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# High Performance Liquid Chromatographic Determination of Naturally Occurring Primary and Secondary Amines with Dabsyl Chloride

**Jen-Kun Lin* and Chen-Ching Lai**

*Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China*

**A high performance liquid chromatographic method has been developed for the determination of ammonia, methylamine, ethylamine, dimethylamine, and diethylamine in fish and shrimp samples. These aliphatic amines are modified by pre-column derivatization with dabsyl chloride which stabilizes the molecules, facilitates extraction, and improves detection of nanogram amounts. The levels of these amines in 18 kinds of dried fish and shrimp, 17 kinds of fresh fish, and 9 kinds of canned fish were analyzed. Dimethylamine was found to be abundantly distributed in various kinds of shrimp and fish. The complete assay procedure takes about 150 min. Recovery from the sample extract is varied between 94 to 98% depending on which of the four aliphatic amines or ammonia was studied. Crystalline dabsyl derivatives of 14 amines were synthesized and their physicochemical properties including colors, melting points, electronic absorption maxima, molar absorptivities, and infrared spectra are described.**

Recent developments in environmental carcinogenesis have demonstrated that *N*-nitroso compounds are a major class of chemical carcinogens which lead to a wide variety of tumors in many animals (*1*). These compounds may be formed in vitro by reaction of nitrite with secondary or tertiary amines and in vivo in the stomach or small intestine of experimental animals following the concurrent ingestion of nitrite and precursor amines (*2, 3*). Some carcinogenic nitrosamines have been demonstrated in normal human feces indicating that they have been produced endogenously in the gastrointestinal tract (*4*). These findings suggested that the naturally occuring amines in daily foods might play a significant role in the incidence of some forms of human cancer. Because of the wide distribution of these amines in various foods, data are urgently needed to see whether these compounds constitute a biohazard problem in the environment.

Recently, several high performance liquid chromatographic (HPLC) methods for the determination of amines such as a metal–amine complex procedure (*5*), a copper(II)-bonded phase (*6*), fluorimetric derivatization with *o*-phthalaldehyde (*7*), and an iodine–amine transfer complex method (*8*) have been described. However, these procedures have been designed mainly for the separation and identification of aromatic amines. A new HPLC procedure was needed for the determination of naturally occurring amines such as methylamine, ethylamine, dimethylamine, and diethylamine.

In this paper, an HPLC procedure has been developed for the determination of naturally occurring amines with dabsyl chloride, a chromophoric labeling reagent for amino compounds (*9*). The levels of aliphatic amines and ammonia are reported in 18 kinds of dried fish and shrimp, 17 kinds of fresh fish, and 9 kinds of canned fish.

## EXPERIMENTAL

**Apparatus.** High performance liquid chromatography was accomplished with a μBondapak $C_{18}$ reverse-phase column (30 cm × 3.9 mm i.d.) on a Waters ALC 201 liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model U6K universal injector, a Model 660 solvent programmer, and a Model 450 variable wavelength photometer set at 425 nm (all products of Waters Associates, Milford, Mass.). The recorder was a OmniScribe strip chart recorder, Model B 5000 (Houston Instruments, Austin, Tex.).

**Chemicals.** Dabsyl chloride (4-dimethylaminoazobenzene-4′-sulfonyl chloride, mp 186–188 °C) was synthesized by reacting sodium 4-dimethylaminoazobenzene-4′-sulfonate with phosphorus pentachloride (*9*), or by chlorosulfonation of 4-dimethylaminoazobenzene (*10*). The dabsyl chloride was also purchased from Polysciences, Inc. (Warrington, Pa.).

Methylamine, *n*-propylamine, *n*-butylamine, dimethylamine, piperazine, and morpholine were purchased from Wako Pure Chemical Industries, Inc. (Tokyo). Diethylamine, sarcosine, and ethylenediamine were purchased from Sigma Chemical Co., (St. Louis, Mo.). Piperidine, diphenylamine, ammonia, and ethylamine were purchased from E. Merck Co., (Darmstadt, Germany); and *N*-methylaniline was purchased from Fluka AG (Switzerland).

**Sample Collection.** Canned fish were purchased from the supermarket in Taipei. Dried shrimp and fish samples were purchased from the local markets and fisheries at Taipei, Taitung, Taichung, Chiayi, Tainan, Kaoshiung, and Su-ou. The samples were placed in plastic bags and stored at −20 °C. Fresh fish were obtained from fisheries and immediately packed in dry ice. The samples were analyzed as soon as possible.

**Sample Dabsylation for HPLC Analysis.** Up to 1 g of sample was placed in 10 mL of 0.12 N hydrochloric acid overnight and then homogenized. The homogenate was centrifuged at 2000 rpm for 30 min and the supernatant (2 mL) was taken and mixed thoroughly with 2 mL of acetone. This mixture was then centrifuged at 2000 rpm for 30 min, and then a 1-mL aliquot of the supernatant was pipetted into a 15-mL test tube and mixed with 30 mg of sodium carbonate and 1.6 mg of dabsyl chloride in 1 mL of acetone. The mixture was Vortex-mixed thoroughly and allowed to stand at ambient temperature (25–26 °C) for 30 min.

