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NOVEL DERIVATIZATION REAGENT WITH TETRATHIAFULVALENE AS AN ELECTROPHORE FOR PRE-COLUMN LABELING OF AMINES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A novel derivatization reagent with tetrathiafulvalene as an electrophore, N-succinimidyl tetrathiafulvalene-2-carboxylate, was prepared and evaluated for pre-column labeling of amine by using β-phenethylamine as a model compound in high-performance liquid chromatography with electrochemical detection. The reagent reacted readily with the primary amine at room temperature for 1 h, providing the amide which was highly responsive to an electrochemical detector (detection limit = 21 fmol, signal to noise ratio = 5) at a relatively low applied potential (+700 mV vs. Ag/AgCl). The characteristic two-stage oxidative pattern appeared on the hydrodynamic voltammogram of the derivative, which helped to identify the peak on the chromatogram.

KEYWORDS tetrathiafulvalene; electrophore; pre-column labeling; N-succinimidyl tetrathiafulvalene-2-carboxylate; amine; high-performance liquid chromatography; electrochemical detection

High-performance liquid chromatography (HPLC) with electrochemical detection (ED) is a useful way to determine trace components in biological fluids because it is selective and sensitive. Recently many pre- and post-column labeling methods have been developed to extend the applicability of HPLC-ED to electrochemically inactive compounds.1) In our previous papers, we developed pre-column labeling reagents with guaiacol, aromatic amine or ferrocene as an electrophore to determine hydroxy, carboxy, thiol and amino compounds in HPLC-ED.2) Recently much interest is focused on tetrathiafulvalene (TTF) and its derivatives whose charge transfer salts show metal-like properties such as high electroconductivity,3) and many charge transfer salts of these compounds have been developed as organic superconductors.4) We studied the intriguing electrochemical properties of TTF which is a two-stage redox system.5, 6) The present paper deals with the preparation and properties of a novel derivatization reagent with TTF as an electrophore to determine amine in HPLC-ED.

A novel reagent, N-succinimidyl tetrathiafulvalene-2-carboxylate (**1**; **Chart 1**) was prepared from tetrathiafulvalene-2-carboxylic acid6) by condensation with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide. Properties of 1: dark purple crystals from benzene. mp 172.5-173.5°C. ^{1}H-NMR ($CDCl_3$)δ : 2.87 [4H, s, $CO(CH_2)_2CO$], 6.35 and 6.36 (each 1H, each d, J=each 6.6 Hz, TTF-H), 7.69 (1H, s, TTF-H). High-resolution MS m/z: 344.9253 (M^+)(calculated for $C_{11}H_7NO_4S_4$, 344.9256). The reactivity and electrochemical properties of 1 were investigated using β-phenethylamine as a model compound. The apparatus used for HPLC was a TOSOH CCPD chromatograph equipped with an EICOM ECD-100 detector using glassy carbon as a working electrode. HPLC was carried out on a Chemcosorb 5-ODS-H (5 μm) column (15 cm x 0.46 cm i.d.) at a flow rate of 1 ml/min. β-Phenethylamine (10^{-5} M) with 1 (5×10^{-4} M) was quantitatively condensed at room temperature for 1 h in CH_3CN-pyridine-H_2O (5:1:4). This was confirmed by comparison with the peak area of the authentic derivative (**2**) [orange amorphous substance from benzene. mp 114.5-115.5°C. ^{1}H-NMR ($CDCl_3$)δ :2.86 (2H, t, J=6.9 Hz, $NCH_2C\underline{H}_2$-Ar), 3.58 (2H, q, convertible to t with 20% ND_3 in D_2O, J=6.9 Hz, $NC\underline{H}_2CH_2$-Ar), 5.58 (1H, br s, exchangeable with 20% ND_3 in D_2O, NH), 6.32 (2H, s, TTF-H), 7.01 (1H, s, TTF-H), 7.15-7.40 (5H, m, Ar-H). MS m/z: 351 (M^+)]. The derivatized amine showed a single peak of theoretical shape with a k' value of 5 [mobile phase; CH_3CN-H_2O (65:35) containing 0.05 M $NaClO_4$] and was highly responsive to the electrochemical detector with a detection limit of 21 fmol (signal to noise ratio = 5, +700 mV vs. Ag/AgCl), but the excess reagent came out at the solvent front.

