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Note

Synthesis and mass spectral characterization of *p*-acrylamidophenylmercury chloride for high-capacity affinity gel chromatography

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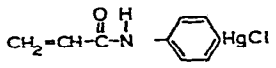
Organomercurial agarose has been used for the selective purification of sulfhydryl proteins[1–4], sulfhydryl nucleotides[5–7] and sulfhydryl substituted ribonucleic acids[5–8]. This affinity matrix has a low organomercurial content and is therefore only suitable for the fractionation of small quantities of thiol-containing compounds. We report here the synthesis and characterization of *p*-acrylamidophenylmercury chloride (I), which may be polymerized to form a polyacrylamide matrix containing very high levels of covalently bound mercury.



(I)

The synthesis of mercury derivatives of methyl acrylamide has been reported previously[9], however, neither details of the synthesis nor characterization of the product was given.

Synthesis of compound I is achieved by direct coupling of acrylyl chloride to *p*-aminophenylmercuric acetate in dimethylformamide.

EXPERIMENTAL

Reagents

Mercuric acetate and aniline (Class II) were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Aniline was distilled prior to use. Acrylyl chloride (98%) was supplied by Polysciences (Warrington, Pa., U.S.A.).

Deuterated benzene (99.9%, d_6) was supplied by Stohler (Waltham, Mass., U.S.A.).

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Instrumentation

Low-resolution electron impact mass spectra (70eV) were recorded on a DuPont Model 491 instrument equipped with a Finnigan/Incos data system. Samples were analyzed by direct insertion probe with the source temperature at 250°. High-resolution analysis was performed on an AEI MS-9 instrument, again using direct insertion at a source temperature of 250°. Peak matching of the molecular ion at *m/z* 383 was carried out against a suitable reference mass from haptacosafluorotributylamine at a resolution of approximately 15,000.

Synthesis of p-acrylamidophenylmercury chloride

It was found necessary to synthesize *p*-aminophenylmercuric acetate by direct mercuration of aniline[10] since commercial preparations are not of sufficient purity.

p-Aminophenylmercuric acetate (5 g) is dissolved in 25 ml of anhydrous redistilled dimethylformamide. This solution is chilled on ice, 1.9 ml of acrylyl chloride is added slowly with stirring and the reaction product is precipitated by the addition of chilled diethyl ether, filtered, washed with ether and dried by desiccation.

p-Acrylamido-d_4-phenylmercury chloride was synthesized by direct mercuration of d_5-aniline[10] (prepared by nitration[11] and reduction[12] of d_6-benzene) which was then coupled with acrylyl chloride as described above. Final deuterium incorporation into compound I was approximately 98%.

RESULTS AND DISCUSSION

Mass spectrometric analysis of product I confirmed that the acetate counter-ion of *p*-aminophenylmercuric acetate is replaced by the chloride ion of acrylyl chloride during the coupling reactions. Presumably the chloride ion displacement which occurs during amide bond formation is accompanied by a rapid chloride ion substitution on the organomercurial moiety. This reaction is consistent with the known high affinity of organomercurials for the chloride ion[11] but contradicts previously published results[9] in which the acetate ion is not displaced.

The mass spectrum of *p*-acrylamidophenylmercuric chloride is shown in Fig. 1. The molecular weight of the product is calculated as 383 based upon the most abundant isotope of mercury, namely mass 202, and the mass 35 isotope of chlorine. The complex nature of the molecular ion region reflects the combination of isotope patterns from mercury, of which there are six stable isotopes and the 3:1 ratio of chlorine 35 and chlorine 37 isotopes.

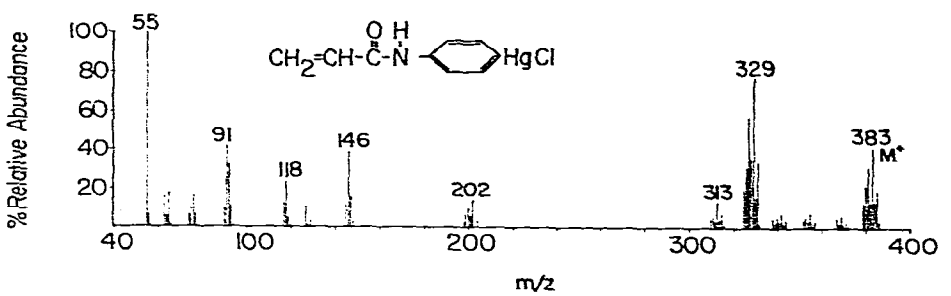
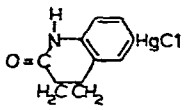


Fig. 1. Electron-impact mass spectrum of *p*-acrylamidophenylmercury chloride.

High-resolution mass spectrometry confirmed the exact molecular weight and molecular formula of our product. The abundant ion at *m/z* 329 arises from transfer of a proton from the acrylyl moiety to nitrogen and subsequent loss of the neutral acrylyl group.

Synthesis of *p*-acrylamido-d_4-phenylmercury chloride aided in the assignment of the composition of ions at lower mass associated with or derived from the phenyl ring.

Fragment ions at masses 369 and 355 are difficult to associate with product I and arise from an interfering secondary product of the synthesis described earlier. Two products were observed to fractionate off the direct insertion probe. The unknown product which evaporates slightly earlier than product I is postulated to be the cyclic adduct of I shown below.



Whether or not this product interferes with the chromatographic properties of the affinity chromatography gel has not as yet been determined.

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