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Table I. Physicochemical Properties of Dabsylamides

| amine | structure $(CH_3)_2NC_6H_4N=NC_6H_4SO_2NR_1R_2$ $R_1$ | $R_2$ | color[a] | yield % | melting point, °C | electronic absorption peaks (nm) and molar absorptivities ($\epsilon \times 10^{-4}$) acetone | ethanol | 0.2 N $NH_4OH$ in ethanol | 0.2 N HCl in ethanol |
|---|---|---|---|---|---|---|---|---|---|
| ammonia | H | H | O | 95 | 274-275 | 435 (2.94) | 430 (3.65) | 429 (2.43) | 512 (4.48) |
| methylamine | H | $CH_3$ | R | 92 | 207-208 | 435 (3.00) | 433 (2.99) | 435 (2.08) | 510 (3.52) |
| ethylamine | H | $C_2H_5$ | R | 91 | 171-172 | 435 (3.07) | 433 (2.99) | 430 (2.48) | 511 (3.30) |
| *n*-propylamine | H | $C_3H_7$ | O | 90 | 178-179 | 434 (2.60) | 433 (2.66) | 433 (2.12) | 510 (3.56) |
| *n*-butylamine | H | $C_4H_9$ | Y-O | 92 | 156-157 | 433 (3.26) | 435 (2.98) | 435 (2.56) | 514 (3.95) |
| dimethylamine | $CH_3$ | $CH_3$ | Y-O | 87 | 204-206 | 440 (3.30) | 439 (2.94) | 439 (2.24) | 504 (3.65) |
| diethylamine | $C_2H_5$ | $C_2H_5$ | Y-O | 85 | 157-159 | 435 (2.99) | 434 (3.02) | 433 (3.02) | 510 (3.78) |
| diphenylamine | $C_6H_5$ | $C_6H_5$ | B-R | - | 189-192 | 467 (6.75) | 453 (4.94) | 435 (4.20) | 503 (7.53) |
| piperazine | -- | -- | O | - | 225-227 | 440 (2.96) | 438 (3.28) | 441 (3.39) | 510 (4.59) |
| piperidine | -- | -- | R-O | - | 222-223 | 447 (2.99) | 439 (3.15) | 440 (3.18) | 508 (4.18) |
| morpholine | -- | -- | O | 91 | 247-248 | 440 (2.85) | 440 (2.64) | 440 (2.37) | 512 (3.23) |
| *N*-methylaniline | $CH_3$ | $C_6H_5$ | B-R | 67 | 200-203 | 441 (3.00) | 440 (2.93) | 440 (2.93) | 511 (3.91) |
| sarcosine | $CH_3$ | $CH_2COOH$ | R | 83 | 190 (dec.) | 466 (2.78) | 458 (4.44) | 435 (3.92) | 502 (6.60) |
| ethylenediamine | H | $CH_2CH_2NH_2$ | R-O | - | 193-195 | 439 (3.94) | 439 (3.31) | 436 (2.86) | 510 (3.72) |

[a] O, orange; R, red; Y-O, yellow orange; B-R, brownish red; R-O, red orange.

Then 0.8 mg of dabsyl chloride in 0.5 mL of acetone was again added to the mixture which was then Vortex-mixed, and allowed to stand at ambient temperature for 30 min. The resultant mixture was extracted twice with 1 mL of *n*-hexane–*n*-butanol (1:1). The organic layers were combined, washed twice with 2 mL of water, and then dehydrated with solid anhydrous sodium sulfate (0.3–0.5 g). A 5–20 μL aliquot of the clear yellow extract was taken for HPLC analysis.

For construction of standard curves, various concentrations of aliphatic amine (0 to 40 ppm of ammonia, methylamine, ethylamine, dimethylamine, or diethylamine) in 1 mL of 0.12 N hydrochloric acid was dabsylated and analyzed as described above.

**Preparation of Dabsylamides.** Dabsyl derivatives of primary and secondary amines (Table I) were prepared by reacting dabsyl chloride (60–100 mg in 70 mL of acetone) with an excess amount of various amines in the presence of sodium bicarbonate (200 mg in 20 mL of water) at ambient temperature for 2 h. After removal of acetone under reduced pressure, the precipitates of dabsylamides were filtered off, washed thoroughly with water, and dried over solid potassium hydroxide in a desiccator. Recrystallization of these dabsylamides was performed by using either a benzene–*n*-hexane system or an ethanol–water system. The melting points of these new compounds were measured in a Thomas Model 40 Micro Hot Stage (Kofler type) and are uncorrected. Electronic spectra were determined on a Shimadzu Double-Beam spectrophotometer (UV-200S) equipped with a Shimadzu U-125MU scanning recorder. Infrared spectra were recorded with a Perkin-Elmer 577 grating spectrophotometer. KBr disks were prepared by pressing 1–2 mg of sample with 200 mg of KBr.

