1*H*)-Pyrazinone; emeheterone; stellatin.

Abstract—Emeheterone, a novel pyrazinone derivative, has been isolated from the culture filtrate of the fungus *Emericella heterothallica*, along with stellatin. Its molecular structure has been investigated by spectroscopic means.

INTRODUCTION

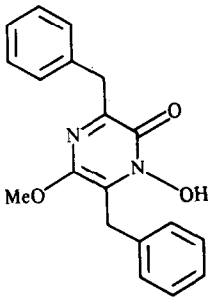
Recently the antifungal epidithiodioxopiperazines, emestrin [2] and dithiosilvatin [3], were isolated from *Emericella striata* (Rai, Tewari & Mukerji) Malloch & Cain and *Aspergillus silvaticus* Fennell & Raper, respectively. In the course of screening for dioxopiperazine derivatives from *Emericella* spp., a novel pyrazinone derivative designated emeheterone (1) was isolated from the dichloromethane extract of the culture filtrate of *Emericella heterothallica* (Kwon, Fennell & Raper) Malloch & Cain (anamorph: *Aspergillus heterothallicus* Kwon, Fennell & Raper) (mating type a), strain ATCC 16824, along with a dihydroisocoumarin, stellatin **(2)**.

RESULTS AND DISCUSSION

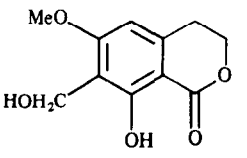
Emeheterone **(1)**, mp 215–217°, gave molecular ions at *m/z* 322 in EIMS and at *m/z* 323 in CIMS, and elemental analysis confirmed the molecular formula as $C_{19}H_{18}N_2O_3$. The strong ion at *m/z* 91 $[C_6H_5CH_2]^+$ in the EIMS suggested the presence of benzyl groups in the molecule of **1**. The ^1H NMR signals at δ 7.20–7.40 (10H), 3.93 (2H), and 4.20 (2H), the 10 ^{13}C NMR signals at δ 126–137 and the two ^{13}C NMR signals at δ 30.39 and 34.01 were assigned to two benzyl groups. The other ^1H NMR signals at δ 3.92 (3H) and 3.73 (1H) in **1** were assigned to a methoxy group, which appeared at δ 61.73 in the ^{13}C NMR spectrum, and a hydroxyl group, respectively.

The IR absorption maximum at 1650 cm^{-1} and the ^{13}C NMR signal at δ158.14 (St) in emeheterone **(1)** suggested the presence of a conjugated carbonyl, probably an amide. Compound **1** gave a positive coloration (greenish brown) with 0.5% aq. copper chloride [4], which suggested the presence of an oxime-like structure. The UV absorption maxima at 228, 276, 334 (sh), and 352 nm of **1** were closely similar to those at 225, 265, 279 (sh), 347 (sh), and 364 nm for deferriastechrome **3** [5], which also has a maximum at 291 nm due to the indole moiety. The above results are consistent with structure **1** for emeheterone, but do not suggest the orientation of the methoxy and hydroxyl groups located at N-1 and C-5.

* Part 20 in the series 'Studies on Fungal Products'. For Part 19 see ref. [1].



1



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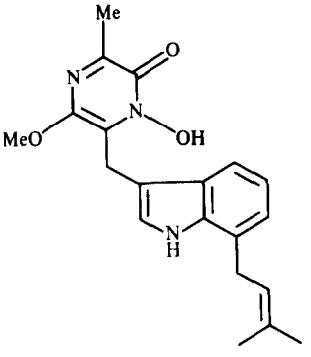

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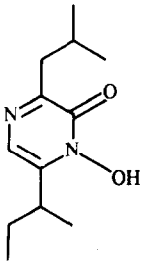

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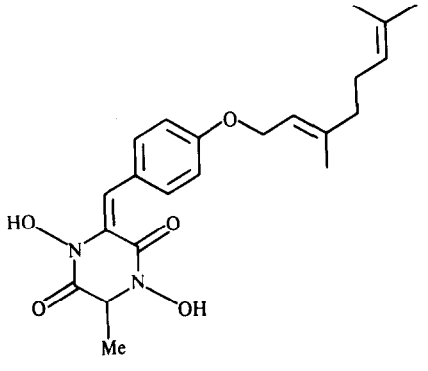

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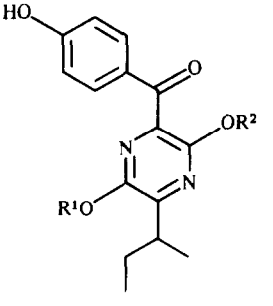

6

(R^1 = H, R^2 = Me or R^1 = Me, R^2 = H)

The carbon signal at δ 144.17 in **1**, which was assigned to the carbon bearing an oxygen function in the pyrazinone ring, was observed to be changed from a multiplet into a triplet by selective irradiation of the methoxy protons (δ 3.92). This fact confirmed that the methoxy group is located at C-5 and therefore that the structure of emeheterone **(1)** is 3,6-bis (phenylmethyl)-1-hydroxy-5-methoxy-2(1*H*)-pyrazinone.

Several pyrazinones related to aspergillic acid **(4)** [6] have been isolated from fungi, mainly from *Aspergillus* spp., but only three pyrazinones derived originally from aromatic amino acids have been reported: Mycelianamide **(5)** and septorine **(6)** were isolated from *Penicillium griseofulvum* Dierckx [7] and *Septoria nodorum* Berk [8], respectively. Astechrome, the iron complex of **3**, was isolated from *Aspergillus terreus* Thom [5]. Emeheterone **(1)** is the first example of a pyrazinone which has been derived from two aromatic amino acid residues.

