N-Hydroxsuccinimide Ester of Chloromercuroacetic Acid, a New Reagent for Preparing Mercury Derivatives of Amino Acids, Proteins and Aminoacyl Transfer Ribonucleic Acids

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Esters of N-hydroxsuccinimide are known to be convenient and specific reagents for free amino groups of amino acids in the free form as well as when present in macromolecules. In a search for new methods of preparing heavy-atom derivatives for X-ray-crystallographic investigations of macromolecules and for use as active site directed enzyme reagents we have prepared the N-hydroxsuccinimide ester of chloromercuroacetic acid. The latter acid, studied recently by Tlinder was esterified with N-hydroxsuccinimide by the method of Anderson et al. The ester was purified by crystallization from 2-propanol and was obtained in more than 90% yield. The structure of the ester given above was confirmed by mass spectrometry.

The preparation of two derivatives shows that this mercury-containing active ester reacts with a free amino acid, L-valine, as well as with the same amino acid esterifying the 3' end of valine-specific tRNA. In the latter case it was observed that the chloromercuroacetylation product of L-valyl-tRNA is strongly retarded on a benzoylated DEAE-cellulose column, i.e. as is the case with phenoxacylated aminoacyl-tRNA (cf. Gillam et al.). The two chloromercuroacetylated L-valine derivatives prepared are of interest in studies of the active site of the enzyme valyl transferribonucleic acid synthetase since most such synthetases are supposed to be sulphhydryl enzymes. The reactivity of the mercury-containing active ester towards free amino groups in proteins will be studied separately in connection with X-ray crystallographic studies on the enzyme carbonic anhydrase.

Experimental. Melting points were determined using a Koelner bench. Rf values refer to thin-layer chromatography on Kieselgel G. The spots on the plates were detected with the chlorine-iodide-starch reaction. IR spectra were recorded on samples in the solid state (KBr pellets) using a Perkin-Elmer 157 apparatus. Mass spectra were obtained using an MS 902 instrument (AEI, Manchester). The unit used to quantitize tRNA, A₄₅₀, was defined as the quantity material which dissolved in 1 ml had an absorbance at 260 nm of 1 with a light path of 1 cm. Assays for ability to form L-valyl-tRNA and enzymatic preparation of the latter were performed by the methods and materials of Lagorkvist et al.

Chloromercuroacetic acid N-hydroxsuccinimide ester. N,N'-Dicyclohexyl carbodiimide (2.05 g, 10 mmole) was added to an ice-cold solution of chloromercuroacetic acid (2.95 g, 10 mmole) and N-hydroxsuccinimide (1.15 g, 10 mmole) in anhydrous dioxane (60 ml). The mixtures were stirred for 3 h at room temperature, and the precipitate of dicyclohexyl urea (2.1 g, m.p. 230°, decomps.) was removed by filtration. The solution was evaporated to dryness in vacuo and the residue crystallized from 2-propanol. Yield 3.0 g (92%); m.p. 172°; Rf 0.67 (1-butanol-acetic acid-water, 4:1:1); Rf 0.28 (light petroleum-tetrahydrofuran-acetic acid 60:40:1). The IR spectrum showed carboxyl absorptions typical for N-hydroxsuccinimide esters, with one band at 5.65 μ (ester carboxyl) and a second band of approximately the double intensity at 5.8 μ (succinimide carboxyls). The mass spectrum showed high peaks corresponding to fragments such as:

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\begin{align*}
\text{m/e} & = 279, [^{35}\text{Cl}^{302}\text{Hg-CH}_2\text{CO}]^+ \quad \text{and} \quad [^{37}\text{Cl}^{302}\text{Hg-CH}_2\text{CO}]^+; \\
\text{m/e} & = 157, [\text{CH}_4\text{CO-O-N(CO-CH}_2\text{H}_3]^+; \quad \text{as well as a series of characteristic molecule ions of m/e 389-397, the highest of which had m/e = 393 (containing } ^{35}\text{Cl}^{302}\text{Hg and } ^{37}\text{Cl}^{302}\text{Hg).}
\end{align*}
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N-Chloromercuroacety-L-valine. L-Valine (235 mg, 2 mmole) was dissolved in water (5 ml) and 1 M NaClCO₃ (4 ml). The solution was cooled to 0° and a solution of chloromercuroacetic acid N-hydroxsuccinimide ester

small volume in vacuo. Water (3 ml) and 1 M HCl (2 ml) was added and the crystalline precipitate was collected by filtration, washed with water, and dried in vacuo over KOH. Yield 0.6 g (76 %), m.p. 230° (decomp.); \( R_F \) 0.73 (1-butanol-acetic acid-water, 4:1:1); \( [\alpha]_D^{27} +16.5 \) (c 4.84 in \( N,N \)-dimethylformamide). The IR spectrum showed a single carbonyl absorption band at 5.9 \( \mu \). (Found: N 3.4. Calc. for \( C_2H_{12}ClHgNO_2 \) (394.2): N 3.5.) Acid hydrolysis of the compound (5 mg in 1 ml of 6 M HCl, 4 h, 110°) gave valine, identified by thin-layer chromatography.

