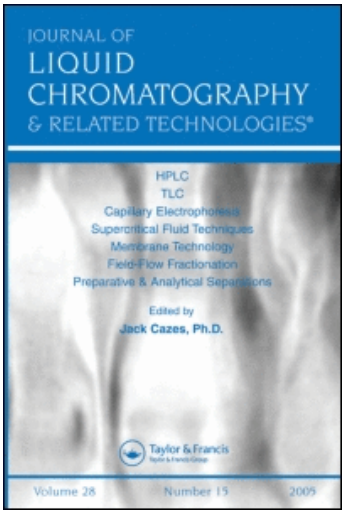

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A NEW FLUOROGENIC REAGENT FOR LABELLING CARBOXYLIC ACIDS IN HPLC

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A NEW FLUOROGENIC REAGENT FOR LABELLING CARBOXYLIC ACIDS IN HPLC

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ABSTRACT

A new fluorescent reagent for carboxylic acids, *N*-(4-bromomethyl-7-hydroxy-2-oxo-2H-6-chromenyl) bromoacetamide (Br-MAMC) was synthesized. The fluorescence properties of Br-MAMC were monitored for a series of polar and non-polar solvents. Its applicability as a precolumn derivatization reagent in high-performance liquid chromatography was examined using undecylenic acid as a model compound.

The derivatization reaction was performed in acetone in the presence of potassium carbonate and the crown ether, 18-crown-6 at 30°C for 20 min. The reaction solution was subjected to a high-performance liquid chromatographic procedure on a reversed-phase ODS C-18 column (150 × 4.6 mm i.d., 5 μm) with a mobile phase of acetonitrile and water (75 : 25, v/v) at a flow rate of 1.0 mL/min.

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The derivative of undecylenic acid was detected fluorometrically; excitation and emission wavelengths were set at 345 and 435 nm, respectively. The detection limit of the method was determined to be 12.5 pg (signal to noise ratio = 3) of the derivatized undecylenic acid on column. The derivatives of butyric, hexanoic, octanoic, decanoic, and dodecanoic acid with Br-MAMC were also well resolved with a mobile phase of acetonitrile and water (60 : 40, v/v).

INTRODUCTION

Carboxylic acid containing substances represent one of the most interesting categories of molecules, as they are present in substances of significant biological interest, such as prostaglandins, free fatty acids, non-steroidal anti-inflammatory drugs (NSAIDs). Their determination in low levels is important and, therefore, sensitive techniques are employed for their analysis. As most of these compounds are non-fluorescent and exhibit usually low UV absorbance, they cannot be determined easily. High-performance liquid chromatography in combination with precolumn fluorescence derivatization is widely employed for their determination (1).

Various fluorescent labeling reagents have therefore been developed for this purpose. Among these, are 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone (Br-DMEQ),(2,4) 9-bromomethylacridine (Br-MA), (5) (*N*-9-acridinyl)-bromacetamide, (6) 9-anthryldiazomethane (ADAM),(7,9) *N*-cyclohexyl-*N*-(1-pyrenyl)carbodiimide, (10) and *N*-(4-nitro-2,1,3-benzoxa-diazoyl-7-yl)-*N*-methyl-2-aminoaceto-hydrazine (NBD−CO−Hz) (11).

Coumarin based reagents represent some of the most succesful probes used for the determination of substances with carboxylic acid moiety. These reagents can be categorised as bromomethyl derivatives, such as 4-bromomethylmethoxy-coumarin (Br-MMC),(12–15) 7-acetoxy-4-bromomethyl coumarin (Br-MAC), (16,17) 4-bromomethyl-6,7-dimethoxycoumarin (Br-MDMC), (18) 4-bromomethyl-6,7-methylendioxycoumarin (Br-MDC), (19) as well as haloacetyl derivatives, such as 3-bromoacetylcoumarin, (20) 3-bromacetyl-6,7-methylendioxycoumarin, (21) and 7-diethylamino-3-((4′-(iodoacetyl)amino)phenyl)-4-methylcoumarin (DCIA) (22).

Comparing the two aforementioned categories of the coumarin based reagents, those containing the haloacetyl group are considered generally more reactive than the corresponding bromomethyl group species. Furthermore, among the various substituents of the coumarin nucleus, the alkylamino, as well as the alkoxy groups, are considered the most beneficial in terms of enhancement of fluorescent intensity. The co-existence of two bulky groups in our molecule

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(a methoxy and a diethylamino) restricts their rotation ability. Thus, the loss of the absorbed energy due to internal conversion is minimized. Furthermore, it can also enhance the charge transfer into the molecule, a property which generally correlates with enhancement of fluorescence.

