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Synthesis and Binding Studies of *N*-(2-Hydroxy-1-phenoxyacetyl)glycylglycine

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Abstract. The synthesis of *N*-(2-hydroxy-1-phenoxyacetyl)glycylglycine **3** from 2-acetoxyphenoxyacetic acid is described. Compound **3**, a simple model for the carboxy-containing ionophore, Lasalocid, binds cations in methanol in the order: $Ca^{2+} \geqslant Ba^{2+} > Sr^{2+} \geqslant Li^{+} > Na^{+}, K^{+}$.

Key words. Glycylglycinamide, carboxy ionophore model, cation binding.

1. Introduction

We have synthesized and studied the properties of neutral dioxydiamide ionophores for a number of years [1, 2]. However, many of the most useful ionophores contain carboxy and hydroxy groups which form internal hydrogen bonded rings upon metal cation binding [3]. These cation complexes are electrically neutral since the carboxyl group loses its proton upon binding and they are more effectively transported through membranes than are the complexes of neutral ionophores which must include an external anion [4].

2. Experimental

The general laboratory techniques used have been described previously [1c]. Proton NMR spectra were recorded on Varian A-60A and T-60 spectrometers at Yeshiva University and on a GE Nicolet (300 MHz) spectrometer at the Lederle Laboratories of American Cyanamid Company. Infrared spectra were recorded on Beckman IR 33 and Perkin Elmer 1420 spectrophotometers at Ramapo College, a Perkin Elmer 457 spectrophotometer at Yeshiva University and a Nicholet FT-IR spectrometer at Lederle Laboratories. Mass spectra were done by Dr. M. Siegel at Lederle Laboratories using a Kratos MS-50 with FAB techniques, xenon, and sulfolane solutions. UV binding studies were done on a Varian Spectroscan 3 spectrophotometer. Elemental analyses were done by Galbraith Laboratories, Knoxville, TN.

2.1. 2-ACETOXYPHENOXYACETIC ACID **1**

Reaction of catechol with chloroacetonitrile (1.4 equiv.), K_2CO_3 gave 2-imino-1,4-benzodioxan [6] which was hydrolyzed with 5N HCl to give 2-hydroxyphenoxy-

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acetic acid [6, 7]. The latter was acetylated with acetic anhydride and H_2SO_4 to give **1**: mp 111–112°C (lit. [8] 110°C); NMR ($CDCl_3$) δ 2.32 (s,3, CH_3), 4.7 (s,2, CH_2), 6.85–7.25 (m,4, aryl), 10.36 (s,1, CO_2H).

2.2. ETHYL *N*-(2-ACETOXY-1-PHENOXYACETYL)GLYCYLGLYCINATE 2

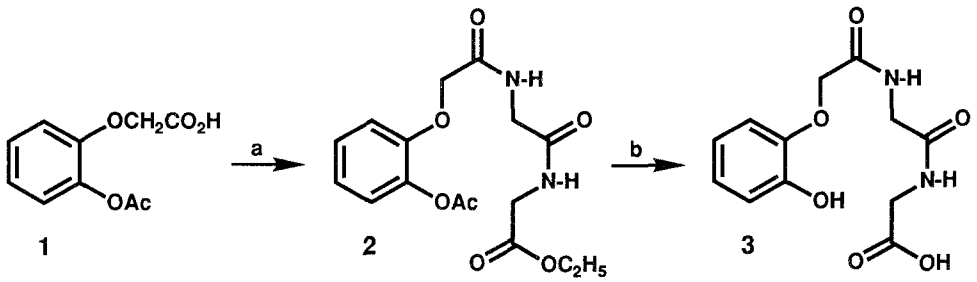
A suspension of **1** (4.2 g, 0.02 mol), ethyl glycylglycinate.HCl(4.0 g, 0.02 mol), *N*-ethylmorpholine (NEM, 9.2 g, 10 mL, 0.08 mol) and 1-hydroxybenzotriazole (4.6 g, 0.03 mol) in anhydrous tetrahydrofuran (80 mL) under flowing nitrogen was cooled in an ice–NaCl bath. Dicyclohexylcarbodiimide (8.66 g, 0.042 mol) was added with stirring and the resultant mixture was stirred at 0°C for 2.5 h, then at 25°C for 2.5 h, and filtered *in vacuo*. The filtrate was evaporated *in vacuo* to leave a yellow residue which was kept under high vacuum for 1–2 h to remove traces of solvent and NEM. The residue was dissolved in EtOAc (100 mL) to give a yellow solution which was filtered to remove a small amount of undissolved solid, and washed with saturated $NaHCO_3$ (3 × 30 mL) and 2N citric acid (3 × 30 mL). Dicyclohexylurea (DCU, mp 231–233°C) was filtered. The filtrate was washed with $NaHCO_3$ (2 × 30 mL), H_2O (2 × 30 mL), saturated NaCl (30 mL) and dried over Na_2SO_4. The mixture was filtered and the filtrate was evaporated *in vacuo* to give a residue (4.8 g) which was dissolved in EtOAc (100 mL). This mixture was again filtered to remove more insoluble DCU. The filtrate was reduced in volume to *ca.* 80 mL and kept at 25°C. The product slowly crystallized to give **2** (3.72 g, 0.011 mol, 53%); mp 117–118°C; IR (1%, KBr) ν 1780 (OAc), 1760 (Et ester), 1710 w, 1660 str (amide) cm^{-1}; NMR ($CDCl_3$) δ 1.25 (t,3, $OCH_2\underline{CH_3}$), 2.35 (s,3, $CH_3C=O$), 3.97 (d,2, NCH_2), 4.05 (d,2, NCH_2), 4.25 (quart,2, $O\underline{CH_2}CH_3$), 6.77, 6.90 (m,2, NH), 7.05 (m,4, aryl).

