oxide solution to 100 ml of fresh 0.2% (w/v) amitriptyline hydrochloride solution. The hydrogen peroxide content was insufficient to cause this marked degree of decomposition by direct oxidation.

Addition of 0.05% (w/v) of the antioxidant propyl gallate to an amitriptyline hydrochloride solution initially caused a 10% fall in drug concentration but subsequently completely stabilized the solution (Fig. 4). Hydroquinone, 0.01% (w/v), was less effective, since it abolished the lag phase and only appeared to slow the reaction after 15 days. The antioxidants appeared to break the chain reaction. With propyl gallate at least, however, there was evidence of a direct reaction with amitriptyline hydrochloride.

The reducing agent sodium metabisulfite was not effective in stabilizing the solution. Figure 4 shows that 0.121% (w/v) sodium metabisulfite produced an immediate fall in the drug concentration and a subsequent acceleration in the decomposition rate. This process did not follow first-order kinetics at any stage. The concentration of sodium metabisulfite was equimolar with amitriptyline hydrochloride (6.37×10^{-3} *M*), and the data fit a simple second-order kinetic plot.

Bisulfite-mediated reactions were reported previously (9–13), and a reaction mechanism similar to the thiamine cleavage shown previously (14, 15) is possible in the drug's side chain. However, using the isolation and identification techniques outlined previously (1), dibenzocycloheptanone was the only decomposition product present, suggesting that direct attack at the olefinic double bond in the molecule is more likely. Such an affinity for these bonds by sodium bisulfite was demonstrated and explained (16) on the basis of a free radical mechanism.

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Polyamine Metabolism I: Synthesis of Dansyl Derivatives of *N*-(Monoaminoalkyl)- and *N*-(Polyaminoalkyl)acetamides and Elucidation in Urine of a Cancer Patient

MAHMOUD M. ABDEL-MONEM [x] and KOSEI OHNO

Abstract □ The dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides were synthesized and unequivocally characterized. TLC of the dansyl derivatives obtained from human urine indicated the presence of *N*-[3-[(4-aminobutyl)amino]propyl]acetamide (N^1-acetylspermidine), *N*-[4-[(3-aminopropyl)amino]butyl]acetamide (N^8-acetylspermidine), and *N*-(4-aminobutyl)acetamide (*N*-acetylputrescine) in appreciable amounts. The dansyl derivatives of N^1-acetylspermidine, N^8-acetylspermidine, and *N*-acetylputrescine were isolated and purified using various chromatographic methods. The mass spectra of these compounds were similar to those of authentic samples, which confirmed the identity of these compounds and established the presence of N^8-acetylspermidine as well as N^1-acetylspermidine and *N*-acetylputrescine in human urine.

Keyphrases □ Polyamines—*N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, determined in human urine, dansyl derivatives synthesized □ *N*-(Aminoalkyl)acetamides—determined in human urine, dansyl derivatives synthesized □ Dansyl derivatives—of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides synthesized □ TLC—determination, dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, human urine □ High-pressure liquid chromatography—determination, dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, human urine

The diamine 1,4-butanediamine (putrescine) (I) and the polyamines *N*-(3-aminopropyl)-1,4-butanediamine (spermidine) (II) and *N,N'*-bis(3-aminopropyl)-1,4-butanediamine (spermine) (III) are present in all animal and plant tissues, and at least one of these is present in all microorganisms (1). Studies of both normal and neoplastic rapid growth systems indicate that the synthesis and accumulation of polyamines are elevated shortly after a stimulus inducing proliferation. Furthermore, the levels of these amines were elevated in the urine of cancer patients (2–10). These amines are excreted in human urine mainly as conjugates, which yield the free amines after hydrolysis (2).

Although several studies determined the levels of total

$H_2N(CH_2)_4NH_2$ (I)

$H_2N(CH_2)_3NH(CH_2)_4NH_2$ (II)

$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ (III)

$CH_3COHN(CH_2)_nNH_2$ IV: $n = 4$; V: $n = 5$

$CH_3COHN(CH_2)_3NH(CH_2)_4NH_2$ (VI)

$CH_3COHN(CH_2)_4NH(CH_2)_3NH_2$ (VII)

polyamines in urine of normal subjects, cancer patients, and noncancer patients after hydrolysis, few studies addressed the identification of the polyamine conjugates present in urine. *N*-(4-Aminobutyl)acetamide (*N*-acetylputrescine) (IV) was identified in the urine of normal subjects (11), and *N*-(5-aminopentyl)acetamide (*N*-acetylcadaverine) (V) was found in the urine of schizophrenic patients (12). *N*-[3-[(4-Aminobutyl)amino]propyl]acetamide (N^1-acetylspermidine) (VI), but not *N*-[4-[(3-aminopropyl)amino]butyl]acetamide (N^8-acetylspermidine) (VII), also was detected in urine of healthy children (13) and a patient with acute myelocytic leukemia (14). Both VI and VII were formed in *Escherichia coli* on storage of a culture in the cold or growth in medium containing high concentrations of spermidine (15).

