

Reaction of amino acids with *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride forms hemi-maleate salts, not maleimides

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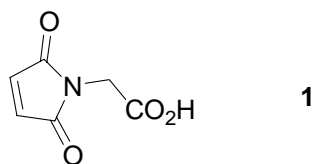
Abstract

Contrary to a recent report, maleimides are not formed by treating *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride with amines in aqueous solution. The true products are hemi-maleate salts of the amines.

Keywords: Hydrolysis, hemi-maleate salts, *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride

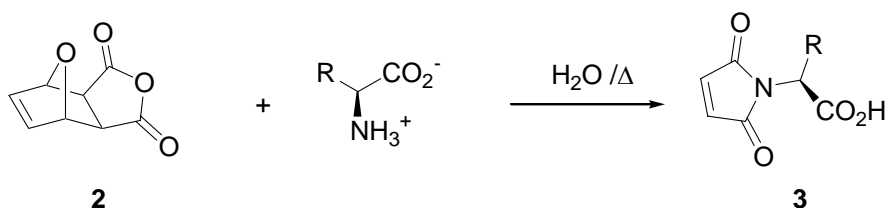
Introduction

Maleimides are useful reactive moieties in bio-organic chemistry, for example in their ability to enable highly site-selective conjugation of fluorophores and other species to proteins by reaction with cysteine side chains.¹⁻³ Although *N*-arylmaleimides are readily formed by cyclization of *N*-arylmaleamic acids, the corresponding *N*-alkyl compounds are often more difficult to access. In particular, *N*-maleoylamino acids such as the glycine derivative **1** are difficult to prepare and largely rely on methods described in the mid-1970s by Keller and Rudinger⁴ and by Rich and co-workers.⁵



In our experience, both methods have drawbacks and our attention was caught by the high yielding and simple procedure recently described in this Journal by Ondruš et al.⁶ in which a range of unprotected α - or ω -amino acids was heated in an aqueous medium (either conventionally or by microwave irradiation) with *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic

anhydride **2**. This reaction was claimed to yield the maleoyl derivatives **3** (shown for α -amino acids in Scheme 1).



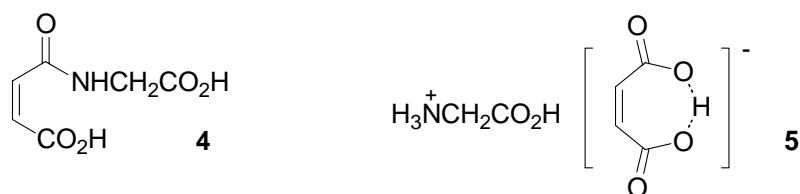
Scheme 1

Results and Discussion

When an aqueous solution of glycine and the anhydride **2** was heated and subsequently evaporated by azeotropic distillation of toluene as described by Ondruš et al., we obtained material which corresponded with that reported by them.⁶ However, upon attempted crystallization from chloroform, as previously reported for **1** by Rich et al.⁵ it became apparent that the isolated material was not *N*-maleoylglycine **1**. The material was instead crystallized from methanol-chloroform and then had m.p. 143-146 °C, substantially above the value of 118-121 °C reported for non-crystallized material.⁶ Its ¹H NMR spectrum (DMSO-*d*₆) was essentially the same as reported by Ondruš et al.⁶ (singlets of equal intensity at δ 3.65 and 6.05 p.p.m.). However, upon addition of authentic **1** (prepared by the method of Rich et al.⁵), two additional singlets also of equal intensity were observed at δ 4.13 and 7.12 p.p.m. The identity of our authentic sample of **1** was secure, as its properties agreed with those reported by Rich et al. and it had previously been used by us for preparation of the corresponding acid chloride, that was further elaborated into compounds of confirmed structures.⁷ We note that the olefinic proton signal of maleimide derivatives has a strong solvent dependence. Rich et al.⁵ reported values of δ 6.67-6.78 in CDCl_3 , 6.78-6.92 in $\text{MeOH-}d_4$ and 7.00-7.13 in $\text{DMSO-}d_6$, while Keller and Rudinger⁴ reported δ 6.95 for **1** in $\text{acetone-}d_6$. The olefinic resonance reported at 6.04 p.p.m. by Ondruš et al. for their material is substantially upfield of this entire range.

