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Synthesis, structure and antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione

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ABSTRACT

Cyclodepsipeptides are known to exhibit a broad spectrum of biological activities and present a great potential for pharmacological application. A novel didepsipeptide member of the family, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione, was synthesized and its structure was confirmed by IR, ^{1}H and ^{13}C NMR spectral data. The structure and relative stability of the diastereoisomers, tautomers and anionic derivatives of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione were studied by DFT. Different conditions were considered by calculations in gas phase at the B3LYP/6-311++G** level and in polar medium utilizing PCM methods at the same level of theory. In all cases the keto forms were found to be more stable and this form should be expected to exist in real systems. Experimental evidence for this statement was found by the IR spectra measured in KBr, polar and nonpolar solvent. The IR spectroscopy was employed to monitor the formation of anion derivative of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione. The corresponding spectral and structural changes, accompanying the molecule → anion conversion were studied by IR spectra and DFT calculations. The 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione showed antimicrobial activity against four of five tested bacterial strains, being the most effective against *Escherichia coli*.

1. Introduction

Cyclodepsipeptides are known to exhibit a broad spectrum of biological activities [1–3]. In this way, they present a great potential for pharmacological application [3,4] yet demonstrated by the clinical trials of Kahalalide [5,6], Romidepsin [7,8], Aplidin [9], PF1022A and Emodepside [10,11], and the laboratory testing of many other compounds [1–4].

Among the large family of cyclodepsipeptides, the simplest members are the cyclodidepsipeptides which have an ester group and an amide group in the same 6-membered ring. They contain only one residue of amino acid and one residue of lactic, α-hydroxyisovaleric or other α-hydroxy acid. Some reports on their immunomodulating [12], anticoagulant [13], and inhibitory activity towards acyl-CoA:cholesterol acyltransferase [14] and α-glucosidase [15–17], were published. However, an extensive study on their structure and biological activity was not carried out until now. In this regard, detailed studies on the conformation and size of the depsipeptide cycle, electronic structure properties, tautomerism, hydrogen bonding and their effect on the biological activities of cyclodidepsipeptide will help to characterize these compounds in view of their pharmacological application.

Many studies relate the mechanism of the biological action of cyclodepsipeptides to their ionophoric properties *i.e.* to the interaction with metal ions [18–23]. The ability to bind to metal ions is important also in view of the interaction of the bioactive compounds with catalytic active sites of metallo-enzymes [24,25], and DNA coordination [26]. On the other side, biological activity of heterocycles depends on prototropic tautomerization [27–30]. All these points should be considered in the characterization of cyclodepsipeptides in order to gain better insight into their biological action and the molecular features governing the biochemical processes involved.

In our previous paper, we reported that three cyclodidepsipeptides containing residues of α-hydroxyisovaleric acid and valine, leucine and isoleucine were found for the first time in natural products, as potential precursors of enniatin B in the pathogenic fungi *Fusarium sporotrichioides*, isolated from the stem of fresh *Hypericum barbatum* Jacq. For identification and confirmation, those compounds were synthesized and studied by IR and DFT methods [31].

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The present work is focused on the synthesis, structure and antimicrobial activity of a novel cyclodidepsipeptide, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione (**5a–6d**, Scheme 1), containing an alanine moiety. It is structurally related to the three abovementioned cyclodidepsipeptides, isolated from *Fusarium sporotrichioides*. Its structure was confirmed by IR, ^{1}H and ^{13}C NMR spectral data. DFT computational methods and experimental IR spectral techniques were employed to investigate the preferred conformations of different diastereomeric structures and the prototropic tautomerism of the compound. The interaction of the cyclodidepsipeptide with sodium ion resulting in the formation of respective anion was monitored by IR spectra in solution. The changes arising from the ion formation were described in terms of structural variations, electron charge distribution over molecular fragments, and IR frequency shifting based on the experimental and theoretical data. The *in vitro* antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione was tested against five bacterial strains.

2. Experimental

2.1. Synthesis

The title compound was synthesized according to the general procedure reported by us earlier [31]. The reaction conditions were slightly modified (Scheme 1) which allowed us to isolate and characterize the noncyclic precursor **3**, as well as to obtain the cyclodidepsipeptide product **5** with higher yields.

The structures of both compounds were confirmed by IR, ^{1}H and ^{13}C NMR spectral data. The ^{1}H spectra indicated the presence of a mixture of two diastereomes in a 3:2 ratio for **3** and in a 4:3 ratio for **5**, respectively. It was previously reported that synthesis of optically active morpholine-2,5-diones, via N-(α-halogenoacyl)-α-amino acids, may result in racemic product [32, and references therein]. During cyclization the stereogenic center adjacent to the halogen atom is involved leading to racemization at C^{6} of the heterocycle [32]. In our case, diastereomeric mixture is present for both the 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5** and its noncyclic precursor **3**. It is an indication that the racemization occurred in a preceding synthetic step – the introduction of the bromine atom in the isovaleryl chloride.

