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Anion-Exchange Resin Catalyzed Derivatization of Fatty Acids with 3-Bromoacetyl-6, 7-methylenedioxycoumarin

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Summary

Anion-exchange resins, Duolite A-375, A-561 and Amberlite IRA-904, were applied to the fluorescent derivatization of fatty acids with 3-bromoacetyl-6, 7-methylenedioxycoumarin (I) for high-performance liquid chromatographic analysis. The derivatization proceeded rapidly and efficiently by stirring a mixture of fatty acids and I in acetone with a weakly basic resin, Duolite A-375, at room temperature. The procedure using the resins is of practical value and is applicable to the derivatization of free fatty acids in human blood plasma.

The development of convenient methods for the 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 known to be excellent catalysts in many organic reactions, for instance, in esterification of carboxylic acids (1), in *O*-benzylation of phenols (2) and in cyanohydrin formation (3). 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 the derivatization in HPLC.

In a preceding communication (4), we reported 3-bromoacetyl-6, 7-methylenedioxycoumarin (**I**) as a new fluorescence derivatization reagent which reacts with fatty acids to afford highly fluorescent fatty acid esters (**IIa-g**). This reaction, however, requires suitable catalysts; such as 18-crown-6 and potassium

bicarbonate for effective derivatization.

The present paper deals with a convenient derivatization of fatty acids with **I** by use of an anion-exchange resin as substitute for such catalysts.

Experimental

Anion-Exchange Resins

Anion-exchange resins (Duolite A-375, A-561 and Amberlite IRA-904) were purchased from Sumitomo Kagaku Kogyo Co. (Osaka) and Organo Co. (Tokyo). These resins obtained as quaternary ammonium chloride were converted to the hydroxyl form by washing with 5% sodium hydroxide solution and then washed with distilled water until the washings became neutral.

Derivatization Procedure

Stock solution of stearic acid (10 μM) and **I** (100 μM) were prepared in reaction solvents. A mixture of each stock solution (1 ml) and Duolite A-375 resin (25 mg) was stirred at room temperature for 20 min. An aliquot (5 μl) of the reaction mixture was injected into the liquid chromatograph.

Derivatization of Free Fatty Acids in Human Blood Plasma

The plasma (100 μl) obtained from fresh human blood was treated in the usual manner (5). The stock solution of **I** (5 ml) and Duolite A-375 resin (200 mg) was added to a plasma sample in a reaction vial. The mixture was treated in the manner described above.

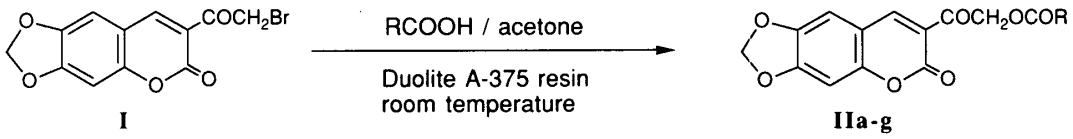
Apparatus and Measurements

All melting points were measured with a Yanagimoto micro-melting point apparatus and are uncorrected. ^{1}H-NMR spectra were taken with a JEOL JNM-GSX 500FT-NMR spectrometer employing tetramethylsilane as an internal standard. Absorption and fluorescence spectra were measured with a Hitachi 150-20 spectrophotometer and a Hitachi F-4000 fluorescence spectrophotometer, respectively. Fluorescence quantum yields were determined according to the method of Paker and Rees (6), quinine sulfate in 0.5M H_2SO_4 was used as a standard. Mass spectra were taken with a JEOL JMXDX303 spectrometer. HPLC was carried out at room temperature on a Hitachi 655A high-performance liquid chromatograph equipped with a Hitachi F-1050 fluorescence spectrophotometer (excitation wavelength 388 nm; emission wavelength 475 nm) and a Hitachi L-6200 solvent gradient device. A Unisil Pack 5C18-250A column (250 $\times$ 4.6 mm i.d.; particle size, 5 μm; GL Science Ltd., Tokyo) was used in a Hitachi 655A-52 column oven (ca. 30°C).

