

## Sensitive and Mild Fluorogenic Reagents for Biogenic Carboxylic Acids in HPLC

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**Abstract:** Five acid hydrazides as precolumn fluorescence derivatization reagents for carboxylic acids in HPLC, which have the benzofuran or benzothiazole moiety conjugated to a furan, thiophene or oxazoline ring, were synthesized and examined in view of reactivity, separability and sensitivity. Of these hydrazides, 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole (BTOH) was most favorable. The detection limit of lauric acid as a model acid was 0.1 pmol per 10- $\mu$ l injection volume at S/N=3, which was roughly equal to that of an analogous compound, 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole. The reagent allowed rapid assays of carboxylic acids (C<sub>12:0</sub>-C<sub>20:4</sub>) within 20 min with satisfactory separability. The method was applied to the determination of fatty acids in human sera from healthy volunteers as well as from patients with diabetes or thyroid dysfunction.

**Keywords:** High performance liquid chromatography, Acid hydrazide  
Fluorescence derivatization reagents, Biogenic carboxylic acids.

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### 1. Introduction

In previous works [1-4], we synthesized novel fluorescence derivatization reagents, 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (OMBH) [1], 2-(5-hydrazinocarbonyl-2-thienyl and furyl)-5,6-methylenedioxybenzofurans (TMBH and

FMBH, respectively) [2], and 2-(5-hydrazinocarbonyl-2-furyl and oxazolyl)-5,6-dimethoxybenzothiazoles (BTFH [3] and BTOH [4], respectively) for the determination of carboxylic acids in HPLC, and applied these compounds to the assay of biological samples.

These fluorogenic acid hydrazides have the advantage compared to other derivatization reagents for carboxylic acids that the derivatization can be performed

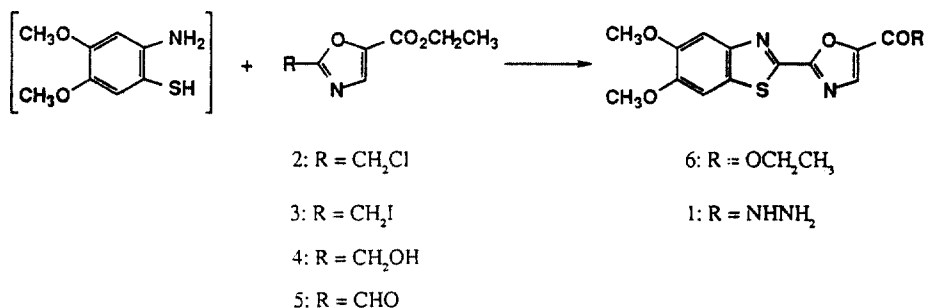
carboxylic acids that the derivatization can be performed under mild conditions in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) [5,6]. The methods are selective and sensitive toward carboxylic acid and the derivatives are stable. The detection limits of lauric acid using OMBH, TMBH, FMBH, and BTFH were in the range 0.1 - 0.05 pmol per 10- $\mu$ l injection volume at S/N=3.

The investigation on the fluorescence property and the chromatographic behavior of these compounds revealed that the benzothiazole derivative (BTFH) exhibits a higher detection sensitivity for carboxylic acids than does the corresponding benzofuran derivative (FMBH) with similar separability and that the heteroaromatic moiety contributes primarily to the separation. The OMBH-derivatized acids (oxazolyl derivatives) are more polar than the corresponding FMBH- or TMBH-derivatized acids (furyl or thienyl derivatives, respectively) and they tend to be less retained on a reversed-phase column, resulting in a rapid elution. It was therefore envisioned that an acid hydrazide which incorporated both benzothiazole and oxazole core structures had the merit of high sensitivity. In this report we describe the synthesis, fluorescence properties and its application to the assay of fatty acids in serum of such a novel acid hydrazide, BTOH (compound 1).

