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Short Communication

Novel ferrocene reagent for pre-column labelling of amines in high-performance liquid chromatography with electrochemical detection

MAKOTO TANAKA, KAZUTAKE SHIMADA and TOSHIO NAMBARA*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980 (Japan)

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High-performance liquid chromatography (HPLC) is a useful tool for the separation and determination of various compounds having a wide range of polarities and molecular weights. In order to extend the applicability of HPLC, numerous labelling reagents for fluorescence or ultraviolet detection have been developed^{1,2}. It has recently been demonstrated that electrochemical detection (ED) is extremely favorable for the analysis of electroactive compounds in biological fluids with respect to its sensitivity and selectivity^{3,4}.

Derivatization reagents of potential utility for HPLC-ED have been reviewed⁵. In reductive ED, the aromatic nitro group is used as an 'electrophore', *i.e.*, a functional group with a high ED response⁶⁻⁸, and labelling with this group enables the determination of amino acids at the picomole level. The presence of oxygen gas, however, exerts significant interferences and hence, a tedious and time-consuming procedure is required for the removal of oxygen dissolved in the mobile phase and sample^{9,10}. On the contrary, oxidative ED is unaffected by dissolved oxygen and is as sensitive as the reductive mode. Several labelling reagents have been developed for amines in oxidative ED, involving the aromatic amino^{11,12} and guaiacol groups¹³ as electrophores. We wish to report the development of a highly selective and sensitive derivatization reagent which possesses the ferrocenyl group as an electrophore. The ferrocenyl moiety can easily be oxidized and can be detected selectively in the presence of other electroactive compounds such as phenols and aromatic amines.

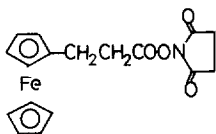


Fig. 1. Structure of N-succinimidyl 3-ferrocenylpropionate.

A new reagent, N-succinimidyl 3-ferrocenylpropionate (I), was prepared from 3-ferrocenylpropionic acid¹⁴ by condensation with N-hydroxysuccinimide in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Properties of compound I: m.p. 141-142°C. ¹H-NMR (C²HCl₃) δ: 2.79 (4H, s, ferrocenyl-CH₂CH₂-), 2.85 (4H, s, -COCH₂CH₂CO-), 4.15 (9H, broad s, ferrocenyl H). Elemental analysis: calculated for C₁₇H₁₇FeNO₄: C, 57.49; H, 4.82; N, 3.94; found: C, 57.38; H, 4.76; N, 3.94. Mass spectrometry: *m/z*: 355 (M⁺).

The reactivity and electrochemical properties of this pre-column labelling reagent were then investigated. The apparatus used was a Kyowa KHP-010 chromatograph (Kyowa Seimitsu, Tokyo, Japan) equipped with a Yanagimoto VMD-501 electrochemical detector (Yanagimoto, Kyoto, Japan) and a μ Bondapak C₁₈ column (30.5 cm \times 0.39 cm I.D.) (Waters Assoc., Milford, MA, U.S.A.). A mixture of acetonitrile and 0.5% ammonium dihydrogen phosphate (pH 3.0) containing 0.3% sodium perchlorate was used as the mobile phase at a flow-rate of 1 ml/min. Quantitative condensation of arylalkylamines such as phenethylamine and tryptamine with N-succinimidyl 3-ferrocenylpropionate was effected at room temperature for 20 min in acetonitrile-0.05 M borate buffer (pH 8.0) (1:1). A hydrodynamic voltammogram of the resulting derivatives indicated that the maximum sensitivity would be obtainable at +0.40 V vs. Ag/AgCl reference electrode with a detection limit of 0.2 pmole (signal-to-noise ratio = 2 at 5 nA full scale). At this applied potential, other electroactive compounds such as phenols, guaiacols, and aromatic amines gave no response to the electrochemical detector. Furthermore, the resulting ferrocene derivatives could be oxidized much more readily ($E_{1/2} = +0.28$ V) than a catechol, 2-hydroxyestrone ($E_{1/2} = +0.44$ V).

An oxidative and reductive series electrode detection system¹⁵ was then used. The applied potentials of upstream and downstream working electrodes were set at +0.6 V and 0 V, respectively. The collection efficiency (the ratio of the current at the downstream detector to that at the upstream detector) of ferrocene derivatives was found to be 0.289, which is higher than that of 2-hydroxyestrone (0.259). It is to be noted that the ferrocenyl group is more reversible in the redox reaction than the catechol group.

The present results show that the ferrocenyl derivative can be detected with high selectivity in the presence of other electroactive compounds. The newly developed reagent, N-succinimidyl 3-ferrocenylpropionate, has proved to be of great use for the selective and sensitive analysis of amino compounds in a complex matrix, particularly in biological fluids. Application of this method to the determination of arylalkylamines in urine is being conducted in these laboratories, and the details will be reported elsewhere.

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