Anal. Calcd for $C_{16}H_{20}N_2O_7$:C, 54.54; H, 5.72; N, 7.95. Found: C, 54.62; H, 5.87; N, 8.05.

2.3. *N*-(2-HYDROXY-1-PHENOXYACETYL)GLYCYLGLYCINE 3

Compound **2** (1.0 g, 0.0028 mol) was treated with 1N NaOH (12 mL) in methanol (50 mL) with stirring at 37°C for 10 min, followed by cooling and acidification to a pH of 3 with 2N NCl (6.2 mL). This solution was evaporated *in vacuo* to give a white solid which was dissolved in cold 0.5N NaOH (200 mL), washed with EtOAc (2 × 40 mL), reacidified with 2N HCl (50 mL) and evaporated again. The residue was dissolved in water (100 mL) and kept at 5–10°C for 15 h to give **3** as needles. The product was recrystallized from hot water (20 mL) to give 0.36 g (two crops, 0.0013 mol, 46%): mp 163–165°C; FT-IR (KBr) ν 3600 br (CO_2H, OH), 1740 (acid C=O), 1660 (amide C=O), 740 (ortho-disubst. benzene) cm^{-1}; NMR (300 MHz, $CDCl_3$) δ 3.95 (d,2, NCH_2), 4.05 (d,2, CH_2) 4.55 (s,2, OCH_2), 6.8–6.9 (m,2, aryl), 7.5 (m,2, aryl), 8.9 (br s,1, ArOH); mass spectrum (70 eV) *m/e* (rel. intensity) 150 (71, M-132), 132 (3), 122 (58), 121 (100), 114 (9), 63 (21).

Anal. Calcd for $C_{12}H_{14}N_2O_6$: C, 51.07; H, 5.00; N, 9.92. Found: C, 50.97; H, 5.04; N, 9.80.



Scheme I. (a) $NH_2CH_2C(=O)NHCH_2CO_2Et$, DCC, HO-Benzotriazole. (b) 1: 1N NaOH, CH_3OH, 37°C, 10 min; 2: 2N HCl.

3. Results and Discussion

We now describe the synthesis of *N*-(2-hydroxy-1-phenoxyacetyl)glycylglycine **3**, from 2-acetoxyphenoxyacetic acid **1** in a 24% overall yield. Compound **3** is a simple analog of Lasalocid and related carboxy ionophores [5]. The preparation of **1** from catechol has been previously described [6–8]. Condensation of **1** with ethyl glycylglycinate by the use of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HO–BT) [9] gives the acetoxycarbethoxy ester **2**. The acetoxy and ethyl ester groups are removed by brief treatment with cold 1N NaOH in methanol [10] to give **3** (Scheme 1). Although CPK models suggest that **3** might exhibit intramolecular hydrogen bonding, its low solubility in $CDCl_3$ or CH_3CN precluded confirmation by IR studies. Hydrogen bonding in **3** is present in its IR spectrum in a KBr pellet but is presumably intermolecular.

The binding of **3** with metal cations in methanol is being studied with our previously described UV method utilizing Scatchard plots [1c, 2a]. Compound **3** (conc. $= 2-4 \times 10^{-5}$ M) is found to bind $Ba^{2+} \geqslant Ca^{2+}$ ($K_{app} = 3.5 - 4.0 \times 10^4\ M^{-1}$) $> Sr^{2+}$ (1.7×10^3) $\gg Li^+ > Na^+, K^+$. The stoichiometry of binding (n) $= 1.0$ (cation/ligand) for all of the cations except Sr^{2+} ($n = 1.7$). Correlation coefficients (R) $= 0.9-0.99$. The reproducibility of the K_{app} values is $\pm 10-20\%$. Whether **3** is complexing in its neutral or proton dissociated form is not yet known. Compound **3** is a weaker binder than some of our previously described neutral ionophores such as *N,N,N',N'*-tetrakis-(*n*-propyl)-1,2-phenylenedioxydiacetamide (K_{app} up to 7×10^4) [1, 2a]. Studies on the pH dependence of the binding of **3** with cations and attempted isolation of cation complexes are in progress.

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