The **Fig. 1** shows the hydrodynamic voltammograms of β-phenethylamine- together with the secondary amine-derivative [piperidine derivative (**3**): yellow amorphous substance from benzene. mp 149.5-151.0°C. ^{1}H-NMR

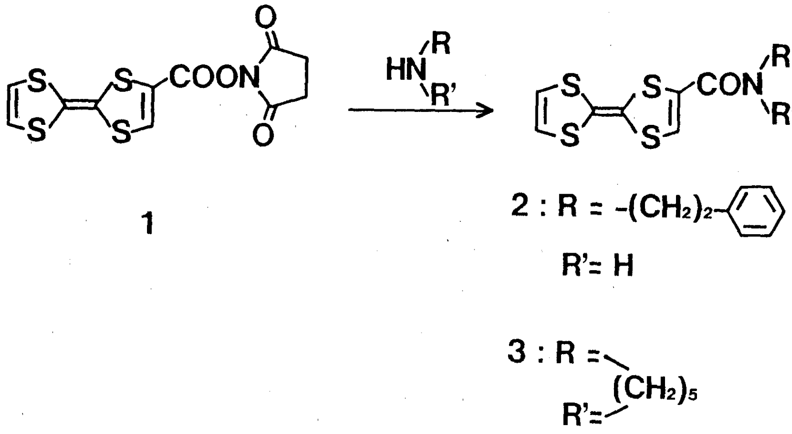

Chart 1

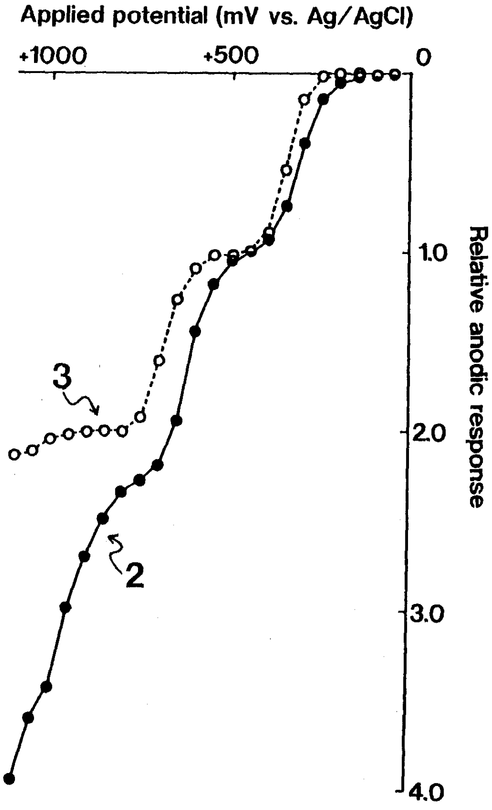

Fig. 1. Hydrodynamic Voltammograms of TTF Derivatives
Mobile phase: CH_3CN-H_2O (2, 70:30; 3, 65:35) containing 0.05 M $NaClO_4$. The response (peak height) of each compound at +450 mV was taken as 1.0.

($CDCl_3$) δ:1.50-1.75 (6H, m, piperidine-H), 3.56 (4H, br t, J= 5.4 Hz, piperidine-H), 6.32 (2H, s, TTF-H), 6.53 (1H, s, TTF-H). MS m/z: 315 (M^+)]. Both derivatives exhibited two-stage oxidative voltammograms due to the TTF moiety, which helped the selective detection of the TTF derivatives. In addition, derivative 2 showed a further increasing oxidative curve above +800 mV. This also occurred in the glycine ethyl ester derivative and may be due to the oxidation of amido and/or sulfur residues. These characteristic hydrodynamic voltammograms resulting from the amine moiety may be used to differentiate analytes having amine moieties. Although TTF has a redox system, these derivatives could not be detected by reductive ED after first oxidizing with a coulometric dual electrode detector (ESA 5100A). Such a phenomenon also occurs on polarographic reduction of TTF-radical cations in aqueous solution.[7] The details of this are now under investigation in our laboratories.

In conclusion, the newly developed derivatization reagent having tetrathiafulvalene as an electrophore has useful electrochemical properties for the sensitive analysis of biological substances. The development of this type of reagent having the reacting group toward the other functional group is now being conducted in our laboratories and the details will be reported elsewhere in near future.

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