Journal of Organometallic Chemistry 724 (2013) 88–94

Contents lists available at SciVerse ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

A sweeter way to combat *Helicobacter pylori*? Bismuth(III) complexes and oxido-clusters derived from non-nutritive sweeteners and their activity against *H. pylori*

Philip C. Andrews [a,*], Richard L. Ferrero [b], Peter C. Junk [c], Roshani M. Peiris [a]

[a] School of Chemistry, Monash University, P.O. Box 23, Melbourne, Vic. 3800, Australia
[b] Centre for Innate Immunity and Infectious Diseases, Monash Institute of Medical Research, Clayton, Melbourne, Vic., 3168, Australia
[c] School of Pharmacy and Molecular Sciences, James Cook University, Townsville, Qld. 4811, Australia

ARTICLE INFO

Article history:
Received 3 August 2012
Received in revised form
8 October 2012
Accepted 12 October 2012

Keywords:
Bismuth(III)
Non-nutritive sweeteners
Acetosulfame
Cyclamate
Oxido-clusters
H. pylori

ABSTRACT

Eight new homo-and hetero-leptic bismuth(III) complexes and two new polynuclear bismuth(III) oxido clusters derived from acetosulfame (AceH) and cyclamic acid ($CycH_2$) have been synthesised and characterised. Complexes, [Ph2Bi(Ace)] **1**, [Bi(Ace)3] **2**, [$PhBi(Ace)_2$] **3**, [$Bi(CycH)_3$] **6**, [$Ph_2Bi(CycH)$] **7** and [$PhBi(CycH)_2$] **8** were synthesised by treating $BiPh_3$ with the appropriate acid in 1:1, 1:2 and 1:3 stoichiometric ratios under solvent-free or solvent-mediated conditions. Complex **4**, [$Bi(OH)(Ace)_2$], was obtained from the hydrolysis of **3**. [$Bi2(Cyc)_3$] **9** was obtained from the reaction of cyclamic acid with ($Bi(O^tBu)_3$) in a 3:2 ratio under inert conditions. The polynuclear bismuth oxido clusters, [$Bi_{50}O_{64}(Ace)_{22}(H_2O)_{10}$] **5** and [$Bi_{38}O_{45}(CycH)_{24}(H_2O)_{14}$] **10** were obtained using Bi_2O_3 under sonication in water and their composition confirmed through elemental and thermogravimetric analyses. The DMSO soluble complexes, **1**, **2**, **4**, **5**, **6**, **7** and **9**, were all assessed for their in-vitro activity against three strains of *H. pylori* (251, 26695 and B128). All compounds gave an MIC value of 6.25 μg/mL, indicating that bactericidal activity is insensitive to increased substitution by acetosulfamate or cyclamate at the Bi(III) centre.

1. Introduction

Helicobacter pylori, a Gram-negative bacterium found in the stomach, is responsible for gastritis, peptic and duodenal ulcers, and gastric cancers [1–3]. Bismuth compounds have been used to treat gastrointestinal disorders for centuries, even before the link between *H. pylori* and above diseases was established [4]. Triple therapy, a combination therapy of two antibiotics (commonly amoxicillin and clarithromycin) and a proton pump inhibitor (omeprazole), is the regimen most commonly recommended for treatment of *H. pylori* but this has shown a failure rate in more than 20% of patients for many years [5]. The failure is mainly due to increasing antibiotic resistance, especially against clarithromycin [6]. Therapies in which a bismuth compound, usually the *sub*-salicylate or *sub*citrate, is included as part of the treatment regimen have greater success rates since *H. pylori* strains resistant to bismuth compounds have not yet been reported [7]. They also have the advantage of not requiring a neutral stomach pH to be effective [8].

A significant drawback for the bismuth-based therapies is the amount of bismuth which is ingested. While intoxication during treatment is rare and bismuth is considered a relatively safe heavy metal, overdosing and *in vivo* biomethylation can increase the possibility of toxic effects [9]. Greater activities at lower concentrations, combined with benign ligands, are principal targets in the development of new bismuth-based drugs.