#### Apparatus

Fluorescence spectra were measured on a Hitachi 650-60 spectrometer in 10 x 10 mm quartz cells (Tokyo, Japan); spectral bandwidths of 5 nm were used in both the excitation and emission monochromators. Proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) spectra were obtained on a Bruker AC-200P spectrometer (Rheinstetten, Germany), operating at 200 MHz, using tetramethylsilane as an internal standard. The splitting patterns were designated as follows: s, singlet; t, triplet; q, quartet. Infrared spectra were taken on a Hitachi 270-30 spectrometer. Fast atom bombardment mass spectra (FAB-MS) were measured on a JEOL JMS-AX 505W mass spectrometer (Tokyo, Japan) using 2-nitrobenzyl alcohol as a matrix. Uncorrected melting points were obtained on a Yamato MP-21 melting point apparatus (Tokyo, Japan). HPLC analyses were performed with a Shimadzu LC-4A liquid chromatograph (Kyoto, Japan), equipped with a RF-530 fluorescence detector (with a 1- $\mu$ l flow cell) operating at an excitation wavelength of 369 nm and an emission wavelength of 451 nm. The column was a Wakosil-II 5C18 HG (4.6 x 250 mm i.d.; particle size 5  $\mu$ m; Osaka, Japan) and the temperature was 40  $^\circ\text{C}$ . The mobile phase was aqueous acetonitrile which was pumped at a flow rate of 1.0 ml min $^{-1}$ .

Chart 1



### Reagents and materials

Caprylic (C<sub>8:0</sub>), capric (C<sub>10:0</sub>), lauric (C<sub>12:0</sub>), myristic (C<sub>14:0</sub>), palmitic (C<sub>16:0</sub>) and stearic (C<sub>18:0</sub>) acids were purchased from Wako Pure Chemical Industries (Osaka, Japan), margaric (C<sub>17:0</sub>) and nonadecanoic (C<sub>19:0</sub>) acids were from Tokyo Chemical Industry (Tokyo, Japan) and myristoleic (C<sub>14:1</sub>), palmitoleic (C<sub>16:1</sub>), linoleic (C<sub>18:2</sub>), oleic (C<sub>18:1</sub>), arachidonic (C<sub>20:4</sub>) and linolenic (C<sub>18:3</sub>) acids from Sigma (St. Louis, MO, U.S.A.). Prostaglandins (PG) F<sub>1α</sub>, F<sub>2α</sub>, E<sub>1</sub> and E<sub>2</sub> were also purchased from Sigma. All the fatty acids were used without purification. Stock solutions of these acids (1 mM) were prepared in methanol and diluted with water to give required concentrations prior to use. The solution of BTOH (15 mM) was prepared in dimethyl sulfoxide (DMSO). Serum specimen were obtained from healthy volunteers in our laboratory; those from patients with diabetes or thyroid dysfunction were obtained from Kumamoto University Hospital (Kumamoto, Japan).

### Synthesis

#### Ethyl 2-chloromethyl-5-oxazole carboxylate (2)

Compound **2** was prepared from ethyl chloroglyoxalate and diazomethane in 18 % yield as a colored oil by the method described in a patent literature [6]. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.40(t, 3H, CH<sub>3</sub>, J = 7.1 Hz), 4.41(q, 2H, CH<sub>2</sub>, J = 7.1 Hz), 4.66(s, 2H, CH<sub>2</sub>Cl), 7.73(s, 1H, oxazolyl-H). IR (neat, cm<sup>-1</sup>): 2996 (oxazoline), 1742 (ester), 1594, 1550. FAB-MS: *m/z* = 190 [M+1]<sup>+</sup>.

#### Ethyl 2-iodomethyl-5-oxazole carboxylate (3)

A mixture of chloride **2** (5.0 g, 26.4 mmol) and sodium iodide (11.7 g, 78.1 mmol) in acetone (80 ml) was refluxed for 1 h, during which time the chloride **2** disappeared (as judged by thin layer chromatography). The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo* to give iodide **3** quantitatively as a reddish brown oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.39(t, 3H, CH<sub>3</sub>, J = 7.2 Hz), 2.05(s, 2H, CH<sub>2</sub>I), 4.43(q, 2H, CH<sub>2</sub>, J = 7.1 Hz), 7.70(s, 1H, oxazolyl-H). IR (neat, cm<sup>-1</sup>): 2996 (oxazoline), 1728 (ester), 1590, 1534. FAB-MS: *m/z* = 282 [M+1]<sup>+</sup>.

