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A Convenient Derivatization with Anion Exchange Resin Catalysts for High-Performance Liquid Chromatographic Analysis. I. Derivatization of Estrogens with Dansyl Chloride

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Anion exchange resins, Duolite A-101D, A-102D, and A-109, were applied to the fluorescent derivatization of estrogens with dansyl chloride (DNS-Cl) for high-performance liquid chromatographic analysis. The derivatization proceeded rapidly and efficiently on passing a mixed solution of estrogens and DNS-Cl in acetone through a small column packed with Duolite A-102D (OH^- form) to give the corresponding DNS–estrogens in excellent yields. The procedure using the resin column is of practical value and may be applicable to the derivatization of other analytical samples.

Keywords——anion exchange resin; estrogen; dansyl chloride; column operation; fluorescent derivatization; HPLC

The development of convenient and effective methods for derivatization of analytical samples in high-performance liquid chromatographic (HPLC) analysis is as important as the development of new derivatization reagents. Anion exchange resins have been shown to be excellent catalysts in many organic reactions, for instance, in cyanohydrin formation,[1] Knovenagel[2] and Michael[3] condensations, in esterification of carboxylic acids,[4] and in *O*-benzylation of phenols.[5] These reactions were generally carried out at room temperature with stirring in the presence of anion exchange resins in organic solvents or water–organic solvent mixture. Such reactions under mild conditions should be useful for derivatization in HPLC analysis, but have not yet been applied. The present paper deals with the applicability of anion exchange resins to the fluorescent derivatization of estrogens with 5-dimethylaminonaphthalene-1-sulfonyl chloride (DNS-Cl).

Experimental

Materials——Anion exchange resins, Duolite A-101D, A-102D, and A-109, were kindly donated by Sumitomo Kagaku Kogyo Co. (Osaka). α-Estradiol was purchased from Sigma Chemical Co. (St. Louis, Mo.) and other estrogens were from Tokyo Kasei Co. (Tokyo). DNS-Cl was prepared in our laboratory by known methods.[6]

Preparation of Anion Exchange Resin Column——Anion exchange resins obtained as quaternary ammonium chloride salts were converted to the hydroxyl form by washing with 5% sodium hydroxide solution and then washed with distilled water until the washings were neutral. The resins (300 mg) were packed into a column (90 × 5 mm i.d.) and flushed with acetone.

Derivatization Procedure——Stock solutions (10 ml each) of DNS-Cl (73.5 μmol) and estrogen (7.35 μmol) in acetone were prepared in volumetric flasks and equal portions were mixed prior to use. The mixed solution (100 μl) was applied to the column, and eluted with 1 ml of acetone after 5 min. The eluate was evaporated to dryness by purging with nitrogen and the residue was dissolved in acetone (100 μl). A 10 μl aliquot of acetone solution was injected into the HPLC column.

HPLC——A model 655 HPLC machine (Hitachi Ltd., Tokyo) equipped with a model 650-10S fluorescence detector (Hitachi, Ltd.) was used throughout this work. A reversed-phase liquid chromatographic column, Unisil Pack Column (type 5C18-250A), commercially available from Gaskuro Kogyo Inc. (Tokyo), was used under ambient

conditions. Methanol–water–acetic acid (50 : 20 : 1, v/v) was used as the mobile phase at a flow rate of 0.8 ml/min. The fluorescence was monitored at 546 nm with excitation at 345 nm.

Results and Discussion

Anion exchange resins, Duolite A-101D, A-102D, and A-109, were usually purchased as quaternary ammonium chloride salts. These resins were converted to the hydroxyl form by treating them with dilute sodium hydroxide solution before use. The fluorescent derivatization of estrogens with DNS-Cl has been carried out in the presence of bases such as sodium bicarbonate, sodium carbonate, and sodium hydroxide as catalysts to increase the nucleophilicity of the phenolate ion.[7] Thus, preliminary attempts to apply these resins instead of such bases to DNS derivatization of estrogens were done on a preparative scale. Estrogens were easily derivatized with DNS-Cl in the presence of resins by batch operation to give the corresponding DNS–estrogens in 48—93% yields. The compounds obtained were purified by recrystallization and stored for use as standard samples. The structures of the DNS–estrogens were confirmed by the elemental analysis and ^{1}H-nuclear magnetic resonance (NMR) spectral data (shown in Table I).