Results and Discussion

Preparation of Standard Samples

Standard samples for seven fatty acids (lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids) were synthesized on preparative scale according to the method of derivatization procedure (Chart 1). The obtained fatty acid coumarinacyl esters (**IIa-g**) were recrystallized from ethanol after isolation by thin layer chromatography (solvent system, benzene-ethyl acetate=5 : 1 v/v). The structures of **IIa-g** were confirmed by elemental analysis, ^{1}H-NMR and mass spectral data (Table 1 and 2). But the suitable values in elemental analysis of **IIg** could not be obtained in spite of careful treatment. This may be because the double bonds in acid moiety are easily oxidized.



I **IIa-g**

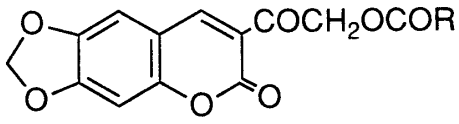
IIa : R = -$C_{11}H_{23}$ (laurate)
IIb : R = -$C_{13}H_{27}$ (myristate)
IIc : R = -$C_{15}H_{31}$ (palmitate)
IId : R = -$C_{17}H_{35}$ (stearate)
IIe : R = -$C_{17}H_{33}$ (oleate)
IIf : R = -$C_{17}H_{31}$ (linoleate)
IIg : R = -$C_{17}H_{29}$ (linolenate)

CHART 1.

TABLE 1. *Physical properties of 6, 7-methylenedioxycoumarin-3-acetyl carboxylates*

Compound	mp (°C)	Formula	Analysis (%) Calcd		Found	
			C	H	C	H
IIa	135-137	$C_{24}H_{30}O_7$	66.96	7.02	66.93	6.82
IIb	137-138	$C_{26}H_{34}O_7$	68.10	7.47	68.03	7.37
IIc	131-133	$C_{28}H_{38}O_7$	69.11	7.87	69.17	7.79
IId	133-134	$C_{30}H_{42}O_7$	70.01	8.23	69.86	8.20
IIe	114-115	$C_{30}H_{40}O_7$	70.29	7.87	70.05	7.73
IIf	109-110	$C_{30}H_{38}O_7$	70.56	7.50	70.52	7.47
IIg	94-95	$C_{30}H_{36}O_7$	70.84	7.14	—	—

TABLE 2. ^1H-NMR spectral data of



Compoud	R	^{1}H-NMR($CDCl_3$) δ				
		C_4-H	C_5-H	C_8-H	C_3-$COCH_2$-	C_6-OCH_2O-C_7
IIa	$C_{11}H_{23}$	8.53	6.97	6.87	5.36	6.15
IIb	$C_{13}H_{27}$	8.53	6.97	6.87	5.36	6.15
IIc	$C_{15}H_{31}$	8.54	6.97	6.87	5.36	6.15
IId	$C_{17}H_{35}$	8.53	6.97	6.87	5.36	6.15
IIe	$C_{17}H_{33}$	8.53	6.97	6.87	5.35	6.15
IIf	$C_{17}H_{31}$	8.53	6.97	6.87	5.37	6.15
IIg	$C_{17}H_{29}$	8.54	6.97	6.87	5.37	6.15

Fluorescent Derivatization of Fatty Acids with I

The derivatization of a test compound, stearic acid, with **I** in the presence of various anion-exchange resins was carried out at room temperature in acetone. The derivatization efficiency of stearic acid was estimated from the fluorescent peak height of product (**IId**) at 475 nm (Fig. 1).

In preliminary experiments, the blank tests were run at room temperature by stirring **I** with various resins, such as Duolite A-101D, A-102D, A-109, Amberlite IRA-904 (strongly basic resins) and Duolite A-340, A-375, A-561 (weakly basic resins). The use of strongly basic resins except for Amberlite IRA-904 revealed large peaks due to the decomposition of reagent in the region which overlapped with those of the derivatization products on the chromatograms. On the other hand, Duolite A-375, A-561 and Amberlite IRA-904 among these resins, particularly Duolite A-375 not only minimized the undesired peaks in that region but also effectively catalyzed the derivatization (Fig. 1). Duolite A-375 resin, therefore, was used as a catalyst for further experiments.

