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

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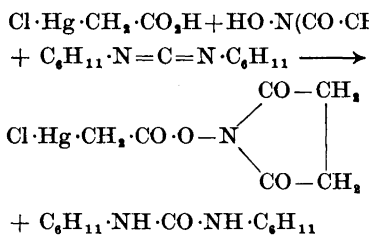
Esters of *N*-hydroxysuccinimide are known to be convenient and specific reagents for free amino groups of amino acids in the free form as well as when present in macromolecules.[1] In a search for new methods of preparing heavy-atom derivatives for X-ray-crystallographic investigations of macromolecules and for use as active site directed enzyme reagents we have prepared the *N*-hydroxysuccinimide ester of chloromercuriacetic acid. The latter acid, studied recently by Tilander [2] was esterified with *N*-hydroxysuccinimide by the method of Anderson *et al.*[1]



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 chloromercuriacetylation product of L-valyl-tRNA is strongly retarded on a benzoylated DEAE-cellulose column, *i.e.* as is the case with phenoxyacetylated aminoacyl-tRNA (*cf.* Gillam *et al.*[1]).

The two chloromercuriacetyl-L-valine derivatives prepared are of interest in studies of the active site of the enzyme valyl transferribonucleic acid synthetase [3] since most such synthetases are supposed to be sulphydryl 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.[4]

Experimental. Melting points were determined using a Kofler bench. R_F values refer to thin-layer chromatography on Kieselgel G. The spots on the plates were detected with the chlorine-iodide-starch reaction.[5] 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, A_{260}, 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 Lagerkvist *et al.*[3]

Chloromercuriacetic acid N-hydroxysuccinimide ester. *N*,*N*′-Dicyclohexyl carbodiimide (2.05 g, 10 mmoles) was added to an ice-cold solution of chloromercuriacetic acid [2] (2.95 g, 10 mmoles) and *N*-hydroxysuccinimide (1.15 g, 10 mmoles) 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°, decomp.) was removed by filtration. The solution was evaporated to dryness *in vacuo* and the residue crystallized from 2-propanol. Yield 3.6 g (92 %); m.p. 172°; R_F 0.67 (1-butanol-acetic acid-water, 4:1:1); R_F 0.28 (light petroleum-tetrahydrofuran-acetic acid 60:40:1). The IR spectrum showed carbonyl absorptions typical for *N*-hydroxysuccinimide esters, with one band at 5.65 μ (ester carbonyl) and a second band of approximately the double intensity at 5.8 μ (succinimide carbonyls). The mass spectrum showed high peaks corresponding to fragments such as:

m/e = 279, $[^{35}Cl\cdot^{202}Hg\cdot CH_2\cdot CO]^+$ and $[^{37}Cl\cdot^{200}Hg\cdot CH_2\cdot CO]^+$;

m/e = 157, $[CH_2\cdot CO\cdot O\cdot N(CO\cdot CH_2)_2]^+$; as well as a series of characteristic molecule ions of m/e 389–397, the highest of which had m/e = 393 (containing $^{35}Cl^{202}Hg$ and $^{37}Cl^{200}Hg$).

N-Chloromercuriacetyl-L-valine. L-Valine (235 mg, 2 mmoles) was dissolved in water (5 ml) and 1 M $NaHCO_3$ (4 ml). The solution was cooled to 0° and a solution of chloromercuriacetic acid *N*-hydroxysuccinimide ester

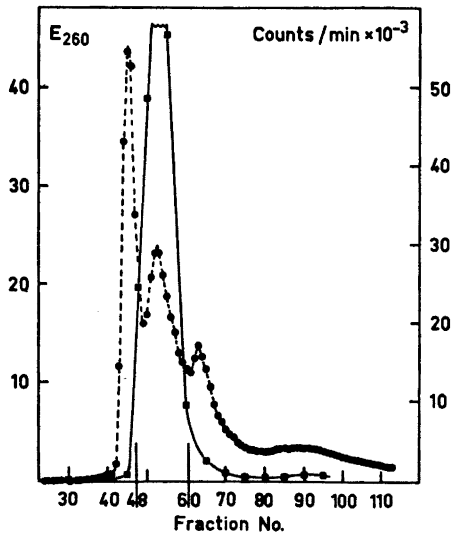


Fig. 1. Chromatography of commercial tRNA on benzoylated DEAE-cellulose. Material eluted with 0.8 M NaCl (Gillam's Solution B; *cf.* the text). ● UV absorption. ■ ^{14}C cpm obtained in the standard assay for $tRNA^{Val}$.