EXPERIMENTAL

General. Mps: uncorr. Low pressure LC (LPLC) was performed on a Chemco Low-Prep 81-M-2 in a glass column (200 × 10 mm) packed with silica gel CQ-3 (30–50 μ; Wako).

Isolation of emeheterone **(1)**. *Emericella heterothallica*, strain ATCC 16824, was cultivated at 27° for 21 days in Czapek–Dox medium. The culture filtrate (50 l) was extracted with CH_2Cl_2 at pH 2, and the organic layer was dried (Na_2SO_4) and evapd. The residue (9.9 g) was chromatographed on silica gel with $CHCl_3$–MeOH (100:1) followed by LPLC using $CHCl_3$ to give emeheterone **(1)** (50 mg), and with $CHCl_3$–MeOH (50:1) followed by LPLC using C_6H_6–Me_2CO (50:1) to obtain stellatin **(2)** (200 mg).

Emeheterone **(1)**. Needles (C_6H_6); mp 215–217°; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (CON); UV λ_{max}^{MeOH} nm (log ε): 228 (4.45), 276 (3.84), 334 (sh) (3.96), 352 (3.99); EIMS (probe) 70 eV, *m/z* (rel. int.): 322 $[M]^+$ (2), 305 $[M-OH]^+$ (100), 290 (16), 91

$[C_6H_5CH_2]^+$ (91); CIMS (*iso*-butane, probe) 200 eV, *m/z* (rel. int.): 323 $[M+1]^+$ (39), 307 (100); (Found: C, 70.7; H, 5.6; N, 8.6. Calc. for $C_{19}H_{18}O_3N_2$: C, 70.8; H, 5.6; N, 8.7 %); ^1H NMR (99.60 MHz, $CDCl_3$, TMS as int. std): δ3.73 (1H, *s*, OH), 3.92 (3H, *s*, OMe), 3.93 (2H, *br s*, CH_2), 4.20 (2H, *br s*, CH_2), 7.20–7.40 (10H, *m*, aromatic H); ^{13}C NMR (100.40 MHz, $CDCl_3$,TMS): δ 30.39 (Tt), 34.01 (Tt), 61.73 (Q), 126.83 (2C, Dt), 127.64 (2C, Dt), 128.49 (2C, Dd), 129.10 (2C, Dd), 129.43 (St), 129.57 (2C, Dm), 135.68 (Sm), 136.47 (Sm), 140.60 (St), 144.17 (Sm), 158.13 (St).

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^{13}C NMR SPECTRA OF 4-KETO STEROIDAL ALKALOIDS

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Key Word Index—Alkaloids; ^{13}C NMR; 4-keto-steroidal alkaloids; solaphyllidine; deacetylsolaphyllidine; deacetoxysolaphyllidine; solamaladine; dihydrosolaphyllidine.

Abstract—Natural abundance ^{13}C NMR spectra were recorded of four naturally occurring 4-keto steroidal alkaloids, their acetylated derivatives, and of dihydrosolaphyllidine. The assignments were made with the help of APT spectra, based on chemical shifts arguments, and by comparison with the values reported for other steroidal alkaloids.

INTRODUCTION

As part of our investigation of the Andean flora several 4-keto steroidal alkaloids have been isolated [1–4]. To the best of our knowledge the literature lacks information on the ^{13}C NMR spectra of such compounds. This paper reports the ^{13}C NMR chemical shift assignments of solaphyllidine (**1a**), deacetylsolaphyllidine (**1b**), deacetoxysolaphyllidine (**2a**), solamaladine (**3a**), their acetylated derivatives (**1c**, **2b**, **3b**, **4**), and dihydrosolaphyllidine (**5**).

RESULTS AND DISCUSSION

The chemical shifts of the various carbon resonances for compounds **1**–**5** are listed in Table 1. The ^{13}C NMR resonances have been assigned on the basis of chemical shift theory and comparison with the published assignments of dihydro-25-isosolafloridine, and dihydrosolacongestidine [5]. Discrimination among carbon types was performed by direct comparison of Proton Noise Decoupled and APT spectra [6].

In these types of compounds quaternary carbons (C-10 and C-13) are easily distinguished from methylenes by their lower intensity in APT conditions. The resonance of carbon C-13 is affected by substitution at C-16. The presence of a 16 α-acetoxy group as in **1a** and **5** produces a paramagnetic shift of 1.2 ppm, while compounds with no substituent at C-16 (**2a**, **2b**, **3a**, **3b**, and **4**) show the C-13 signal at δ 42.5.

The comparison of the quaternary carbon signals in all alkaloids containing the 3-hydroxy-4-keto moiety (**1a**, **2a**, and **3a**) permitted the assignment of the signal at δ 43.1 to C-10. In the case of **1b** the small difference (0.8 ppm) could be attributed to a solvent effect. Acetylation of the 3β-hydroxy group produces a small diamagnetic shift in all cases (**1c**, **2b**, **3b**, and **4**). The presence of the carbonyl at C-4 is responsible for shifting the C-10 resonance about 7 ppm downfield from its normal range [5]. When the carbonyl is reduced, as in dihydrosolaphyllidine (**5**), C-10 appears at δ 35.5.

The most shielded methine is C-25 on the side chain. The resonance of C-25 appears at δ 30–31 in all com-