The present paper reports the synthesis and unequivocal characterization of the dansyl [5-(dimethylamino)-1-naphthalenesulfonyl] derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides and the isolation and unambiguous elucidation of the *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides from the urine of one cancer patient who had not received therapy. The approach used in identifying the polyamine conjugates in human urine involved the separation and identification of their dansyl derivatives. This approach has several advantages: (*a*) the dansyl derivatives of the polyamines and their conjugates are fluorescent, which permits the detection of minor components; (*b*) chromatographic separation of the dansyl derivatives of polyamines is easier than the separation of the free amines; and (*c*) the use of the dansyl derivatives permits the quantitation of the identified polyamines.

EXPERIMENTAL

Synthesis of Dansyl Derivatives of *N*-(Monoaminoalkyl)acetamides (VIII–XI)—The diamines were treated with dansyl chloride according to the method of Nilsson *et al.* (16) to provide the monodansyl derivatives in 60–80% yields (Scheme I).

A solution of the monodansyl derivative in a mixture of chloroform (10 ml) and pyridine (2 ml) was cooled in an ice bath and treated dropwise with acetic anhydride (1.5 molar equivalents) in chloroform (8 ml) and pyridine (2 ml). The reaction mixture was stirred for 1 hr at room temperature and then treated with ice water. The chloroform layer was separated, washed with water, dried, and evaporated *in vacuo*. The yellow residue was purified by column chromatography on silica gel with chloroform–ethanol (97:3) to provide pure VIII–XI in 90–95% yields. The products were recrystallized from benzene–chloroform.

$$H_2N(CH_2)_nNH_2 \xrightarrow{(CH_3)_2NC_{10}H_6SO_2Cl} (CH_3)_2NC_{10}H_6SO_2HN(CH_2)_nNH_2$$

$$\xrightarrow{(CH_3CO)_2O} (CH_3)_2NC_{10}H_6SO_2HN(CH_2)_nNHCOCH_3$$

VIII: $n = 2$; IX: $n = 3$; X: $n = 4$; XI: $n = 5$

Scheme I

$$I + CH_2{=}CHCN \longrightarrow H_2N(CH_2)_4NHCH_2CH_2CN \xrightarrow{(CH_3)_2NC_{10}H_6SO_2Cl}$$

XII

$$(CH_3)_2NC_{10}H_6SO_2HN(CH_2)_4NCH_2CH_2CN \xrightarrow{B_2H_6}$$

$$\big|$$

$$SO_2C_{10}H_6N(CH_3)_2$$

XIII

$$(CH_3)_2NC_{10}H_6SO_2HN(CH_2)_4N(CH_2)_3NH_2 \xrightarrow{(CH_3CO)_2O}$$

$$\big|$$

$$SO_2C_{10}H_6N(CH_3)_2$$

XIV

$$(CH_3)_2NC_{10}H_6SO_2HN(CH_2)_4N(CH_2)_3NHCOCH_3$$

$$\big|$$

$$SO_2C_{10}H_6N(CH_3)_2$$

XV

Scheme II

Synthesis of Dansyl Derivative of *N*-[3-[(4-Aminobutyl)amino]propyl]acetamide (XV)—*N*-2-Cyanoethylbutane-1,4-diamine (XII) was prepared according to the method of Israel *et al.* (17) (Scheme II). The mixture of products was fractionally distilled three times *in vacuo* to provide XII, bp 87–92°/0.01 mm Hg [lit. (17) bp 88°/0.02 mm Hg]. A solution of triethylamine (10 mmoles) and XII (0.688 g, 4.88 mmoles) in chloroform (15 ml) was treated with a solution of dansyl chloride (2.69 g, 10.0 mmoles) in chloroform (25 ml), and the reaction mixture was stirred at room temperature for 4 hr. The usual workup of the reaction mixture provided a yellow oil (3.7 g), which was purified by column chromatography on silica gel. Elution with chloroform–benzene (7:3) gave an uncharacterized oil (0.21 g), and elution with chloroform provided 2.58 g (yield 87%) of XIII. The nitrile group of XIII was reduced with diborane using the method of Brown *et al.* (18).