#### Ethyl 2-hydroxymethyl-5-oxazole carboxylate (4)

A mixture of iodide **3** (7.4 g, 26.3 mmol) and silver carbonate (21.5, 78.0 mmol) in 70 % (v/v) aqueous tetrahydrofuran (THF; 100 ml) was refluxed for 4 h. The reaction mixture was filtered through Celite and the filtrate was concentrated *in vacuo* to leave solids which was chromatographed on silica gel (5 % ethyl acetate in benzene), affording 2.04 g (45 %) of alcohol **4** as off-white crystalline solids. mp: 60.5 - 62.0 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.39(t, 3H, CH<sub>3</sub>, J = 7.1 Hz), 2.73(t, 1H, OH, J = 6.4 Hz), 4.40(q, 2H, CH<sub>2</sub>, J = 7.1 Hz), 4.81(d, 2H, CH<sub>2</sub>OH, J = 6.1 Hz), 7.72(s, 1H, oxazolyl-H). IR (KBr, cm<sup>-1</sup>): 3348 (OH), 3136, 3004 (oxazoline), 1726 (ester), 1588, 1544. FAB-MS: *m/z* = 172 [M+1]<sup>+</sup>.

#### Ethyl 2-formyl-5-oxazole carboxylate (5)

To a stirred CH<sub>2</sub>Cl<sub>2</sub> solution (15 ml) of oxalyl chloride (2.04 g, 16.0 mmol) was added DMSO (1.54 g, 19.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 ml) at -50 °C under nitrogen. After 5 min, alcohol **4** (1.4 g, 8.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 ml) was added and the solution was stirred for 15 min at this temperature. Triethylamine (5.7 ml, 40.9 mmol) was added to the solution which was then allowed to warm to room temperature. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo*. Chromatography on silica gel (5 % ethyl acetate in benzene) yielded 465 mg (33 %) of aldehyde **5**. mp: 71.5 - 73.0 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.42(t, 3H, CH<sub>3</sub>, J = 7.1 Hz), 4.45(q, 2H, CH<sub>2</sub>, J = 7.2 Hz), 7.97(s, 1H, oxazolyl-H), 9.85(s, 1H, CHO). IR (KBr, cm<sup>-1</sup>): 3164 (oxazoline), 1736 (ester), 1716 (aldehyde), 1566, 1504. FAB-MS: *m/z* = 170 [M+1]<sup>+</sup>.

#### 5-(5',6'-Dimethoxybenzothiazolyl)oxazolyl-2-carboxylic acid, ethyl ester (6)

Concentrated hydrochloric acid (11.3 ml) was added to a stirred ethanol solution (100 ml) containing di(4,5-dimethoxy-2-nitrophenyl)sulfide [**5**] (0.75 g, 1.8 mmol) and tin powder (3.3 g, 27.8 mmol) at 40 - 45 °C. After being stirred for 1 h at this temperature, the reaction mixture was diluted with water (~100 ml). Hydrogen sulfide gas was bubbled through the solution, which resulted in precipitation. The solution was filtered, and the filtrate was concentrated *in vacuo* to give 4,5-dimethoxy-2-aminothiophenol as an off-white solid which was used without purification for the following

condensation reaction. A 2 : 1 (v/v) mixture (30 ml) of ethanol and acetic acid containing 4,5-dimethoxy-2-aminothiophenol and aldehyde 5 (0.75 g, 5.0 mmol) was refluxed for 1 h. The resulting precipitates were collected and recrystallized from ethanol to afford 381 mg (32 % over 2 steps) of ester 6 as yellow needles. mp: 200.0 - 201.0 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.43(t, 3H, CH<sub>3</sub>, J = 7.2 Hz), 3.99(s, 3H, OCH<sub>3</sub>), 4.01(s, 3H, OCH<sub>3</sub>), 4.45(q, 2H, CH<sub>2</sub>, J = 7.2 Hz), 7.35(s, 1H, Ar), 7.66(s, 1H, Ar), 7.93(s, 1H, oxazolyl-H). IR (KBr, cm<sup>-1</sup>): 3444 (aromatic), 2992, 1734 (ester), 1716 (aldehyde), 1584, 1558, 1496. FAB-MS: *m/z* = 335 [M+1]<sup>+</sup>.

