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Original article:

A NEW SULFOXIDE ANALOG OF 1,2,3,6-TETRAHYDROPHENYLPYRIDINE AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

Bioactivities of thiotetrahydropyridines were previously described. Herein, a novel bioactive sulfoxide analog; *N*-acetyl-2-(1-adamantylsulfoxo)-3-acetoxy-4-phenyl-6-hydroxy-1,2,3,6-tetrahydropyridine (**3**) from the deoxydative substitution of 4-phenylpyridine 1-oxide is reported. Its structure was elucidated using spectral data including 2D-NMR, MS, IR and UV. The sulfoxide **3** exhibited antibacterial activity against *Moraxella catarrhalis* and *Streptococcus pyogenes* with minimum inhibitory concentration of 128 and 256 μg/mL, respectively.

Keywords: tetrahydropyridine; sulfoxide; sulfonium ion; 4-phenylpyridine l-oxide; antimicrobial activity

INTRODUCTION

Tetrahydropyridines are a class of compounds that possess a vast array of bioactivities. In particular, synthesis and bioactivities of thiotetrahydropyridines were previously reported (Prachayasittikul et al., 1985, 1991, 2009a). The tetrahydropyridines are *N*-acetyl-1,2,3,4- and 1,2,3,6- isomers (**1** and **2**, respectively) containing 1-adamantylthio and hydroxyl (acetoxyl) groups (Figure 1) (Egan et al., 1969; Kokosa et al., 1975; Hershenson and Bauer, 1969; Prachayasittikul et al., 1985, 1991, 2009a). These tetrahydropyridines were isolated from the deoxydative substitution reaction of pyridine 1-oxides by thiols in the presence or absence of triethylamine (Egan et al., 1969; Kokosa et al., 1975; Hershenson and Bauer, 1969; Prachayasittikul et al., 1985, 1991, 2009a). According to our experience working on such reactions, it occurred in mind that more and new interesting tetrahydropyridines remained to be explored. As part of our continuing study, isolation and characterization of new tetrahydropyridines from 4-phenylpyridine 1-oxide and its antimicrobial activity have been reported.

MATERIAL AND METHODS

General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 300 NMR spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively). Infrared spectra (IR) were obtained on a Perkin Elmer System

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2000 FTIR. Ultraviolet (UV) spectra were recorded on a Milton Roy Spectronic 300 Arrays. Mass spectra were recorded on a Finnigan INCOS 50 and a Bruker Daltonics (micro TOF). Column chromatography was carried out using silica gel 60 (0.063–0.200 mm). Analytical thin layer chromatography (TLC) was performed on silica gel 60 PF_{254} aluminium sheets (cat. No. 7747 E., Merck). Solvents were distilled prior to use. Reagents for cell culture and assays were of analytical grade. All chemicals for reaction were used as supplied. 1-Adamantanethiol (1-AdmSH) was prepared by the literature method (Khullar and Bauer, 1971).

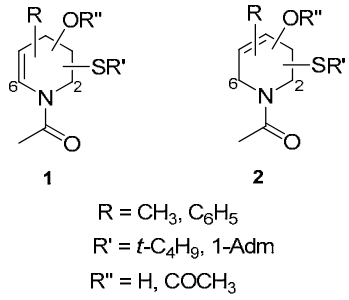

Figure 1: Chemical structures of 1,2,3,4- and 1,2,3,6-tetrahydropyridines

Synthesis of N-acetyl-2-(1-adamantyl-sulfoxo)-3-acetoxy-4-phenyl-6-hydroxy-1,2,3,6-tetrahydropyridine (3)