The present paper describes the synthesis of *N*-(4-bromomethyl-7-hydroxy-2-oxo-2H-6-chromenyl) bromoacetamide (Br-MAMC), which is planned to be used as a coumarin based fluorescence probe. The fluorescent properties of the synthesized molecule were monitored for a series of polar and non-polar solvents. Furthermore, the applicability of Br-MAMC to the HPLC analysis of carboxylic acids was evaluated using undecylenic acid as a model compound. The undecylenic acid (also called 10-undecenoic acid) is an unsaturated hydroxy acid with anti-fungal properties (23,24). The choice of this acid as a model was based on the fact that it exhibits low UV absorbance and has no natural fluorescence, due to the absence of a strong chromophore or fluorophore.

These properties, in combination with the fact that it does not possess any significant stereochemical hindrance, makes undecylenic acid a good candidate for testing the efficiency of the synthesized reagent. Moreover, only a few methods have been reported for the assay of this compound, including potentiometric titration, (25) high-performance liquid chromatography with either direct UV detection at 200 nm very close to the cut-off of the mobile phase, (26) or at 265 nm after derivatization with 4′ nitrophenacyl bromide, (27) and gas chromatography (28).

The derivatization reaction was performed in acetone in the presence of potassium carbonate and the crown ether, 18-crown-6. The optimum reaction conditions (reaction time, temperature, concentration of Br-MAMC, solvent, presence or absence of catalyst, etc.) were also investigated. Furthermore, the derivatization procedure was successfully applied to the fluorescent labelling of a mixture of butyric, hexanoic, octanoic, decanoic, and dodecanoic acid.

EXPERIMENTAL

Materials and Reagents

All solvents used were of HPLC grade and were purchased from Lab-Scan Science Ltd., Ireland. Potassium carbonate was purchased from Merck (Darmstandt, Germany). Water was deionized and further purified by means of a Milli-Q Plus Water Purification System, Millipore Ltd. Undecylenic acid, butyric acid, hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid were purchased from Fluka Chemie, Switzerland. All reagents used for the synthesis of Br-MAMC along with 18-crown-6 were obtained from Sigma-Aldrich Chemie

(Steinheim, Germany). 2,4-dimethoxy aniline was obtained also from Sigma-Aldrich Chemie (Steinheim, Germany) and was recrystallized prior to use.

Apparatus

The chromatographic system comprised of a Spectra Physics, Model SS8810, pump and a Rheodyne, Model 7725i, injector with a 20-μL loop, which were coupled to a Perkin-Elmer, Model LS-30, spectrofluorometer, equipped with a total emission accessory, and a Spectra Physics Model SP4270 integrator. The mobile phase was filtered through a 0.45 μm Millipore filter and degassed under vacuum prior to use.

Fluorescence measurements were performed on the Perkin Elmer, Model LS-30, spectrofluorometer that is equipped with a peristaltic pump for the introduction of the sample (using spectral bandwidths of 2 nm for the acquirement of the spectra). UV-vis spectra were recorded on a Perkin Elmer, Model Lambda 7 spectrophotometer.

^{1}H-NMR spectra were recorded on a Brucker, Model AC-200 spectrometer (200 MHz). IR-spectra were recorded on a Perkin-Elmer, Model 883 infrared spectrophotometer as nujol mulls. Melting points were determined with a Buchi melting-point apparatus and are uncorrected.

Synthesis

N-(2,4-dimethoxyphenyl) acetamide (I): An aqueous potassium carbonate solution (20 gr. K_2CO_3 in 77 mL water) was added to a solution of 2,4-dimethoxyaniline (23 gr. 0.15 mol) in 50 mL chloroform and the bilayer mixture was stirred vigorously. An acetyl chloride solution (15.7 gr—0.17 mol in 15 mL chloroform) was added drop wise and the stirring continued for another 30 min. The aqueous layer was discarded and the organic was washed with 5% aqueous HCl and brine. Evaporation of the solvent in vacuum gave 29.3 gr of a brownish solid (yield 100%). Recrystallization from ethanol gave colorless needles (mp 117°C).

N-(2,4-dihydroxyphenyl) acetamide (II): A mixture of (*I*) (20.3 gr—0.104 mol), $AlCl_3$ (125.5 gr—0.936 mol) and NaCl (48.7 gr) were heated in an oil bath at 140°C under rigorous mechanical stirring, until the evolution of gas has been ceased (approx. 2 hrs). The resulting green liquid, while hot, was poured into an ice–water mixture. The resulting solution was extracted exhaustively with ethyl acetate. The solvent was removed under reduced pressure and the resulting brown solid (17.4 gr. yield 100%) was recrystallized from water (mp 180°C)

IR (nujol mull), cm^{-1}: 3369, 3528–2539, 1638.

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^{1}H-NMR (dmso-d_6), ppm: 2 (3H, s, $-CH_3$), 6.1–6.2 (1H, q, arom.), 6.3 (1H, d, arom.), 7.1–7.2 (1H, d, arom.), 9.2 (2H, s, OH), 9.6 (1H, s, NH).