*2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole (1)*

A solution of ester 6 (381 mg, 1.14 mmol) in ethanol (20 ml) containing dimethylformamide (DMF; 3 ml) and hydrazine hydrate (5 ml) was refluxed for 1 h. The resulting precipitates were collected, washed with ethanol, and dried *in vacuo* to give 243 mg (63 %) of BTÖH as a yellow powder. mp: 255.5 - 280.0 °C (decomp.). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ 3.89(s, 6H, OCH<sub>3</sub>), 4.63(s, 2H, NH<sub>2</sub>), 7.68(s, 1H, Ar), 7.80(s, 1H, Ar), 7.97(s, 1H, oxazolyl-H), 10.12(s, 1H, NH). IR (KBr, cm<sup>-1</sup>): 3316 (aromatic), 3096, 1634 (ester), 1560, 1496, 1442, 1432. FAB-MS: *m/z* = 321 [M+1]<sup>+</sup>. Anal. Calcd. for C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>O<sub>4</sub>S: C, 48.75; H, 3.78; N, 17.49. Found: C, 48.70; H, 3.82; N, 17.67.

*Derivatization reaction with lauric acid*

A mixture containing compound 1 (50 mg, 0.16 mmol), lauric acid (31 mg, 0.16 mmol), 45 ml of 100 mM EDC in methanol (0.23 mmol), and pyridine (10 ml) in 3 : 1 (v/v) mixture of methanol and DMSO (4 ml) was incubated at 37 °C for 2 h. The resulting mixture was diluted with ethyl acetate (100 ml), and washed successively with water and 5 % NaOH. After being dried over Na<sub>2</sub>SO<sub>4</sub>, the organic phase was concentrated and the residue was chromatographed on SiO<sub>2</sub> (50 → 100 % ethyl acetate in chloroform) to give 30 mg (38 %) of the derivatized product as a yellow solid. mp: 143.5 - 153.5 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 0.88 (t, 3H, CH<sub>3</sub>, J = 6.7 Hz), 1.25(m, 16H, CH<sub>2</sub>), 1.65(m, 2H, CH<sub>2</sub>), 2.36(t, 2H, COCH<sub>2</sub>, J = 7.6 Hz), 3.98(s, 3H, OCH<sub>3</sub>), 4.01(s, 3H,

OCH<sub>3</sub>), 7.28(s, 1H, Ar), 7.61(s, 1H, Ar), 7.95(s, 1H, oxazolyl-H), 9.07(s, 2H, NH). IR (KBr, cm<sup>-1</sup>): 3268 (aromatic), 2928, 2860, 1718, 1698, 1682, 1670, 1648, 1558, 1498, 1282. FAB-MS: *m/z* = 503 [M+1]<sup>+</sup>.

*Derivatization*

To 100 µl of a test solution containing fatty acids (C<sub>12:0</sub> - C<sub>20:4</sub>, 10 mM each) in methanol were sequentially added 100 µl of 1 % (v/v) pyridine in methanol, 100 µl of 15 mM compound 1 in DMSO and 100 µl of 100 mM EDC in methanol. The mixture was incubated at 37 °C for 60 min; a 10-µl aliquot of the resulting mixture was injected into the HPLC.

*Assay of fatty acids in serum*

Fifty µl of human serum was extracted with 100 µl of the Dole solution [7] (isopropanol - heptane - 1N H<sub>2</sub>SO<sub>4</sub>, 40 : 10 : 1, v/v); the organic phase (50 µl) was separated and concentrated *in vacuo* to dryness. To this was added 50 µl each of 15 mM compound 1, 100 mM EDC, 1 % (v/v) pyridine and 100 mM nonadecanoic acid (as an internal standard). After the mixture was incubated at 37 °C for 60 min, a 10-µl aliquot was injected into the HPLC.