A solution of 4-phenylpyridine 1-oxide (17.1 g, 0.1 mol) in acetic anhydride (75 mL) containing triethylamine (0.35 mol, 50 mL) was added dropwise (30 min) a solution of 1-AdmSH (16.8 g, 0.1 mol) in acetic anhydride (25 mL). The reaction mixture was heated at 95°C for 3 h, then evaporated *in vacuo*, neutralized with 50 % Na_2CO_3 and extracted with toluene (3 × 100 mL). After washing with water (50 mL), dried (anh. Na_2SO_4), the solvent was removed *in vacuo* to give a dark-brown solid (55 g). The solid was chromatographed on a silica gel (500 g) column. Elution with petroleum ether : toluene (1:1, 1.5 L) then toluene: $CHCl_3$ (4:1, 11 L) gave a mixture of 1-Adm$SCOCH_3$ (14.3 g) and pyridyl sulfides (10.6 g). Further elution with $CHCl_3$ (7 L) gave a mixture of tetrahydropyridines which was rechromatographed on the silica gel (120 g) to yield 3.2 g solid of tetrahydropyridines from petroleum ether: $CHCl_3$ (1:1, 1.1 L). The 3.2 g solid was re-isolated on the silica gel (120 g) column to afford tetrahydropyridine 2.3 g from $CHCl_3$ (4.5 L). The product was recrystallized from ether to provide 0.55 g of *N*-acetyl-2-(1-adamantylsulfoxo)-3-acetoxy-4-phenyl-6-hydroxy-1,2,3,6-tetrahydropyridine (**3**); mp 158-159 °C, UV (95 % ethanol) λ_{max} (log ε) 205 (4.31) and 245 (4.02) nm, IR (UATR) 3257, 2909, 2852, 1741, 1672, 1498, 1448, 1390, 1370, 1299, 1217, 1067, 1013, 987 cm^{-1} , ^{1}H-NMR (300 MHz, $CDC1_3$): δ = 1.55, 1.62, 1.82, 1.85, 2.07, (5s, 15H, 1-Adm*H*), 1.85 (s, 3H, NCOC*H*$_3$), 2.33 (s, 3H, OCOC*H*$_3$), 5.50-5.56 (m, 2H, H-3, H-6), 5.63 (d, J = 11.4 Hz, 1H, OH), 6.28 (d, J = 2.1 Hz, 1H, H-2), 6.35 (d, J = 3.0 Hz, 1H, H-5), 7.25 (s, 5H, Ph*H*). ^{13}C-NMR (75 MHz, $CDC1_3$) : δ = 20.8 ($NCOCH_3$), 21.7 ($OCOCH_3$), 29.3 (3 × CH-1-Adm), 35.0, 36.0 (6 × CH_2-1-Adm), 57.8 (Cqua-1-Adm), 61.8 (C-2), 67.7 (C-3), 73.7 (C-6), 125.6, 129.0, 136.6 (Ph-C), 132.1 (C-5), 136.6 (C-4), 170.5 ($NCOCH_3$), 172.7 ($OCOCH_3$). LRMS (EI):m/z (%) = 457 (6.30) $[M - H]^+$, 456 (30.56), 427 (17.50), 426 (18.03), 348 (14.67), 256 (25.52), 215 (24.24), 214 (100.00), 172 (12.92), 156 (13.75), 135 (3.03). HRMS (TOF): m/z $[M+Na]^+$ calcd for $C_{25}H_{31}NSO_5Na$: 480.1815; found : 480.1815.

Antimicrobial assay

Antimicrobial activity of the tested compound was performed using agar dilution method as previously described (Prachayasittikul et al., 2009b). Briefly, the tested compound dissolved in DMSO was individually mixed with 1 mL Müller Hinton (MH) broth. The solution was then transferred to the MH agar solution to yield the final concentrations of 64-256 μg/mL. Twenty-one strains of microorganisms, cultured in MH broth at 37 °C for 18-24 h, were diluted with 0.9 % normal saline solution to adjust the cell density to 1×10^8 cells/mL compared with 0.5 McFarland. The organisms were inoculated onto each plate and further incubated at 37 °C for 24-

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48 h. Compounds which possessed high efficacy to inhibit bacterial cell growth were analyzed.

RESULTS AND DISCUSSIONS

Chemistry

Reaction of 4-phenylpyridine 1-oxide with 1-AdmSH in acetic anhydride containing triethylamine was performed. The products were isolated by repeated silica gel column chromatography to give first 1-Adm$SCOCH_3$ (Prachayasittikul and Bauer, 1985) and a mixture of α- and β-pyridyl sulfides from toluene : $CHCl_3$ (4:1 elution) (Prachayasittikul et al., 1991). From more polar $CHCl_3$ elution afforded a mixture of tetrahydropyridines (based on ^{1}H-NMR spectra). Upon extensive rechromatographic separation, a new tetrahydropyridine (**3**) was found as a crystalline solid of m.p. 158-159°C. Structure of compound **3** was elucidated using 2D-NMR; ^{1}H- and ^{13}C-NMR, LRMS, HRMS, IR and UV spectra. Its IR spectra showed the presence of OH group (3257 cm^{-1}), strong ester and amide CO at 1741 and 1672 cm^{-1}, respectively. The very strong S = O moiety was observed at 1013 cm^{-1}. The UV spectra displayed λ_{max} at 245 nm indicating an unconjugated alkene amide of 1,2,3,6-tetrahydropyridine (Prachayasittikul et al., 1991, 2009a).

