Chromatographic Separation and Identification of Nylon-9 Intermediates and Coproducts Derived from Soybean Oil[1]

W.L. KOHLHASE, W.E. NEFF, R.A. AWL, E.H. PRYDE, and J.C. COWAN,
Northern Regional Research Laboratory[2], Peoria, Illinois 61604

ABSTRACT

9-Aminononanoic acid, 9-aminononanamide, and related compounds derived from soybean oil by reactions, including reductive ozonolysis and reductive amination, were separated on an analytical scale either by gas liquid chromatography of trifluoroacetylated or trimethylsilylated derivatives or by thin layer chromatography and on a preparative scale by ion-exchange chromatography. Comparative analyses also were carried out with certain homologous ω-amino acids and amines.

INTRODUCTION

In previous work, soybean oil was reported to be a good source from which to prepare the polyamide nylon-9; 9-aminononanoic acid (ANAC) (1) was preferred to 9-aminononanamide (ANAM) as the monomer (2). Various routes for monomer synthesis from soybean oil were explored; all involved reductive ozonolysis, reductive alkylation of ammonia with the product aldehydes (3) (also called reductive amination), and hydrolysis or ammonolysis (4), but in different sequences. A number of by-products were formed that required isolation and identification. Gas liquid chromatography (GLC) and thin layer chromatography (TLC) procedures were developed for analytical purposes, and ion-exchange procedures for preparative and purification purposes. This article describes all three.

EXPERIMENTAL PROCEDURES

Instrumentation

GLC analyses were performed either with an F&M model 500 chromatograph having a thermal conductivity detector operated at 350 C or with an F&M model 810 chromatograph having a flame ionization detector with a helium flow rate of 50-60 ml/min at 40-50 psi. The model 810 chromatograph had a glass-lined injection port at 290 C and detector inlet at 300 C. The detector was operated with 600 cc/min air, 100 cc/min hydrogen, sensitivity range switch at 10^3, and attenuation 1. Peak areas and absolute retention times were measured with a Hewlett-Packard model 3370A electronic integrator. The integrator settings were: recorder 1 or 2 mv full scale, 0.1 mv/min front and rear slope sensitivity, 0.4 min base line reset and all other settings at the off position. Internal standards generally were used for calculating composition (5). Routine quantitative analyses require that the runs be made in triplicate and that the response factor be measured daily.

Differential thermal analysis (DTA) was performed on a Du Pont model 900 differential thermal analyzer, using microsamples, a heating rate of 5 C/min, a Y-axis sensitivity of 0.2 C/in. and an X-axis resolution of 20 C/in. The reported DTA melting points (mp) are endotherm peak temperatures.

GLC

Trifluoroacetylation: From 30-50 mg dry solid was placed in, or a similar amount of liquid sample was injected into, a dry 12 x 35-mm vial having a silicone rubber septum. If desired, a weighed amount (30-50 mg) of an internal standard (dimethyl azelate or phthalate) also was added. Trifluoroacetic (TFA) anhydride (0.2 ml) then was injected; and, after the sample was dissolved completely, the solution was mixed, and an excess of 1-butanol was added immediately before GLC analysis (6). The 1-butanol converted ompounds, with free carboxyl groups to their more volatile butyl ester derivatives, quenched excess TFA anhydride, and esterified TFA acid. Excess TFA anhydride was not removed by volatilization to avoid loss of low boiling components in the samples. Free TFA acid usually produced a large, very broad peak in the zone of major interest. Immediate injection after addition of 1-butanol was necessary to avoid alcoholysis of the TFA esters of other hydroxy compounds. Excess 1-butanol also resulted in alcoholysis of amide N-TFA derivatives in the injection port. To measure amide content accurately, chromatograms had to be made before, as well as immediately after, addition of the 1-butanol. The column used was 3/16 in. x 4 ft aluminum packed with 20% Versamid 900 polyamide resin on 80-100 mesh Gas Chrom P, a neutral washed calcined diatomaceous earth.

