MASS SPECTRA OF THE REACTION PRODUCTS OF SOME BIOLOGICALLY IMPORTANT PRIMARY AMINES AND FLUORESCAMINE

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SUMMARY

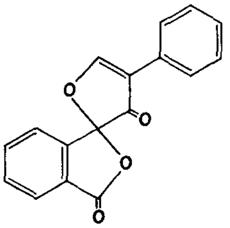
The fluorophors obtained by reaction of primary amines with fluorescamine are useful derivatives for mass spectrometric identification of biologically important amines. The mass spectra of a few typical phenethylamines, amphetamines, tryptamine and amino acid esters are given and the characteristic fragmentation mechanism is discussed. The advantages of this fluorophor over the dansyl derivatives are also discussed.

Fluorescamine (I) is a new reagent recently developed for the detection and fluorometric assay of amino acids, peptides, proteins and primary amines in the picomole range (1,2). The reaction between this reagent and primary amines is almost instantaneous at room temperature and has also been reported to be quantitative (1,2). The hydrolysis products of the excess reagent and the reagent itself are not fluorescent, but the reaction product (II) of the primary amine and fluorescamine is highly fluorescent. In a series of communications the use of these reagents in automated analysis of peptides, amino acids and also as a spray reagent for TLC has been reported (1,2,3,4). In earlier work dansylation has been very widely used to produce a fluorophor. Several biologically important compounds have been identified by TLC of dansylated compounds (5,6).

In our efforts to provide sensitive and specific methods for biologically important compounds we have now investigated the mass spectra of the fluorophors formed from primary amines and fluorescamine (II).

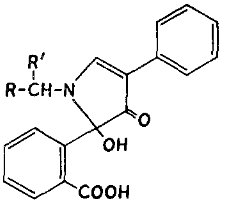
MATERIALS AND METHODS

A few typical examples of different types of biologically important primary amines were chosen for derivatization, phenylethylamine, 3- and 4-O-methyl ethers of dopamine, 3:4-dimethoxyphenethylamine (DMPEA), *p*-MeO-phenethylamine,



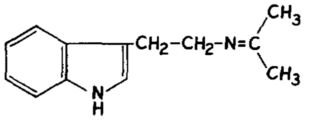
I

FLUORESCAMINE
(FLURAM™)



II

R' = H PHENETHYLAMINES
R' = CH_3 AMPHETAMINES
R' = COOEt AMINO ACID ESTERS



III

p-tyramine D-amphetamine, *p*-MeO-amphetamine, tryptamine, glycine ethyl ester and phenylalanine ethyl ester.

Reagents

Fluorescamine reagent (30 mg of fluorescamine in acetone or alcohol), ethyl acetate (nanograde), borate buffer 0.2 M (pH 9.0), 0.5 N dihydrochloric acid.

Fluorophor Preparation

Method I. The primary amines as their hydrochlorides were dissolved in water at a concentration of 1 mg/ml. Similarly solutions of the free amines in ethyl acetate were obtained by extracting the aqueous solution at pH 10. The aqueous solution of the amine (100 µl) was diluted with 0.5 ml borate buffer, pH 9.0, treated with 0.5 ml of the fluorescamine reagent and the mixture was stirred for 2 min on a virtis mixer. The mixture showed an intense fluorescence under UV, the fluorescence maxima was checked on an Aminco-Bowman spectrofluorometer, excitation wavelength at 390 nm and emission at 475 nm. The reaction mixture was then acidified with 0.5 ml of 0.5 N HCl and shaken with 10 ml of ethyl acetate. When viewed under UV, the fluorescence was now found in the upper organic layer. After shaking for 5 min, the mixture was centrifuged, the ethyl acetate layer separated, dried over sodium sulfate and evaporated to dryness under vacuum. The residue was redissolved in 100 µl of ethyl acetate and a 2-µl aliquot was used for mass spectrometry.

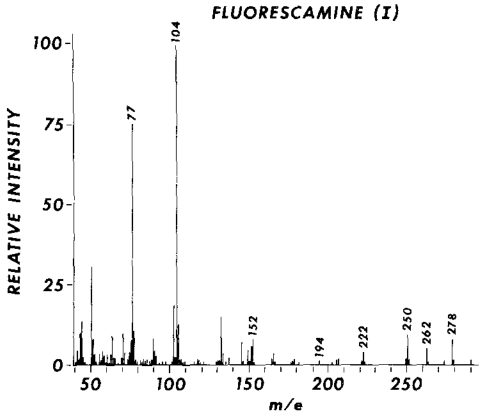


Fig. 1 Mass spectrum of fluorescamine.

