Some New Fluorescent Derivatives for the Mass Spectrometric Quantitation of Biogenic Amines

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The 5-dimethyl-, diethyl-, dipropyl-, dibutyl-, and dipentyl-aminonaphthalene-1-sulfonyl (dansyl, ethansyl, propansyl, bansyl and pentansyl respectively) derivatives of tyramine and other biogenic amines were prepared and their mass spectra recorded. The relative intensity of the largest unique ion increased with increasing length of the alkyl group. Several *O*-alkyl-*N*-propansyl- and *N,O*-dialkyl-*N*-propansyl-, bansyl- and pentansyl-tyramines were also synthesized and their mass spectra recorded. Dimethylbansyl- and dimethylpentansyl-tyramines exhibited the largest unique ions in their mass spectra and the greatest sensitivity in quantitation by the integrated ion current method. Procedures for preparing these derivatives in amounts ranging from nanograms to milligrams are presented and their thin-layer chromatographic behavior in three solvent systems is described.

INTRODUCTION

Mass spectrometric identification and quantitation of biogenic amines as their dansyl (dimethylamino-naphthalenesulfonyl) derivatives has proven to be very successful.[1-6] The high sensitivity, specificity and accuracy of the method and the capability of using stable isotope labeled internal standards are its major advantages. In order that the results be unambiguous it is important that the mass spectrometer be focused on a unique ion, that is, one which retains the complete information of the derivatized molecule. Unfortunately, the largest unique ions of most dansyl derivatives, the molecular ion, particularly the bis and tris derivatives of the phenol- and catecholamines, are very small. The mass spectrometric behavior of a number of dansylated biogenic amines has been investigated in depth by Durden *et al.*[3] Two characteristics of these derivatives tend to reduce their usefulness for quantitation by mass spectrometry. First, most of the ion current is carried by nonunique ions, mainly m/z 170, invariably the base peak, due to fragmentation of the naphthalene–sulfur bond with the charge remaining on the naphthalene fragment. Second, the bis-dansyl derivatives of the phenolic amines exhibit much smaller (usually a factor of 10 or more) molecular ions than do the monodansyl derivatives of nonphenolic amines, suggesting that the bis derivatives either decompose thermally to some extent in the mass spectrometer or fragment more readily, particularly at the sulfate ester bond.

Recently, Seiler has succeeded in producing derivatives of amines with unique ions as the base peak by employing *N,N*-dibutylaminonaphthalenesulfonyl (bansyl) chloride as reagent.[7,8] The longer alkyl group opened the way to a more favorable fragmentation, namely cleavage between the carbon atoms α and β to the nitrogen atom. A recent paper by Lehmann and co-workers[9] described the electron impact and field desorption mass spectrometry of trisbansyldopamine and some of its metabolites.

There is no immediately obvious reason why butyl should be preferable to ethyl, propyl or pentyl groups, and in this paper the mass spectra of some amines derivatized with *N,N*-dialkylaminonaphthalenesulfonyl chloride reagents containing alkyl groups from C_1 to C_5 are compared. *p*-Tyramine is used as an example since the molecular ion of bis-dansyltyramine is very small, and as an important trace amine an improvement in its detection limit would be useful.

In order to reduce the probability of thermal decomposition and sulfate ester fragmentation, mixed derivatives, in which the fluorescent moiety is attached only to the nitrogen atom and various alkyl groups are attached to the oxygen or oxygen and nitrogen atoms, have been prepared and their mass spectra compared.

The sensitivity of the mass spectrometer to those derivatives which appeared most promising on the basis of their spectra has been determined by measuring the integrated ion current arising from the evaporation of 1.00×10^{-10} mol and has been compared with bis-dansyltyramine.

EXPERIMENTAL

Nomenclature

Because the names dansyl and bansyl are well established in the literature and therefore cannot be changed, the nomenclature employed for related compounds cannot be entirely consistent. In this paper, the names adopted are dansyl, ethansyl, propansyl, bansyl and pentansyl respectively for the *N,N*-dimethyl, diethyl, dipropyl, dibutyl and dipentylamino-naphthalenesulfonyl compounds.

Materials

Reagent grade solvents (Fisher) were used in all experiments except those requiring integrated ion current analyses, in which cases solvents distilled in glass (Caledon Laboratories Ltd, Georgetown, Ontario, Canada) were employed.