Trimethylsilylation: Weighed amounts (10-20 mg) of sample and methyl stearate internal standard were placed in a clean pressure-reaction vial with a Teflon-lined screw cap (Regis no. 975401), and ca. 0.5 ml N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, Regisil) was added (7,8). The sample mixture was refluxed for 15 min by heating the lower part of the tightly capped vial in an oil bath at 155 C. The mixture was cooled at 22-25 C for 5 min before injection into the glass-lined injection port at 290 C. The 2 mm inside diameter x 4 ft stainless steel column was packed with 2.8 g 80-100 mesh high-performance Chromosorb G (acid-washed, treated with dimethylchlorosilane) coated with 4% SE-30 methyl silicone fluid.

TLC

Samples (usually 5-15 μl of alcohol/water solution containing 0.02-0.05 mg of sample) were applied to commercial precoated plates in 5-μl increments from a 0.3-mm bore capillary. For some analyses of basic compounds of low solubility, the solution was made 0.1 N in hydrochloric acid to permit higher concentrations. Although 10 different developing solvents were tested, most work was done with 1-butanol/water mixtures containing a minor amount of acetic acid. Vertical development, generally for 16 cm, was carried out at 22-24 C in both lined and unlined covered tanks.

Spots were visualized temporarily under UV light or by exposure to iodine vapor at 22-24 C and permanently with ninhydrin (9) (0.2% in 95% ethanol) plus heating at 125 C for 5-10 min, bromcresol green (0.1% aqueous solution of the sodium salt), by $(NH_4)_2SO_4$ charring at 200 C, or by lightly spraying with 40% $(NH_4)_2SO_4$ followed by a 1% solution of 2,3-dichloro-1,4-naphthoquinone (10) and baking at 200 C for 15-25 min.

Ion-Exchange Procedures

Resins included the weakly acidic polymethacrylic CG-50, the strongly basic modified styrene-divinylbenzene CG-400 with quaternary nitrogen functional groups, the weakly basic phenol-formaldehyde polyamine CG-4B, and

[1]Presented at the AOCS Meeting, Chicago, September 1973.
[2]ARS, USDA.

the strongly acidic styrene-divinylbenzene IR-120 with sulfonic acid groups—all from Rohm and Haas, Philadelphia, Pa., (Amberlite analytical reagent grade). Ion-exchange chromatography was carried out in all glass equipment with columns having inner diameters of 1.2-2.5 cm. A plug of 1.8 denier polypropylene wool supported the resin bed, because silica tended to leach out from glass wool.

Application of ion-exchange chromatography to the complex reaction products obtained from different starting materials is illustrated in the following examples.

ANAC-ANAM separation: The waxy solid (6.86 g) from simultaneous reductive amination and ammonolysis of methyl azelaaldehydate (MAZ, 5.00 g), in the presence of ANAC as catalyst (1.16 g) (2), was hydrolyzed on a steam bath for 6 hr to convert the intermediate unstable methyl 9-aminononanoate to ANAC; under these conditions, hydrolysis of ANAM is negligible. The mixture was cooled to 0 C and filtered to remove 0.48 g insolubles. Amino compounds were separated from nonbasic impurities in the colorless filtrate by passing it through a 2.5 x 14.2 cm bed of CG-50(H^+). Elution with H_2O and evaporation of the 640 ml eluate produced a white solid (mp 85-107 C, 0.16 g) believed to be impure 9-hydroxynonanamide (estimated 1-2% yield, based upon MAZ). Elution with 5% NH_3/H_2O (560 ml) and evaporation produced a 5.38 g mixture of ANAC and ANAM. The solids were redissolved in H_2O, and the filtered solution (0.14 g insolubles) was passed through a 2.5 x 16 cm bed of CG-400(OH^-) resin. Elution with H_2O (260 ml) and evaporation of the solution produced 2.52 g ANAM (56% yield based upon MAZ) as a white microcrystalline powder having the correct elemental and molecular wt analyses. A further H_2O wash only isolated a minor amount of ANAM; a 10% NH_3/H_2O wash did not elute ANAC, but one of 5% HCOOH/H_2O (420 ml) did. On the basis of nitrogen analyses, the total ANAC recovered was 1.92 g (17% yield based upon MAZ, after correcting for the amount added as catalyst for the ammonolysis reaction).