**Chloromercureicatlylation of \( ^{14}C \)-L-valyl-tRNA\(^{Val} \).** Commercial transfer-ribonucleic acid (tRNA; 1 g: 18 370 \( A_{250} \) units; purchased from Boehringer & Söhne, Mannheim, Germany) was chromatographed on benzoylated diethylaminoethyl cellulose (BD-cellulose) by the method of Gillam et al.\(^1 \), using a 2.8 \( \times \) 85 cm column. A valine-accepting fraction (7620 \( A_{250} \) units) containing most of the tRNA\(^{Val} \) present in the starting material was obtained as indicated in Fig. 1. The tRNA was precipitated by adding 3 volumes of cold ethanol and a part of it (2740 \( A_{250} \) units) was then esterified enzymatically with \( ^{14}C \)-L-valine (14.9 mC/mole; 23.3 \( \times \) 10\(^6 \) cpm/\( \mu \)mole) in the presence of valyl transfer-ribonucleic acid synthetase, and the mixture of \( ^{14}C \)-L-valyl-tRNA\(^{Val} \) and unesterified bulk tRNA isolated again by ethanol precipitation. This precipitate (12.2 \( \times \) 10\(^6 \) cpm corresponding to 520 \( \mu \) moles of \( ^{14}C \)-L-valine) was dissolved in 7 ml of 0.1 M triethanolamine-HCl buffer of pH 4.3 and containing 0.01 M MgCl\(_2\), and was

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**Fig. 1.** Chromatography of commercial tRNA on benzoylated DEAE-cellulose. Material eluted with 0.8 M NaCl (Gillam’s Solution B; cf. the text). ● UV absorption. ■ \( ^{14}C \) cpm obtained in the standard assay for tRNA\(^{Val} \).

(750 mg, 2 mmoles) in anhydrous dioxane (4 ml) was added. The solution was stirred for 2 h at room temperature. 1 M HCl (2 ml) was added and the solution evaporated to a

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**Fig. 2.** Chromatography on benzoylated DEAE-cellulose of tRNA esterified with \( ^{14}C \)-L-valine and treated with chloromercureic acid \( N \)-hydroxysuccinimide ester. For complete description of eluents, see Gillam et al.\(^1 \) and the text. ● UV absorption. ■ \( ^{14}C \) radioactivity.

reacted at pH 8 with a solution of 200 mg chloromercuriacetic acid N-hydroxysuccinimide ester in 1 ml of anhydrous tetrahydrofuran, as described for the corresponding phenoxacyctylation procedure (Gillam et al.\textsuperscript{1})

The reaction product isolated by ethanol precipitation was dissolved in 10 ml of Gillam's solution A and was applied (2800 $A_{260}$ units, $11.4 \times 10^4$ cpm) to the previously mentioned BD-cellulose column. The column was eluted with Gillam's solutions A, B, E, and finally with 1.5 M NaCl solution containing 10% ethanol, 0.01 M MgCl$_2$ and 0.01 M sodium acetate buffer pH 4.5. The chromatogram obtained is shown in Fig. 2. It is evident from the figure, that non-radioactive material, i.e. unesterified tRNA is eluted with the solution B at the same position as in Fig. 1 (peak I)). No $^{14}$C-valyl-tRNA seems to be present in the product, since we have found such aminoacyl-tRNA to be eluted with the solution B immediately after the unesterified tRNA.

The material eluted with 1.5 M NaCl should be chloromercuriacetylated $^{14}$C-valyl-tRNA because of its coinciding UV-absorption and radioactivity, and because of the similar high affinity to the column that has been found for all N-acylated aminoacyl-tRNA's studied by Gillam et al.\textsuperscript{1}

The fraction (II) indicated in Fig. 2 was precipitated by 3 volumes of ethanol, collected by centrifugation and dissolved in 4 ml of water. This solution contained 310 $A_{260}$ units (560 mmole, calculated with the value of 1.8 mmole tRNA per $A_{260}$ units\textsuperscript{4}) and $10.2 \times 10^8$ cpm (440 mmole) giving a ratio tRNA/$^{14}$C-valine of 1.27.

Acknowledgements. The author is indebted to Professor Ulf Lagerkvist for guidance and support in the tRNA part of this work, to Miss Vaike Kaak and Mrs. Monica Olsson for technical assistance and to Dr. Bengt Andersson for running the mass spectra. The investigation was supported by a research grant from the Swedish Medical Research Council (B 70-13X-175-06B).


Received March 24, 1970.

Naphthylmethylseleno-substituted Alkanoic Acids

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In the course of current work on the biological effects of organoselenium compounds the acids I, II and 18 homologues (Tables 1 and 2) have been prepared. The end in view was to test the growth-regulating activity, the factor-3-effect and possibly other biological effects. The work on the growth-regulating activity, which also included some acids with selenium attached directly to the naphthalene ring prepared in this institute,\textsuperscript{12} was carried out by Professor B. Åberg.* It has in part been published\textsuperscript{13} and indicates that the selenium compounds have an anti-auxin effect, more pronounced than that of the corresponding sulphur compounds. The acid II is perhaps the most powerful anti-auxin known.\textsuperscript{3}

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\[\text{CH}_2\text{Se-CH}_2\text{-COOH} \quad \text{I} \quad \text{CH}_2\text{Se-CH}_2\text{-COOH} \quad \text{II}\]