### 3. Results and Discussion

*Synthesis and fluorescence properties*

Compound 1 was synthesized by a similar method to that for BTFH as shown in Chart 1. The key intermediate, aldehyde 5, was prepared in 3 steps from the known compound 2 [6]. Since, unlike BTFH, direct conversion of 2 to alcohol 4 resulted in a poor yield (< 5 %), the alcohol was prepared via the corresponding iodide. The next oxidation reaction to the aldehyde under a variety of conditions resulted mainly in a complex product mixture presumably due to decomposition of the oxazoline ring. Swern oxidation [8] was eventually found to be effective, giving aldehyde 5 in 15 % overall yield from 2. The subsequent condensation reaction with 4,5-dimethoxy-2-aminothiophenol under acidic conditions, which was achieved in a similar manner to

that for BTFH with slight modification, afforded **6** as a single product. The transformation to the corresponding acid hydrazide readily proceeded to give compound **1**. The emission wavelengths of OMBH, TMBH, FMBH and BTFH were 450 nm (excited at 350 nm), 483 nm (excited at 373 nm), 462 nm (excited at 362 nm) and 452 nm (excited at 363 nm), respectively.

The fluorescence emission maximum ( $\lambda_{em}$ ) of **1** in acetonitrile was 451 nm at the excitation maximum ( $\lambda_{ex}$ ) of 369 nm; these were roughly the same as those of BTFH. There was not a large wavelength shift in the  $\lambda_{em}$  of lauric acid derivatized with **1** (454 nm in acetonitrile), which was synthesized with 1.0 equiv. of **1** and 1.4 equiv. of EDC at 37 °C for 2 h in a moderate yield, whereas the  $\lambda_{ex}$  shifted substantially (369 nm  $\rightarrow$  412 nm). The  $\lambda_{em}$  shifted to a longer wavelength (473 nm) in water (at  $\lambda_{ex}$  = 369 nm) with an intensity being increased (1.25-fold). The fluorescence intensity (at the concentration of  $10^{-7}$  M in water) is 0.45 times less than that of BTFH under identical conditions.

#### Derivatization

The derivatization conditions were optimized using lauric acid. The fluorescence peak height increased with increasing concentrations of **1** in the range 0 - 15 mM in DMSO, in the derivatization reaction carried out with 100  $\mu$ l each of compound **1**, 10  $\mu$ M lauric acid, 100 mM EDC and 1 % (v/v) pyridine in methanol for 60 min at 37 °C. The reagent **1** precipitated in the reaction medium at concentrations above 15 mM. The concentration of 15 mM of the reagent was thereafter used in the following experiments as the optimum value.

When the EDC concentration was varied in the range 0 - 400 mM in the derivatization with 15 mM reagent, while other conditions remained in the same as above, the fluorescence response increased with increasing EDC concentrations and reached a maximum and constant at 100 mM.

The pyridine concentration was next varied in the range 0 - 10 % (v/v) under the same conditions with 15 mM reagent and 100 mM EDC; the pyridine concentration of 1 % (v/v) gave the maximum derivatizing efficiency. Thus, the concentrations of 100 mM and 1

% (v/v) were used as the standard protocol for EDC and pyridine, respectively.

The derivatizations performed at different temperatures did not yield a substantial difference in the peak heights, whereas the yield of the reaction depended substantially on the reaction time, reaching the maximum at 120 min; the following assays of fatty acids were carried out by derivatizing them at 37 °C for 60 min. The reaction yield of the acid after a 2-h incubation at 37 °C was calculated to be 73 % based on the standard curve ( $Y = 44.45 X - 92.11$ ;  $r = 0.999$ ) between the fluorescence peak height ( $Y$ ) and the amount of the derivative of **1** in pmol per 10- $\mu$ l injection volume ( $X$ ). The within-run repeatability of the derivatization yield at 37 °C was within 5 % as the relative standard deviation ( $n = 5$ ).