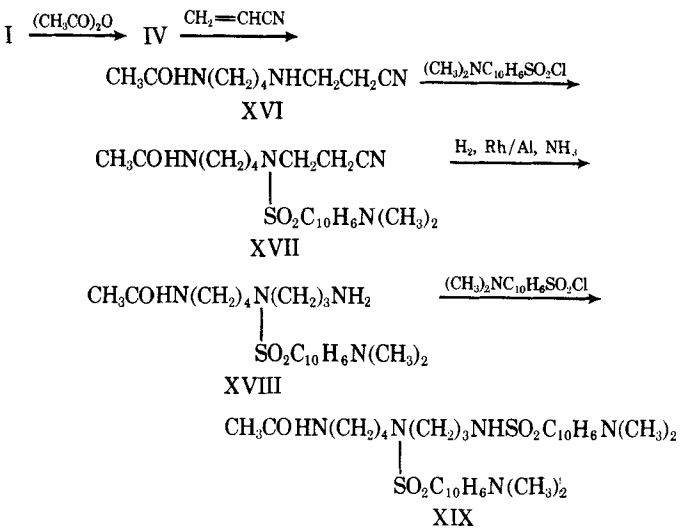
A solution of crude XIV (0.6 g, 1.13 mmoles) in a mixture of chloroform (10 ml) and triethylamine (2 ml) was treated dropwise with acetic anhydride (1.5 ml) in chloroform (10 ml) and stirred for 2 hr. The usual workup of the reaction mixture provided a crude product, which was purified by column chromatography on silica gel using chloroform–ethanol (98:2). The first fraction provided XV as a yellow oil, 63% yield based on the amount of XIII used.

Synthesis of Dansyl Derivative of *N*-[4-[(3-Aminopropyl)amino]butyl]acetamide (XIX)—*N*-(4-Aminobutyl)acetamide hydrochloride (IV) was obtained using the method of Tabor *et al.* (19), mp 135–138° [lit. (19) mp 136–139° and (20) 140–141°]. TLC of the product on silica gel plates, using 1-butanol–acetic acid–pyridine–water (4:1:1:2) and visualization with ceric sulfate, indicated two major products in a 2:1 ratio. A solution of the crystalline product (10 g) in water was adjusted to pH 8.0 with ammonium hydroxide and added to an ion-exchange column[1]. The water eluate was evaporated to dryness *in vacuo* to provide diacetylputrescine (2.39 g), mp 137–138°. The column was washed with 1 *M* ammonium hydroxide, the eluate was concentrated and treated with ethanolic hydrochloric acid, and the product was recrystallized from 2-propanol to provide IV (5.0 g), mp 141–143° [lit. (20) mp 140–141° and (19) 135–138°].

N-[4-[(2-Cyanoethyl)amino]butyl]acetamide (XVI) was obtained from crude IV using the method of Tabor *et al.* (19) (Scheme III). The crude product, XVI hydrochloride, was dissolved in water, made basic with sodium bicarbonate, and extracted with chloroform. The chloroform extract was concentrated to dryness, and the residue was applied to a silica gel column and eluted with chloroform containing different amounts of ethanol. A fraction eluted with 2–5% ethanol in chloroform contained 5.4 g of *N*-[4-[bis(2-cyanoethyl)amino]butyl]acetamide, mp 55–58°. The second fraction, eluted with 5–15% ethanol in chloroform, contained a mixture of diacetylputrescine and XVI. The crude fraction was extracted with hot benzene; the benzene solution, on concentration, provided XVI, mp 35–37°. The benzene-insoluble solid was recrystallized from chloroform to provide diacetylputrescine, mp 137–138°.

A solution of XVI (1.83 g, 10 mmoles) and triethylamine (1.8 g, 17.8 mmoles) in chloroform (30 ml) was treated with a solution of dansyl chloride (2.8 g, 10.4 mmoles) in chloroform (20 ml), and the mixture was

[1] Amberlite IR-120 (H^+).



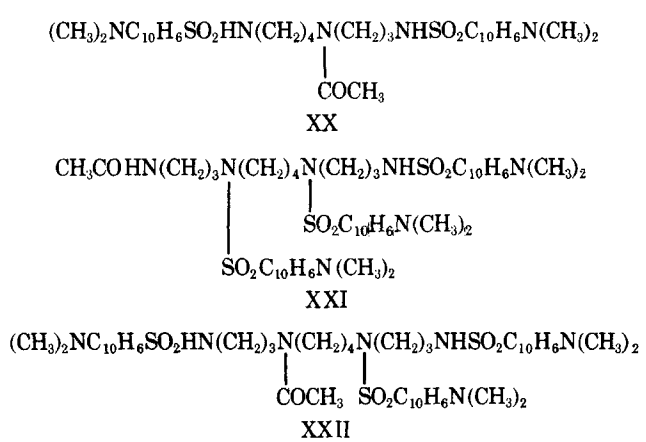
Scheme III

stirred at room temperature for 2 hr (Scheme III). The reaction mixture was filtered, the filtrate was washed with saturated sodium carbonate and then brine, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was purified by column chromatography on silica gel with chloroform to afford *N*-[4-[*N'*-(2-cyanoethyl)-*N'*-dansylamino]butyl]acetamide (XVII), 0.12 g (yield 99%).