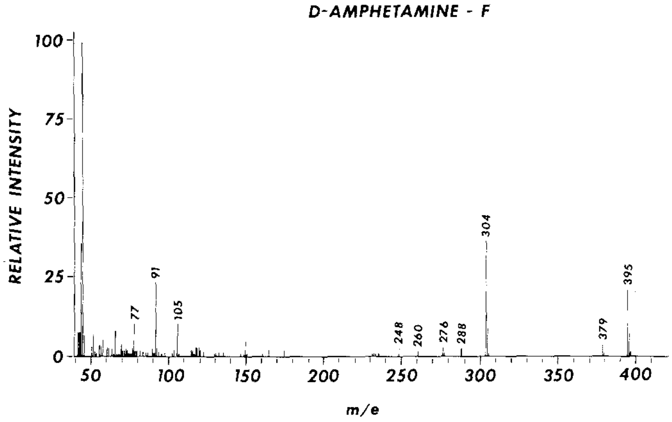


Fig. 2 Mass spectrum of the fluorophor(F) from D-amphetamine and fluorescamine.

Method II. Ethyl acetate solution (100 μl) of the primary amines containing 1 μg/μl of the base was stirred on a virtis mixer with 0.5 ml of the reagent solution in alcohol for 5 min. When viewed under UV an intense fluorescence was seen. The solution was evaporated to dryness and redissolved in 0.1 ml of ethyl acetate. A 2-μl aliquot was used for mass spectrometry.

Mass Spectrometry

The mass spectra of the fluorophors were obtained by introducing the sample directly to the ion source, through the solid sample probe (gold cup),

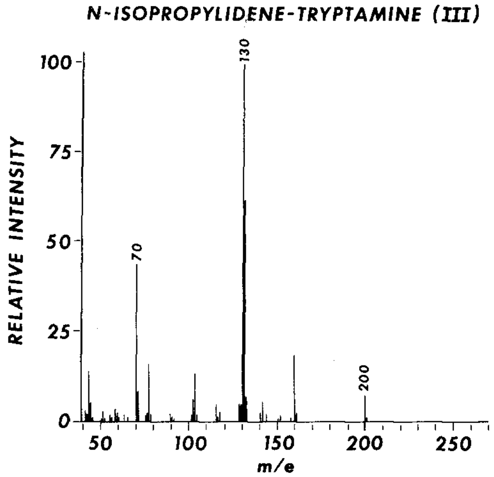


Fig. 3 Mass spectrum of N-isopropylidene-tryptamine.

of a CH-7 Varian mass spectrometer. The ionizing potential was 70 eV, the ion source temperature 250°C, the probe temperature was raised to 250°C. As a reference spectrum, the mass spectrum of the reagent was also obtained.

RESULTS

The mass spectra of the reagent and of the fluorophors are comparable and are in agreement with the structures I and II (Figs. 1 and 2). As the temperature of the solid probe is raised, total ion current increases to an initial maximum at about 150°C, falls and again increases to a maximum at 250°C. When acetone solution of fluorescamine was used to form the fluorophor, spectra recorded at the lower temperature showed small traces of the acetone condensation product (isopropylidene derivative) (7) of the amine. The mass spectra of the two products in the reaction with tryptamine are shown in Figs. 3 and 4. The spectrum shown in Fig. 3 agrees with the structure III, the molecular ion at *m/e* 200 being 40 amu higher than that of the amine.

The mass spectra of the fluorphors obtained from the amines by Methods I and II are identical. The molecular ion from structure II is not seen in the spectrum, but the highest mass that is seen is the $(M\text{-}18)^{+\cdot}$ ion. Evidently at the temperature where the sample is volatilized, there is, as could be expected, an easy dehydration.

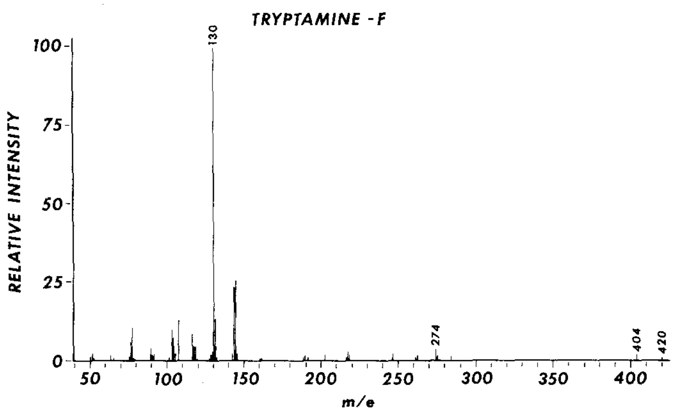


Fig. 4 Mass spectrum of the fluorophor(F) from tryptamine and fluorescamine.