By derivatizing lauric acid at trace level (40 nM - 1  $\mu$ M in the reaction mixture) under the optimized conditions, the detection limit of the acid was determined to be 0.1 pmol (per 10- $\mu$ l injection volume) at  $S/N=3$ , which was roughly equal to that of BTFH; the derivatized lauric acid was stable for weeks when refrigerated. A linear relationship between the peak height and the lauric acid concentration was observed in the range 1 pmol to 25 nmol (per 10- $\mu$ l injection volume). A linear regression analysis yielded the values of  $a$  and  $b$  as  $4.20 \times 10^{-4}$  and  $1.58 \times 10^{-2}$ , respectively, in the linear equation  $Y = aX + b$  ( $r = 0.998$ ), where  $X$  and  $Y$  are the amount of the acid in pmol (per 10- $\mu$ l injection volume) and the relative fluorescence peak height (measured with nonadecanoic acid as the internal standard), respectively.

It was also confirmed that compound **1** did not yield any fluorescent products with amino acids and  $\alpha$ -ketoglutaric acid under these conditions. The reactivity of compound **1** with aldehydes was examined using hexanal, octanal, decanal and dodecanal as representative ones. The each aldehyde of the amount down to 2 - 3 pmol (per 10- $\mu$ l injection volume) was detectable in the derivatization reaction using 5 mM compound **1** in DMF at 90 °C for 20 min, but the peak reproducibility was not satisfactory compared to **1** (data not given). Ketones were not derivatized under the conditions.

#### Assay of fatty acids

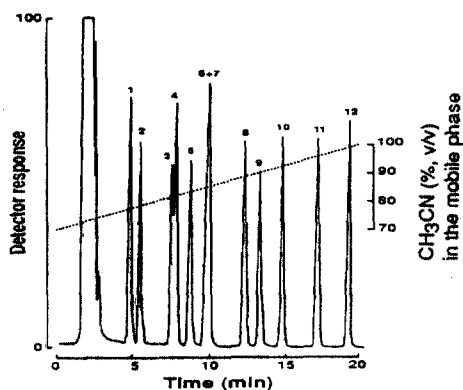


Fig. 1 A chromatogram obtained with a standard mixture of carboxylic acids (25 nmol each per 10- $\mu$ l injection volume) derivatized with compound 1. The derivatization was carried out as described in the experimental section. Peaks: 1 = C12:0; 2 = C14:0; 3 = C16:0; 4 = C18:0; 5 = C20:0; 6 = C22:0; 7 = C24:0; 8 = C26:0; 9 = C28:0; 10 = C30:0; 11 = C32:0; 12 = C34:0.

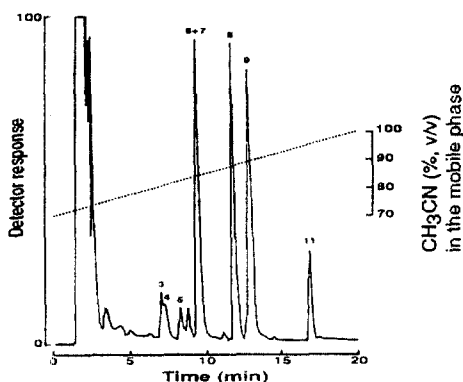


Fig. 2 A chromatogram of a normal human serum derivatized with compound 1.

A 50- $\mu$ l aliquot of the serum was derivatized as detailed in the experimental section. The HPLC conditions and the peak identification were identical to those in Fig. 2.

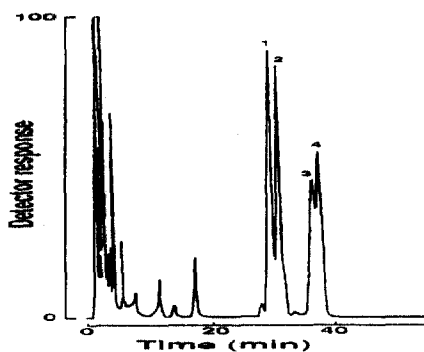


Fig. 3 A chromatogram of PGs derivatized with compound 1. PGs (0.25 pmol each in a 10- $\mu$ l injection volume) were derivatized as described in the experimental section. The HPLC conditions were identical to those in Fig. 2. Peaks: 1 = PGF<sub>1</sub> $\alpha$ ; 2 = PGF<sub>2</sub> $\alpha$ ; 3 = PGE<sub>2</sub>; 4 = PGE<sub>1</sub>.