(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 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 μ. (Found: N 3.4. Calc. for $C_7H_{13}ClHgNO_3$ (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.

Chloromercuriacetylation of ^{14}C-L-valyl-$tRNA^{Val}$. Commercial transfer-ribonucleic acid (tRNA; 1 g: 15 370 A_{260} 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 × 85 cm column. A valine-accepting fraction (7620 A_{260} 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_{260} units) was then esterified enzymatically with ^{14}C-L-valine (14.9 mC/mmole; 23.3×10^6 cpm/μmole) 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 (12.2×10^6 cpm corresponding to 520 mμ 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

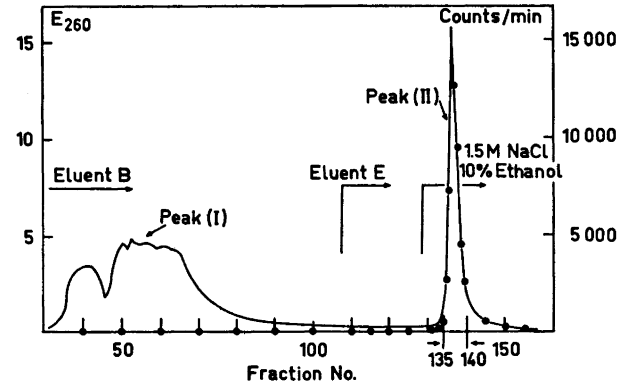


Fig. 2. Chromatography on benzoylated DEAE-cellulose of tRNA esterified with ^{14}C-L-valine and treated with chloromercuriacetic acid *N*-hydroxysuccinimide ester. For complete description of eluents, see Gillam *et al.*[1] and the text. ——— UV absorption. ● ^{14}C radioactivity.

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 phenoxyacetylation procedure (Gillam *et al.*[1]) The reaction product isolated by ethanol precipitation was dissolved in 10 ml of Gillam's[1] solution A and was applied (2800 A_{260} units, 11.4×10^6 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.*[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 mμmoles, calculated with the value of 1.8 mμmoles tRNA per A_{260} units[6]) and 10.2×10^6 cpm (440 mμmoles) giving a ratio tRNA/^{14}C-L-valine of 1.27.

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1. Anderson, G. W., Zimmerman, J. E. and Callahan, F. M. *J. Am. Chem. Soc.* **86** (1964) 1839; Lapidot, Y., De Groot, N., Weiss, M., Peled, R. and Wolman, Y. *Biochim. Biophys. Acta* **138** (1967) 241; Lapidot, Y., Rappoport, S. and Wolman, Y. *J. Lipid Res.* **8** (1967) 142; Gillam, I., Blew, D., Warrington, R. C., von Tigerstrom, M. and Tener, G. M. *Biochemistry* **7** (1968) 3459.
2. Tilander, B. *Arkiv Kemi* **25** (1966) 459.
3. Lagerkvist, U. and Rymo, L. *J. Biol. Chem.* **244** (1966) 2476 and previous papers.
4. Strandberg, B. *Arkiv Kemi* **28** (1967) 1; Fridborg, L., Kannan, K. K., Liljas, A., Lundin, J., Strandberg, B., Strandberg, R., Tilander, B. and Wirén, G. *J. Mol. Biol.* **25** (1967) 505.
5. Rydon, H. N. and Smith, P. W. G. *Nature* **169** (1952) 922.
6. Holley, R. W., Everett, G. A., Madison, J. T. and Zamir, A. *J. Biol. Chem.* **240** (1965) 2122.

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Naphthylmethylseleno-substituted Alkanoic Acids

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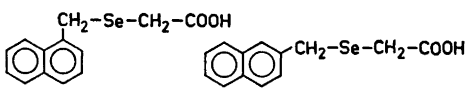
In the course of current work on the biological effects of organoselenium compounds the acids I, II and 18 homologues (Tables 1 and 2) have been prepared. The end in view was to test the growth-regulating activity, the factor-3-effect and



I II

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,[1,2] was carried out by Professor B. Åberg.* It has in part been published[2-4] 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.[4]

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