Figure 2 shows the time course of derivatization of stearic acid in various solvents in the presence of Duolite A-375 resin. The derivatization, as shown in Fig. 2, was accomplished after 20 min at room temperature in acetone or acetonitrile. Furthermore, the effects of concentration of reagent and amount of catalyst were examined by HPLC method. Fluorescent peak height of the products showed the constant values in the ranges of 5 to 10 fold molar excess of reagent in concentration and 20–100 mg in the amount of catalyst.

From these results, the derivatization of fatty acids was carried out by stirring them with more than 10-fold moles of **I** and 25 mg of Duolite A-375 resin

6, 7-methylenedioxycoumarin-3-acetyl carboxylates

^{1}H-NMR($CDCl_3$) δ			
R			
2.48 (-$OCOCH_2$-)	1.06-1.56 (-CH_2-)	0.88 (-CH_3)	
2.47 (-$OCOCH_2$-)	1.20-1.73 (-CH_2-)	0.88 (-CH_3)	
2.47 (-$OCOCH_2$-)	1.20-1.78 (-CH_2-)	0.88 (-CH_3)	
2.47 (-$OCOCH_2$-)	1.13-1.62 (-CH_2-)	0.88 (-CH_3)	
2.48 (-$OCOCH_2$-)	1.20-1.78 (-CH_2-)	0.87 (-CH_3)	5.30-5.39 (-CH=CH-)
2.47 (-$OCOCH_2$-)	1.30-2.79 (-CH_2-)	0.89 (-CH_3)	5.35-5.37 (-CH=CH-)
2.48 (-$OCOCH_2$-)	1.30-2.83 (-CH_2-)	0.97 (-CH_3)	5.33-5.40 (-CH=CH-)

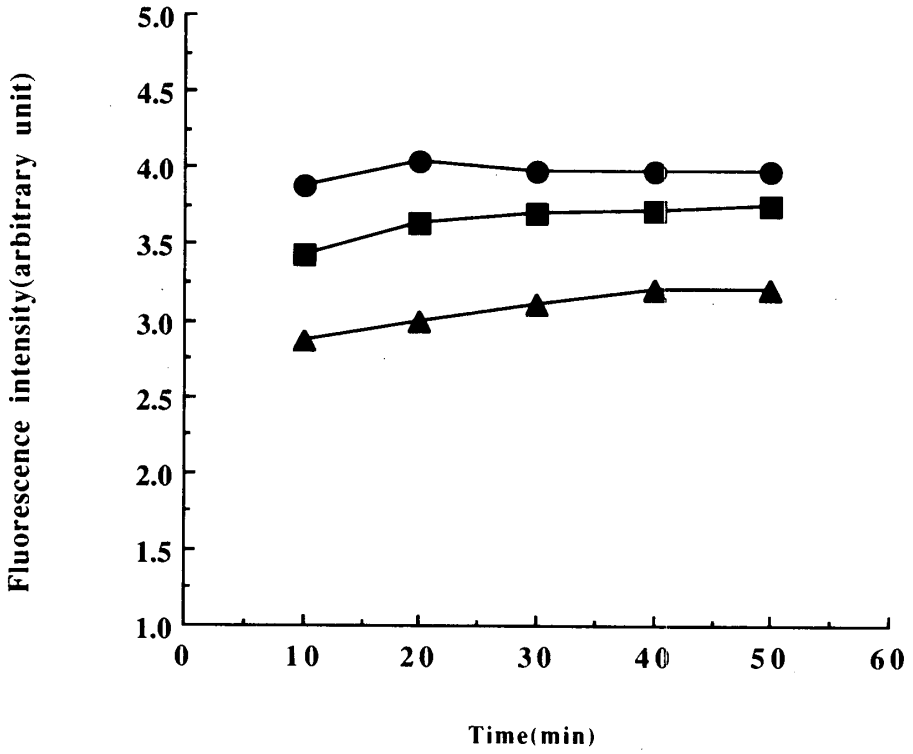


FIG. 1. Effect of anion-exchange resin catalyst for derivatization of stearic acid in acetone
concentration : stearic acid, 10 nmol/ml ; I, 100 nmol/ml.
catalyst : ●, Duolite A-375 (25 mg) ; ■, Amberlite 904 (10 mg) ; ▲, Duolite A-561 (25 mg).