Compound XVII was catalytically reduced using the method of Freifelder (21) (Scheme III). A solution of XVII (2.08 g, 5 mmoles) in ethanol (30 ml) containing ammonia (1.5 g) was hydrogenated with rhodium-on-alumina (10 g) catalyst. The catalyst was removed by filtration, and the filtrate was concentrated to dryness *in vacuo* to provide crude XVIII. A solution of crude XVIII (2.1 g, 5 mmoles) in triethylamine (2 ml) and chloroform (25 ml) was treated with a solution of dansyl chloride (1.35 g, 5 mmoles) in chloroform (15 ml), and the mixture was stirred at room temperature overnight. The usual workup of the reaction mixture provided a residue. This residue was purified by column chromatography on silica gel with chloroform–ethanol (97:3) to provide 2.55 g of pure XIX (yield 78%).

Preparation of Dansyl Derivatives of N^1-, N^8-, and N^4-Acetylspermidines (XV, XIX, and XX)—Spermidine (II) (1.45 g, 10 mmoles) was acetylated according to the method of Tabor *et al.* (19). The crude mixture of products was dissolved in triethylamine (5 ml) and chloroform (50 ml) and treated dropwise at room temperature with a solution of dansyl chloride (4.5 g, 17 mmoles) in chloroform (30 ml). Then the mixture was stirred at room temperature for 2 hr and concentrated *in vacuo*. The residue was treated with water (30 ml) and extracted with chloroform (3 × 50 ml). The chloroform solution was washed, dried, and evaporated to dryness.

The fluorescent oil obtained was purified by column (45 × 4.0 cm i.d.) chromatography on silica gel[2]. Elution with chloroform (containing 0.75% ethanol)–ethanol (99:1) provided tridansylspermidine (0.64 g, 5.4% yield). Elution with chloroform–ethanol (97:3) provided the mixture of the three



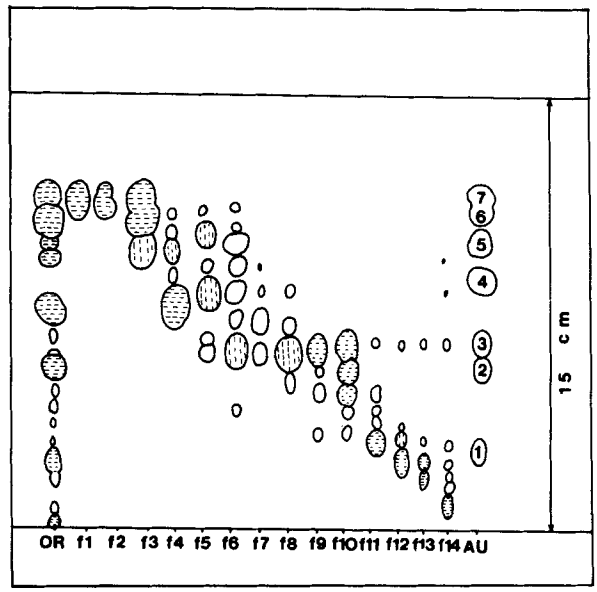


Figure 1—*TLC of the fractions obtained by column chromatographic separation of the dansyl derivatives from the urine of a patient with hepatoma. Solutions tested were: OR, solution of the dansyl derivatives obtained from urine before application to the column; f1–f14, fractions 1–14 obtained after column separation; and AU, mixture of the authentic samples of the dansyl derivatives of IV (1), VI (2), VII (3), ammonia (4), I (5), II (6), and III (7).*

isomeric didansylmonoacetylspermidines (1.48 mg, 22.61% yield), and elution with chloroform–ethanol (90:10) provided a mixture of the dansyldiacetylspermidines (2.18 g, 47.1% yield). Finally, elution with chloroform–ethanol (80:20) provided an unknown fluorescent substance (0.88 g). The fraction containing the didansylmonoacetylspermidines was chromatographed on a column of neutral alumina[3] (28 × 3.0 cm i.d.). Elution with chloroform–0.75% ethanol provided a mixture of XV and XIX (0.76 g) in approximately a 1:6 ratio. Elution with chloroform–ethanol (97:3) gave XX (0.52 g, 40% of total didansylmonoacetylspermidines).

Preparation of Dansyl Derivatives of *N*-[3-[[4-(3-Aminopropyl)aminobutyl]amino]propyl]acetamide (XXI) and *N*-[4-[(3-Aminopropyl)amino]butyl]-*N*-(4-aminobutyl)acetamide (XXII)—A solution of III (0.25 g, 1.21 mmoles) and triethylamine (0.12 g, 1.21 mmoles) in chloroform (15 ml) at 0° was treated dropwise with a solution of acetic anhydride (0.123 g, 1.21 mmoles) in chloroform (10 ml) and then allowed to stand at room temperature for 2 hr. The reaction mixture was treated dropwise with a solution of triethylamine (0.366 g, 3.6 mmoles) and dansyl chloride (1.03 g, 3.82 mmoles) in chloroform (5 ml), allowed to stand overnight, treated with aqueous sodium carbonate, and stirred for 1 hr.