^{1}H-NMR spectra showed δ 6.28 of H-2 as a doublet with a coupling constant (J) of 2.1 Hz. In addition, H-3 and H-6 were a multiplet at δ 5.50-5.56, H-5 at δ 6.35 was noted as a doublet with the J value of 3.0 Hz. The signal at δ 5.63 as a doublet was collapsed by D_2O exchange, suggesting the presence of hydroxyl group at C-6 with the large J value of 11.4 Hz. The stereochemistry at C-2 and C-3 was assigned using the J value between H-2 and H-3 and the Karplus relationship. The coupling constant of 2.1 Hz suggested that the H-2 and H-3 are *trans*-quasidiequatorial. Hence, the sulfoxide at C-2 and acetate at C-3 are *trans*-quasidiaxial. Comparision with the well established tetrahydropyridine (Prachayasittikul et al., 1985, 1991, 2009a; Egan et al., 1969), the OH group at C-6 is assigned to have the same stereochemistry as C-2. The two substituents at C-2 and C-6 are *cis*-quasidiaxial in twist chair form. Furthermore, the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) experiment was also employed to confirm the assignments of protons in the sulfoxide **3** through a long-range coupling of protons and carbons (Table 1). The results showed the correlations between H2 with C-3, C-6, C-4, $NCOCH_3$ and quaternary carbon of 1-Adm; H3 with Ph-C-1′ and $OCOCH_3$; H5 with C-3 and Ph-C-1′ and H6 with C-4. Mass spectra showed a low intensity fragment of $[M-H]^+$ with m/z 457 including a base peak at m/z 214 resulting from the loss of 1-AdmSO, CH_3CO and H_2O from the molecule of **3**. The presence of amide and ester carbonyls was confirmed by ^{13}C-NMR showing δ 170.5 and 172.7 ppm, respectively. Based on the spectral data, thus, compound **3** was identified to be the new sulfoxide analog of tetrahydropyridine; *N*-acetyl-2-(1-adamantylsulfoxo)-3-acetoxy-4-phenyl-6-hydroxy-1,2,3,6-tetrahydropyridine. Its molecular formula was confirmed by HRMS as $C_{25}H_{31}NSO_5$.

Analogously, such 1,2,3,6-tetrahydropyridine from 4-phenylpyridine 1-oxide was reported previously (Prachayasittikul et al., 1991), but as a sulfide derivative (**4**). When the reaction was studied without inclusion of triethylamine, 1,2,3,6-tetrahydropyridine (**4a**) was found in which the sulfide group was deposited at C-3 (Prachayasittikul et al., 2009a). The isolated sulfoxide of 1,2,3,6-tetrahydropyridine (**3**) has been proposed to be formed *via* episulfonium ion intermediate (**a**) (Prachayasittikul et al., 1985, 1991, 2009a) to give first sulfide **4**. Finally, oxidation of the sulfide function by acetic anhydride in triethylamine furnished the sulfoxide product **3** (Figure 2).

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Table 1: HMBC spectroscopic data of the sulfoxide **3** in $CDCl_3$

δ_H (ppm)	Carbon correlated with δ_H (ppm)
6.35 (H-5)	67.7 (C-3), 136.6 (Ph-C-1′)
6.28 (H-2)	57.8 (Cqua-1-Adm), 67.7 (C-3), 73.7 (C-6), 132.1 (C-4), 170.5 ($NCOCH_3$)
5.50-5.56 (H-3)	136.6 (Ph-C-1′), 172.7 ($OCOCH_3$)
5.50-5.56 (H-6)	132.1 (C-4)