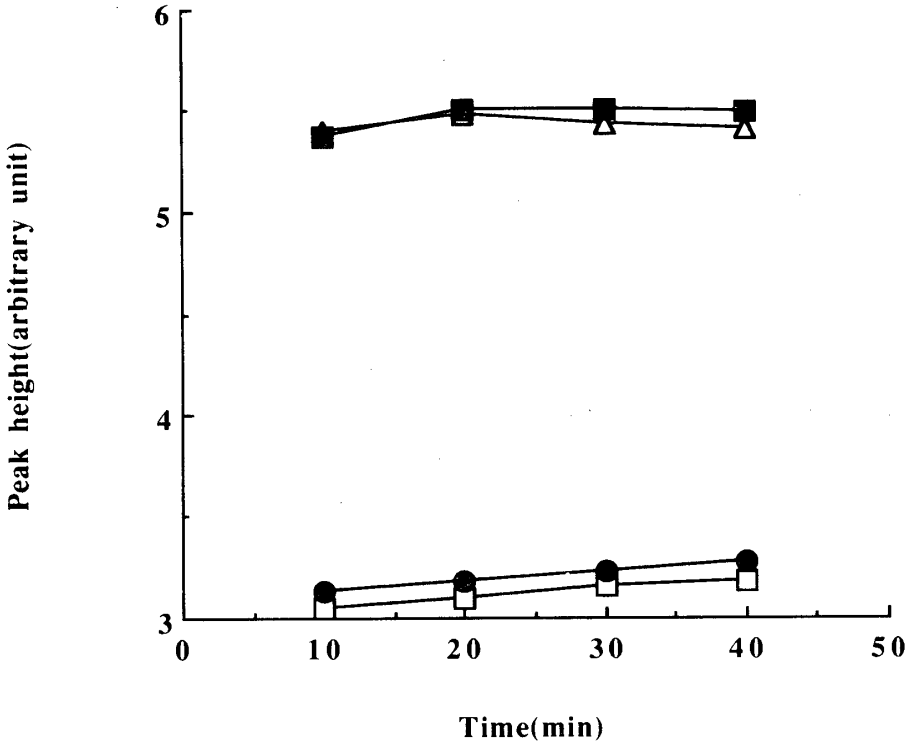


FIG. 2. Time course of derivatization of stearic acid with **I** at room temperature in various solvents
solvents: ■, acetone; △, acetonitrile; ●, benzene; □, dichloromethane.
concentration: stearic acid, 10 nmol/ml; **I**, 100 nmol/ml.
catalyst: Duolite A-375, 25 mg.

for 20 min in acetone at room temperature.

HPLC

The HPLC chromatogram of the derivatives obtained by derivatizing a mixture of seven fatty acids under the given condition coincided with that shown in the preceding paper (4). A linear relationship for stearic acid was also obtained in the range of 10 to 300 pmol/injection and its detection limit was 500 fmol (S/N=3). The coefficient of variation (C.V.) of the derivatization for stearic acid (1 nmol/ml) was 3.0% (n=7). When this method was applied to the derivatization of free fatty acids (FFAs) in human blood plasma, HPLC peaks originated from FFAs in plasma were clearly separated as well as those given by another method (4). Incidentally, the fluorescence quantum yields of **IIa-g** in methanol and acetonitrile were found to be in the range of 0.51 to 0.86.

The present method should be useful as a convenient derivatization procedure in HPLC analysis for monitoring FFAs or drugs in blood and determining carboxylic acids in foods.

References

1) G. Caineli and F. Maneschalchi, *Synthesis*, **1975**, 723 (1975).
2) E.J. Row, K.L. Kaufman and C. Piantadosi, *J. Org. Chem.*, **23**, 1622 (1958).
3) C.J. Schmidle and R.C. Mansfield, *Ind. Eng. Chem.*, **44**, 1388 (1952).
4) A. Takadate, T. Masuda, C. Murata, C. Haratake, A. Isobe, M. Irikura and S. Goya, *Anal. Sci.*, **8**, 695 (1992).
5) H. Tsuchiya, T. Hayashi, M. Sato, M. Tatsumi and N. Takagi *J. Chromatogr.*, **309**, 43 (1948).
6) C.A. Parker and W.T. Rees, *Analyst* [London], **85**, 587 (1960).