N-(4-bromomethyl-7-hydroxy-2-oxo-2H-6-chromenyl) acetamide (III): 17.5 gr. (0.1 mol) *II* and 13.65 gr. (0.1 mol) ethylacetoacetate were mixed thoroughly until a uniform paste was obtained and the mixture was placed in an ice–salt bath. Consequently 30.5 mL of cold 70% aqueous sulphuric acid was added and the mixture was left to stand at 5°C overnight. The yellow-green liquid was poured into an ice water mixture and the resulting precipitate was collected with suction filtration and was washed with cold water until all washings became neutral (16 gr yield 67.6%). Recrystallization from DMF afforded yellow solid (mp 297°C) IR (nujol mull), cm^{-1}: 3410, 1735, 1665.

^{1}H-NMR (dmso-d_6), ppm: 2.1 (3H, s, CH_3-CO), 2.3 (3H, s, $-CH_3$), 6.2 (1H, s, $-CH=$), 6.8 (1H, s, arom.), 8.1 (1H, s, arom.), 9.4 (1H, s, OH), 11 (1H, s, NH).

N-(7-methoxy-4-methyl-2-oxo-2H-6-chromenyl) acetamide (IV)*:* 2.32 gr. *III* were suspended in 150 mL anhydrous acetone and 3.8 gr. (0.03 mol) dimethylsulfate and 3 gr. K_2CO_3 were added. The mixture was heated under reflux for 5 hrs, the solvent was removed and 100 mL of cold water were added. The resulting solid (1.8 gr. yield 73.2%) was recrystallized from acetone (mp 232–234°C).

IR (nujol mull), cm^{-1}: 3450, 1690, 1655.

^{1}H-NMR (dmso-d_6), ppm: 2.1 (3H, s, $-CO-CH_3$), 2.4 (3H, s, $-CH_3$), 3.9 (3H, s, CH_3O), 6.3 (1H, s, $-CH=$), 7.1 (1H, s, arom.), 8.3 (1H, s, arom.), 9.4 (1H, s, NH).

Elemental analysis: calc. C 63.15%, H 5.30%; found C 63.38%, H 5.37%.

6-amino-7-methoxy-4-methyl-2H-2-chromenone (V): 1.5 gr. (0.061 mol) is suspended in an ethanol/hydrochloric acid mixture 1 : 2 (27 mL) and the suspension is refluxed for 45 min. The resulting solution is refrigerated overnight and the resulting precipitate is collected, dried, and dissolved again in the minimum required water volume. The solution is neutralized with ammonium hydroxide and the resulting yellow crystals (1 gr. yield 82%) were recrystallized from acetone/DMF (mp 223–225°C).

IR (nujol mull), cm^{-1}: 3340, 3355, 1705.

^{1}H-NMR (dmso-d_6), ppm: 2.4 (3H, s, $-CH_3$), 3.8 (3H, s, $-OCH_3$), 4.9 (2H, s, $-NH_2$), 6.2 (1H, s, $-CH=$), 6.8 (1H, s, arom), 6.9 (1H, s, arom).

Elemental analysis: calc. C 64.38%, H 5.4%; found C 64.19%, H 5.39%.

N-(7-methoxy-4-methyl-2-oxo-2H-chromenyl)-2-bromoacetamide *(VI)*: 0.5 gr. (0.00243 mol) *V* was dissolved in acetone and 0.41 gr. (0.00027 mol) of bromoacetylchloride along with 0.25 gr. K_2CO_3 were added. After 2 hrs refluxing the solvent was removed and 100 mL cold water was added. The resulting yellow solid (0.45 gr. yield 56.6%) was recrystallized from acetone (mp 224–225°C).

IR (nujol mull), cm^{-1}: 3420, 1710, 1670.

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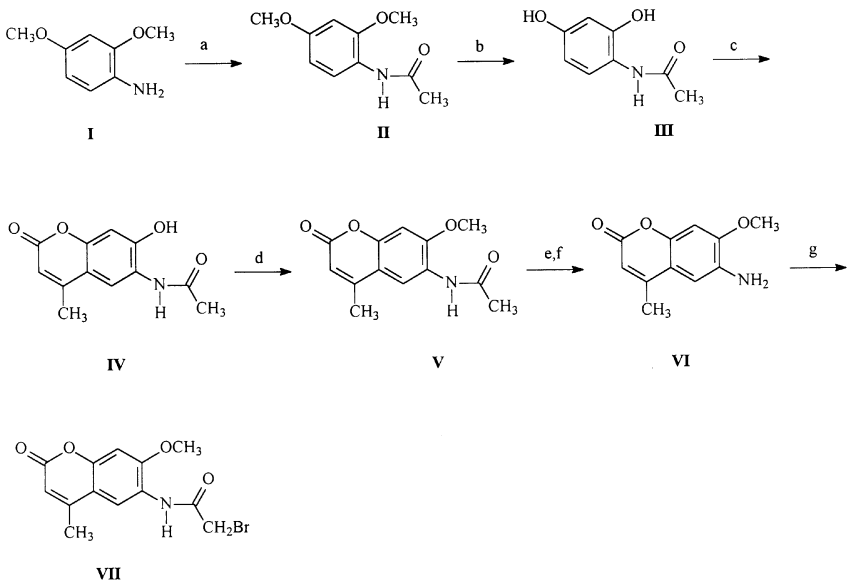