## RESULTS AND DISCUSSION

**Identification of Dabsylamides.** The structures and physicochemical properties of 14 dabsylamide derivatives including colors, melting points, electronic absorption maxima, and molar absorptivities are given in Table I. The typical electronic absorption spectra of dabsylamide in various media are illustrated in Figure 1.

The presence of sulfonamide groups (—$SO_2$—N=) in these dabsylamides was revealed by their infrared absorption around 1141–1130 $cm^{-1}$ and 1370–1360 $cm^{-1}$ (Table II). There have been several reports that the sulfonamide groups absorb at 1178–1159 $cm^{-1}$ and 1370–1333 $cm^{-1}$ (*9*, *11*). The presence of the azo linkage (—N=N—) and the dimethylamino group was demonstrated by their absorptions around 1570–1510 $cm^{-1}$ and 1433–1410 $cm^{-1}$ and 1370–1360 $cm^{-1}$ and 1315–1312 $cm^{-1}$, respectively. Silverstein et al. (*12*) have assigned 1576 and 1429 $cm^{-1}$ for the —N=N— group and 1360–1310 $cm^{-1}$ for the C–N stretch of tertiary aromatic amines.

Primary sulfonamide shows strong N–H stretching bands at 3325 and 3228 $cm^{-1}$ in the solid state (Table II, 1), while secondary sulfonamides absorb near 3362–3233 $cm^{-1}$ (Table II, 2, 3, 4, 5, and 14). Similar assignments for primary and secondary sulfonamides have been reported in the literature (*12*).

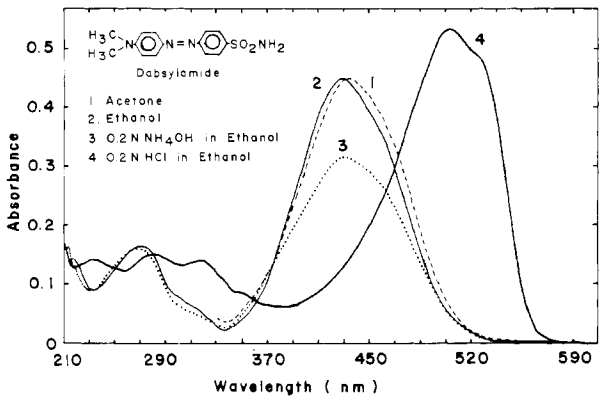


**Figure 1.** Electronic absorption spectra of 4-dimethylaminoazobenzene-4′-sulfonamide (dabsylamide). (1) $1.7 \times 10^{-5}$ M dabsylamide in acetone; (2) $1.2 \times 10^{-5}$ M dabsylamide in ethanol; (3) $1.2 \times 10^{-5}$ M dabsylamide in 0.2 N ammonium hydroxide; (4) $1.2 \times 10^{-5}$ M dabsylamide in 0.2 N HCl

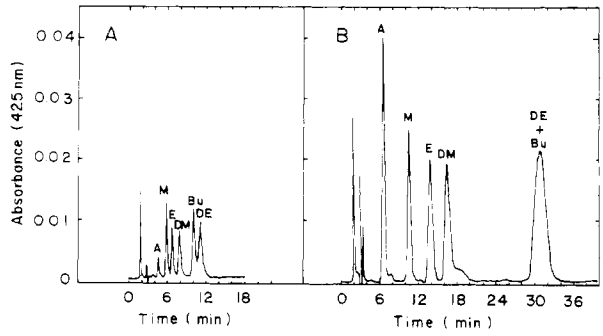


**Figure 2.** HPLC separation of six aliphatic amine standards, derivatized by the reaction with dabsyl chloride. Amines: A, ammonia; M, methylamine; E, ethylamine; DM, dimethylamine; Bu, *n*-butylamine; and DE, diethylamine. Column: μBondapak $C_{18}$; AUFS, 0.1; flow rate, 1.0 mL/min. Profile A: sample, 10 μL; standards, 2 ng for ammonia and 10 ng for other amines; mobile phase, 95% ethanol:acetonitrile:water (6:6:7). Profile B: sample, 10 μL; standards, 40 ng each; mobile phase, 95% ethanol:water (1:1)

**HPLC Analysis of Dabsylamides.** Figure 2A shows the separation by HPLC isocratic elution of 6 aliphatic amines (2–10 ng of each) in a standard solution. The separation took less than 12 min. Figure 2B shows that better resolution of the four amines and ammonia can be achieved with another