ANAM-NH_4OAc separation: The crude product from the reductive amination-ammonolysis of MAZ (5.00 g) with ammonium acetate (2.5 g) as the ammonolysis catalyst (4) was extracted with H_2O leaving 0.22 g insolubles. The aqueous extract was evaporated, and the residual wax (6.11 g) was extracted with benzene to remove intermediate amino ester. The benzene-insoluble solid (3.89 g) was redissolved in H_2O; the solution was filtered and passed through a 2.5 x 15 cm bed of CG-50(H^+). The bed was washed free of acetic acid with H_2O, and the ANAM was eluted with 5% NH_3/H_2O (280 ml) to give 2.00 g product (87% purity, 59% yield based upon MAZ). The crude ANAM was purified by preparing the highly insoluble crystalline monooxalate. After recrystallization from aqueous acetone, the oxalate (mp 117-125 C, decomposition) gave the correct elemental analysis.

Analysis: Calculated for $C_{11}H_{22}N_2O_5$: C, 50.37; H, 8.45; N, 10.68. Found: C, 50.35, H, 8.72; N, 10.58.

Glycerol-ANAM separation: Aldehyde oil (17.5 g, 2.56 meq carbonyl/g) from partial ozonolysis of soybean oil (11) was subjected to simultaneous reductive amination-ammonolysis in 1-propanol containing ammonium acetate as the ammonolysis catalyst (4.4 g) and Raney Ni (1.0 g). A 17.3% portion of the paste was extracted with a total of 100 ml H_2O, the solution filtered through diatomaceous earth, and the filtrate evaporated to leave 2.18 g oil. The oil was redissolved in 10 ml H_2O and fractionated in a CG-50(H^+) column. Evaporation of the first 100 ml H_2O eluate yielded 0.84 g glycerine containing a small amount of ammonium acetate, n_D^{30} literature: 1.4679; found, 1.4714; N = 1.6%. After the resin had been washed with 135 ml 10% H_2O in methanol, it was eluted further with 250 ml 1% NH_3/H_2O to give 0.15 g resinous solid. Elution with 250 ml 5% NH_3/H_2O gave crude ANAM (0.75 g). Extraction of ANAM into 60 ml acetone and addition of a solution of 0.60 g oxalic acid in 10 ml acetone produced a precipitate (0.90 g) of ANAM monooxalate (78% yield, based upon aldehydic content of the aldehyde oil). The oxalate was recrystallized twice from CH_3OH/H_2O to give 0.44 g pure salt having the correct elemental analysis and melting at 105-150 C, decomposition (2).

Glycerol-ANAC-ANAM separation: The H_2O-soluble products (8.4 g in a total of 26.8 g product mixture) from the reaction of 20 g soybean aldehyde oil (2.51 meq-CHO/g, 1.12 meq-COOH/g) (11) with H_2 and NH_3 at 60 C in CH_3OH containing Raney Ni were predominantly glycerol, ANAM, ANAC, and NH_4^+ carboxylates. An 8.00 g portion of this soluble product in 110 ml H_2O was passed through a 2.5 x 26 cm bed of CG-40 resin (OH^--form), followed by H_2O washes. Evaporation of the first 460 ml eluate left a 4.15-g mixture of glycerol and ANAM. These were separated by passing 4.03 g mixture in 70 ml H_2O through a 2.5 x 19 cm bed of weak acid resin (H^+ form), followed by H_2O washes. Evaporation of the first 140 ml eluate left 1.53 g colorless 98% glycerine (n_D^{27} = 1.4665, N-free). The ANAM then was removed from the resin by washing with 5% NH_3/H_2O. The first 140 ml contained 0.57 g 84% pure ANAM carbamate salt, and evaporation of the next 140 ml gave 1.86 g 97% pure ANAM (DTA 74 C).

Analysis: Calculated for $C_9H_{20}N_2O$: C, 62.75; H, 11.70; N, 16.26. Found: C, 62.47; H, 11.41; N, 15.83.

ANAC and other acids still remaining on the strong base resin then were eluted with 10% HCOOH/H_2O. Evaporation of the 440 ml eluate left 4.12 g solid acids, including ANAC, azelamic acid, the mono-NH_4^+ salt of azelaic acid, and other components. These were partially separable with CG-4B resin and H_2O or 5% NH_3/H_2O elution.

ANAC-azelaic acid separation: A synthetic mixture of 3.52 g crude ANAC (1) (mp 165-173 C) and 2.12 g commercial azelaic acid (Eastman no. 1421) in 900 ml H_2O was passed through a 2.0 x 12.5 cm bed of CG-4B resin followed by H_2O washes. Evaporation of the first 1200 ml eluate left 3.26 g recovered ANAC (mp 175-179 C).