The reaction mixture was extracted with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was chromatographed on a silica gel column and eluted with chloroform containing increasing amounts of ethanol. Elution with chloroform alone provided the unreacted dansyl chloride (0.12 g) followed by a fluorescent uncharacterized compound (0.135 g). Elution with chloroform–ethanol (99:1) provided tetradansylspermine (0.05 g), and elution with chloroform–ethanol (98:2) provided the tridansylmonoacetylspermines (0.132 g, 11.7% yield). Elution with chloroform–ethanol (95:5) provided the didansyldiacetylspermines (0.285 g, 31.3%).

The fraction containing the tridansylmonoacetylspermines was chromatographed on a neutral alumina column. Elution with chloroform–petroleum ether (8:2) provided XXI (one part), and elution with chloroform provided XXII (0.65 part).

Extraction and Dansylation of IV, VI, and VII from Human Urine—A 24-hr urine (1680 ml) was collected from one patient with diagnosed hepatoma who had not received therapy. The urine was collected over toluene, refrigerated during collection, and stored at −20° until analysis. An aliquot of urine (1580 ml) was adjusted to pH 10–11 with 2 *N* NaOH (about 50 ml) and extracted with 3-methyl-1-butanol (4 × 300 ml). Nitrogen was bubbled through the organic solvent extract

[2] Baker analyzed reagent, silica gel powder, 60–200 mesh.

[3] Woelm neutral alumina oxide, Activity IV.

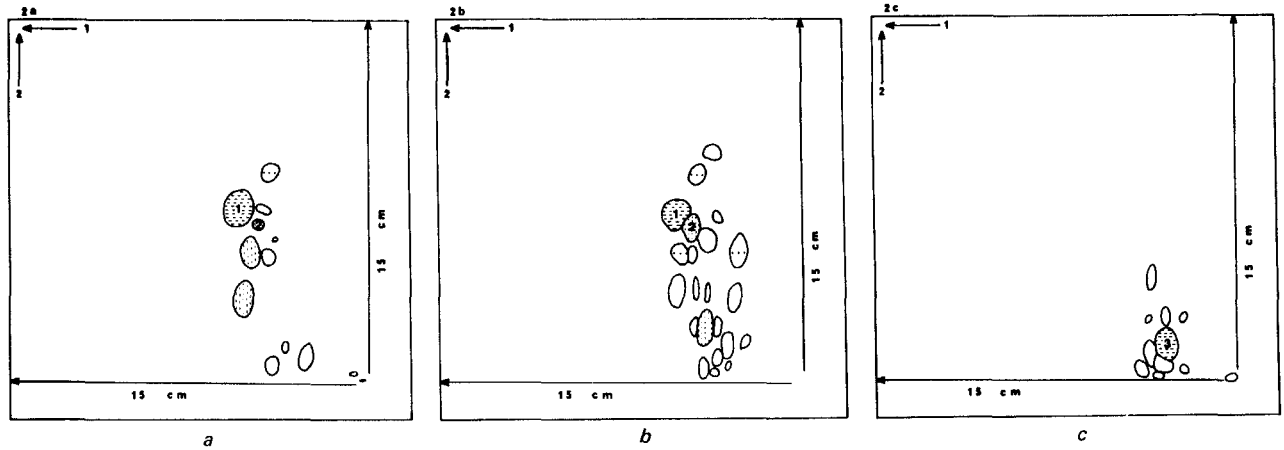


Figure 2—*Two-dimensional TLC of selected fractions obtained by column chromatographic separation of the dansyl derivatives from the urine of a patient with hepatoma. Fractions 9 (2a), 10 (2b), and 11 (2c) were examined. Key: spot 1, XV; spot 2, XIX; and spot 3, X.*

to remove ammonia. Concentrated hydrochloric acid (30 ml) was added to the extract, and the mixture was concentrated to dryness *in vacuo* at 45°.

The residue was dissolved in 5% HCl (10 ml), and the flask was rinsed with additional water (25 ml) and acetone (20 ml). Then the solution was adjusted to pH 8.5–9 with anhydrous sodium carbonate and treated with a solution of dansyl chloride (0.538 g, 2 mmoles) in acetone (20 ml). The mixture was stirred at room temperature for about 12 hr in a covered flask and was concentrated to remove the acetone. Water (70 ml) was added to dissolve the precipitated solids, and the reaction mixture was extracted with chloroform (3 × 100 ml). The combined chloroform extract was washed with saturated sodium bicarbonate solution (80 ml) and water (50 ml) and dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness *in vacuo* to give a dark-brown oil.

Isolation and Purification of X, XV, and XIX—*Column Chromatography*—The residue obtained from the dansylation reaction was dissolved in chloroform, applied on a column (30 × 2.4 cm i.d.) of silica gel[4], and eluted with chloroform–ethanol. The elution on the column was monitored with a long wavelength UV lamp, and 15 fluorescent fractions were collected. The solvent composition and volumes (milliliters) of the fractions were: chloroform (containing 0.75% ethanol), 200, 300, 300, 400, and 500; chloroform–ethanol (98:2), 150, 100, 300, and 200; chloroform–ethanol (95:5), 500, 300, 300, 400, and 200; and chloroform–ethanol (8:2), 300.