A standard mixture of fatty acids (10 mM each in methanol) was derivatized with compound 1 under the optimized conditions and the derivatized acids were separated by reversed-phase HPLC (Fig. 1). All acids except for arachidonic and linoleic acids (peaks 6 and 7, respectively) were well separated by linear gradient elution with aqueous acetonitrile (70  $\rightarrow$  100 %, v/v) within 20 min; the elution time was less than a half of that using BTFH. The values of relative standard variation for both within-run peak intensity and retention time did not exceed 2 % for each acid in 10 separate runs.

Figure 2 shows a typical chromatogram obtained with a serum specimen from a healthy volunteer in our laboratory, where the HPLC conditions were identical to those for the standard mixture. The serum specimen was extracted by the Dole's method [7] and the extract was derivatized as described in the experimental section. The peaks were identified based on co-chromatography with each authentic acid. Both dicarboxylic acids (oxalic, malonic, succinic and adipic acids) and hydroxycarboxylic acids (lactic and malic acids) yielded the fluorescent products by this method, but they did not interfere with the chromatography because of their retention times that were less than 5 min. The short run-time within 20 min was less than a half of that of BTFH which normally required more than 40 min in the assay of serum samples. The recoveries (mean  $\pm$  S.D.;  $n = 5$ ) of fatty acids (2.5 nmol each added to 50 ml of serum) were  $84.9 \pm 6.5$  % (C<sub>16:0</sub>),  $79.8 \pm 7.3$  % (C<sub>18:1</sub>) and  $87.8 \pm 2.1$  % (C<sub>18:0</sub>).

The method was then applied to the determination of fatty acids in human sera from healthy volunteers as well as from patients with diabetes or thyroid dysfunction (hyper- and hypothyroidism). The concentrations of major four acids (C<sub>16:0</sub>, C<sub>18:0</sub>, C<sub>18:1</sub> and C<sub>18:2</sub>) were determined by using a working curve, in which linearity was observed in the range 10 pmol to 2 nmol (per 10- $\mu$ l injection volume) for each acid. Since linoleic (C<sub>18:2</sub>) and arachidonic (C<sub>20:4</sub>) acids are not resolved under the HPLC conditions, the concentration of linoleic acid possibly contains some of arachidonic acid (although it is much smaller). The increased concentration of fatty acids in sera from patients with diabetes was reported

elsewhere [9]. The increased or decreased concentration in the case of hyperthyroidism or hypothyroidism, respectively, might be explained in relevance to the disorder in the metabolic use of glucose [10].

A standard PG mixture of F<sub>1α</sub>, F<sub>2α</sub>, E<sub>1</sub>, and E<sub>2</sub> was also tested by this method (Fig. 3). All the PGs were separated under the same HPLC conditions as above although the separation between E<sub>1</sub> and E<sub>2</sub> was not satisfactory. Compared to BTFH, the separation was very similar, but the overall elution time of less than 40 min was significantly shorter (> 50 min for BTFH) under similar HPLC conditions. Changing the HPLC conditions did not result in better separation.

In summary, we have synthesized a unique acid hydrazide, compound **1**, that is characterized by its benzothiazole core structure conjugated to an oxazoline residue, and examined the applicability of this compound as a precolumn derivatization reagent for carboxylic acids in HPLC. With its short run-time and reasonable separability, compound **1** permits rapid assays for fatty acids; this is due to the presence of the oxazoline moiety because an analogous furan-containing compound, BTFH, requires much longer elution time (more than 2-fold) under similar HPLC conditions with a sensitivity being similar to **1**. We believe that the method using **1** is of particular value when a large number of samples are assayed.

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