Analysis: Calculated for $C_9H_{19}NO_2$: C, 62.39; H, 11.05; N, 8.08. Found: C, 61.98; H, 10.98; N, 8.34.

After an additional 1060 ml H_2O wash, the resin was washed with 3 times the theoretical amount of NH_3/H_2O (1150 ml, 0.6 N). Evaporation of the eluate at 40 C/0.1 mm Hg left 2.51 g crude mono-NH_4^+ salt of azelaic acid (mp 160-166 C) containing a minor amount of di-salt.

Analysis: Calculated for $C_9H_{19}NO_4$: C, 52.67; H, 9.33; N, 6.82. Found: C, 52.89; H, 9.66; N, 7.58.

Interconversion of ANAM oxalate salts: A solution of 0.46 g ANAM monooxalate in 80 ml H_2O was passed through a 2.5 x 13.4 cm bed of CG-4B resin followed by a 220 ml H_2O rinse at 6 ml/min. Evaporation of the eluate at 25 C/0.01 mm Hg left 0.33 g (87% recovery) ANAM having the correct elemental analysis for the hemioxalate (2). The hemioxalate was converted readily back to the monooxalate (mp 105-120 C) by mixing with an equal wt of $H_2C_2O_4 \cdot 2H_2O$ in 2:3 methanol:acetone, evaporating and extracting excess $H_2C_2O_4$ from the residual solids with acetone.

RESULTS AND DISCUSSION

GLC

The products in our reaction mixtures had to be converted to more volatile derivatives for GLC analysis; trifluoroacetylation and silylation were tested. Relative retention times are shown in Table I for both derivatives of the various intermediates, monomers, and by-products.

Although trifluoroacetylation of labile hydrogen (12) gave suitably volatile derivatives and has been used successfully with 12-aminostearic acid (9), problems in reproducibility were encountered. Darbre and Blau (13) noted that

TABLE I

Relative Retention Times for Gas Liquid Chromatographic Derivatives[a]

Compound	Formula	TFA derivative	Silyl derivative
1-Butanol	$CH_3(CH_2)_3OH$	0.32	0.02
Methyl pelargonate	$CH_3(CH_2)_7COOCH_3$	0.66	0.15[b]
1-Nonanol	$CH_3(CH_2)_7CH_2OH$	0.74	0.21
Methyl 9-hydroxynonanoate	$HO(CH_2)_8COOCH_3$	0.83	0.49
Butyl pelargonate	$CH_3(CH_2)_7COOC_4H_9$	0.91	0.38[b]
9-Hydroxynonanamide	$HO(CH_2)_8CONH_2$	0.91	0.78, 0.81[c]
Nonylamine	$CH_3(CH_2)_7CH_2NH_2$	0.95	0.25
Dimethyl azelate	$CH_3OOC(CH_2)_7COOCH_3$	1.00	0.44[b]
Butyl 9-hydroxynonanoate	$HO(CH_2)_8COOC_4H_9$	1.07	—
9-Hydroxynonanoic acid	$HO(CH_2)_8COOH$	—	0.63
Nonanamide	$CH_3(CH_2)_7CONH_2$	1.17	0.43, 0.45[c]
Methyl 9-aminononanoate	$H_2N(CH_2)_8COOCH_3$	1.21	0.76
9-Aminononononanoic acid	$H_2N(CH_2)_8COOH$	—	0.70
9-Aminononononanamide	$H_2N(CH_2)_8CONH_2$	1.24	0.85
Palmitamide	$CH_3(CH_2)_{14}CONH_2$	1.26	0.78, 0.83[c]
Butyl 9-aminononanoate	$H_2N(CH_2)_8COOC_4H_9$	1.30	—
Stearamide	$CH_3(CH_2)_{16}CONH_2$	1.35	0.96, 1.00[c]
Dibutyl azelate	$C_4H_9OOC(CH_2)_7COOC_4H_9$	1.35	—
Methyl stearate	$CH_3(CH_2)_{16}COOCH_3$	—	1.00

[a]Gas liquid chromatograms run on F&M model 810 chromatograph, programed for the 1-butanol quenched trifluoroacetyl (TFA) derivatives at 70-290 C at 10 C/min and for the silyl derivatives (relative retention time standard deviation ±0.01) at 100-300 C at 8 C/min.