Despite their widespread availability and use, still little is known about the biological action of bismuth and its compounds. This, combined with their potential medicinal and antimicrobial applications, warrants continuing research into their synthesis, stability and biological activity. Over the past few years our group has been successful in developing and assessing the activity against *H. pylori* of a range of homo-and heteroleptic bismuth(III) complexes containing different ligands such as aryls, carboxylates, sulfonates, saccharinates and thiosaccharinates [10–14]. The variance in observed activities against the three different bacterial strains indicates that the nature of the ligand(s) is important in moderating the bactericidal activity of the compounds. For example, the inclusion of a single sulfonate, saccharinate or thiosaccharinate ligand onto a *di*-phenylbismuth(III) centre through protolysis reactions with $BiPh_3$, giving compounds of general formula [Ph_2BiL], can magnify the bactericidal properties significantly relative to $BiPh_3$.

As bismuth(III) complexes of saccharin and thiosaccharin showed promising activity against *H. pylori* (Minimum Inhibitory

* Corresponding author.
E-mail address: phil.andrews@monash.edu (P.C. Andrews).

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http://dx.doi.org/10.1016/j.jorganchem.2012.10.024

Concentration; 6.25 μg/mL), we turned our attention to other common non-toxic non-nutritive sweeteners; namely acetosulfame (6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide) and cyclamate (N-cyclohexylsulfamic acid/cyclamic acid).

Acetosulfame bears some structural similarity to saccharin (Fig. 1) and has been used as a non-nutritive sweetener since 1988. The potassium salt can be found in, for example, sweets, soft drinks, cosmetics, and toothpastes [15]. Cyclamate, in the form of its sodium or calcium salt, is used in more than 50 countries in both food and pharmaceuticals [16].

Surprisingly, the metal-organic and organometallic chemistry of acetosulfame and cyclamate is not well developed. Among the reported complexes of acetosulfame many are transition metal complexes involving Pt(II), Ag(I), Cu(II), Pd(II), Co(II) and Zn(II) [15,17–21]. There is no single complex of the p-block metals yet reported. The Ag(I) complex, $[Ag(Ace)]_n$, has shown good activity against *Mycobacterium tuberculosis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* [19]. The Pt(II) complex, $K_2[PtCl_2(Ace)_2]$, has shown some cytotoxicity towards human cervix cancer cells, and has shown good activity against dengue virus type 2 [19]. The complexes of cyclamate are limited only to s-block metals; Na, K, Rb and Ca [16], and to Ag(I). Not surprisingly, the Ag(I) cyclamate complex also shows some activity against *M. tuberculosis* [22].

The acetosulfamate and cyclamate anions have heteroatoms which are capable of forming ionic, covalent or dative bonds with metals, and can thus act as polyfunctional complexing agents. However, crystallographic studies have shown the most common binding mode in acetosulfame is monodentate through the nitrogen atom [15,17,20,21] and in cyclamate through the sulfonyl oxygen atom [16]. The exceptions are the Cu(II), Pt(II) and Ag(I) complexes which have the acetosulfamate ligand binding in a bidentate manner. In the Cu complex it binds through the nitrogen and carbonyl oxygen atoms, whereas in the Pt(II) and Ag(I) complexes it is through the nitrogen and sulfonyl oxygen atoms [19].

In this paper we describe the synthesis and characterisation of ten new homo-and heteroleptic bismuth(III) compounds bearing acetosulfamate and cyclamate ligands; $[Ph_2Bi(Ace)]$ **1**, $[Bi(Ace)_3]$ **2**, $[PhBi(Ace)_2]$ **3**, $[Bi(OH)(Ace)_2]$ **4**, $[Bi(CycH)_3]$ **6**, $[Ph_2Bi(CycH)]$ **7**, $[PhBi(Cyc\text{-}H)_2]$ **8**, $[Bi_2(Cyc)_3]$ **9**, and the polynuclear bismuth-oxido clusters; $[Bi_{50}O_{64}(Ace)_{22}(H_2O)_{10}]$ **5** and $[Bi_{38}O_{45}(CycH)_{24}(H_2O)_{14}]$ **10**. The bactericidal activity of compounds **1**, **2**, **4**–**7** and **9** against *Helicobacter pylori* has been assessed and is reported.

2. Results and discussion

2.1. Acetosulfame

Acetosulfamate complexes of bismuth(III) were synthesised by both solvent-mediated and solvent-free reactions with $BiPh_3$. The solvent-mediated reactions were conducted in ether at room temperature for a period of 4 h or in ethanol at reflux temperature for a period of 8–12 h. The solvent-free reactions involved heating a mixture of the reactants, which had been ground together, to a temperature of 80–90 °C for a period of 30–60 min.