In order to find a convenient derivatization procedure for HPLC analysis, small columns packed with Duolite resins were tested (Fig. 1). The resin amounts in the columns were changed in the range of 100 to 500 mg, and the derivatization of the test compounds, β-estradiol, with DNS-Cl was examined. Namely, the mixed solution of β-estradiol and DNS-Cl in acetone was applied to a column and then eluted with acetone. The eluate was evaporated, the residue was redissolved in acetone, and an aliquot was injected into a HPLC instrument equipped with a fluorescence detector (Ex. 345 nm, Em. 546 nm). DNS–estrogens formation

Table I. Preparation of DNS–Estrogens and Analytical Data

Compound[a]	Yield[b] (%)	mp (°C)	Formula	Analysis (%) Calcd (Found)			^{1}H-NMR ($CDCl_3$) δ[c]
				C	H	N	
DNS–α-estradiol	93	92—94	$C_{30}H_{35}NO_4S$	71.26 (71.95	6.98 7.13	2.77 2.63)	0.60 (3H, s, CH_3), 1.03—2.80 (16H, m, aliphatic-H), 2.96 (6H, s, $N(CH_3)_2$), 6.40—8.60 (9H, m, aromatic-H)
DNS–β-estradiol	92	183—184	$C_{30}H_{35}NO_4S$	71.26 (71.09	6.98 6.70	2.77 2.87)	0.71 (3H, s, CH_3), 0.85—2.76 (16H, m, aliphatic-H), 2.88 (6H, s, $N(CH_3)_2$), 6.36—8.60 (9H, m, aromatic-H)
DNS–ethynylestradiol	83	96—98	$C_{32}H_{35}NO_4S$	72.57 (72.00	6.66 7.04	2.64 2.28)	0.84 (3H, s, CH_3), 0.90—2.83 (15H, m, aliphatic-H), 2.63 (1H, s, C≡CH), 2.90 (6H, s, $N(CH_3)_2$), 6.40—8.63 (9H, m, aromatic-H)
DNS–estriol	48	217—219	$C_{30}H_{35}NO_5S$	69.08 (68.91	6.76 6.78	2.69 2.46)	0.74 (3H, s, CH_3), 1.10—2.80 (15H, m, aliphatic-H), 2.90 (6H, s, $N(CH_3)_2$), 6.37—8.70 (9H, m, aromatic-H)
DNS–estrone	90	184—185	$C_{30}H_{33}NO_4S$	71.55 (71.51	6.61 6.49	2.78 2.56)	0.86 (3H, s, CH_3), 0.97—2.86 (15H, m, aliphatic-H), 2.90 (6H, s, $N(CH_3)_2$), 6.40—8.70 (9H, m, aromatic-H)

a) Prepared by the use of Duolite A-102D resin as a catalyst. *b*) Isolated yields. *c*) Tetramethylsilane was used as an internal standard. Abbreviations: s, singlet; m, multiplet.

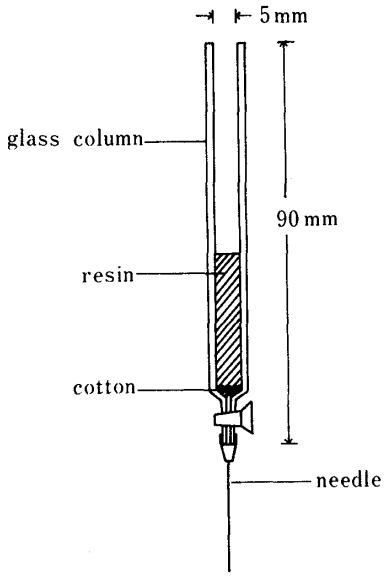

Fig. 1. Dimensions of Derivatization Column

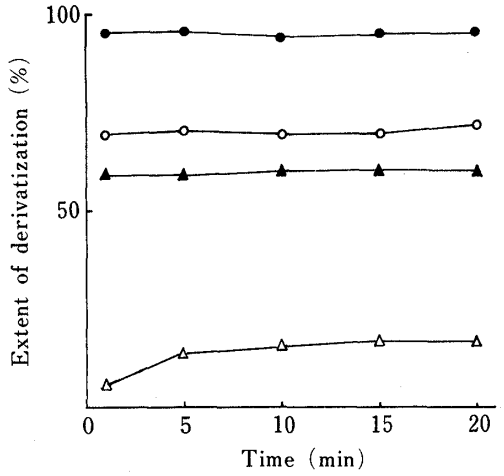

Fig. 2. Time Courses of Derivatization of β-Estradiol with DNS-Cl Using a Duolite A-102D Resin Column in Various Solvents