CCC-0306-042X/79/0006-0149$04.00

The five dialkylaminonaphthalenesulfonyl chlorides were prepared by heating 5-amino-1-naphthalenesulfonic acid (0.05 mol), the alkyl iodide (0.10 mol) and anhydrous potassium fluoride (0.10 mol) in dimethylformamide (60 ml) at 130–140 °C for 18 h with vigorous agitation. The reaction mixture was poured into six volumes of cold water, the suspension was cooled and allowed to settle. The dark brown aqueous solution was poured off leaving the desired product, a cake of yellow-brown material. More dialkylaminonaphthalenesulfonic acid could be obtained from the mother liquor by allowing it to concentrate slowly by evaporation in the fume hood. The product was washed with acetone and air dried, for a yield of 60%. Conversion to the sulfonyl chloride was achieved as described previously.[7,10]

The bis-dansyl, ethansyl, propansyl, bansyl and pentansyl derivatives of tyramine and the mono derivatives of phenylethylamine and tryptamine were synthesized by a method similar to that developed by Seiler for the synthesis of dansyl amines.[11] Approximately 2×10^{-5} mol (about 1 mg) of the amine hydrochloride in 1 ml of 10% sodium carbonate was treated with 3 ml of an acetone solution of the reagent (3 mg ml^{-1}), the mixture was shaken vigorously and allowed to stand overnight. An equal volume of ethyl acetate was added to precipitate water and sodium carbonate, the organic layer was removed and concentrated to about 1 ml, diluted with ether, extracted with 5% sodium bicarbonate, dried over sodium sulfate, and the ether evaporated. The product was purified by TLC (E. Merck, Darmstadt, Germany, distributed in Canada by BDH Chemicals, Toronto), using benzene + triethylamine 5:1 as developer. The appropriate zones were removed, extracted and prepared for mass spectrometric analysis as described previously.[12]

The alkylated derivatives of tyramine were prepared in a two-step synthesis in which the nitrogen atom is first derivatized with the dialkylaminonaphthalenesulfonyl chloride, using sodium acetate as base (in order to prevent simultaneous derivatization of the phenolic group), followed by alkylation of the oxygen or oxygen and nitrogen atoms with the appropriate alkyl halide and potassium carbonate, catalyzed by 18-crown-6. The following alkyl halides were employed: methyl, ethyl, 1-propyl, 1-butyl, 1-pentyl, 1-hexyl, 1-heptyl, 2-propyl, 2-butyl, 3-pentyl, 2-heptyl and 4-octyl; all were bromides except methyl which was iodide. For example, 2×10^{-5} mol of tyramine hydrochloride in 1 ml of 10% sodium acetate was treated with 3 ml of reagent solution and isolated as described above for the bis derivatives. The product was transferred to a 0.3 ml Reacti-vial (Pierce Chemical Co., Rockford, Illinois, USA), dissolved in 100 μl of a 0.3% solution of 18-crown-6 in benzene, 25 μl of the alkyl halide and a few mg of powdered anhydrous potassium carbonate were added, and the mixture was heated overnight at 65 °C. The length of time required for complete reaction is proportional to the quantity being derivatized. Thus, a few hundred nanograms require less than an hour, whereas 50 mg requires two days. The reaction proceeds faster in acetonitrile or dimethylformamide, but these solvents are less suitable than benzene for spotting on TLC plates and therefore were not used. The entire product mixture (excluding undissolved potassium carbonate) was applied to the origin of a thin-layer plate and developed with benzene + triethylamine, 20:1 (v/v). The appropriate zone was removed, extracted and analyzed by mass spectrometry.

Instrumentation

Spectra and integrated ion current curves were recorded on an AEI MS 902S double focusing mass spectrometer equipped with a VG 2050 Data system and a DEC Lab 8/e minicomputer. The ion source electron beam was set at its highest current (500 μA) during recording of integrated ion current curves in order to maximize the number of ions. The resolution was set at 1500 for recording spectra and 7000 for the integrated ion current measurements. Samples were introduced into the mass spectrometer on a direct insertion probe of fixed length. Probe tips were made from borosilicate capilllary tubes (1.8 mm o.d., 1.4 mm i.d.), 25 mm long, sealed about 8 mm from one end. During recording of the integrated ion current curves the temperature was set so that the sample evaporated in less than 60 s. For spectra, the required temperature of the source ranged from 250 °C for the alkylated derivatives to 300 °C for bis-dansyltyramine. About 1–2 μg of each derivative was used for determining spectra.

Procedure for the determination of relative sensitivities

Five derivatives were chosen for comparison with bis-dansyltyramine: bis-propansyltyramine, bis-bansyltyramine, *O*-(4-octyl)-*N*-bansyl-tyramine, *N,O*-dimethyl-*N*-bansyltyramine and *N,O*-dimethyl-*N*-pentansyltyramine. An amount of each of these compounds corresponding to $1.00\pm0.05\times10^{-5}$ mol was weighed and dissolved in 5 ml ethyl acetate to give the stock solution with a concentration of 1.00×10^{-8} mol per 5 μl; 5 μl is the amount introduced onto the probe tip for the integrated ion current analysis. The solutions were diluted successively by factors of 10 to give five dilutions with concentrations from 10^{-13} to 10^{-9} mol per 5 μl. The 10^{-10} and 10^{-11} mol solutions were analyzed by the integrated ion current method for all derivatives; dimethylbansyl- and dimethylpentansyltyramines were measured over the entire concentration range of 10^{-13} to 10^{-9} mol. Each measurement was carried out three times and the results averaged. The ions on which the mass spectrometer was focused are recorded in Table 5.

