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Short Note

3-(1-Ethylamino-ethylidene)-1-methyl-pyrrolidine-2,4-dione

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Abstract: 3-(1-Ethylamino-ethylidene)-1-methyl-pyrrolidine-2,4-dione was obtained as an unexpected product in a three-step synthesis starting with *o*-nitrobenzoyl sarcosine, acetoacetanilide and ethylamine. The compound showed moderate antibacterial activity against *S. aureus* and *E. coli*.

Keywords: tetramic acid; enamine; pyrrolidine-2,4-dione; antibacterial

1. Introduction

The pyrrolidine-2,4-diones, also known as tetramic acids, and their 3-acyl derivatives in particular, are a large group of naturally occurring compounds [1,2]. Although the spectrum of biological activities shown by these compounds is quite broad, the most notable representatives of this group are known for their antimicrobial and antifungal properties [3,4]. Compounds such as equisetin [5], trichosetin [6], reutericyclin [7] and magnesidin [8] are effective inhibitors of *S. aureus* and other Gram-positive bacteria. Of particular interest are synthetic analogs effective against methicillin-resistant *S. aureus* [9]. The enamines of 3-acyltetramic acids are much less abundant in nature [10,11] but are nevertheless of interest as potentially bioactive aza-analogues of the 3-acyltetramic acids [12,13]. Some synthetic enaminotetramic derivatives are reported to exhibit an interesting combination of herbicidal and antifungal activities [14].

2. Results

We have previously demonstrated a novel synthesis of 1-alkoxycarbonyl-3-enaminotetramic acid derivatives, based on α-C-acylation of β-enamino amides with *N*-protected α-amino acids [15]. Currently, while working on a methodologically related approach towards benzodiazepine enamines, we came across an unexpected formation of a hitherto unknown 1-methyl-3-enamino tetramic derivative **4**. At the start, we prepared the intermediate **3**, following our well-established procedure for acylation of β-enamino amides with *N*-protected amino acids [15]. In this particular case, compound **3** was obtained in a 85% yield by acylation of enamino amide **1** with *o*-nitrobenzoylsarcosine **2**, using the mixed carbonic anhydride method (Scheme 1).

Compound **3** was expected to undergo a decarbamoylative C-C bond cleavage [16] in the next step of our planned synthesis—a reactivity observed by us in other derivatives of this type possessing a tertiary nitrogen in their amino acid moiety (Scheme 2). Here, however, the *o*-nitrobenzoyl group proved unstable under the reaction conditions (H_3PO_4 at 60 °C), and compound **3** underwent a cyclisation to the tetramic derivative **4** as a single product—a reactivity common for analogous compounds with secondary nitrogen in the amino acid residue [15]. Compound **4** was isolated in an 82% yield after column chromatography. Although unexpected, this result indicates a possible extension of our previously published procedure, giving access to enaminotetramic derivatives with methyl, rather than an alkoxycarbonyl substituent, at the *N*-atom of the tetramic ring.

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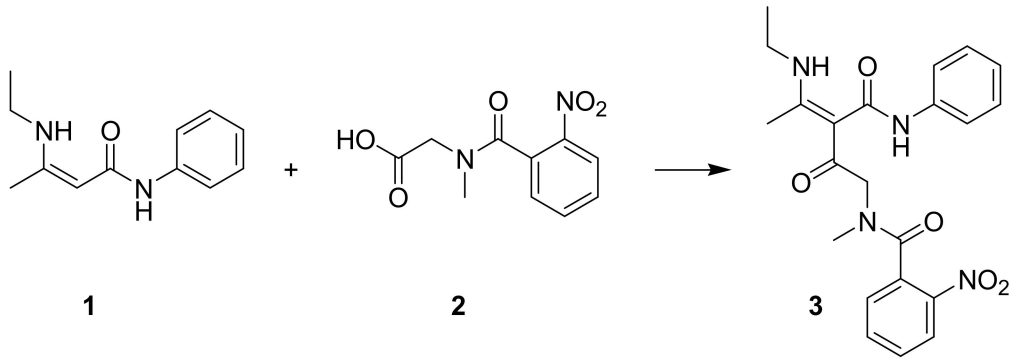
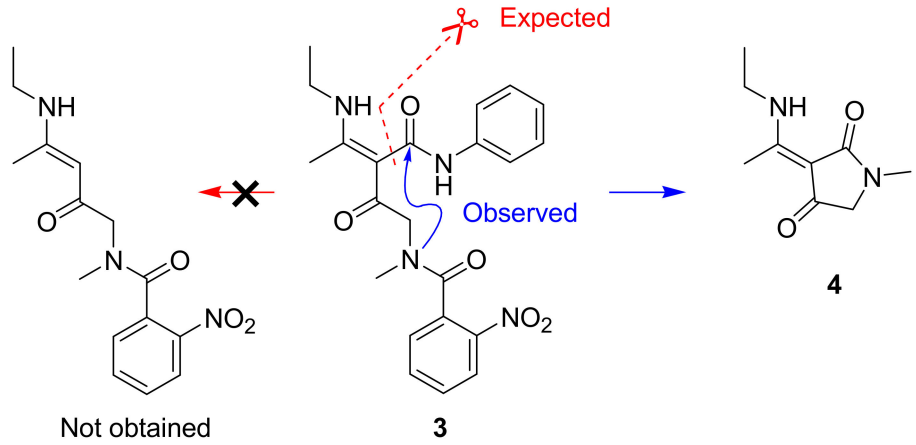