[b]Underivatized compound.

[c]Lower value for N-monosilyl higher for N,N-disilyl derivatives.

TABLE II

Accuracy of Gas Liquid Chromatographic Assay of Trimethylsilylated Synthetic Mixtures

Compound[a]	Composition of synthetic mixture, wt %					Absolute retention time, min
ANAC	100.0	72.6	45.2	30.3	25.1	9.15
ANAM	0.0	3.0	16.3	2.2	57.8	11.18
9-Hydroxynonanoic acid	0.0	7.4	10.7	5.6	12.1	8.15
Methyl 9-hydroxynonanoate	0.0	13.3	10.5	33.4	3.2	6.34
Nonylamine	0.0	3.7	17.3	28.5	1.8	3.28
	Relative error of gas liquid chromatographic assay					
ANAC[a]						
Internal standard method (5)	-1.9	-0.6	-0.9	+2.3	-3.2	
Area normalization method	0.0	-0.8	-0.4	-1	-0.4	

[a]ANAC = 9-aminononanoic acid and ANAM = 9-aminononamide.

[b]Response factor = 1.02 (σ = ±0.03)

TABLE III

Thin Layer Chromatography of 9-Aminononanoic Acid and Related Compounds

Compound	R_f values[a]				
	Cellulose-coated		Silica-coated[b]		
	A[c]	B	A	C	D
$H_2N(CH_2)_8COOH$	0.51	0.21	0.31, —; 0.46	0.68, 0.51; 0.60[d]	—, 0.60; —
$H_2N(CH_2)_8COOCH_3$	—	—	—	—, 0.74; —	—, 0.79; —
$H_2N(CH_2)_8CONH_2$	0.34	0.41	0.16, —; 0.27	0.60, —; 0.43	—, 0.54; 0.65
$H_2N(CH_2)_8CONH_2 \cdot HCl$	—	—	—	0.55, 0.33; —	—
$HN[-(CH_2)_8COOH]_2$	0.33	0.25	—	0.93, —; —	—
$HN[-(CH_2)_8COOCH_3]_2$	0.90	0.90	0.43, —; —	—;	—
$HN[-(CH_2)_8CONH_2]_2$	0.57	0.53	—	—	—
$HO(CH_2)_8COOH$	—	—	—; —; 0.77	—, 0.82; 0.76	—, 0.85; 0.91
$HO(CH_2)_8CONH_2$	—	—	—; —; 0.68	—, 0.71; 0.69	—, —; 0.88

[a]Developing solutions: A = 1-C_4H_9OH:H_2O:CH_3COOH (50:10:1), B = 1-C_4H_9OH:H_2O (5:1), C = 1-C_4H_9OH:H_2O:CH_3COOH (8:2:2) and D = O(-CH_2CH_2-)$_2$O:H_2O:CH_3COOH (8:2:2).

[b]Where three R_f values are listed, the first is for data from 1 month old E. Merck Brinkmann plates in unlined tanks; and the second is for the same lot of plates 46 months later in lined tanks. The third is for Analtech Uniplates in lined tanks.

[c]Under these conditions the mono-, di-, and trioctylamines had R_f values of 0.72, 0.92, and 0.95, respectively.

[d]In an unlined tank the R_f was 0.70.

TABLE IV

Thin Layer Chromatography of Homologous ω-Amino Acids

Compound	Thin layer chromatography results[a]						
	R_f[b]		Shape[b,c]		Response[b,c]		Resolution[e]
H_2NCH_2COOH	0.53[f],	0.47	—,	1.1	—,	15	(0.5)
$H_2N(CH_2)COOH$	0.40[f],	0.35	—,	1.1	—,	23	(-0.4)
$H_2N(CH_2)_3COOH$	0.36[g],	—	1.3,	—	61,	—	0.2
$H_2N(CH_2)_4COOH$	0.45[h],	—	1.1,	—	62,	—	0.8
$H_2N(CH_2)_5COOH$	0.53[i],	0.56	0.9,	1.1	53,	12	0.6
$H_2N(CH_2)_6COOH$	0.60[j],	—	0.8,	—	46,	—	1.5
$H_2N(CH_2)_8COOH$	0.68[k],	0.60	1.0,	1.2	41,	17	(0.1)
$H_2N(CH_2)_{10}COOH$	0.75[f],	0.66	—,	0.9	—,	11	

[a]On 20 x 20 cm glass plates with 0.25 mm silica coating developed with 1-C_4H_9OH:H_2O:CH_3COOH (8:2:2). Values are averages from two to eight runs.