The integrated ion current values were obtained using the peak switching facilities of the mass spectrometer. A relatively constant pressure of the reference compound heptacosafluoro-tri-*n*-butylamine was maintained in the ion source. The magnetic field was adjusted so that a reference compound peak lower in mass than the ion to be analyzed appeared on the oscilloscope, and the peak-switching decade was set to the ratio of the two masses so that the largest unique ion of the derivatized amine appeared in the high mass position. With the electron multiplier gain set to its maximum value, a sample of the least concentrated solution (5 μl, dried) on the probe was inserted into the ion source. The reference signal remained constant, whereas the derivatized amine signal

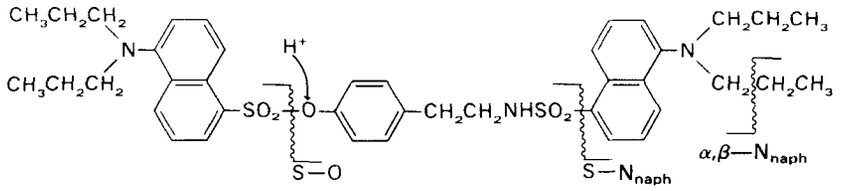

Figure 1. Major fragmentations of bis-propansyltyramine.

varied in a Gaussian manner as the sample evaporated. The signals from the derivatized amine were integrated by computer, and the result is directly proportional to the absolute value of the ion current. For different derivatives of the same quantity of an amine, the absolute values of the ion current then represent absolute differences in the sensitivity of the instrument to those derivatives. These differences are evident from the values in Table 9 (Column 2) for the integrated ion currents. A fuller description of this procedure has been published previously.[3,13]

RESULTS AND DISCUSSION

Spectra

The spectra of the bis-(*N,N*-dialkylaminonaphthalenesulfonyl)-tyramines exhibit marked changes as the length of the alkyl chain increases from methyl through to pentyl. Although the relative intensity of the molecular ion does not change much, the ion resulting from α,β-cleavage of the alkyl chain attached to the aminonaphthalene moiety (hereafter referred to as α,β-N_{naph}) is greater than 75% for the propansyl, bansyl and pentansyl derivatives (Table 1). Other major fragmentations include breakage of the sulfate–oxygen bond (S—O) with rearrangement of a hydrogen atom to the charged species, and cleavage of the sulfur–naphthalene (S—Naph) bond (Fig. 1). Both these fragments undergo further fragmentation by α,β-N_{naph} cleavage. There is considerable variability in the relative intensities of the ions from one scan to another, suggesting that decomposition is occurring to some extent in the ion source, and that the extent of decomposition depends critically on a number of factors which cannot be controlled precisely. Therefore, one probably would not read too much significance into the fact that the $[M-29]^+$ ion of bis-propansyltyramine is the base peak whereas the $[M-57]^+$ ion of bis-pentansyltyramine is 77% of base.

Another trend worth noting is the increase of S—O fragmentation (which includes the subsequent or previous α,β-fragmentation) as the alkyl chain length increases (Table 1). For dansyl, this is 6% of base, whereas for pentansyl the two signals combined are 96% of base. Obviously, if this fragmentation did not occur, the molecular ion and its α,β-cleavage fragment would be much larger.

It was for this reason and also to reduce or eliminate thermal decomposition, which seems to be associated with the sulfate ester linkage, that studies into mixed derivatives were undertaken, in which the oxygen of the phenol (and in some cases also the nitrogen) would bear an alkyl group and the nitrogen atom would bear one of the fluorescent groups. In an earlier paper, the alkylation of phenolic acids using potassium carbonate and 18-crown-6 in several organic solvents was described.[14] Application of this method to the *O*- and *N,O*-alkylation of *N*-dansyltyramine proved to be successful for a number of alkyl halides.