Scheme 1. Reagents and conditions: **2**, *N*-methylmorpholine, EtOCOCl, 5 min at 0–5 °C in CH_2Cl_2. Then, **1**, DMAP (0.2 equiv.) in CH_2Cl_2, 1 h at r.t.



Scheme 2. Reagents and conditions: neat H_3PO_4, 60 °C, 30 min.

The unexpected product 3-(1-Ethylamino-ethylidene)-1-methyl-pyrrolidine-2,4-dione (**4**) showed NMR spectral characteristics typical for the enamines of 3-acyltetramic acids, which are known to equilibrate between the *Z* and *E* forms in solution [13,17,18]. Interestingly, in the case of compound **4**, clearly separated NMR signals of the different π-diastereomers were observed in DMSO-d_6, but not in $CDCl_3$ (see Supplementary Materials).

Even though compound **4** was obtained as an unexpected product, it was interesting to test its antibacterial activity in comparison with similar enaminotetramic derivatives, obtained by us earlier [15]. The antimicrobial bioassays of **4**, along with two structural analogs, were conducted against *S. aureus* and *E. coli*, using the hole plate method in Mueller–Hinton agar with 100 µL loading of 2 mg/mL solutions in DMSO. While the *N*-alkoxycarbonyl analogs were completely inactive, compound **4** showed moderate antibacterial activity against both strains, with inhibition zones of 17 mm and 15 mm for *E. coli* and *S. aureus*, respectively (Table 1).

Table 1. Antibacterial activity of compound **4** (R = CH_3) and *N*-alkoxycarbonyl analogs.

General Formula	R	Inhibition Zone (mm)	
		E. coli ATCC 25922	*S. aureus* ATCC 25923
	CH_3	17	15
	$COOCH_2CH_3$	-	-
	$COOCH_2CCl_3$	-	-

3. Materials and Methods

All reagents and solvents were purchased from Sigma-Aldrich, Darmstadt, Germany and were used as supplied. NMR spectra were run on a Bruker Avance AV600 (600/150 MHz $^{1}H/^{13}C$) or Bruker NEO 400 (400/100 MHz $^{1}H/^{13}C$) spectrometer at BAS-IOCCP, Sofia. Chemical shifts (δ, ppm) are downfield from TMS. IR spectra were measured on a Bruker Alpha II FT IR spectrometer. High resolution mass spectral measurements were performed on a Thermo Scientific Q Exactive hybrid quadrupole-orbitrap mass spectrometer. TLC was done on aluminum-backed Silica gel 60 sheets (Merck, Rahway, NJ, USA) with $KMnO_4$ staining; Melting point measurement was done on a Boëtius hot stage apparatus and is not corrected.

Synthesis of 3-Ethylamino-but-2-enoic acid phenylamide (**1**)*:* Ethylamine (70% aq. solution, 1.6 mL, 20 mmol) was added to a solution of acetoacetanilide (3.54 g, 20 mmol) in 50 mL DCM. The mixture was stirred intensely in a sealed flask for 2 h; then, anhydrous sodium sulfate was added, and the stirring was continued overnight at r.t. Then, the sulfate was filtered off, and the solvent was evaporated under reduced pressure. The oily residue crystallized upon trituration with a small amount of diethyl ether to give the β-enamino amide **1** as a white solid in a nearly quantitative yield (3.85 g, 95%), m.p. 90–92 °C. ^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 1,24 (t, *J* = 7.0, 3H), 1.95 (s, 3H), 3.27 (m, 2H), 4.43 (s, 1H), 6.65 (br s, 1H), 7.03–7.46 (m, 5H), 9.16 (br s, 1H); ^{13}C-NMR ($CDCl_3$, δ ppm): 15.8, 19.5, 37.7, 84.4, 119.7, 122.8, 128.9, 139.3, 160.4, 169.2; IR (KBr, cm^{-1}): 3298, 3130, 3061, 2997, 1631, 1608, 1593, 1524; HRMS *m*/*z* (ES+): calcd. for $C_{12}H_{16}N_2NaO^+$ $[M + Na]^+$ 227.1155, found 227.1157.