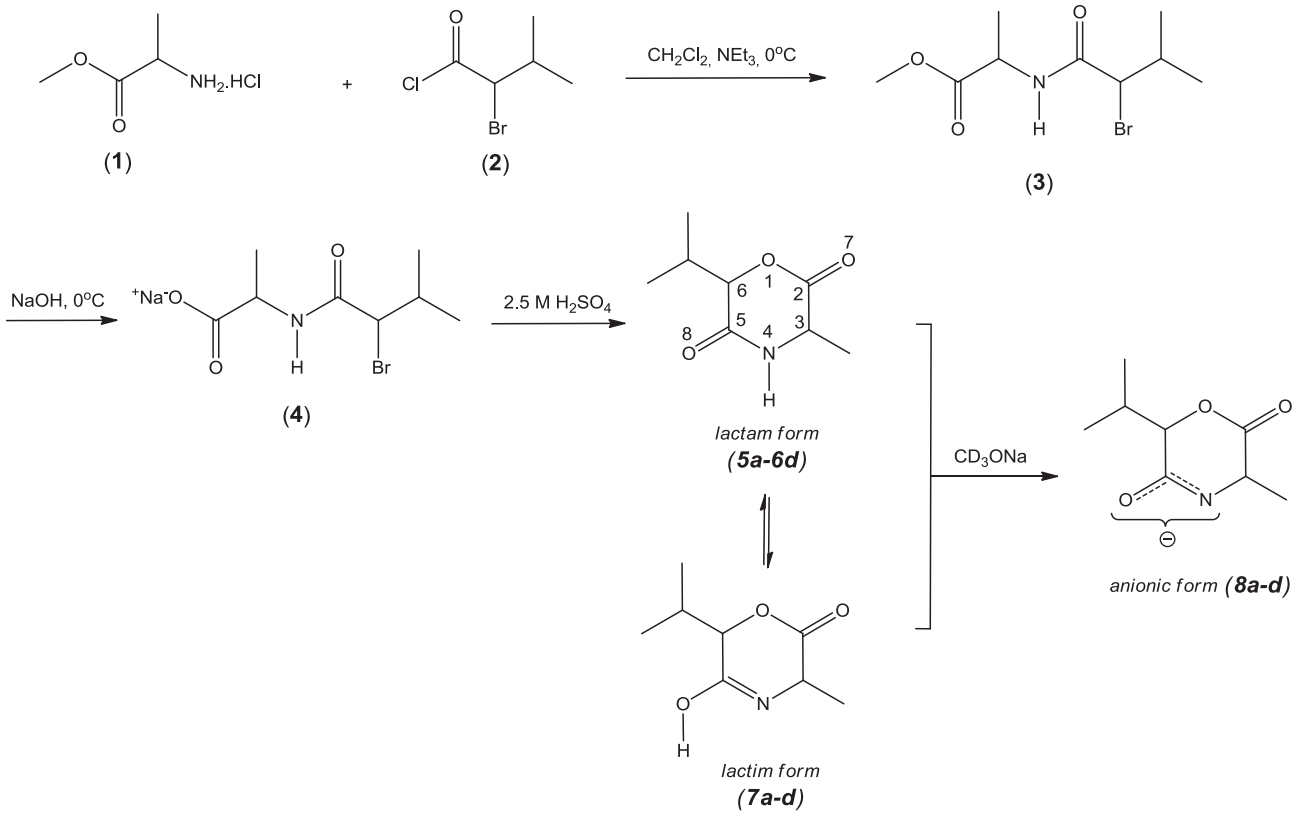
2.1.1. Synthesis of noncyclic methyl 2-(2-bromo-3-methylbutanamido)propanoate (**3**)

L-Alanine methyl ester hydrochloride **1** (0.002 mol) was dissolved in 25 ml dry dichloromethane and 0.006 mol of triethylamine was added. The solution was cooled in ice bath, and 0.003 mol of 2-bromoisovaleryl chloride **2** was added dropwise. The mixture was stirred for 2 h, and then the temperature was allowed to rise to RT. The reaction mixture is washed by 0.5 M HCl, 10% $NaHCO_3$ and brine. The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude methyl 2-(2-bromo-3-methylbutanamido)propanoate **3** was recrystallized from water–methanol mixture (1:4) and light yellow crystals as needles were obtained.

Methyl 2-(2-bromo-3-methylbutanamido)propanoate (**3**): $C_9H_{16}BrNO_3$, M = 266.13; yield = 75%; m.p. 58–59 °C; IR (KBr), cm^{-1}: 3294, 3076, 2994, 2967, 2935, 2874, 1745, 1652, 1549, 1452, 1437, 1380, 1342, 1320, 1283, 1226, 1191, 1149, 1115, 1072, 1057, 999, 979, 932, 859, 773, 767, 731, 622, 521.

The 1H spectra indicated the presence of a mixture of two diastereomes in a 3:2 ratio.

2.1.1.1. Major isomer. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H = 7.10 (1H, br s, NH); 4.58 (1H, m, NCH); 4.33 (1H, d, J = 5.2, CHBr); 3.78 (3H, s, OCH_3); 2.41 (1H, m, $CHMe_2$); 1.48 (3H, d, J = 7.2, $NCHC\underline{H}_3$); 1.09



Scheme 1. Synthesis of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione and its anion.