[b]First value with precoated TLC plates from E. Merck/Brinkmann in lined tanks; the second, on "Uniplates" from Analtech.

[c]Ratio of spot height to width, a circle being 1.0.

[d]Ratio of spot area to sample wt in cm^2/mg for ninhydrin visualization of 6-30 μg samples. Response decreased for larger samples and increased for other visualizers in the order: $(NH_4)_2SO_4$ charring < bromcresol green < $(NH_4)_2SO_4$ + 2,3-dichloronaphthoquinone.

[e]Ratio of distance between the nearest edges of adjacent spots to their average height, for 6 μg each component. A negative value indicates spot overlap. Data from Merck/Brinkmann plates. Values in parentheses estimated from single component thin layer chromatographic data on Uniplates.

[f]From data on Uniplates, normalized with the other amino acids.

[g]A second spot usually appeared R_f 0.23 higher than the amino acid in samples applied to 1 month old plates from 0.1 N HCl solutions in 80% ethanol which were at least 1 day old—presumably due to partial formation of the ethyl ester.

[h]Per footnote g, but second spot R_f 0.14 higher than the amino acid.

[i]Per footnote g, but second 0.09 higher.

[j]Per footnote g, but second 0.07 higher.

[k]Per footnote g, but second 0.05 higher; however, plates from the same lot did not give a second spot 46 months later, and the 9-aminononanoic acid R_f was only 0.51.

similar trifluoroacetylated derivatives decomposed over certain polar stationary phases. Since free carboxyl groups in the C_9 compounds were in equilibrium as the anhydride derivative with TFA anhydride (14), the C_9 anhydrides reverted to free carboxyl compounds and volatile TFA anhydride in the hot injection port. To avoid this problem, 1-butanol was added to convert the carboxyl to an ester, the esterification being catalyzed by TFA acid (6). An excess of butanol destroyed excess TFA anhydride, esterified TFA acid, and avoided transesterification and degradation of the polyamide stationary phase by the strongly acidic TFA acid. The degradation was evidenced by a large baseline hump under the main peaks. Although a large excess of butanol generally gave satisfactory chromatograms, variable results with amides indicated alcoholysis of the N-TFA groups. This problem was circumvented reasonably well by chromatographing two samples, one without butanol and the other with an excess.

Trimethylsilylation of ANAC and allied materials with BSTFA (7,8) gave more satisfactory GLC derivatives, provided care was taken to ensure complete reaction as reported by others (15). Accuracy of the BSTFA method for ANAC mixtures (summarized in Table II) was superior to that of Mori, et al., (16) who reported an average relative error of ±4.4% for the silyl derivatives of certain ω-amino acids.

As noted elsewhere (1), some reaction products produced anomalous results, the ANAC content being lower than expected from mp, TLC, and elemental analyses. The discrepancy was ascribed to nickel from the reduction catalyst, which may have catalyzed oligomerization of the bifunctional C_9 compound, caused decomposition of the BSTFA derivative, or simply formed a nonvolatile complex (17).

TLC

This technique was particularly useful for samples of low volatility, e.g. oligomers whose mol wt was too high for GLC. For example, TLC analysis of butyl 9-aminononanoate, which had solidified while stored for 3 years at 3 C, showed two main basic components believed due to a dimer and a trimer.

TLC spots were visualized with several reagents. For carboxylic acids and primary, secondary, or tertiary amines, visualization was instant with bromcresol green spray (18,19). However, the amount of spray had to be controlled carefully for good spot definition, particularly with silica coatings. Primary and secondary amines were revealed with ninhydrin and heating at 125 C, but not tertiary amines. Further heating of ninhydrin plates to 500 C for 5 min often brought out additional spots of higher R_f, presumably due to charring of nonbasic components or tertiary amines, or both. Charrring with $(NH_4)_2SO_4$ alone at 200 C gave light brown spots with all compounds having 9 or more C atoms; although amino acids with 1-6 C atoms did not give spots visible in ordinary light, they were visible under UV at 366 mμ. The $(NH_4)_2SO_4$/2,3-dichloro-1,4-naphthoquinone (10) combination provided the greatest number of spot colors and revealed the greatest variety of compounds.