Synthesis of N-(4-Ethylamino-2-oxo-3-phenylcarbamoyl-pent-3-enyl)-N-methyl-2-nitro-benzamide (**3**)*:* N-methylmorpholine (3 mmol, 0.33 mL) was added to a magnetically stirred suspension of *o*-nitrobenzoylsarcosine **2** (715 mg, 3 mmol) in CH_2Cl_2 (15 mL). The resulting solution was then cooled in an ice bath, and ethyl chloroformate (3 mmol, 0.3 mL) was added dropwise. The mixture was left to stir for 5 min, and after that, a solution of enamino amide **1** (613 mg, 3 mmol) and DMAP (73 mg, 0.6 mmol) in CH_2Cl_2 (45 mL) was added in one go. The ice bath was then removed, the reaction mixture was allowed to warp up to r.t. and was left to stir for one more hour. The reaction mixture was then transferred to a separatory funnel with 20 more mL of CH_2Cl_2 and washed with aqueous (10:1) HCl. The aqueous layer was extracted with 20 more mL of CH_2Cl_2, the combined organic layers were dried with anhydrous sodium sulfate, the drying agent was removed by filtration, and the solvent was distilled off. The crude α-C-acylated product **3** crystallized upon trituration with diethyl ether. Yield: 1.08 g (85%), yellow solid, m.p. 151–153 °C. ^{1}H-NMR (DMSO-*d6*, δ ppm, *J* Hz): 1.21 (t, *J* = 7.0, 3H), 2.02/2.08 (s, 3H), 2.80/2.95 (s, 3H), 3.41 (m, 2H), 4.02/4.39 (s, 2H), 7.01–8.20 (m, 9H, Aryl), 10.05/10.27 (s, 1H, NH), 11.44/11.53 (t, *J* = 5.6, 1H, NH); ^{13}C-NMR (DMSO-*d6*, δ ppm): 15.4/15.6 ($CH_2\underline{C}H_3$), 16.5/16.7 (=$C\underline{C}H_3$), 33.9/37.9 ($N\underline{C}H_3$), 38.1/38.2 ($NH\underline{C}H_2CH_3$), 53.0/56.2 ($CO\underline{C}H_2N$), 106.9/107.0 (C), 119.6, 123.8, 125.1, 128.4, 128.8, 129.1, 129.2, 130.7, 132.8, 133.2, 134.9, 135.3, 139.7, 140.0, 145.5, 145.9 (Aryl C, CH), 164.3/165.0 (CO), 167.4 (<u>C</u>=C-CO) 167.6/167.9 (CO), 186.8/187.4 (CO). Some signals are doubled because of rotamers. IR (KBr, cm^{-1}): 3283, 3060, 3037, 2977, 2936, 2877, 1637, 1595, 1578, 1529, 1347. HRMS *m*/*z* (ES+): calcd. for $C_{22}H_{24}N_4NaO_5^+$ $[M + Na]^+$ 447.1639, found 447.1636.

*Synthesis of 3-(1-Ethylamino-ethylidene)-1-methyl-pyrrolidine-2,4-dione (***4***):* Compound **3** (424 mg, 1 mmol) was mixed with anhydrous ortho-phosphoric acid (H_3PO_4, 5 g) in a glass vial and was stirred intensely at 60 °C until full homogenization. Then, the stirring was continued for 30 more min. at the same temperature. The mixture was then diluted with cold water to 60–70 mL and extracted twice with CH_2Cl_2 (2 × 40 mL). The combined organic layers were dried with Na_2SO_4, the solvent was removed on a rotary evaporator, and the residue was purified by column chromatography on silica gel with diethyl ether as the eluent (increasing polarity with methanol to Et_2O: MeOH = 20:1 by volume). Compound **4** was isolated as a colorless oil (150 mg, 82%). ^{1}H-NMR (DMSO-*d6*, δ ppm, *J* Hz): 1.17/1.18 (t, *J* = 7.0, 3H, $NHCH_2\underline{C}H_3$), 2.47/2.50 (s, 3H, =$C\underline{C}H_3$), 2.80/2.81 (s, 3H, $N\underline{C}H_3$), 3.41 (m, 2H, $NH\underline{C}H_2CH_3$), 3.54/3.61 (s, 2H, $N\underline{C}H_2CO$), 10.36/10.67 (br s, 1H, $\underline{NH}CH_2CH_3$); ^{13}C-

NMR (DMSO-*d*6, δ ppm): 12.7/13.8 (CH_3), 14.9/15.0 (CH_3), 28.2/28.6 (CH_3), 37.2/37.5 (CH_2), 55.2/56.8 (CH_2), 94.8/96.6 (C=C), 167.0/167.6 (C=C), 170.4/173.4 (C=O), 191.7/194.7 (C=O); All signals are doubled because of a *Z/E* equilibrium. HRMS *m/z* (ES+): calcd. for $C_9H_{14}N_2NaO_2^+$ $[M + Na]^+$ 205.0947, found 205.0949.

Supplementary Materials: The spectral data can be downloaded online.

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Conflicts of Interest: The authors declare no conflict of interest.

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