The following conclusions can be drawn from the results of experiments on the alkylation on monodansyltyramine. Both the oxygen and nitrogen atoms will be alkylated if the reaction is allowed to proceed long enough and monoalkylated products are *O*-alkylated rather than *N*-alkylated, probably for steric reasons. The spectra of the dialkyldansylamines were disappointing, in that the molecular ions (the largest unique ions) ranged in relative intensity from only 12 to 30% of the base peak, which was at m/z 170. A significant new fragment having relative intensities ranging from 50 to 90% resulted from fragmentation between the α and β carbons of the tyramine part of the molecule (hereafter referred to as α,β-N_{tyr} cleavage); unfortunately the charge remains with the nonunique fragment. The spectra of the *O*-alkyl-*N*-dansyltyramines (where alkyl is 2-propyl, 2-butyl, 3-pentyl, 2-heptyl and 4-octyl) exhibit molecular ions with relative intensities of about 50%, and α,β-N_{tyr} fragmentation is insignificant, probably because the compounds are secondary amides, which undergo α,β-cleavage less readily that tertiary amides. The disadvantage to the monoalkylated derivatives for

Table 1. Relative intensities and masses of the major ions in the spectra of *N,N*-dialkylaminonaphthalenesulfonyltyramines

Derivative	$[M]^{+\cdot}$[a]	$\alpha,\beta-N_{naph}$	S—O	S—O-$\alpha,\beta N_{naph}$	S—N_{naph}	S—N_{naph}-α,β-N_{naph}
Bis-dansyl	7% (603)	—	6% (370)	—	100% (170)	—
Bis-ethansyl	8% (659)	17% (644)	5% (398)	6% (383)	36% (198)	100% (183)
Bis-propansyl	19% (715)	100% (686)	19% (426)	38% (397)	18% (226)	74% (197)
Bis-bansyl	17% (771)	89% (728)	16% (454)	45% (411)	27% (254)	100% (211)
Bis-pentansyl	10% (827)	77% (770)	13% (482)	83% (425)	23% (282)	100% (225)

[a] Masses are in parentheses.

the purpose of quantification is that they are not formed quantitatively, but always contain some dialkyl compound as contaminant. The tendency to dialkylate increases as the concentration of alkyl halide relative to that of the material to be alkylated increases, and this is usually the case when nanogram rather than microgram or milligram quantities are to be alkylated.

The spectra of the corresponding dialkyl and monoalkyl propansyl, bansyl and pentansyltyramines were recorded. The results are summarized in Tables 2, 3, 4, 5 and 6, and the major fragmentations are depicted in Fig. 2. In these tables the ions listed in columns 2 and 3 are unique to the tyramine derivative.

Some trends manifest themselves in these results. First of all, the spectra of the dialkyl derivatives show that the branched alkyl groups are considerably inferior to the straight chain groups in so far as the relative intensities of unique ions are concerned (cf. Tables 2 and

Table 2. Relative intensities of the major ions in the spectra of *N,O*-dialkyl-*N*-propansyltyramines (straight chain alkyl)

Alkyl group	$[M]^{+\cdot}$	α,β-N_{naph}	α,β-N_{tyr}	S-Naph (*m/z* 226)	S—naph-α,β-N_{naph}[a] (*m/z* 197)
Methyl	40% (454)	86% (425)	53% (333)	100%	95%
Ethyl	23% (482)	30% (453)	68% (347)	100%	54%
Propyl	18% (510)	20% (481)	74% (361)	100%	68%
Butyl	16% (538)	17% (509)	87% (375)	100%	68%
Pentyl	17% (566)	16% (537)	100% (389)	98%	76%
Hexyl	13% (594)	14% (565)	100% (403)	96%	73%
Heptyl	12% (622)	13% (593)	100% (417)	92%	76%

[a] Corrected for isotopic contribution from *m/z* 196.

Table 3. Relative intensities of the major ions in the spectra of *N,O*-dialkyl-*N*-bansyltyramines (straight chain alkyl)

Alkyl group	$[M]^{+\cdot}$	α,β-N_{naph}	α,β-N_{tyr}	S—N_{naph} (*m/z* 254)	S—Naph-α,β-N_{naph}[a] (*m/z* 211)
Methyl	29% (482)	100% (439)	30% (361)	57%	60%
Ethyl	31% (510)	65% (467)	76% (375)	100%	65%
Propyl	18% (538)	42% (495)	80% (389)	100%	85%
Butyl	9% (566)	22% (523)	66% (403)	100%	84%

[a] Corrected for isotopic contribution from *m/z* 210.

Table 4. Relative intensities of the major ions in the spectra of *N,O*-dialkyl-*N*-pentansyltyramines (straight chain alkyl)

Alkyl group	$[M]^{+\cdot}$	α,β-N_{naph}	α,β-N_{tyr}	S—Naph (*m/z* 282)	S—N_{naph}-α,β-N_{naph}[a] (*m/z* 225)
Methyl	24% (510)	100% (453)	21% (389)	35%	44%
Ethyl	42% (538)	100% (481)	71% (403)	72%	72%
Propyl	43% (566)	87% (509)	100% (417)	83%	88%
Butyl	35% (594)	68% (537)	100% (431)	71%	77%

[a] Corrected for isotopic contribution from *m/z* 224.