A sample of each fraction was applied to a TLC silica gel GF plate (250 μm, 20 × 20 cm). A sample of the crude dansylation reaction prior to the column separation and a mixture of the authentic samples of the dansyldiamines, dansyl polyamines, and *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides were also applied to the same plate. The plate was developed in chloroform–2-propanol (10:1) and examined under a long wavelength UV lamp (Fig. 1).

Fraction 9 contained primarily XV and a small amount of XIX together with other fluorescent impurities. Fraction 10 contained a mixture of XV and XIX together with some fluorescent impurities. The dansyl derivative of IV was eluted in fraction 11. Each fraction was examined by two-dimensional TLC (Fig. 2).

Analytical TLC—Silica gel GF plates[5] (250 μm, 20 × 20 cm) were used in one- or two-dimensional analysis. The solvent systems used were: Solvent 1, chloroform–2-propanol (10:1); and Solvent 2, chloroform–triethylamine (10:2). The developed plates were examined under a long wavelength UV lamp. Samples from selected fractions after column separation were applied to the corner of a TLC plate, and the plate was developed in one dimension for 15 cm with Solvent 1. The plate then was dried at room temperature and developed in a second dimension with Solvent 2.

The desired spots were scraped from the plates and immediately extracted with a mixture of equal volumes of triethylamine and 2-propanol (8 ml). The extracts were evaporated to dryness in a nitrogen stream at 40°, and the residues were stored at 4° until analysis by one-dimensional TLC and high-pressure liquid chromatography (HPLC). Immediately before analysis, the residues were dissolved in chloroform, and aliquots were spotted on a TLC plate or injected in the HPLC system. The TLC plates were developed in Solvents 1 and 2 in one dimension.

HPLC[6]—The separation of the dansyl derivatives was achieved, using a modification of a published method (22), on a silica[7] column (120 cm × 2.2 mm i.d.) with chloroform (containing 0.75% ethanol)–triethylamine (100:3).

Preparative TLC—Selected fractions after column separation were concentrated to dryness, and the residue was dissolved in an aliquot of chloroform. The solution was streaked on silica gel GF plates[5] (250 μm, 20 × 20 cm), and the plates were developed in either Solvent 1 or 2. The fluorescent bands corresponding to the desired compounds were scraped off and immediately extracted with a mixture of equal volumes of triethylamine and 2-propanol. The extract was evaporated to dryness in a nitrogen stream at 40°, and the residue was stored at 4°.

Compound XV was obtained in the preparative scale from fraction 9 using Solvent 2. The residue obtained from the fluorescent band corresponding in position to XV was examined by HPLC (22) to confirm its purity and was submitted for mass spectroscopic analysis. The mass spectrum of the residue, together with that of an authentic sample of XV, is shown in Fig. 3.

Compound XIX was obtained from fraction 10 using Solvent 1, which was especially good for the separation of XV and XIV (22). The developed chromatograms contained two intense fluorescent bands. The upper band had the same R_f as XV, and the lower band had the same R_f as XIX. The extract from the lower band (crude XIX) was concentrated and streaked on TLC plates, and the plates were developed in Solvent 2. The residue obtained from the fluorescent band that corresponded in position to XIX was identified as XIX by comparison of its mass spectrum with that of an authentic sample and by its chromatographic mobility on TLC and HPLC. Since it contained some low molecular weight impurities, the residue was purified on a small silica gel column. After evaporation of the solvent, the residue was submitted for mass spectroscopic analysis. This mass spectrum, together with that of an authentic sample of XIX, is shown in Fig. 4.

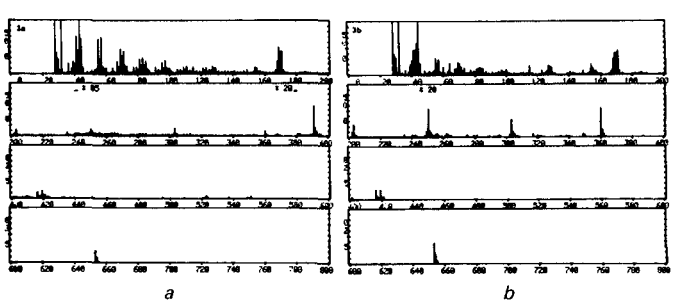


Figure 3—*Mass spectra of the dansyl derivative of VI obtained from the urine of a hepatoma patient (3a) and of the authentic sample of XV (3b).*

[4] E. Merck, Gel-60, 230–400 mesh.
[5] Analtech, Newark, Del.
[6] Model 6000 solvent delivery system, Waters Associates, Milford, Mass., and LDC model 1209 fluoromonitor, Laboratory Data Control, Riviera Beach, Fla.
[7] Corasil II, Waters Associates, Milford, Mass.