Table II. Infrared Spectra of Dabsylamides

| amine | positions and intensities of absorption maxima, $cm^{-1}$ [a] | | | | |
|---|---|---|---|---|---|
| | $-SO_2-N=$ | $-NH-$ | $-N=N-$ | $-N(CH_3)_2$ | others |
| 1. ammonia | 1139s<br>1160s<br>1364s | 3228s<br>3325s | 1421s<br>1558w | 1325s<br>1364s | 493m, 540w, 559s, 602s, 640w, 690s, 749s, 821s<br>843s, 900s, 949m, 1008w, 1062w, 1090s, 1101w, 1229s,<br>1255w, 1301w, 1410w, 1445w, 1510m, 1604s, 2902m. |
| 2. methylamine | 1130s<br>1160s<br>1360s | 3300m | 1410m<br>1512w | 1310s<br>1360s | 470m, 540w, 565s, 599m, 684s, 745m, 828s, 842s, 942m,<br>1088s, 1230m, 1258m, 1326s, 1446w, 1601s. |
| 3. ethylamine | 1140s<br>1165s<br>1369s | 3233m | 1419m<br>1516m | 1328s<br>1369s | 541m, 573s, 602m, 640w, 690s, 749w, 800m, 821s, 855s,<br>948s, 1025w, 1064w, 1088m, 1104w, 1234m, 1262s,<br>1303w, 1315w, 1605s, 2900m, 2931m. |
| 4. *n*-propylamine | 1138s<br>1162s<br>1370s | 3362m | 1420s<br>1560m | 1315s<br>1370s | 492w, 538w, 570s, 598s, 639w, 685s, 748m, 820s, 842s,<br>888w, 916m, 948m, 1000m, 1031w, 1092s, 1230m,<br>1250m, 1300s, 1390w, 1440w, 1515m, 1602s, 2925m. |
| 5. *n*-butylamine | 1135s<br>1165s<br>1362s | 3280m | 1422s<br>1560m | 1315s<br>1362s | 330m, 468m, 540m, 570s, 598m, 684s, 718w, 732w, 746s,<br>824s, 846m, 900m, 945m, 985m, 1032m, 1090s, 1228s,<br>1250m, 1512s, 1601s, 2900m. |
| 6. dimethylamine | 1141s<br>1161s<br>1367s | -- | 1422s<br>1518m | 1318w<br>1367s | 488m, 513w, 543w, 555s, 590s, 637w, 670w, 706s, 720s,<br>736m, 751s, 815s, 848s, 956s, 1088s, 1231m, 1249w,<br>1269w, 1307w, 1318w, 1332s, 1445w, 1462w, 1479w,<br>1601s, 2900m. |
| 7. diethylamine | 1134s<br>1161s<br>1365s | -- | 1433m<br>1518m | 1312w<br>1365s | 500w, 503w, 570s, 592s, 640w, 668w, 700s, 721m, 750s,<br>786m, 830s, 860s, 940s, 1019s, 1068w, 1089w, 1102w,<br>1202s, 1230m, 1251m, 1305w, 1330s, 1445m, 1600s,<br>2930m. |
| 8. diphenylamine | 1130s<br>1180s<br>1360s | -- | 1414s<br>1580s | 1312s<br>1360s | 372m, 479w, 500s, 522w, 540m, 568s, 582s, 639m, 682s,<br>714w, 747s, 824s, 848s, 744s, 760w, 1008w, 1076s,<br>1104w, 1159w, 1229s, 1260m, 1298w, 1335w, 1447m,<br>1518m, 1602s, 2920m. |
| 9. piperazine | 1140s<br>1168s<br>1365s | -- | 1415s<br>1510s | 1315m<br>1360s | 498m, 579w, 550s, 592s, 640w, 679m, 728s, 751s, 800m,<br>820s, 850m, 885s, 940s, 1000w, 1670m, 1090s, 1115m,<br>1200w, 1236m, 1255w, 1390w, 1448s, 1602s, 2900m. |
| 10. piperidine | 1140s<br>1170s<br>1360s | -- | 1410s<br>1518s | 1315m<br>1360s | 315m, 483m, 500m, 546s, 588s, 638w, 678m, 710s, 725m,<br>736s, 752s, 806w, 826s, 840s, 858w, 910w, 939s, 968w,<br>1000w, 1029m, 1051s, 1070w, 1090s, 1105m, 1218w,<br>1231w, 1251m, 1278m, 1303w, 1338s, 1390w, 1441s,<br>1601s, 2840m, 2932m. |
| 11. morpholine | 1140s<br>1170s<br>1363s | -- | 1447s<br>1575m | 1315m<br>1363s | 332m, 499s, 523m, 542m, 558s, 591s, 612m, 640m, 679m,<br>729s, 751s, 821s, 852s, 940s, 1032w, 1068m, 1090s,<br>1112s, 1230m, 1265s, 1300w, 1334w, 1350s, 1390w,<br>1420s, 1602s, 2828m, 2900m. |
| 12. *N*-methylaniline | 1136s<br>1170s<br>1341s<br>1370s | -- | 1419s<br>1510w | 1311m<br>1390m | 450w, 508w, 539m, 570s, 601s, 620w, 638w, 678m, 699s,<br>720m, 749s, 771s, 795w, 820s, 838w, 850s, 864s, 920m,<br>946m, 961w, 1028m, 1066s, 1086m, 1100w, 1159w,<br>1232m, 1252m, 1444m, 1492m, 1605s, 2900m. |
| 13. sarcosine | 1130s<br>1180s<br>1360s | -- | 1414s<br>1580m | 1310s<br>1360s | 500m, 540m, 568s, 584s, 638m, 682s, 715w, 746m, 822s,<br>848s, 942s, 1078s, 1105w, 1156w, 1229m, 1260m,<br>1448m, 1520m, 1602s, 2920m. |
| 14. ethylenediamine | 1134s<br>1159s<br>1366s | 3310m<br>3360m | 1420s<br>1511m | 1314s<br>1366s | 330m, 466m, 500w, 540s, 576s, 600s, 640m, 683s, 791m,<br>820s, 852s, 948s, 1030s, 1060w, 1090s, 1232s, 1252m,<br>1300s, 1390w, 1442m, 1600s, 2855m, 2900m. |