Microcrystalline cellulose coatings separated ANAM and ANAC better than did commercial silica gel plates (Table III), but the latter gave slightly better spot roundness and faster developing. A small amount of acetic acid was needed in the butanol-water mixtures to prevent tailing of the basic C_9 compounds.

All components of a synthetic mixture of homologous ω-amino acids separated well on silica gel plates; their R_f values increased linearly with increasing chain length in the range of C_4-C_{11} (Table IV), but the C_2 and C_3 compounds gave anomalous results. In place of the conventional manner of illustrating TLC spot shape, response, and resolution (for mixtures) by use of photographs or line

TABLE V

Influence of Sample Solvent pH on 9-Aminononanoic Acid R_f Values

Sample solvent	R_f values[a] A	C	E	Probable 9-Aminononanoic acid form
CH_3OH (neutral)	0.49	0.64	0.15	$H_3\overset{\oplus}{N}(CH_2)_8COO^{\ominus}$ (zwitterion)
	0.44	—	0.04	$H_2N(CH_2)_8COOH$
CH_3OH (0.5 M in NH_3)	0.48	0.65	—	$H_2N(CH_2)_8COO^{\ominus}$ (anion)
			0.14	$H_3\overset{\oplus}{N}(CH_2)_8COO^{\ominus}$
	0.42	0.42	0.04	$H_2N(CH_2)_8COOH$
CH_3OH (0.1 M in HCl)	0.60		0.41	$H_3\overset{\oplus}{N}(CH_2)_8COOCH_3$ (protonated ester)
		0.66		
	0.51		0.28	$H_3\overset{\oplus}{N}(CH_2)_8COOH$ (protonated acid)
CH_3OH (neutral, diazomethane)	0.92	—	0.93	Trimer
		—	0.80	Dimer
	0.50	0.89	0.27	$H_2N(CH_2)_8COOCH_3$
	0.46	0.64	—	$H_3\overset{\oplus}{N}(CH_2)_8COO^{\ominus}$
	0.23	—	0.66	$H_2N(CH_2)_8COO^{\ominus}$
CH_3OH (saturated with HCl, neutralized after dissolution of sample)	0.87[b]	—	—	Dimer
		0.66	0.30	$H_2N(CH_2)_8COOCH_3$
	0.48		0.12	$H_3\overset{\oplus}{N}(CH_2)_8COO^{\ominus}$
	0.42	—	0.07	$H_2N(CH_2)_8COOH$

[a]Unlined tanks. Developing solutions: A = 1-C_4H_9OH:H_2O:CH_3COOH (50:10:1), C = 1-C_4H_9OH:H_2O:CH_3COOH (8:2:2), and E = CH_2Cl_2:CH_3OH:H_2O (80:20:3).

[b]Tank lined with solvent-wetted paper.

drawings of actual plates, the Table IV space-cost-saving numerical methods of presenting such information were devised. Comparative data on several of these homologues have been reported previously under other TLC conditions (19-21).

To obtain the desired 1% solutions of higher ω-amino acids, the alcoholic spotting solvent was made 0.1 N in HCl. Unless these acidified solutions were analyzed promptly, each component presented a second spot of higher R_f under certain conditions—presumably due to formation of ethyl esters or an alternate ionic form of the amino acid (9). Using freshly prepared spotting solutions and relatively new (1 month old) precoated plates in an unlined tank, only 1 spot was obtained for each amino acid deposited from solution in neutral or acidic ethanol. However, acidic spotting solutions that stood at room temperature for one