a. CH_3COCl/K_2CO_3
b. $AlCl_3$ / NaCl / 140°C
c. $CH_3COCH_2COOCH_2CH_3$ / H_2SO_4
d. $(CH_3)_2SO_2$ / K_2CO_3
e. HCl
f. Aq. NH_3
g. $BrCH_2COCl$

Scheme 1. Synthesis of *N*-(4-bromomethyl-7-hydroxy-2-oxo-2H-6-chromenyl) bromoacetamide.

^{1}H-NMR (dmso-d_6), ppm: 2.4 (3H, s, $-CH_3$), 4 (3H, s, $-CH_3O$), 4.2 (2H, s, $-CH_2Br$), 6.3 (1H, s, $-CH=$), 7.2 (1H, s, arom), 7.8 (1H, s, arom), 9.8 (1H, s, NH).

Elemental analysis: calc. C 47.88%, H 3.71%; found C 47.82%, H 3.74%.

The synthetic route is represented briefly in Scheme 1.

Stock Standard Solutions

Stock standard solutions of the reagent (Br-MAMC), 40.0 μg/mL and of the 18-crown-6, 1.0 μg/mL, were prepared by dissolving appropriate amounts of the corresponding compounds in acetone. These solutions were stored in the dark

under refrigeration and were found to be stable for a period of at least 3 weeks. Stock standard solutions of undecylenic, butyric, hexanoic, octanoic, decanoic, or dodecanoic acid (1.0 μg/mL), were also prepared in acetone and were found to be stable for several weeks at 4°C.

Derivatization Procedure

An appropriate volume (25–500 μL) of the undecylenic acid solution reacted with 50 μL of the Br-MAMC solution (40.0 μg/mL) and corresponding aliquots of acetone were added to reach a volume of 1000 μL. The reaction occurred in the presence of 15 μL of 1.0 μg/mL 18-crown-6 solution and 2 mg of a fine suspension of K_2CO_3, into an amber-colored micro-reaction vessel (3.0 mL). The reaction mixture was allowed to stand for 20 min at 30°C. Consequently, 100 μL of this reaction mixture were drawn and diluted with 100 μL of the mobile phase; a 20-μL aliquot was injected into the HPLC. The same procedure was repeated for the derivatization reaction of all five fatty acids.

Chromatographic Conditions

All of the chromatographic separations of Br-MAMC esters of the studied carboxylic acids were achieved on a reversed-phase ODS C-18 column (150 × 4.6 mm, 5 μm particle size) Jones Chromatography, USA. The mobile phase used for the separation of Br-MAMC ester of undecylenic acid from the excess of the fluorescent probe was a mixture of acetonitrile and water (75 : 25 v/v) pumped at a flow rate of 1.0 mL/min. The mobile phase was slightly modified in order to achieve complete separation of Br-MAMC esters of the five fatty acids, thus, a mixture of acetonitrile and water (60 : 40 v/v) pumped at a flow rate of 1.0 mL/min was used.

All chromatographic experiments were conducted at ambient temperature. Initially excitation and emission wavelengths were adjusted at 345 and 435 nm, respectively.

RESULTS AND DISCUSSION

One of the most important advantages of Br-MAMC is the coexistence of two electron-donating groups, namely the methoxy and the bromacetyl group. Their charge transfer properties, acting essentially in an additive manner, results to the larger degree of charge transfer compared to that if only one electron donor was present to the coumarin nucleus. Thus, the amplification of polarization

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ability facilitates the movement of charge during the excitation process, thus enhancing its fluorescence intensity.

One of the major ways of relieving the excess of absorbed energy in a fluorescent molecule is the thermal relaxation process, happening through the vibrational movement of flexible moieties existing in it. The simultaneous existence of two bulky groups in Br-MAMC (the methoxy ether and the bromacetamido group) restricts, significantly, their rotation ability. This way, the loss of absorbed energy after the excitation process, through vibrational relaxation, is minimized, increasing the quantum yield of fluorescence.