[a] s, strong; m, medium; w, weak.

solvent system with the drawback that the elution time is increased to about 30 min and that the dabsyl derivative of *n*-butylamine (Bu) is not separated from that of diethylamine (DE). A summary of the retention times of the dabsylamides derived from 8 aliphatic amines in 10 different elution systems is tabulated in Table III.

The determination of most aliphatic amines in biological samples was hindered by the lack of spectrophotometric absorption of these compounds. In this study, these amines were derivatized with dabsyl chloride to form a new series of dabsylamides (Table I). The highly chromophoric property of the dabsyl moiety provides the basis for the detection of these amines.

Response curves for injections ranging from 2 to 5 ng were linear for all five naturally occurring amines (Figure 3). The wide linear range for the different aliphatic amines covers the concentrations of these amines in fish and shrimp samples. For repeated injections of the derivatives from 12.5 ng of ammonia, methylamine, ethylamine, dimethylamine, and diethylamine, the relative standard deviations ($n$ = 6) were 3.5, 4.7, 3.1, 3.3, and 0.9%, respectively.

**HPLC Analyses of Naturally Occurring Amines in Fish and Shrimp.** The HPLC method was applied to the routine analyses of fish and shrimp purchased from the local fisheries; the general procedure was described in the Experimental section. Representative chromatograms illustrating the occurrence of amines in hair-tail fish (*Trichiurus haumela*) and shrimp (*Leander annadalei*) are shown in Figures 4A and 4B, respectively. Fresh hair-tail fish was purchased from a fishery and analyzed immediately, while aged hair-tail fish had been stored at 4–10 °C for 3 days before analysis. The aged hair-tail fish gave higher levels of the aliphatic amines as compared to the fresh sample (Figure 4A). This result suggests that some on-going endogenous biological processes might be responsible for the formation of these aliphatic amines.

Three classes of samples, namely dried, fresh, and canned fish, were analyzed and the results are listed in Tables IV, V, and VI, respectively. In the present study, only 5 common naturally occurring amines were determined. Both di-

Table III. HPLC Data of Dabsylamides

| solvent system[a] | retention time, min | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ammonia | methylamine | ethylamine | morpholine | piperazine | dimethyl-amine | *n*-butyl-amine | diethyl-amine |
| 1. | 4.4 | 5.8 | 6.5 | 7.0 | 7.5 | 7.9 | 9.9 | 11.0 |
| 2. | 4.9 | 6.8 | 8.1 | 9.0 | 9.8 | 9.8 | 14.0 | 15.5 |
| 3. | 4.1 | 5.5 | 6.3 | 6.9 | - | 7.4 | 9.8 | 10.8 |
| 4. | 4.0 | 4.8 | 5.3 | 5.8 | - | 6.2 | 7.0 | 7.9 |
| 5. | 4.3 | 5.8 | 6.5 | 9.0 | - | 8.2 | 10.0 | 12.0 |
| 6. | 4.8 | 6.1 | 7.2 | - | - | 8.3 | 11.2 | 12.1 |
| 7. | 5.1 | 6.0 | 7.0 | - | - | 7.9 | - | 11.2 |
| 8. | 10.1 | 11.0 | 12.0 | - | - | 12.8 | 14.2 | 15.0 |
| 9. | 6.4 | 10.3 | 13.8 | - | - | 16.2 | 30.8 | 30.8 |
| 10. | 5.2 | 7.4 | 9.0 | 11.8 | - | 10.5 | 17.0 | 17.0 |

[a] The solvent systems are: 1, 95% ethanol:acetonitrile:water (6:7:7); 2, 95% ethanol:acetonitrile:water (3:3:4); 3, 95% ethanol:acetonitrile:water (1:1:1); 4, acetonitrile:water (7:3); 5, acetonitrile:water (3:2); 6, methanol:water (4:1); 7, 95% ethanol:water (7:3); 8, 95% ethanol:water (3:2); 9, 95% ethanol:water (1:1); and 10, 95% ethanol:water (11:9). All solvents were made by volume. The flow rate were 0.3 mL/min for solvent 8 and 1.0 mL/min for all other solvents.