Mass Spectral Characteristics

Since the compounds are aromatic in character, we see in the spectra fairly higher relative abundance in the high mass region. The fragmentation at $R\wr CH_2\text{-}N\langle$ gives rise to fragments corresponding to R^+ or $(M\text{-}18\text{-}R)^+$. Further the fragment ions due to loss of 16 amu (spiran O), loss of 28 amu (CO), etc are seen in the spectrum. In addition, the structural characteristics of the parent primary amines are also noticeable from the fragment ion from the structural part R (8). Thus taking the spectrum of the fluorphor obtained from DMPEA, the fragment ion *m/e* 441 corresponds to $(M\text{-}18)^{+\cdot}$, *m/e* 425 to $(M\text{-}18\text{-}16)^{+\cdot}$, *m/e* 290 to $(M\text{-}18\text{-}R)^+$, *m/e* 151 to R^+ and the fragments in the low mass region correspond to those expected of R^+.

As a generalization the mass spectra can be characterized by the following: the $(M\text{-}18)^{+\cdot}$ ion has the numerical value 260 plus the molecular weight of the amine, the ion at *m/e* $(M\text{-}18\text{-}16)^{+\cdot}$, the ion at *m/e* $(M\text{-}18\text{-}R\cdot CH_2)^+$ due to β fission, the ion corresponding to $(R\text{-}CH_2)^+$. Thus the spectrum gives significant information for the full characterization of the amine. The fragmentation is shown in Scheme I. The spectrum of glycine ethyl ester adduct is given as a typical example of the amino acids. The spectrum shows $(M\text{-}H_2O)^{+\cdot}$ $(M\text{-}H_2O\text{-}29)^+$ $(M\text{-}H_2O\text{-}73)$, as characteristic ions for the identification of the amino acid.

In the spectrum of the fluorophor obtained from *p*-tyramine, both the

Table 1

Mass spectral data[a] for fluorescamine derivatives of some biogenic amines,
amphetamines and amino acid esters
(abundances[b] of the most significant ions[c] as percent of base peak)

Phenylethylamine (PEA)	381 (5),	365 (2.4),	290 (31),	91 (100).
p-Tyramine[d]	397 (0.7), 107 (77).	396 (1),	368 (1.1),	120 (100),
p-MeO PEA	411 (9), 91 (40).	395 (1.8),	290 (26),	121 (100),
3-MeO-4-OH PEA	427 (43), 122 (15),	411 (100), 94 (15).	290 (65),	137 (57),
4-MeO-3-OH PEA	427 (24), 122 (22),	411 (12), 94 (22).	290 (55),	137 (100),
DMPEA	441 (13),	425 (7),	290 (9),	151 (100).
Amphetamine	395 (21), 44 (100).	379 (3.8),	304 (37),	91 (24),
p-MeO-Amphetamine	425 (1.7),	304 (5.4),	122 (70),	121 (75).
Tryptamine	420 (1.2),	404 (2.4),	274 (4.0).	130 (100).
Glycine ethyl ester[e]	363 (100), 290 (10),	347 (35), 262 (26).	335 (16),	334 (35),
Phenylalanine ethyl ester[e]	453 (7), 306 (1.3), 102 (86),	437 (1), 278 (28), 91 (100).	380 (3), 250 (2),	362 (5), 120 (83),

[a] *m/e*.

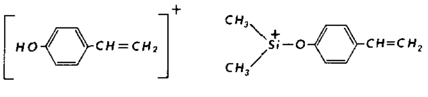
[b] Numerals in parentheses refer to relative abundances of ions of indicated mass.

[c] The first mass in each compound corresponds to the $(M-18)^{+\cdot}$ ion.

[d] Probe temperature 300°C.

[e] Probe temperature 200°C.

$M^{+\cdot}$ and $(M-1)^+$ ions and $(M-1-28)^+$, the ion at *m/e* 368, are all characteristic of the free phenolic fraction. The spectrum is also characterized by the loss of a neutral species by hydrogen transfer from β-carbon atom giving the ion *m/e* 120, corresponding to structure IV. The *p*-tyramine NCS and its



IV m/e 120 m/e 177

Fig. 5 Fragment ions obtained by NCSH loss from *p*-tyramine-NCS and its TMS ether.

TMS derivative give by a similar mechanism *m/e* 120 (unpublished data) and *m/e* 177 ions as shown below (Fig. 5) (8). The mass spectral characteristics of the compounds (fluorophors) are summarized in Table 1.

DISCUSSION

The fluorophors obtained from primary amines of general biological importance and from amino acids can be identified by mass spectrometry. In view of the reaction between acetone and the primary amines, the use of acetone for this reaction is not recommended. The mass spectra are very characteristic of the structures of the amines and hence constitute a convenient and specific diagnostic factor in the identification of these compounds. For this reason and because of the ease and the quantitative character of the reaction with fluorescamine, this reagent is much superior to dansylation procedures. We are currently in the process of studies of TLC and GC separation of these derivatives. Preliminary studies with amines from urine and cerebrospinal fluid indicate that the total mixture of the fluorphor on a direct solid sample probe gives useful information on the amine composition. Full details of this study will be the subject matter of future communications.

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