The solvent-free reaction of AceH (pK_a 2.0) [23] with $BiPh_3$, conducted in a 1:1 stoichiometric ratio at 80 °C for 30 min produced the expected *di*-phenyl product, $[Ph_2Bi(Ace)]$ **1**, in 84% yield (Scheme 1). Changing the stoichiometry to 3:1 produced the *tri*-substituted product, $[Bi(Ace)_3]$ **2**, in 86% yield, after heating at 90 °C for 1 h (Scheme 2). In contrast, the 2:1 solvent-free reaction did not give $[PhBi(Ace)_2]$ **3** as a single isolable product, but instead produced a mixture of all three products **1**–**3**. This reaction therefore was carried out under different conditions, varying the time and temperature in an attempt to maximise the yield of **3**. The best conditions were found to be heating at 65 °C for 60 min which gave a ratio of products of 3:1:1 for **3**, **2** and **1** respectively. Heating at this temperature for 2 h gave the least favourable outcome resulting in **3** and **2** in a 1:4 ratio. Since all three compounds proved to be soluble only in DMSO and DMF, and crystallisation did not occur, it was not possible to isolate **3** as a pure compound. Similar behaviour has been previously observed in heteroleptic bismuth saccharinates [14], sulfonates [10] and thiobenzoates [24], where the 2:1 reaction did not yield the expected *mono*-phenyl product.

The 2:1 reaction was therefore attempted under solvent-mediated conditions, changing the solvents and the reaction conditions to attempt to isolate **3** in a pure form. Again, on almost all occasions only a mixture of products **1**–**3** was isolated, with the best ratio being 1:1:3 respectively.

Only in one instance was **3** ever able to be isolated in pure form, when a homogeneous reaction was carried out in Et_2O at room temperature (RT) for a period of 4 h. With a degree of serendipity this gave a quantitative yield of **3** (based on bismuth).

The single remaining Ph group in **3** appears to be highly sensitive towards hydrolysis and resulted in formation of the unusual bismuth hydroxo compound $[Bi(OH)(Ace)_2]$ **4**, after **3** was left exposed in air for 30 min (Scheme 1). The composition of **4** was confirmed through elemental analysis. The ^1H NMR spectrum of **3**, which was taken immediately after isolation, shows the expected *o*-, *m*-, *p*-Ph protons resonating at 8.74, 8.04 and 7.42 ppm respectively. A small singlet due to benzene is visible at 7.36 ppm, indicative of the extreme lability of the Ph group. Confirmation of this came when the ^1H NMR spectrum of **3** was taken after 30 min standing in air. This showed no resonances due to the phenyl group but a prominent singlet due to benzene. Formation of similar bismuth hydroxo species through the hydrolysis of analogous R_2BiCl species [R = 2,6-diacetylpyridine *bis*-(2-thenoylhydrazone) [25], phenyl-N,N-dimethylmethanamine [26] and 5,6,7,12-tetrahydrodibenz-azabismocine [27]] have been reported previously in the literature, with associated crystallographic data showing a strong Bi–O bond of length 2.08–2.18 Å.

From recent studies we have demonstrated that the reaction of Bi_2O_3 with acids in water can lead to the formation of isolable polynuclear bismuth oxido clusters [28]. Thus, three equivalents of

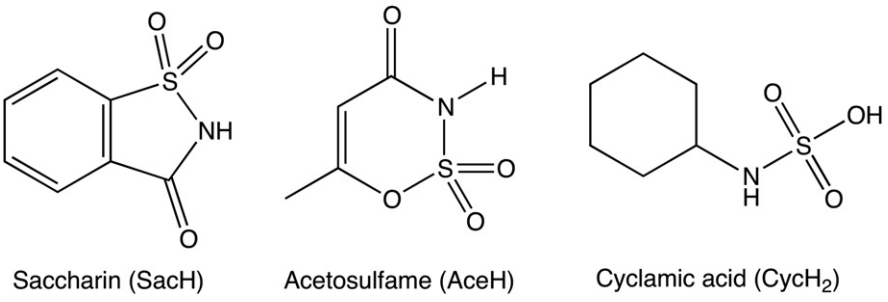

Fig. 1. Structures of saccharin, acetosulfame and cyclamic acid.