Table 5. Relative intensities of the major ions in the spectra of *N,O*-dialkyl-*N*-propansyltyramines (branched chain alkyl)

Alkyl group	$[M]^{+\cdot}$	α,β-N_{naph}	α,β-N_{tyr}	S—Naph (*m/z* 226)	S—N_{naph}-α,β-N_{naph}[a] (*m/z* 197)
2-Propyl	9% (510)	7% (481)	46% (361)	100%	60%
2-Butyl	8% (538)	3% (509)	53% (375)	100%	64%
3-Pentyl	6% (566)	2% (537)	61% (389)	100%	63%
2-Heptyl	6% (622)	4% (593)	75% (417)	100%	71%
4-Octyl	5% (650)	1% (621)	74% (431)	100%	79%

[a] Corrected for isotopic contribution from *m/z* 196.

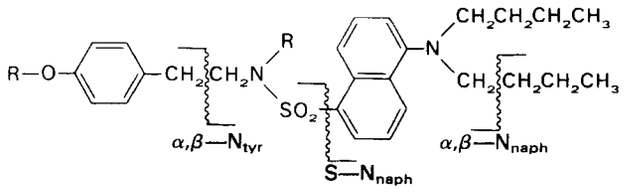

Figure 2. Major fragmentations of dialkylbansyltyramine.

5). Why this should be so is not clear. Second, the relative intensities of the largest unique ions in the spectra of straight chain dialkyl derivatives become smaller as the chain becomes longer, and this is true for the propansyl, bansyl and pentansyl compounds (cf. Tables 2, 3 and 4). Finally, for a particular alkyl group, the relative intensity of the largest unique fragment ($[M-29]^+$, $[M-43]^+$ and $[M-57]^+$ respectively) increases from propansyl to pentansyl. Thus, the $[M-29]^+$ peak of dimethylpropansyltyramine is 85% of base and the $[M-43]^+$ and $[M-57]^+$ peaks of dimethylbansyltyramine and dimethylpentansyltyramine respectively are both the base peak; $[M]^{+\cdot}$ for dimethyldansyltyramine is 31% of base. The trend is even clearer for the diethyl derivatives where the relative intensities are 23, 30, 65 and 100% for dansyl, propansyl, bansyl and pentansyl respectively. Clearly dimethylbansyltyramine and dimethylpentansyltyramines are the best derivatives of the dialkyl group of compounds. An explanation for these trends may be found in the rule of thumb that α,β-fragmentation of tertiary amines and amides occurs in the largest alkyl group.[15] The neutral fragment which is lost in these fragmentations carries off energy leaving a less energetic and therefore more stable charged fragment; the larger the neutral fragment lost, the more energy it will take with it and therefore the more stable will be the remaining charged fragment. One might expect then that the larger neutral fragment A (Fig. 3), the higher will be the relative abundance of the charged fragment C. Therefore, as R is increased in size from methyl through to heptyl (with fragment B left the same size) one would expect the relative intensity of C to increase compared with that of D. This is, in fact, observed (see Table 2). If fragment A is kept the same size (for example, R is ethyl) but fragment B increased (as in dansyl to propansyl, bansyl and pentansyl), one would expect fragment D to increase in size relative to C. This is also observed.

In Table 6, the monoalkylpropansyltyramines are shown to be quite different from their dialkyl counterparts. The length of the alkyl chain has no significant effect on relative intensities. There is no fragmentation α to the tyramine nitrogen, a major fragmentation in the spectra of the dialkyl compounds. Also, sulfur–naphthalene bond fragmentation seems to be less. Most important, in all cases (except pentyl at 93%), the unique $[M-29]^+$ fragment is the base peak. Of the five alkyl groups, the octyl appears to be the most suitable for quantitation purposes since, after completion of the alkylation reaction, a larger fraction of the product mixture is in the form of the monoalkyl compound than is the case with the other alkyl groups. Unfortunately, this promising derivative has the major disadvantage of not being formed quantitatively; substantial quantities of the dialkyl derivative are always found.

Other amines, except octopamine, exhibit similar mass spectral characteristics, as shown in Table 7. The base peak for octopamine is at m/z 319, which results from fragmentation between the nitrogen and sulfur atoms with transfer of a hydrogen atom to the charged species.

The spectra of several derivatives are shown in Fig. 4.

Thin-layer chromatography

Because the alkylated bansyl amines are not very polar, better separations can be effected on the monobansylamines prior to alkylation. This also permits non-

Table 6. Relative intensities of the major ions in the specta of *O*-alkyl-*N*-propansyltyramines (branched chain alkyl)

Alkyl group	$[M]^{+\cdot}$	α,β-N_{naph}	α,β-N_{tyr}	S—Naph (m/z 226)	S—N_{naph}-α,β-N_{naph} (m/z 196)
2-Propyl	24% (468)	100% (493)	0% (319)	5%	73%
2-Butyl	22% (482)	100% (453)	0% (333)	7%	97%
3-Pentyl	19% (496)	93% (467)	0% (347)	8%	100%
2-Heptyl	22% (524)	100% (495)	0% (375)	7%	67%
4-Octyl	23% (538)	100% (509)	0% (389)	8%	69%

