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Synthesis, mass spectral characterization, NMR analyses, and DFT calculations of 1-desoxymaquindox and 4-desoxymaquindox

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ABSTRACT

Maquindox, 3-methyl-2-acetylquinoxaline-1,4-dioxide, is a quinoxaline-N,N-dioxide used in veterinary medicine as a feed additive. 1-Desoxymaquindox and 4-desoxymaquindox, two novel deoxidized metabolites of maquindox are synthesized from their parent drug. This study deals with the structural and spectral properties of the maquindox metabolites by employing experimental and theoretical methods. The investigation, using ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry, shows independent proof of the structures. Gauge-including atomic orbital NMR chemical shifts are calculated for isomeric quinoxaline metabolite pairs and several different parameters (correlation coefficient, mean absolute error, and corrected mean absolute error) are investigated. Comparison of the experimental and calculated ^{1}H and ^{13}C NMR chemical shifts allows the reliable assignment of the isomeric quinoxaline compound pairs.

1. Introduction

Among the various classes of nitrogen-containing heterocyclic compounds, quinoxaline-1,4-dioxide (QdNO) derivatives exhibit notable biological properties, especially antibacterial and antimicrobial ones. Several QdNO derivatives are potent synthetic veterinary drugs widely used at subtherapeutic levels to promote growth and improve the efficiency of animal feed conversion [1]. Maquindox (2-methyl-3-acetylquinoxaline-1,4-dioxide) is one of the new QdNO derivatives by the Lanzhou Institute of Animal Husbandry and Veterinary of the Chinese Academy of Agriculture Sciences and used as an antibacterial for pig treponeme dysentery which is a mucohemorrhagic diarrheal disease caused by *Treponema hyodysenteriae*. Although it has been recognized as a good antibacterial and shows growth enhancing activity, the metabolomics and toxicity of maquindox have not been determined. QdNO derivatives used as veterinary drugs can be easily metabolized by the reduction of one of the N-oxide groups at positions 1 and 4 on the quinoxaline ring in vivo [2]. Over the last two decades, many mono-N-oxides derivatives of this heterocyclic system have been produced and their biological activities have aroused increased interest [3]. In our previous research, the toxicity of QdNO derivatives were determined to be closely associated with the production of its metabolites, especially the deoxidized ones [4]. Therefore, obtaining more detailed information about the isomeric 1- and 4-mono-N-oxides of maquindox is necessary.

Ultrahigh-performance liquid chromatography and electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC/ESI-QTOF-MS) have evolved into powerful modern analytical tools for the identification and structural characterization of drug metabolites in biological matrices because of their superior sensitivity and specificity [5]. UPLC, using a column packed with porous particles about 1.7 μm in size, provides better chromatographic separation with increased sensitivity and resolution much faster than previous methods [6]. The unique feature of ESI-QTOF-MS is its capability to identify families of metabolites using neutral-loss and precursor ion scans [7].

NMR spectroscopy is another efficient and convenient method for determining the structures of complex organic compounds. Nevertheless, even with the development of 1D and 2D NMR techniques, the determination of isomeric structures remained a challenging and vital task in problems related to natural product chemistry, medicinal chemistry, and so on. Therefore, the application of theoretically computed chemical shifts to link experimental data was developed. The technique was pioneered by Bifulco [8,9] and has since played key roles in structural assignment [10].

In this study, 1-desoxymaquindox and 4-desoxymaquindox are synthesized from maquindox and their mass spectral characteristics are determined by UPLC/ESI-QTOF-MS. The structures of the maquindox metabolites are optimized by the density functional theory (DFT) Becke 3-parameter, Lee–Yang–Parr (B3LYP)/

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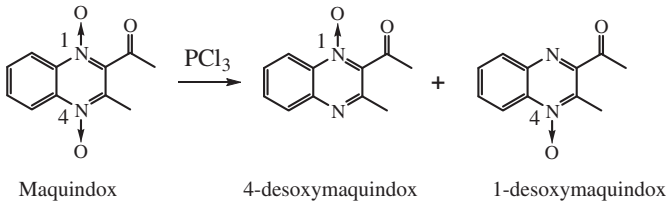

Fig. 1. The reaction and structures of the studied compounds with nitrogen numbering.

6-311++g(2df,2pd) methods and obtained gauge-including atomic orbital (GIAO)-derived ^{1}H and ^{13}C shifts at the same level. Comparison of experimental and calculated NMR chemical shifts provide the reliable assignment of isomeric compound pairs.

2. Experimental

2.1. Methods and calculations

Maquindox (98% purity) was provided by the College of Veterinary Medicine, Huazhong Agricultural University (Wuhan, China). Other chemicals used in the synthesis were purchased from the Guoyao Chemical Co. (Shanghai, China). Acetonitrile (pesticide grade) was purchased from Fisher Chemical Co. (New Jersey, USA). Chromatographic separations were carried out on an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 μm particle size; Waters Co., Milford, MA, USA) using an ACQUITY UPLC™ system (Waters Co.). The UPLC system was coupled to a hybrid SYNAPT™ HDMS™ (Waters, Manchester, UK) system fitted with an electrospray ionization (ESI) source and controlled by MassLynx™ software. ^{1}H and ^{13}C NMR spectra for the $CDCl_3$ solutions were recorded on a Bruker DSX-300 instrument at 100.6 and 40.6 MHz, respectively.