A solution of β-estradiol (10 μg) and DNS-Cl (99 μg) in each solvent (100 μl) was used in the derivatization.
●, acetone; ○, tetrahydrofuran; ▲, acetonitrile; △, benzene.

TABLE II. Effect of Resin Amount on the Derivatization of β-Estradiol[a]

Resin[b]	Extent of derivatization (%)[c]				
	A	B	C	D	E[d]
Duolite A-101D	57	59	60	63	60
Duolite A-102D	96	95	97	97	97
Duolite A-109	86	85	88	84	90

a) β-Estradiol (10 μg) was derivatized with DNS-Cl (99 μg) in acetone (100 μl) using various resin columns and a 10 μl aliquot of reaction mixture was injected into the HPLC column. *b*) Active group (exchange capacity): R–$C_6H_5CH_2\overset{+}{N}(CH_3)_3$ for Duolite A-101D (4.1 meq/g) and A-109 (3.6 meq/g), R–$C_6H_5CH_2\overset{+}{N}(CH_3)_2$ $\underset{CH_2OH}{|}$ for A-102D (3.7 meq/g). *c*) Average values of experiments repeated three or more times. *d*) Resin amounts in columns: A, 100 mg; B, 200 mg; C, 300 mg; D, 400 mg; E, 500 mg.

was estimated from the peak height ratios of standard and injected samples (Table II). It was found that Duolite A-102D among these resins quite effectively catalyzed the derivatization. On the other hand, almost no effect of resin amount in the columns was observed in this derivatization. Therefore, the column packed with Duolite A-102D resin (300 mg) was used in later experiments. Time courses of derivatization of β-estradiol with DNS-Cl using this column were examined in various solvents, acetone, tetrahydrofuran, acetonitrile, and benzene. As can be seen in Fig. 2, the derivatization in acetone gave a higher yield (97%) than those in other solvents. The effect of DNS-Cl concentration on the derivatization was also examined under the conditions defined above (Table III). About 10-fold molar excess of DNS-Cl was suitable for the derivatization. Furthermore, 1 ml of acetone was sufficient for the elution of DNS–β-estradiol from the column. Thus, the procedure in the experimental section was established as a suitable method for the derivatization of estrogens. Derivatization rates of all estrogens obtained by this method are summarized in Table IV. These estrogens other than estriol were derivatized with DNS-Cl in almost quantitative yields. The coefficients of variation (C.V.) of the derivatization for each estrogen (36.75 nmol) were 4.15—5.75%

Table III. Effect of DNS-Cl Concentration on the Derivatization of β-Estradiol[a]

Mol ratio (DNS-Cl/β-Estradiol)[b]	Extent of derivatization (%)
1	55
2	87
4	83
6	97
10	97
20	97

a) Derivatization was carried out using Duolite A-102D. *b*) β-Estradiol (10 μg) in acetone (100 μl) in each case.

Table IV. Rate of DNS–Estrogens Formation Using a Duolite A-102D Resin Column

Compound	Extent of derivatization[a] (%)	C.V.[b] (%)
DNS–α-estradiol	99	5.34
DNS–β-estradiol	97	4.35
DNS–ethynylestradiol	98	4.37
DNS–estriol	64	5.75
DNS–estrone	99	4.15

a) Measured by HPLC. *b*) Values ($n=8$) for 36.75 nmol of each estrogen in the reaction mixture.

($n=8$). The reason for the poor yield in the case of estriol is not yet clear. In the present study, it was found that the derivatization of estrogens with DNS-Cl was rapidly and efficiently accomplished under mild conditions by the use of an anion exchange column without addition of bases, heating of the reaction mixture, or extraction of products such as is required in the known methods.[7]

This method is, therefore, not only useful for the derivatization of estrogens, but also may be applicable to that of other samples for HPLC analysis.

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