Fluorescence Properties

In order to examine the fluorescence properties of the synthesized molecule, its relative fluorescence intensity (water = 100) was monitored for a series of polar and non-polar solvents with varying hydrogen bond donor–hydrogen bond acceptor properties. The results are presented in Table 1. It can be clearly anticipated that hydrogen bond donor solvents, such as water and methanol, enhance fluorescence intensity, presumably through hydrogen bond formation between the solvent and the unshared pair of electrons of the oxygen

Table 1. Relative Fluorescent Intensities of *N*-(4-Bromomethyl-7-hydroxy-2-oxo-2H-6-chromenyl) Bromoacetamide in Various Solvent Mixtures

Solvent Mixture	IF[a]
Methanol	45.3
Methanol and water (75 : 75, v/v)	52.9
Methanol and water (50 : 50, v/v)	59.2
Methanol and water (25 : 75, v/v)	72.6
Acetonitrile	22.5
Acetonitrile and water (75 : 25, v/v)	39.5
Acetonitrile and water (50 : 50, v/v)	47.6
Acetonitrile and water (25 : 75, v/v)	57.8
Tetrahydrofuran	9.8
Tetrahydrofuran and water (75 : 25, v/v)	19.6
Tetrahydrofuran and water (50 : 50, v/v)	27.3
Tetrahydrofuran and water (25 : 75, v/v)	36.9
Chloroform	15.4
Heptane	2.9
Water	100

[a]Relative fluorescence intensity (water = 100).

and nitrogen moieties of the Br-MAMC molecule. Tetrahydrofuran and acetonitrile have a higher polarity index compared to *n*-heptane and form hydrogen bonds only as hydrogen bond acceptors. The significant loss of fluorescence, in the case of *n*-heptane, compared with that observed for the more polar solvents tetrahydrofuran or acetonitrile, reveals that the excited state possesses significant charge transfer character compared to the ground state.

Effort has been made to use a mobile phase with the characteristics described. According to this, the most polar mobile phase that could provide an adequate separation was found to be a mixture water/acetonitrile.

Optimization of the Derivatization Procedure

The precolumn derivatization procedure of undecylenic acid with Br-MAMC was examined. In order to estimate the factors that affect the reaction kinetics, the fluorescence intensities vs. various factors (temperature, reaction time, presence of catalyst, concentration of Br-MAMC) were estimated. In every step of the optimisation procedure, all the contributing factors but one remain constant and the optimised value is used for the next experiment.

Fluorescence intensity was assessed initially for 20 min at 25, 30, 40, 50, and 55°C. The maximum reaction yield was achieved at 30°C. Lower temperatures were inadequate, whereas at temperatures higher than 50°C, the signal degraded slowly, perhaps through thermal decomposition of the product (Figure 1). Consequently, the time profile of the reaction yield was monitored for 60 min, keeping the optimised temperature value of the reaction. The ester

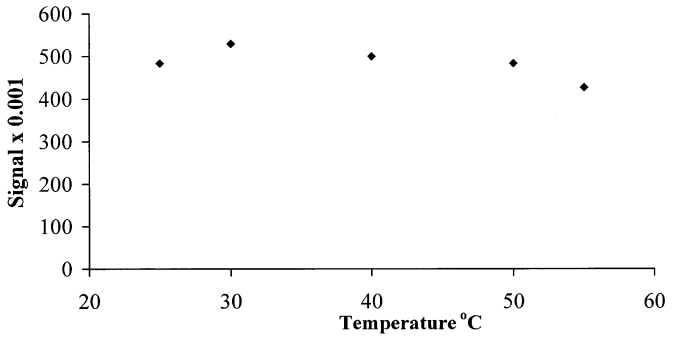

Figure 1. Influence of temperature on the derivatization of undecylenic acid with Br-MAMC; reaction time: 20 min; Br-MAMC-Undecylenic acid: 1/8 M/M; in the presence of 18-crown-6 ether.

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formation was completed within 20 min, while further heating did not increase the reaction yield (Figure 2).

The stoicheiometric ratio between the reagent and undecylenic acid was also examined and it was found that the reaction yield reached to a plateau for ratios greater than 1 : 8, M/M (Figure 3). Finally, the contribution of the catalyst, namely the 18-crown-6 ether, was evaluated. The role of 18-crown-6 ether is to solvate K^+ in its polar cavity formed by the 6 oxygen atoms, leaving the anion of the acid countered by the opposite charge and, thus, more nucleophilic. As it can

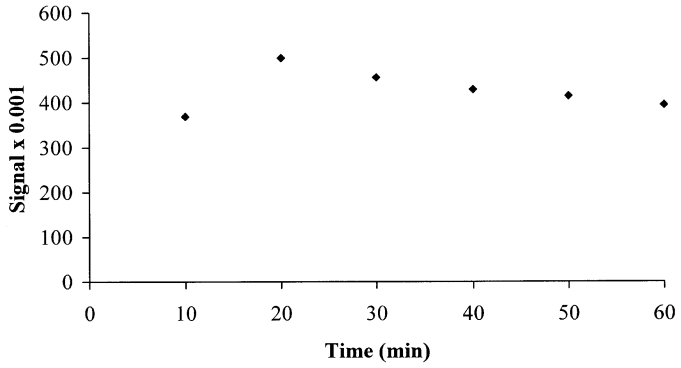

Figure 2. Influence of the reaction time on the derivatization procedure of undecylenic acid with Br-MAMC; temperature: 30°C; Br-MAMC-Undecylenic acid: 1/8 M/M; in the presence of 18-crown-6 ether.