Table IV. Naturally Occurring Amines in Dried Shrimp and Fish

| sample | no. of samples | amines, ppm mean (range) | | | | |
|---|---|---|---|---|---|---|
| | | ammonia | methylamine | ethylamine | dimethylamine | diethylamine |
| 1. shrimp (*Leander annadalei*) small, dried | 9 | 19.9 (8.9-51) | 0.44 (0-4.0) | 4.8 (0-31) | 122.5 (50-172) | 0.24 (0-1.1) |
| 2. shrimp (*Leander annadalei*), medium, dried | 17 | 18.5 (6.2-125) | 4.4 (0-47) | 1.4 (0-10) | 177.6 (66-274) | 0.3 (0-1.7) |
| 3. round herring (*Spratelloides gracilis*) | 10 | 44.5 (17.5-97) | 0.7 (0-3.1) | 0.5 (0-3.2) | 70.6 (35-114) | 0.5 (0-3.4) |
| 4. anchovies (mixed species) | 6 | 49.5 (7.3-125) | 0.6 (0-4.0) | 0 | 132.8 (58-173) | 0.4 (0-2.9) |
| 5. anchovies (post laval) | 3 | 39.5 (13-71) | 0 | 0 | 59.2 (35-92.5) | 0 |
| 6. squid (*Loligo bleekeri*) | 3 | 21.8 (18-29) | 0 | 0 | 179.7 (118-190) | 0 |
| 7. deep flounder (*Pseudorhombus arsius*) | 2 | 27.2 (19-35.5) | 1.5 (0-3.0) | 29 (0-58) | 54.7 (43-66) | 5.1 (0-10.2) |
| 8. gizzard shad (*Nematolosa nasus*) | 1 | 15.2 | 0 | 0 | 212.5 | 0 |
| 9. butterfly fish (*Microcanthus strigatus*) | 1 | 22.6 | 0 | 0 | 45.2 | 0 |
| 10. eel (*Anguilla japonica*) | 1 | 125 | 0 | 24.1 | 0 | 6.8 |
| 11. deep sea bass (*Cephalopholis miniatus*) | 1 | 125 | 0 | 0 | 258 | 3.4 |
| 12. mullet (*Muquil cephalus*) | 1 | 88.2 | 0 | 0 | 93.8 | 0 |
| 13. white bait (*Allanetta bleekeri*) | 1 | 71 | 0 | 0 | 55 | 0 |
| 14. silver anchovy (*Spratelloides gracilis*) | 1 | 125 | 8.5 | 0 | 156.4 | 2.3 |
| 15. sea bass (*Epinephelus areolatus*) | 1 | 8.2 | 0 | 0 | 28.8 | 0 |
| 16. Spanish mackerel (*Scomberomorus commerson*) | 1 | 29.3 | 0 | 10.8 | 36.8 | 0 |
| 17. crab (*Eriokeia chinensis*), dried | 1 | 108 | 0 | 0 | 30.7 | 13.5 |
| 18. oyster (*Crassostrea gigas*), dried | 1 | 82.2 | 71.5 | 122.4 | 39.0 | 0 |

methylamine and ammonia were found in most samples, while methylamine and diethylamine were detected in a few samples (Tables IV and V). The juice and solid parts of the canned fish were analyzed separately; in most cases, solid parts contained somewhat higher levels of dimethylamine and ammonia (Table VI).

The recovery percentages of ammonia, methylamine, ethylamine, dimethylamine, and diethylamine added to 0.5-mL samples of shrimp extract were found to have values, with a percent relative deviation, of 94.6 ± 1.6, 95.3 ± 1.9, 95.6 ± 1.4, 97.0 ± 1.9, and 97.6 ± 1.0%, respectively ($n$ = 6). Recoveries were determined by comparing chromatographic peak heights from supplemented shrimp extract with heights generated by direction injection of the derivatives from these 5 aliphatic amines (Figure 3), recovery being calculated as the difference. Each of the standard aliphatic amines was added to make 10 μg/L of shrimp extract.

There are normally lower concentrations of other amines such as ethanolamine, diethanolamine, ethylmethylamine, tyramine, phenylethylamine, and histamine, so these amines may not be detected by this procedure. Amino compounds with an acidic functional group such as amino acids, phenolic amines (adrenaline, noradrenaline, dopamine, and serotonin) could react with dabsyl chloride to form the corresponding dabsylamides which should stay in the alkaline aqueous medium after *n*-hexane–*n*-butanol (1:1) extraction.

Fluorescence detection coupled with HPLC separation has been reported to be a specific and sensitive method for analyzing biogenic amines (7). To enhance the natural fluorescence of biogenic amines, derivatization reagents such as