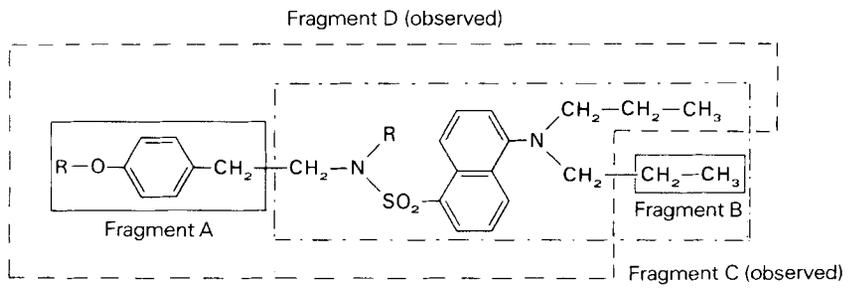

Figure 3. α,β-Fragmentations of alkylated derivatives.

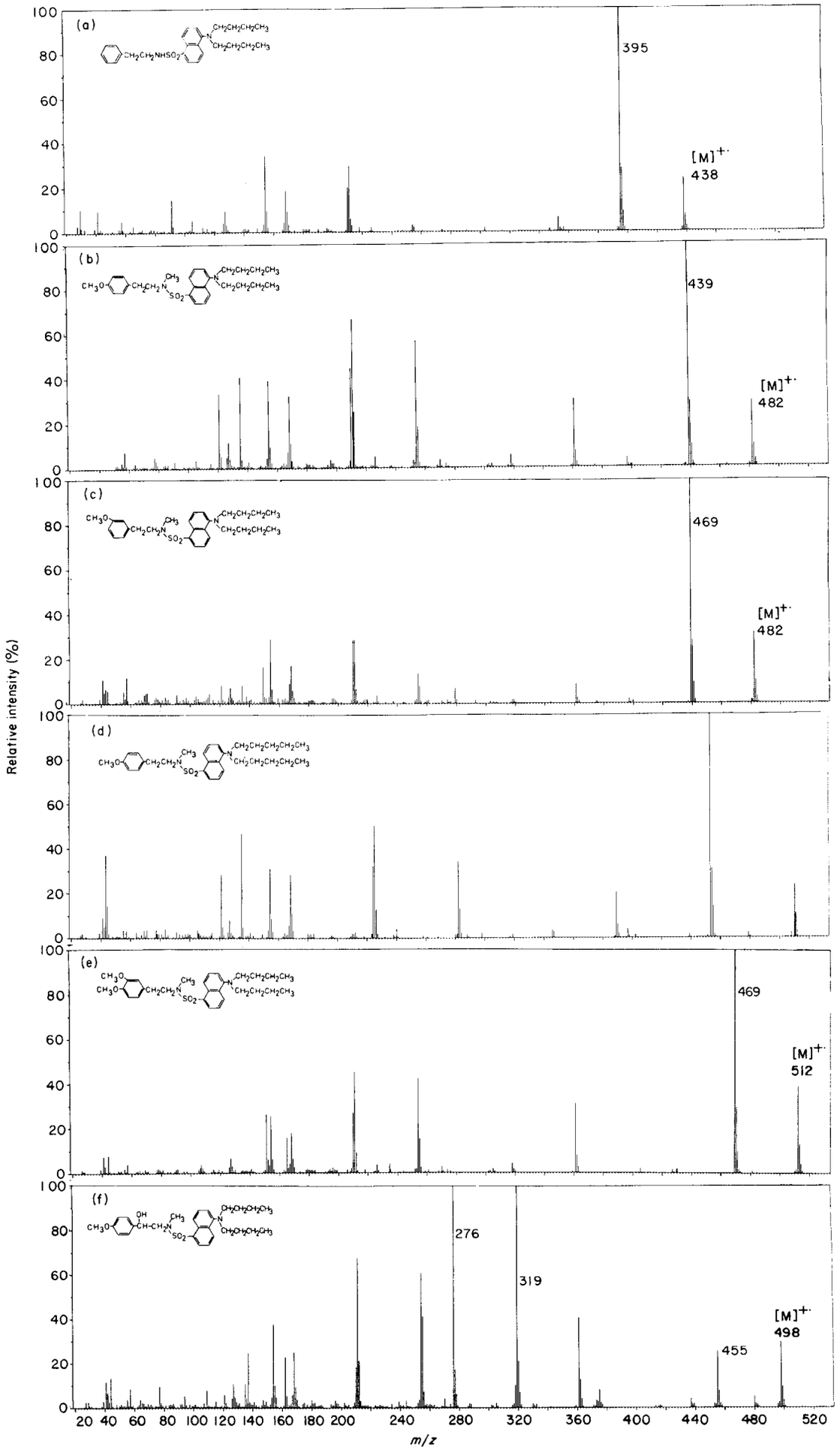

Figure 4. Mass spectra of some bansylamines: (a) bansylphenylethylamine; (b) dimethylbansyl-*p*-tyramine; (c) dimethylbansyl-*m*-tyramine; (d) dimethylpentansyl-*p*-tyramine; (e) trimethylbansyldopamine; (f) dimethylbansyl-*p*-octopamine.