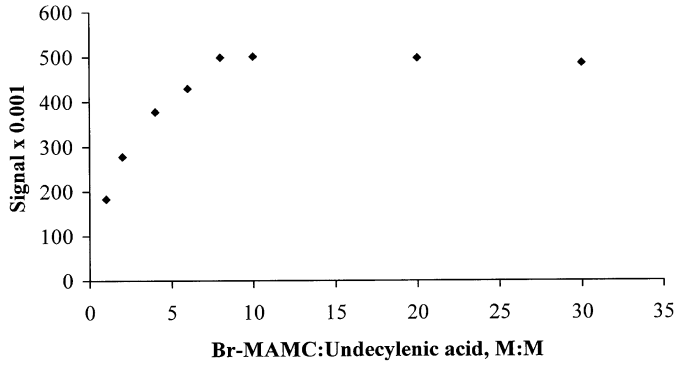

Figure 3. Influence of stoicheiometric ratio of Br-MAMC–undecylenic acid on the derivatization of undecylenic acid with Br-MAMC; reaction time: 20 min; temperature: 30°C; in the presence of 18-crown-6 ether.

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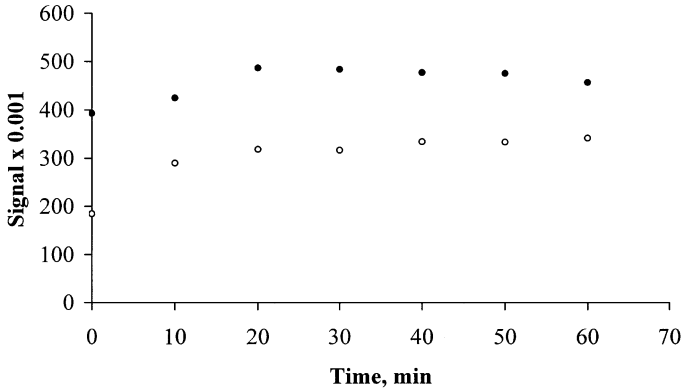

Figure 4. Influence of the presence (●), and the absence, (○), of 18-crown-6 ether on the derivatization of undecylenic acid with Br-MAMC reaction time: 20 min; temperature: 30°C; Br-MAMC–undecylenic acid: 1/8 M/M.

be seen in Figure 4, the derivatization proceeds much slower and in significantly lower yield, about 60% in the absence of 18-crown-6 from the reaction mixture.

The optimum conditions were the following: temperature 30°C, reaction time: 20 min, ratio of undecylenic acid and Br-MAMC (1/8, M/M). In Figure 5 is presented a typical chromatogram of the separation of the excess of Br-MAMC and the ester of Br-MAMC, with undecylenic acid eluting at retention times 2.05 and 5.32 min, respectively.

Statistical Analysis of Chromatographic Data

A calibration curve was constructed by assaying solutions containing various concentrations of undecylenic acid in acetone, under the optimum derivatization conditions described above. To evaluate the linearity of the proposed procedure, the concentration ranges covered from 25 to 250 ng/mL. Results presented in Table 2 indicate that linear relationship was found between the peak area of the ester of Br-MAMC with undecylenic acid and the corresponding concentration. The correlation coefficient, along with the standard deviation of the slopes and intercepts are also presented.

The precision (% R.S.D.) and the accuracy (E_r%) of the proposed derivatization procedure were also evaluated. Thus, ten replicate determinations were carried out on a 100 ng/mL of undecylenic acid solution in acetone, giving a mean value of 100.12 ± 1.20 with a relative standard deviation of 1.2% and a relative percentage error of 0.1%. The detection limit of the method was