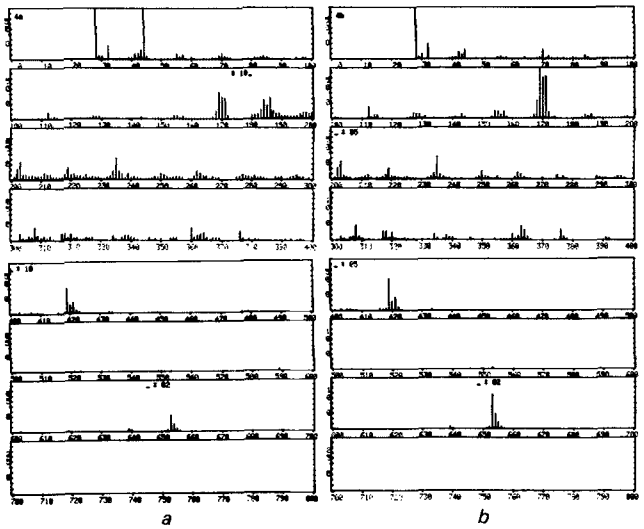


Figure 4—*Mass spectra of the dansyl derivative of VII obtained from the urine of a hepatoma patient (4a) and of the authentic sample of XIX (4b).*

Compound X was purified from fraction 11 using Solvent 1. The concentrated extract from the fluorescent band corresponding to X was rechromatographed on silica gel plates with Solvent 2. The residue obtained from the fluorescent band corresponding to X was submitted for mass spectroscopic analysis. Figure 5 represents the mass spectrum of X obtained from urine (5*a*) and that of an authentic sample of X (5*b*).

Column Chromatographic Purification of XIX—The residue obtained after preparative TLC was dissolved in chloroform, applied to a column (12 × 1.5 cm i.d.) of silica gel[4], and eluted with chloroform–2-propanol (97:3). The central fraction was collected and concentrated to dryness, and the residue was stored at 4°.

RESULTS AND DISCUSSION

All target compounds were verified by their elemental analysis (Table I) and spectral properties. IR, PMR, and electron-impact mass spectra of these compounds were in accord with the proposed structures. IR and PMR spectra of XV and XIX were identical. However, these compounds showed different chromatographic mobilities on TLC and HPLC and different electron-impact mass spectra. IR spectra of the monosubstituted amides (VIII–XI, XV, XIX, and XXI) showed the characteristic band near 1550 cm^{-1} (amide II), which was absent in the spectra of the disubstituted amides (XX and XXII).

The electron-impact mass spectra of the dansyl derivatives (Figs. 3–5) showed relatively intense molecular ions at *m/e* 653 for XV and XIX and at *m/e* 363 for X. These spectra also showed the expected fragments at *m/e* 170 and 234 due to the dimethylaminonaphthyl and dansyl ions, respectively. The fragment corresponding to $(M - C_{12}H_{12}NO_2S)^+$ appeared at *m/e* 419 for XV and XIX and at *m/e* 126 for X.

CONCLUSIONS

TLC and HPLC examination of the dansyl derivatives from the urine of a cancer patient (hepatoma) indicated the presence of VI, VII, and IV

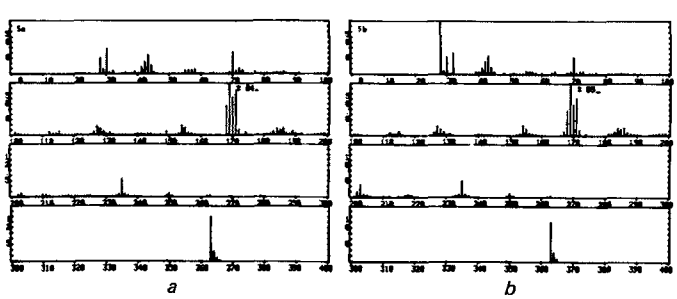


Figure 5—*Mass spectra of the dansyl derivative of IV obtained from the urine of a hepatoma patient (5a) and of the authentic sample of X (5b).*

Table I—Elemental Analyses of the Dansyl Derivatives of *N*-(Monoaminoalkyl)- and *N*-(Polyaminoalkyl)acetamides

Compound	Molecular Formula		Analysis, % Calc.	Found	Melting Point
VIII	$C_{16}H_{21}N_3O_3S$	C	57.29	57.17	102–103°
		H	6.31	6.22	
		N	12.53	12.61	
IX	$C_{17}H_{23}N_3O_3S$	C	58.43	58.60	97°
		H	6.63	6.59	
		N	12.02	11.93	
X	$C_{18}H_{25}N_3O_3S$	C	59.48	59.26	100–101°
		H	6.93	6.74	
		N	11.56	11.41	
XI	$C_{19}H_{27}N_3O_3S$	C	60.45	60.23	Oil
		H	7.21	7.48	
		N	11.13	10.89	
XV	$C_{33}H_{43}N_5O_5S_2$	C	60.62	60.46	Oil
		H	6.63	6.51	
		N	10.71	10.60	
XIX	$C_{33}H_{43}N_5O_5S_2$	C	60.62	60.44	Oil
		H	6.63	6.77	
		N	10.71	10.45	
XX	$C_{33}H_{43}N_5O_5S_2$	C	60.62	60.72	Oil
		H	6.63	6.57	
		N	10.71	10.48	
XXI	$C_{48}H_{61}N_7O_7S_3$	C	61.06	60.80	Oil
		H	6.51	6.52	
		N	10.38	10.19	
XXII	$C_{48}H_{61}N_7O_7S_3$	C	61.06	61.00	Oil
		H	6.51	6.63	
		N	10.38	10.14	