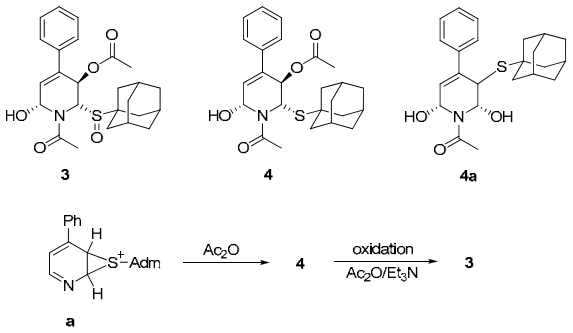

Figure 2: Chemical structure of the sulfoxide **3** and related compounds

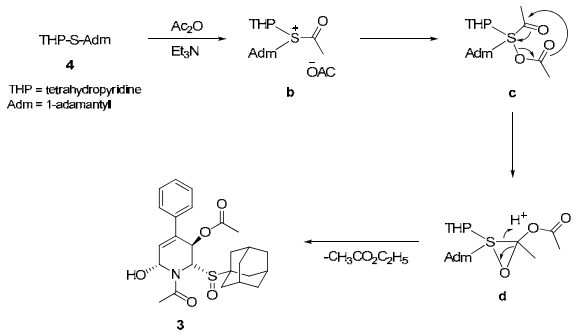

Figure 3: Proposed transformation of sulfide **4** to sulfoxide **3**

Conversion of the sulfide **4** to sulfoxide **3** is likely to occur through *S*-acylation of **4** to give acetylsulfonium salt (**b**) which was attacked by an acetate anion to produce acetoxythiol acetate (**c**). Further intramolecular nucleophilic attack by carbonyl oxygen to afford three membered ring intermediate (**d**) with subsequent cleavage of C-S and C-O bonds to generate the sulfoxide **3** (Figure 3). Oxidation of sulfides to sulfoxides was reviewed in the literatures using a variety of oxidants (Kowalski et al., 2005; Kaczorowska et al., 2005) such as halogen derivatives e.g. molecular halogens and hypervalent iodine (III and IV) reagents (Kowalski et al., 2005) as well as hydrogen peroxide in various solvents and in the presence of catalyst e.g. $TiCl_3$, SeO_2 and TeO_2 (Kaczorowska et al., 2005). This study illustrates the use of Ac_2O/Et_3N as an oxidant converting the sulfide **4** to sulfoxide **3**.

Antimicrobial activity

The sulfoxide **3** was tested for antimicrobial action using the agar dilution method against twenty-one microorganisms (gram-positive and gram-negative bacteria and diploid fungus). It was found that the tetrahydropyridine **3** inhibited the growth of *Moraxella catarrhalis* and *Streptococcus pyogenes* with minimum inhibitory concentration (MIC) of 128 and 256 μg/mL, respectively (Table 2). In addition, the growth of *Corynebacterium diphtheriae* NCTC 10356 was partially inhibited (80 %) at 256 μg/mL. However, the sulfides of 1,2,3,6-tetrahydropyridines (**4** and **4a**) were reported to be an inactive antimicrobial agents (Prachayasittikul et al., 2009a). The result showed that the sulfoxide function provide the compound with bioactivity. This oxidation could possibly be extended to other sulfides in order to improve their bioactivities.

Table 2: Antimicrobial activity[a] of tetrahydropyridines

Compound	Microorganism	Minimum inhibitory concentration (MIC, μg/mL)
3[b]	*M. catarrhalis*	128
	S. pyogenes	256
4[c]	Inactive	—
4a[c]	Inactive	—

[a]Twenty-one strains of tested microorganisms were **gram-negative bacteria**: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Serratia marcescens* ATCC 8100, *Shigella dysenteriae, Salmonella typhi, Vibrio cholerae, Aeromonas hydrophila, Plesiomonas shigelloides, Acinetobacter calcoaceticus, Moraxella catarrhalis and Neisseria mucosa,* **gram-positive bacteria***; Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Corynebacterium diphtheriae* NCTC 10356, *Staphylococcus epidermidis* ATCC 12228*, Streptococcus pyogenes, Enterococcus* group D, *Micrococcus flavus and Listeria monocytogenes and* **diploid fungus***; Candida albicans*. Ampicillin at 10 μg/mL was used as a control of antibacterial testing system; it showed 100 % inhibition on *S. aureus* ATCC 25923*, B. subtilis* ATCC 6633, *S. epidermidis* ATCC 12228, *S. pyogenes* and *M. catarrhalis*. [b]At 256 μg/mL showed partial inhibition on *C. diphtheria* NCTC 10356 (80 %). [c]Prachayasittikul et al., 2009a.

CONCLUSION

The deoxydative substitution of 4-phenylpyridine 1-oxide by 1-AdmSH in acetic anhydride containing triethylamine was reinvestigated to afford the new sulfoxide analog of 1,2,3,6-tetrahydropyridine (**3**) as a unique by-product. The sulfoxide **3** exerted its antibacterial activity against *M. catarrhalis* and *S. pyogenes* with MIC of 128 and 256 μg/mL, respectively. Partial growth inhibition (80 %) of **3** was noted against *C. diphtheriae* NCTC 10356 at 256 μg/mL. This study leads to the discovery of new bioactive sulfoxide of 1,2,3,6-tetrahydropyridine from 4-phenylpyridine 1-oxide.

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