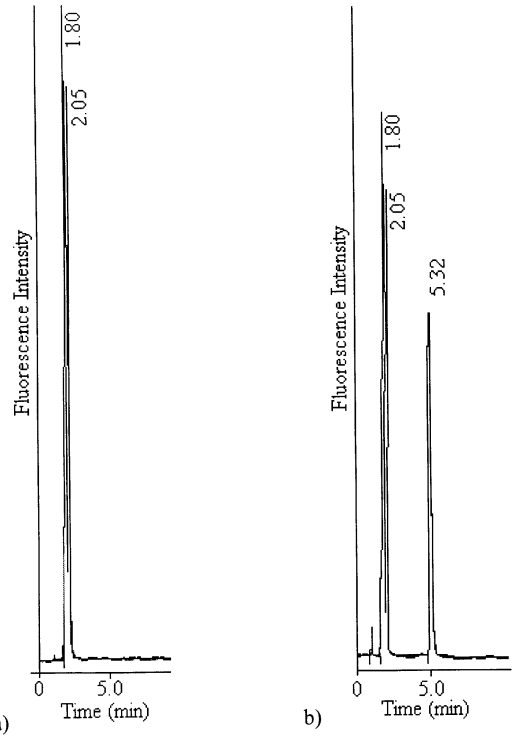

Figure 5. a) Representative chromatogram of Br-MAMC alone; b) Chromatographic separation of the excess of Br-MAMC and the ester of Br-MAMC with undecylenic acid eluted at retention times 2.05 and 5.32 min, respectively. Chromatographic conditions: reversed-phase HPLC on an ODS C-18 column; mobile phase acetonitrile and water (75 : 25, v/v); flow rate 1.0 mL/min, fluorescence detection with $\lambda_{ex} = 345$ nm and $\lambda_{em} = 435$ nm.

Table 2. Analytical Data of the Calibration Graphs Obtained by the HPLC-Fluorescence Method for Undecylenic Acid Derivatized with Br-MAMC

Linearity Range (ng/mL)	Calibration Equation[a]	r[b]
25–250	$S = 0.277(\pm 0.012) \times C_{und} + 0.065\ (\pm 0.073)$	0.9991

[a]Peak area amplitude of the undecylenic acid ester with Br-MAMC $\times$ 0.001, S, vs. concentration of undecylenic acid, C_{und}, in ng/mL; six standards.
[b]Correlation coefficient.

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determined to be 12.5 pg (signal to noise ratio = 3) of the derivatized acid on column, which indicates that the reagent possesses excellent sensitivity.

Separation of Five Fatty Acids

In order to further evaluate the efficiency of Br-MAMC multiple derivatizations of five fatty acids (namely butyric, hexanoic, octanoic, decanoic, and dodecanoic acid) were performed at 100 ng/mL. The mobile phase used for the separation is acetonitrile and water (60 : 40, v/v) in an effort to shorten the analysis time. A typical chromatogram obtained from the analysis of all five fatty acids according to the aforementioned procedure, is illustrated in Figure 6. It is obvious that complete separation of the esters of Br-MAMC with all five fatty acids was achieved in less than 45 min. The logarithms of the capacity factors,

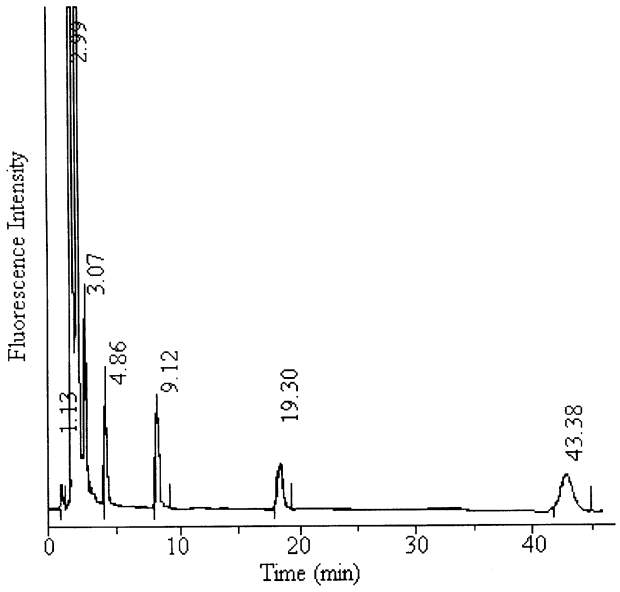

Figure 6. Chromatographic separation of esters of Br-MAMC with five fatty acids. Esters of Br-MAMC with butyric, hexanoic, octanoic, decanoic, and dodecanoic acid were eluted at 3.07, 4.86, 9.12, 19.30, and 43.38 min, respectively. Chromatographic conditions: reversed-phase HPLC on an ODS C-18 column; mobile phase acetonitrile and water (60 : 40, v/v); flow rate 1.0 mL/min, fluorescence detection with $\lambda_{ex} = 345$ nm and $\lambda_{em} = 435$ nm.

log k, were in linear relationship with the number of carbon atoms, n, according to the equation:

$$\log k = 0.167(\pm 0.004) \times n - 0.461(\pm 0.033), \quad r = 0.992$$

This linear relationship reflects, in this way, the increase of lipophilicity with the extension of the carbon chain.