in appreciable amounts. The dansyl derivatives of VI, VII, and IV were isolated and purified using a variety of chromatographic methods. The mass spectra of these compounds were similar to those of authentic samples (Figs. 2, 4, and 5). These data confirmed the identity of these compounds and established the presence of VII as well as VI and IV in human urine.

After the completion of the work described, a report appeared describing the identification of both VI and VII in the urine of normal subjects and cancer patients (23). In that study, the authors isolated these compounds from the urine and established their identity on the basis of IR spectra, elemental analysis, and the analysis of products obtained by acid hydrolysis. The findings reported in the present paper corroborate those of Tsuji *et al.* (23) and provide an unambiguous identification of IV in human urine.

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Dissolution Kinetics of Cholesterol in Simulated Bile I: Influence of Bile Acid Type and Concentration, Bile Acid–Lecithin Ratio, and Added Electrolyte

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Abstract □ A physical model approach was utilized to investigate cholesterol monohydrate dissolution kinetics in simulated bile. The static pellet method and the Berthoud theory were employed to assess the contributions of the diffusion–convection mass transfer resistance and those of the interfacial resistance to the overall kinetics. For almost all situations studied, the interfacial resistance was the dominant rate-determining factor. The effects of four bile acids and their concentrations, the bile acid–lecithin ratio, and the added electrolytes and their concentrations on the interfacial resistance were examined. The results were correlated with those obtained with human bile samples, and the indications were that the kinetics of cholesterol dissolution in bile may be explainable on the basis of the principal bile acids, lecithin, and the electrolytes in the bile.

Keyphrases □ Cholesterol monohydrate—pellets, dissolution kinetics in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes □ Dissolution kinetics—cholesterol monohydrate pellets in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes □ Bile acids—effect of type and concentration on dissolution of cholesterol monohydrate pellets in simulated bile □ Lecithin—ratio to bile acid concentration, effect on dissolution kinetics of cholesterol monohydrate pellets in simulated bile □ Electrolytes—effect on dissolution kinetics of cholesterol monohydrate pellets in simulated bile □ Gallstones, model—cholesterol monohydrate pellets, dissolution kinetics in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes □ Steroids—cholesterol monohydrate pellets, dissolution kinetics in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes

During the past decade, major advances have been made in understanding the cholesterol gallstone problem. But, until recently, the only treatment for cholesterol gallstones was surgery. Recent studies (1–4) demonstrated that oral administration of chenodeoxycholic acid, a naturally occurring bile acid, to patients with cholesterol gallstones decreased the relative concentration of cholesterol in bile and induced dissolution of stones in 6–36 months.

While much is known (5–8) about the thermodynamic factors governing cholesterol gallstone formation and dissolution *in vivo*, there is relatively little information on the kinetics of gallstone dissolution. Such studies could be important, since a relatively slow rate of dissolution of cholesterol gallstones was observed in several clinical studies. A theoretical treatment by Higuchi *et al.* (9) led to the proposal that *in vivo* dissolution of cholesterol gallstones occurred at rates much slower than anticipated when dissolution was solubility–diffusion controlled; therefore, the anomalously slow rates for gallstone dissolution observed previously (1) indicated that interfacial factors might be important *in vivo*.

Indeed, experimental studies (10, 11) showed that *in vitro* dissolution of cholesterol gallstones in simulated bile was dominated by an interfacial barrier at the crystal–solution interface. Subsequent dissolution rate experiments with model gallstones (compressed pellets of cholesterol monohydrate crystals) yielded comparable results and suggested that cholesterol monohydrate pellets were valid model gallstones in studies of cholesterol gallstone dissolution kinetics.

Analyses of biliary lipids in patients showing gallstone dissolution during chenodeoxycholic acid treatment (3) confirmed that desaturation of bile occurs in most instances. Preliminary experiments on the *in vitro* dissolution of cholesterol gallstones, as well as cholesterol monohydrate pellets in micellar bile acid solutions, showed (10, 11) that added lecithin significantly decreased the dissolution rate, even though its addition enhanced equilibrium cholesterol solubility. A review of the chemical composition of human gallbladder bile (12) along with these observations suggested that the major determinants of the dissolution rate process would be the bile acid type and its concentration, the bile acid–lecithin molar ratio, and the electrolytes and their concentrations. This paper reports a systematic study of the effects of these factors on