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New Derivatization of Amines for Electrochemical Detection in Liquid Chromatography

New derivatization of primary and secondary amines for electrochemical detection in liquid chromatography has been developed. The derivatization reagents were prepared from vanillic acid and homovanillic acid by condensation with N-hydroxysuccinimide. Of the two reagents the activated ester of homovanillic acid reacted more readily with amines, providing the amides which were highly responsible for an electrochemical detector in reversed phase chromatography. The detection and quantitation limits determined with ethyl phenylalaninate were 200 pg and 1 ng, respectively.

Keywords——derivatization; electrochemical detection; primary and secondary amines; homovanillic acid; N-hydroxysuccinimide; activated ester method; reversed phase chromatography

In recent years, a variety of excellent derivatization methods have been developed to extend the applicability of liquid chromatography.[1] The labeling with the chromophore or fluorophore can provide the sensitivity or selectivity needed for many practical problems. Liquid chromatography with electrochemical detection (LC/EC) is currently recognized as a useful tool for the selective determination of trace components in the complex matrix.[2] Although the utilization of chemical reactions has been attempted to extend the scope of EC,[3] the derivatization reagents characteristic for LC/EC have not previously been developed. As a series of our studies on derivatization for liquid chromatography,[4-6] the preparation of new reagents for use in LC/EC of primary and secondary amines was undertaken.

The design of the promising reagent requires the incorporation of suitable structural features, *i.e.* functions reactive toward the amino group and responding for an electrochemical detector (ECD) with the high sensitivity. In the preceding paper of this series we reported LC/EC of catechol estrogens and their monomethyl ethers which show the detection response with the nearly identical sensitivity.[7] The catechol monomethyl ether function seems to be much more suitable than the free catechol because of its higher stability. Accordingly, the preparation of the activated esters of vanillic acid and homovanillic acid was carried out. Condensation of vanillic acid with N-hydroxysuccinimide was effected by the presence of N,N'-dicyclohexylcarbodiimide. Recrystallization of the product from ethyl acetate gave the desired ester (I) as colorless prisms in a fairly good yield. mp 173—174°. *Anal.* Calcd. for $C_{12}H_{11}NO_6$: C, 54.34; H, 4.18; N, 5.28. Found: C, 54.47; H, 4.14; N, 5.10. NMR (2.5% solution in $CDCl_3$) δ: 2.90 (4H, s, $-CO(CH_2)_2CO-$), 3.95 (3H, s, $-OCH_3$), 6.89 (1H, d, J=10 Hz, 5-H), 7.55 (1H, d, J=2 Hz, 2-H), 7.75 (1H, q, J=10, 2 Hz, 6-H). MS m/e: 265 (M^+). In similar fashion, condensation of homovanillic acid with N-hydroxysuccinimide followed by recrystallization from ethyl acetate gave the ester (II) as colorless needles. mp 141—142°. *Anal.* Calcd. for $C_{13}H_{13}NO_6$: C, 55.92; H, 4.69; N, 5.02. Found: C, 55.96; H, 4.74; N, 4.86. NMR (2.5% solution in $CDCl_3$) δ: 2.84 (4H, s, $-CO(CH_2)_2CO-$), 3.88 (2H, s, $-CH_2CO-$), 3.92 (3H, s, $-OCH_3$), 6.88 (3H, m, aromatic H). MS m/e: 279 (M^+).

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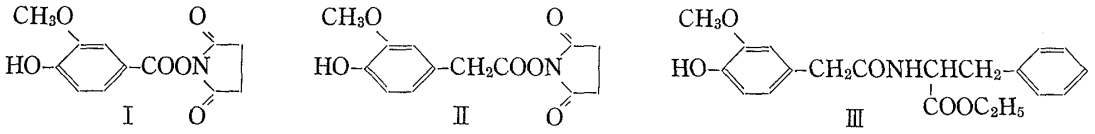

Chart 1

The apparatus used in high-performance liquid chromatography (HPLC) was a Waters Model ALC/GPC 202 chromatograph equipped with a Yanagimoto Model VMD-101 detector. HPLC was carried out on a μBondapak C_{18} column using 0.5% $NH_4H_2PO_4$(pH 3.0)/acetonitrile (2: 1) as a mobile phase at a flow rate of 1 ml/min. The potential of the detector was set at +0.8 volt *vs.* the silver-silver chloride reference electrode.

A preliminary experiment revealed that both derivatization reagents were reactive toward amines but acylation with I needed more prolonged reaction time than with II. Accordingly, derivatization was investigated in detail with II employing ethyl phenylalaninate as a model compound. Reaction of ethyl phenylalaninate with II was effected in pyridine at 60° for 30 min, providing the desired amide (III). The derivatized substrate showed a single peak of theoretical shape with the k' value of 5.0 and was highly responsible for ECD with a detection limit of *ca.* 200 pg. The formation rate of III under the above condition was determined to be *ca.* 83%. The linear response was obtained in the range of 1 to 25 ng of ethyl phenylalaninate. This derivatization method was similarly effective for LC/EC of secondary amines such as piperidine.

It is hoped that the planned synthesis of ECD-positive derivatization reagents will extend and improve the analytical capability, in particular sensitivity, reliability and automation. Further development of suitable derivatization methods for various functions other than amines is being conducted in this laboratory and the details will be reported in the near future.

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Pharmaceutical Institute
Tohoku University
Aobayama, Sendai 980
Japan

KAZUTAKE SHIMADA
MAKOTO TANAKA
TOSHIO NAMBARA

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