In conclusion, the proposed fluorogenic reagent, Br-MAMC, is a highly fluorescent probe that can be used for the trace analysis of carboxyl containing substances. The molecule is under evaluation for the determination of valproic acid in human plasma, exhibiting promising results.

REFERENCES

1. Lingeman, H.; Underberg, W. J. M. *Detection Oriented Derivatization Techniques in Liquid Chromatography*; Marcel Dekker, Inc.: New York, 1990.
2. Yamaguchi, M.; Matsunaga, R.; Fukuda, K.; Nakamura, M. J. Chromatogr. **1987**, *414* (2), 275–284.
3. Kamimori, H.; Hamashima, Y.; Konishi, M. Anal Biochem. **1994**, *218* (2), 417–424.
4. Nakashima, K.; Okamoto, M.; Yoshida, K.; Kuroda, N.; Akiyama, S.; Yamaguchi, M. J. Chromatogr. **1992**, *584*, 275–279.
5. Van der Horst, F. A. L.; Post, M. H.; Holhtius, J. J. M.; Brinkmann, U. A.Th. J. Chromatogr. **1990**, *500*, 443–452.
6. Allenmark, S.; Chelminska-Bertilsson, M.; Thompson, R. A. Anal Biochem. **1990**, *185* (2), 279–285.
7. Roemen, T. H. M.; van der Vusse, G. H. J. Chromatogr. **1991**, *570* (2), 243–251.
8. Tagawa, K.; Hayashi, K.; Mizobe, M.; Noda, K. J. Chromatogr. **1993**, *617* (1), 95–103.
9. Yoshioka, K.; Shimojo, N.; Nakanishi, T.; Naka, K.; Okuda, K. J. Chromatogr. B, Biomed. Appl. **1994**, *655* (2), 189–193.
10. Mitra, B. B.; Hammes, G. G. Biochemistry **1989**, *28*, 3063–3069.
11. Santa, T.; Takeda, A.; Uchiyama, S.; Fukushima, T.; Homma, H.; Suzuki, S.; Yokosu, H.; Lim, C. K.; Imai, K. J. Pharm. Biomed. Anal. **1998**, *17*, 1065–1070.
12. Cohen, H.; Boutin-Muma, B. J. Liq. Chromatogr. **1991**, *14* (2), 313–326.
13. Secrist, J. A., III; Barrio, J. A.; Leonard, N. J. Biochem. Biophys. Res. Commun. **1981**, *45* (5), 1262–1270.
14. Horst, F. A.; Eikelboom, G. G.; Holtius, J. J. M. J. Chromatogr. **1988**, *456*, 191–199.

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15. Dunges, W. Anal. Chem **1977**, *49* (3), 442–445.
16. Tsutciya, H.; Hayashi, T.; Naruse, H.; Takagi, N. J. Chromatogr. **1983**, *234*, 121–130.
17. Tsutciya, H.; Hayashi, T.; Sato, M.; Tatsumi, M.; Takagi, N. J. Chromatogr. **1984**, *309*, 43–52.
18. Farrinoti, R.; Siard, P.; Brouson, T.; Kirkacharian, S.; Valeur, B.; Mahuzier, G. J. Chromatogr. **1983**, *269*, 81–90.
19. Nagamura, N.; Kawahara, Y. J. Chromatogr. **1989**, *478*, 149–158.
20. Takadate, A.; Masuda, T.; Tajima, C.; Murata, C.; Irikura, M.; Goya, S. Anal. Sci. **1992**, *8*, 663–668.
21. Takadate, A.; Masuda, T.; Murata, C.; Haratake, C.; Isobe, A.; Irikura, M.; Goya, S. Anal. Sci. **1992**, *8*, 695–698.
22. Grayeski, M. L.; De Vasto, J. K. Anal. Chem. **1987**, *59*, 1203–1212.
23. Battistini, F.; Cordero, C.; Urcuyo, F. G.; Royas, R. F.; Ollague, W.; Zaias, N. Int. J. Dermatol. **1997**, *22* (6), 388–399.
24. McLain, N.; Ascanio, R.; Baker, C.; Stroharer, R. A.; Dolan, J. W. Antimicrob. Agents and Chemother. **2000**, *44* (10), 2873–2875.
25. Fendin, R.; Puchenkina, F. F.; Fedin, I. M.; Zhoglo, F. A. Farmatsiya **1987**, *36* (2), 66–68.
26. Leung, C. P.; Leung, W. K. H. Anal. Chim. Acta **1990**, *229* (2), 255–259.
27. Lau-Cam, C. A.; Roops, R. W. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21* (4), 519–533.
28. *The United States Pharmacopeia*, Pharmacopeial Convention: Rockville, MD, 1985, 1110.

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