Table V. Naturally Occurring Amines in Fresh Fish

| fish | amines, ppm ammonia | methyl-amine | ethyl-amine | dimethyl-amine | diethyl-amine |
|---|---|---|---|---|---|
| 1. marlin spike fish (*Kajikia mitsukurii*) | 17.5 | 0.8 | 0 | 34.5 | 0 |
| 2. white pomfret (*Stromateoides argenteus*) | 26.7 | 0.9 | 0 | 80.4 | 0 |
| 3. common carp (*Cyprinus carpio*) | 9.1 | 0 | 0 | 2.7 | 0 |
| 4. chub (*Hypophthalmichthys molitrix*) | 17.1 | 0 | 0 | 0 | 0 |
| 5. common mullet (*Mugil cephalus*) | 14.9 | 1.45 | 0 | 76.8 | 0 |
| 6. red shark (*Acanthogobius ommaturus*) | 25.6 | 0.6 | 0 | 64.8 | 0 |
| 7. ginkgo fish (*Gymnocranius griseus*) | 48.7 | 1.0 | 0 | 49.3 | 0 |
| 8. hair-tail (*Trichiurus haumela*), fresh | 16.6 | 1.6 | 0 | 28.8 | 1.1 |
| 9. hair-tail (*Trichiurus haumela*), aged | 67.3 | 3.5 | 0 | 73.8 | 2.9 |
| 10. shark skin (*Scoliodon walbeemii*) | 125 | 1.45 | 0 | 3.1 | 0 |
| 11. golden skinned pargo (*Evynnis cardinalis*) | 28.2 | 0 | 0 | 46.8 | 0 |
| 12. spotted mackerel (*Scomber scombrus japonicus*) | 1.6 | 0.3 | 3.4 | 42.8 | 0 |
| 13. nemipterid (*Nemipterus virgatus*) | 12.3 | 0 | 0 | 14.8 | 0 |
| 14. silver carp (*Psenopsis anomala*) | 20.9 | 1.45 | 0 | 135.9 | 0 |
| 15. silver perches (*Gerres filamentosus*) | 20.7 | 0 | 0 | 4.0 | 0 |
| 16. sea bass (*Psammoperca waigiensis*) | 14.1 | 0.9 | 0 | 76.5 | 0 |
| 17. yellow croaker (*Pseudosciaena crocea*) | 41.5 | 1.8 | 0 | 201 | 0 |

Table VI. Naturally Occurring Amines in Canned Fish

| canned fish | juice or solid | amines, ppm ammonia | methyl-amine | ethyl-amine | dimethyl-amine | diethyl-amine |
|---|---|---|---|---|---|---|
| 1. marlin spike fish (*Majikia mitsukurii*), made in Taiwan | juice | 5.1 | 1.8 | 0 | 37.4 | 2.1 |
| | solid | 15.5 | 0.9 | 0 | 41.2 | 0 |
| 2. pehmoa (*Anguilla japonica*), made in Taiwan | juice | 12.0 | 1.8 | 0 | 48.2 | 1.3 |
| | solid | 33.1 | 0.5 | 0 | 66.6 | 1.5 |
| 3. swamp eel (*Anguilla marmorata*), made in Taiwan | juice | 19.5 | 2.7 | 0 | 1.4 | 1.0 |
| | solid | 14.0 | 0.8 | 0 | 4.3 | 1.4 |
| 4. sardine (*Sardinella sindensis*), made in Japan | juice | 13.8 | 1.2 | 0 | 16.2 | 0 |
| | solid | 7.0 | 0 | 0 | 16.4 | 1.8 |
| 5. sweet fish (*Plecoglossus altivelis*), made in Japan | juice | 18.8 | 1.2 | 0 | 9.1 | 0 |
| | solid | 32.0 | 2.5 | 0 | 16.9 | 1.4 |
| 6. chub mackerel (*Scomber scombrus tapeinocephalus*), made in Japan | juice | 15.5 | 1.5 | 0 | 18.2 | 1.8 |
| | solid | 16.0 | 2.5 | 0 | 26.6 | 1.5 |
| 7. tuna (*Thunnus thynnus*), made in Japan | juice | 3.0 | 0.3 | 0 | 0.7 | 3.4 |
| | solid | 11.5 | 0 | 0 | 16.9 | 0 |
| 8. pond murrels (*Mogurnda obscura*) made in Norway | juice | 24.0 | 0.3 | 0 | 36.3 | 0 |
| | solid | 25.2 | 1.0 | 0 | 39.2 | 1.4 |
| 9. fresh water trout (*Oncorhynchus masou*), made in Iceland | juice | 5.1 | 0.6 | 0 | 24.1 | 1.8 |
| | solid | 15.6 | 0 | 0 | 40.6 | 2.0 |

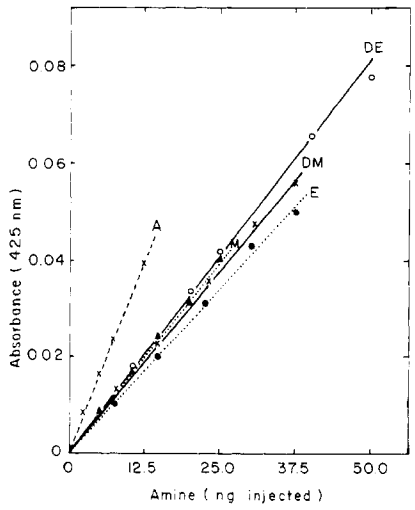


**Figure 3.** Linearity of detection for five aliphatic amine standards. Sample, 5 μL; standards, 2 to 50 ng each. All other conditions as in Figure 2, profile A