Table 7. Relative intensities of the major ions in the spectra of the bansyl and methylated bansyl derivatives of some other amines

Derivative	$[M]^{+\cdot}$	α,β-N_{naph}	α,β-N_{tyr}	S—Naph (*m/z* 254)	S—Naph-α,β-N_{naph}[a] (*m/z* 211)
Bansylphenylethylamine	23% (438)	100% (395)	—	3%	29%
Bansyltryptamine	34% (477)	100% (434)	—	1%	24%
N,O-Dimethyl-*N*-bansyloctopamine	30% (498)	26% (455)	41% (361)	61%	65%
N,O-Dimethyl-*N*-bansyl-*m*-tyramine	32% (482)	100% (439)	9% (361)	13%	24%
N-O,O-Trimethyl-*N*-bansyl-dopamine	49% (512)	100% (469)	31% (361)	42%	41%

[a] Corrected for isotopic contribution from *m/z* 210.

Table 8. R_f values for some bansyl derivatives[a]

Derivative	R_f value in solvent system No. 1[b]	R_f value in solvent system No. 2[c]	R_f value in solvent system No. 3[d]
Monobansyl-*m*-tyramine	0.20	0.28	0.48
Monobansyl-*p*-tyramine	0.20	0.34	0.57
Monabansyl-*p*-octopamine	0.05	0.13	0.16
Monobansyldopamine	0.08	0.04	0.01
Bansylphenylethylamine	0.62	0.81	0.93
Bansyltryptamine	0.28	0.29	0.83
Bis-Bansyl-*m*-tyramine	0.62	0.89	0.99
Bis-Bansyl-*p*-tyramine	0.54	0.85	0.99
N,O-Dimethyl-*N*-bansyl-*p*-tyramine	0.76	0.99	0.96
N,O,O,-Trimethyl-*N*-bansyldopamine	0.51	0.93	0.94

[a] TLC plates: Pierce LQD Quantum.
[b] Solvent System 1: Cyclohexane + ethyl acetate, 3 : 1.
[c] Solvent System 2: Benzene + triethylamine, 5 : 1.
[d] Solvent System 3: Chloroform + ethyl acetate, 4 : 1.

Table 9. Integrated ion current for some tyramine derivatives

Derivative	Integrated ion current for 10^{-10} mol[a]	Ion measured	Integrated ion current for 10^{-10} mol dansyl-tyramine	Integrated ion current for 10^{-10} mol derivative
Bis-dansyltyramine	109	603.1861 $[M]^{+\cdot}$	1.0	
Bis-propansyltyramine	231	686.2722 $[M-29]^{+}$	2.1	
Bis-bansyltyramine	316	728.3192 $[M-43]^{+}$	2.9	
N,O-Dimethyl-*N*-bansyl-tyramine	1069	439.2055 $[M-43]^{+}$	9.7	
N,O-Dimethyl-*N*-pentansyl-tyramine	848	453.2212 $[M-57]^{+}$	7.8	
O-(4-Octyl)-*N*-bansyl-tyramine	584	523.2994 $[M-43]^{+}$	5.4	

[a] Arbitrary units.

phenolic amines such as phenylethylamine and tryptamine which do not need to be alkylated (and should not be alkylated) to be separated from those amines which benefit from alkylation. Table 8 lists the R_f values for a number of bansyl derivatives in three solvent systems.

Integrated ion current studies

On the basis of the foregoing discussion of relative intensities, which can only be a very rough guide to the actual sensitivity of the mass spectrometer to a given derivative, five derivatives were chosen for further study.

Integrated ion current measurements were made on 10^{-10} and 10^{-11} mol of each of the derivatives selected. In Table 9 are given the average (of three) integrated ion current measurements for the 10^{-10} mol samples, the exact masses of the ions measured, and the ratio of the integrated ion current of the derivative to that of bis-dansyltyramine. Calibration curves for dimethylbansyltyramine and dimethylpentansyltyramine were prepared (Fig. 5) by measuring the integrated ion current when five solutions differing in concentration by factors of 10 (containing from 10^{-13} to 10^{-9} mol) were evaporated into the ion source.