As a possible alternative structure, we considered the product of partial cyclization, i.e. the maleamic acid **4**, but the ¹H NMR spectrum (DMSO-*d*₆) of this compound (prepared as described by Rich et al.⁵) had its olefinic protons as an AB quartet (δ 6.32 and 6.39, $J = 12.7$ Hz) and the methylene protons as a doublet (δ 3.91, $J = 5.7$ Hz), coupled to the NH proton (triplet at δ 9.20). Since the NMR spectrum of the Ondruš et al. product indicated a symmetrical structure for the olefinic protons, the most likely contender was the hemi-maleate salt **5**. An authentic sample was prepared by mixing aqueous solutions of glycine and maleic acid and removing the solvent by azeotropic distillation with toluene. The ¹H NMR spectrum of this product was identical to that of the Ondruš et al. material, and a mixture of the two samples showed no additional peaks. Further confirmation that both samples were the salt **5** came from analytical

anion-exchange chromatography, which showed a single peak of identical retention time to a standard prepared by dissolving maleic acid in HPLC mobile phase (pH 6). Authentic *N*-maleoylglycine **1** eluted as a distinct peak with an earlier retention time. Control experiments confirmed that crystallization of the crude reaction product from methanol-chloroform did not cause any chemical change.



Inspection of the data of Ondruš et al.⁶ showed that the ¹H NMR spectra of all the compounds reported by them as maleimides had their olefinic protons in the range 6.04–6.08 p.p.m., consistent with the value found here for the hemi-maleate **5**. Furthermore, the elementary analytical data for every one of their compounds indicated the presence of two molecules of water, assigned as solvent of crystallization. In the light of our findings, it seems clear that in practice this molecular stoichiometry arises because none of the products is a maleimide but instead represents the product of salt formation from hydrolyzed maleic anhydride that is generated from **2**. Note that the first ionization of maleic acid has pK_a 1.83 while that for glycine is 2.35,⁸ so the protonation state shown for **5** conforms with that expected from the relative acidities of the two components that form the salt. We did not attempt to determine whether hydrolytic opening of the anhydride ring took place before or after its retro-Diels-Alder dissociation from furan. However, from a synthetic standpoint, it is evident that this apparently attractive procedure does not in fact yield the maleoyl amino acids that are claimed but instead generates only the hemi-maleate salt of the relevant amino acid.

Experimental Section

General Procedures. ¹H NMR spectra were recorded in DMSO-*d*₆ on a JEOL FX90Q spectrometer. Anion-exchange HPLC was performed on a Whatman SAX column (Catalogue No. 4621-0505) with a mobile phase of 25 mM Na phosphate, pH 6–methanol (100:13 v/v) at a flow rate of 1.5 ml min⁻¹. Detection was at 254 nm. Melting points were determined on a Kofler hot stage instrument.

Reaction of *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride with glycine. This procedure was performed as described⁶ and the solid residue obtained after trituration with acetone was crystallized from methanol-chloroform, m.p. 144–147 °C. The ¹H NMR spectrum is

described in the Results and Discussion section. On anion-exchange HPLC the compound had t_R 4.3 min, identical to maleate dianion. Authentic *N*-maleoylglycine had t_R 1.5 min.

Glycine hemi-maleate (5). A solution of glycine (0.75 g, 10 mmol) in water (10 ml) was mixed with a solution of maleic acid (1.16 g, 10 mmol) in water (20 ml) at room temperature and the solution was evaporated under reduced pressure, then re-evaporated from toluene (2 × 20 ml) to leave a semi-solid. This was triturated with acetone (30 ml) and filtered, and the solid was crystallized from methanol-chloroform to give **5**, (1.42 g, 75%), m.p. 146-149 °C. $^1\text{H NMR}$ δ 6.05 (s, 2H), 3.66 (s, 2H). Its retention time on anion-exchange HPLC was 4.3 min.

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