In the UPLC/ESI-QTOF-MS analysis, the column was maintained at 30 °C and the mobile phase consisted of 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B. The gradient elution program used was as follows: 0–2.5 min, linear gradient from 2% to 5% B; 2.5–7.5 min, linear gradient to 30% B; and 7.5–9.0 min, linear gradient to 100% B. For the parent drug, the ESI source was operated in the positive ionization mode with 3.0 kV capillary voltage and data was acquired from 100 to 1000 Da.

For the NMR calculations, the geometries of 1-desoxymaquindox and 4-desoxymaquindox were pre-optimized at B3LYP/6-311++g(2df,2pd). Subsequently, the ^{1}H and ^{13}C NMR chemical shifts were calculated at the same level. All of the calculations were performed with the DFT within the Gaussian 03 code [11]. The hybrid exchange correlation B3LYP [12] functional was used 6-311++g(2df,2pd) basis set. Components of the shielding tensors were computed by the GIAO method [13] with the same DFT function. The relative chemical shifts δ (in ppm) were computed as differences between atomic isotropic shielding and the corresponding reference atoms in tetramethylsilane (TMS). In this study, the calculated ^{1}H and ^{13}C isotropic chemical shielding for TMS at the B3LYP/6-311++g(2df,2pd) were 31.81 and 183.72 ppm, respectively.

2.2. Synthesis

Phosphorus trichloride (7.5 mL, 0.086 mol) was added into a stirred solution of 2.0 g (0.009 mol) maquindox in 30 mL chloroform. The reaction mixture was stirred for 12 h at room temperature, and then poured into an ice bath, after which excess aqueous sodium hydroxide was added to it. The chloroform layer was separated and the aqueous phase was extracted with chloroform three times. The combined chloroform layers were dried with $MgSO_4$ and evaporated to give a residue which was then purified by column chromatography on silica gel. Elution of the column with 1:3 acetic ester-light petroleum (60–90 °C) gave 490 mg of Metabolite I (R_F = 0.52) and 910 mg of Metabolite II (R_F = 0.36). From a study by EI-Abadelap et al., Metabolites I and II are both mono-N-oxides metabolites (1-desoxymaquindox and 4-desoxymaquindox, respectively) of maquindox [14]. The reaction and structures of the studied compounds with nitrogen numbering are illustrated in Fig. 1.

3. Results and discussion

3.1. Mass spectral characteristics of the maquindox metabolites

The mass spectral analysis of the maquindox metabolites was carried out by UPLC/QTOF-MS. The retention times of Metabolites I and II are 7.38 and 6.70 min, respectively. The product ions of the protonated metabolites are shown in Figs. 2 and 3. Both Metabolites I and II have an elemental composition ($[M+H]^+$) of $C_{11}H_{11}N_2O_2^+$, show a protonated molecule at m/z 203, and feature a loss of 16 Da compared to the protonated molecule of maquindox, suggesting that they are N → O group reduction metabolites of the parent compound. The difference between Metabolites I and II is the position of the N → O group in the quinoxaline ring. In terms of the electronic interaction between N → O group at position 1 and the adjacent acetyl group, 4-desoxymaquindox has two competitive fragmentation pathways. One of the fragmentation pathways is the loss of an OH radical to form the product ion

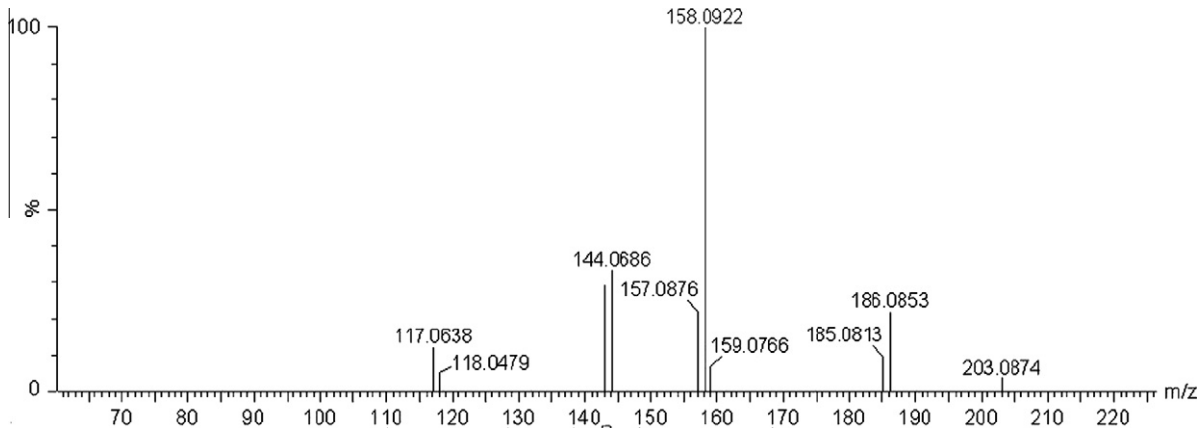

Fig. 2. Accurate MS^2 spectra of Metabolite I of maquindox.