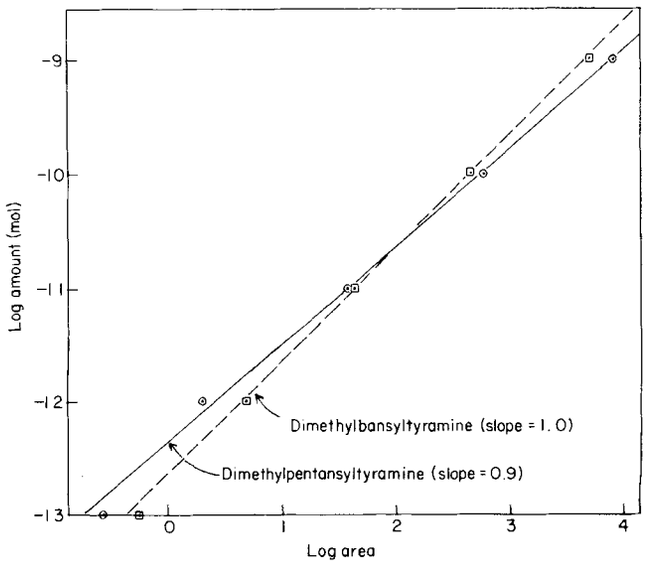

Figure 5. Calibration curves of dimethylbansyltyramine and dimethylpentansyltyramine.

It has been reported previously that the calibration curve for bis-dansyltyramine exhibits two linear but discontinuous sections, the discontinuity occurring between 10^{-10} and 10^{-11} mol. Bis-propansyltyramine and bis-bansyltyramine behave very similarly to bis-dansyltyramine in this regard. Dimethylbansyltyramine, on the other hand, exhibits a continuously linear calibration curve with slope of 1.0. The calibration curve of dimethylpentansyltyramine is also linear but has a slope of only 0.90. This would seem to confirm suspicions that decomposition and loss of sensitivity in bis-dansyltyramine is associated at least in part with the sulfate ester bond.

In conclusion, use of propansyl, bansyl or pentansyl chloride as reagent for the derivatization of amines results in a substantial increase in the relative intensity of unique ions and a modest improvement in sensitivity. The mixed derivatives not only exhibit a unique ion as base peak, but also result in a substantial improvement in sensitivity. Dimethylbansyltyramine seems to be the best of the derivatives investigated.

Since there are some *N*- and/or *O*-methylated amines endogenous in biological materials, an adequate separation of the monobansylamines by TLC prior to methylation must be achieved if methylated derivatives are to be used for the quantitation. Alternatively, iodotrideuteromethane can be used as methylating reagent, thereby eliminating all ambiguity.

Acknowledgements

I should like to thank E. Johnson and C. Nicholaichuk for expert technical assistance, and the Psychiatric Services Branch, Province of Saskatchewan, for continuing financial support.

REFERENCES

1. N. Seiler and A. Askar, *J. Chromatogr.* **62**, 121 (1971).
2. D. A. Durden, S. R. Philips and A. A. Boulton, *Can. J. Biochem.* **51**, 995 (1973).
3. D. A. Durden, B. A. Davis and A. A. Boulton, *Biomed Mass Spectrom.* **1**, 83 (1974).
4. S. R. Philips, B. A. Davis, D. A. Durden and A. A. Boulton, *Can. J. Biochem.* **53**, 65 (1975).
5. T. J. Danielson, B. A. Davis and A. A. Boulton, *Can. J. Physiol. Pharmacol.* **55**, 439 (1977).
6. N. Seiler and L. Demisch, in *Handbook of Derivatives for Chromatography*, ed. by K. Blau and G. King, p. 349. Heyden, London (1977) (and references therein).
7. N. Seiler, T. Schmidt-Glenewinkel and H. H. Schneider, *J. Chromatogr.* **84**, 95 (1973).
8. N. Seiler and H. H. Schneider, *Biomed. Mass Spectrom.* **1**, 381 (1974).
9. W. D. Lehmann, H. D. Beckey and H.-R. Schulten, *Anal. Chem.* **48**, 1572 (1976).
10. G. Weber, *Biochem. J.* **51**, 55 (1952).
11. N. Seiler and M. Wiechmann, in, *Progress in Thin-Layer Chromatography and Related Methods*, Vol. 1, ed. by A. Niederweisser and G. Pataki, p. 95. Ann Arbor-Humphrey Science Publishers, Ann Arbor, Michigan (1970).
12. S. R. Philips, D. A. Durden and A. A. Boulton, *Can. J. Biochem.* **52**, 366 (1974).
13. D. A. Durden, in, *Research Methods in Neurochemistry*, Vol. 4. ed. by N. Marks and R. Rodnight, p. 205. Plenum Press, New York (1978).
14. B. A. Davis, *Anal. Chem.* **49**, 832 (1977).
15. H. Budzikiewicz, C. Djerassi and D. H. Williams, in, *Mass Spectrometry of Organic Compounds*, p. 297. Holden-Day, San Francisco (1967).

Received 8 August 1978