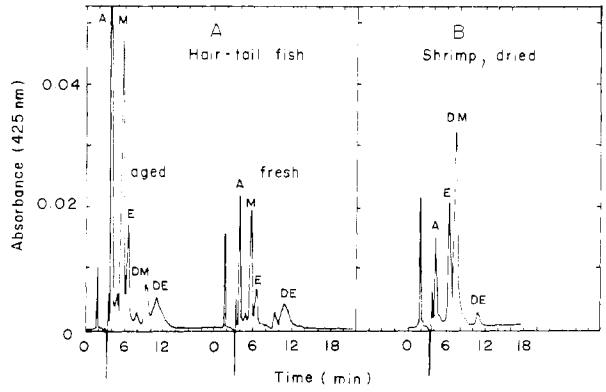


**Figure 4.** Profile of the derivatized aliphatic amines extracted from fish and shrimp samples. Chromatographic conditions: (A) Hair-tail fish sample; mobile phase, 95% ethanol:acetonitrile:water (6:6:7). (B) Shrimp sample; mobile phase, 95% ethanol:acetonitrile:water (1:1:1). All other conditions as in Figure 2. See Experimental section for the handling of the fish or shrimp sample

fluorescamine and *o*-phthalaldehyde have been used to react with primary amines (*13*, *14*). The published methods (*7*, *13*, *14*) appeared to be unsuitable for the detection of secondary amines in biological samples. The chromophoric detection coupled with HPLC separation described in the present study provides a sensitive and rapid method for determining aliphatic primary and secondary amines quantitatively in nanogram amounts. Another advantage of the present method is that the dabsyl derivatives of amines are determined spectrophotometrically at 425 nm; therefore, the interferences

caused by UV-absorbing materials from the biological samples can be avoided. The high efficiency of the HPLC separation combined with the enhanced sensitivity of chromophoric detection allows an extremely low detection limit, less than 1 ng, for most aliphatic amines. The high resolution achieved with the common naturally occurring amines shows the excellent sensitivity of our method, a sensitivity due in part to our use of pre-column derivatization with dabsyl chloride rather than derivatization of the eluate.

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# Vacuum Ultraviolet Circular Dichroism Spectrometer and Its Application to *N*-Acetylamino Saccharides

**Anthony J. Duben[1] and C. Allen Bush***

*Chemistry Department, Illinois Institute of Technology, Chicago, Illinois 60616*

**To provide for the convenient study of aqueous solutions of molecules of biological interest, a vacuum ultraviolet circular dichroism spectrometer has been constructed in which the sample compartment is not enclosed in the vacuum. The instrument features a wide aperture, short focal length monochromator. The light source is a sealed 200-W deuterium lamp with a quartz window. Optical elements in the vacuum include a $MgF_2$ Rochon prism polarizer, a stress plate optical modulator with a $CaF_2$ element, and two $CaF_2$ lenses. The small sample compartment is purged with nitrogen at atmospheric pressure. The circular dichroism is detected electronically by a lock-in amplifier keyed to the frequency of oscillation of the stress plate optical modulator as a small ac signal. The wavelength range of the instrument (350 to 155 nm) is limited at short wavelength by the quartz windows of the sealed lamp and of the photomultiplier tube. In sample spectra of *N*-acetylamino sugars and of glycoprotein linkage compound analogues, the wavelength (240-170 nm) is limited at short wavelength by the absorption of the solvent water in 10-$\mu$m pathlength cells.**

For a molecule to show detectable circular dichroism (CD), it must possess two properties. The first requirement, that of molecular asymmetry, is a property common to most compounds of biological origin. The second requirement is that of light absorption in the wavelength region accessible to the CD instrument. Although molecules having aromatic functionality or other conjugated double-bond systems absorb in the near ultraviolet, some molecules of biological interest absorb only below 200 nm, a range in which light absorption by oxygen in air poses technical limitations.

These limitations may be overcome by vacuum ultraviolet technology in which the optical system is evacuated (*1*). Successful vacuum ultraviolet CD instruments have been described in the literature, several of which have employed 1-m focal length vacuum monochromators capable of moderately high resolution and wavelengths as short as 130 nm (*2-7*). For spectroscopy of molecules in solution, the wavelength range below 160 nm is not generally accesssible owing to light absorption by the solvent. Furthermore, spectral resolution better than a few nanometers is not especially useful since molecules in solution do not generally show the sharp spectral features characteristic of molecules in the gas phase.

In choosing a short focal length monochromator for the instrument described in this paper, we have sacrificed the possibility of high spectral resolution in the interest of enhanced light throughput and economy. In addition, our choice of a sealed quartz lamp has eliminated far ultraviolet penetration below 160 nm in favor of greater light output and stability. By opting for higher light throughput to the detector, we have improved the signal-to-noise ratio, often a limiting problem in CD experiments.

## THE SPECTROMETER

A schematic diagram of our vacuum ultraviolet CD spectrometer for routine biochemical use is shown in Figure 1. The light source is a Hannau sealed deuterium lamp (purchased through Scientific Cell Co., New York). The water cooling jacket of the lamp is mounted directly on the 0.3-m McPherson model 218 monochromator by means of a sliding flange which can be moved to align the lamp with the monochromator optics. Since the lamp arc is not situated at

[1] Present address: Chemistry Department, South Dakota School of Mines and Technology, Rapid City, S.D. 57701.

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