TABLE VI

Properties of 9-Aminononamide and Derivatives

Formula	Description	Elemental analysis, % Theory C	H	N	Found C	H	N
$O{=}CH(CH_2)_7CONH_2$	Unstable paste	63.13	10.01	8.18	63.73	10.24	7.18
$HON{=}CH(CH_2)_7CONH_2$	Microcrystalline	58.04	9.74	15.04	58.33	9.76	14.88
$H_2NCONH\text{-}N{=}CH(CH_2)_7CONH_2$	Short needles	52.61	8.83	24.54	53.17	8.83	24.17
$H_2N(CH_2)_8CONH_2$	Waxy, soluble polar solvents	62.75	11.70	16.26	62.37	11.44	16.14
$0.5\ CO_2 \cdot H_2N(CH_2)_8CONH_2$	Powder	58.73	10.38	14.42	59.44	10.14	14.74
$HCl \cdot H_2N(CH_2)_8CONH_2 \cdot 0.5\ H_2O$	Microcrystalline	49.65	10.18	12.86	50.12	10.62	12.24
$0.5\ (\text{-}COOH)_2 \cdot H_2N(CH_2)_8CONH_2$	Flakes	55.28	9.74	12.89	55.28	9.52	12.75
$(\text{-}COOH)_2 \cdot H_2N(CH_2)_8CONH_2$	Microcrystalline	50.37	8.45	10.68	50.29	8.57	10.81
$CH_3COOH \cdot H_2N(CH_2)_8CONH_2$	Needles	56.87	10.41	12.06	57.07	10.39	12.15
$CH_3CONH(CH_2)_8CONH_2$	Granules	61.65	10.35	13.07	61.60	10.45	13.20
$HO(CH_2)_8CONH_2$	Microflakes	62.39	11.05	8.08	62.59	11.03	8.17
$[H_2NCO(CH_2)_8]_2NH$	Powder	66.01	11.39	12.83	65.82	11.28	12.45
$CH_3OOC(CH_2)_8NH(CH_2)_8CONH_2$	Microflakes	66.63	11.18	8.18	67.26	11.07	8.06
$0.5\ CO_2 \cdot H_2N(CH_2)_8CONH(CH_2)_8COOCH_3$	Microflakes	64.25	10.51	7.68	62.57	10.56	7.97
$0.5\ CO_2 \cdot H[\text{-}NH(CH_2)_8CO\text{-}]_2NH_2$	Powder	63.57	10.67	12.02	63.14	11.17	12.01
$(CH_3COOH)_2 \cdot H[\text{-}NH(CH_2)_8CO\text{-}]_2NH_2$	Powder	59.03	10.13	9.38	58.83	10.45	10.45
$CH_3CO[\text{-}NH(CH_2)_8CO\text{-}]_2NH_2$	Granules	64.83	10.61	11.61	64.36	10.46	10.71
$(\text{-}COOH)_2 \cdot H_2N(CH_2)_8COOCH_8$	Microcrystalline	51.97	8.36	5.05	51.94	8.18	4.98

day or more gave the second spot under the same TLC conditions. This dual spot effect could not be repeated with less active plates. Presumably the second spot was due to the cationic form and to the greater resolving ability of fresh plates; ANAC methyl ester (freshly made from ANAC and CH_2N_2) had an R_f 0.23 higher than ANAC, whereas the second spot from acidic ethanol solutions was only 0.05 higher. A second spot that appeared at higher R_f in TLC of 12-aminostearic acid when acid was added to the methanol spotting solution was said to be due to the protonated (cationic) form (9); the developing solvent was $CH_2Cl_2/CH_3OH/H_2O$.

As many as four spots could be obtained from pure ANAC, depending upon the pH of the sample solvent (Table V). The extra spots can be attributed to ionic forms, ester formation, and oligomer formation. Similar behavior was noted for pure glycine and β-alanine. The ANAC esters are very reactive and readily self-condense to form various oligomers.

Ion-Exchange Separations

Ion-exchange chromatography permitted preparative scale separations of ANAM and ANAC from each other, from the coproducts formed in the reductive alkylation of ammonia with soybean derived aldehydes, and from the ammonium carboxylates used as ammonolysis catalysts. Various compounds and some of their derivatives produced in earlier research (2) are repeated in Table VI because the "Description and Elemental Analysis" in Table I of reference 2 is incorrect. Note under structure I of Figure 1 in the 1970 publication that an O atom is missing at the left side of the formula. Both ANAM and ANAC could be removed from aqueous solutions by carboxylic acid-type ion-exchange resins (H^+ form), and both could be eluted therefrom with 5% NH_3/H_2O. By this means, ANAM could be freed from its acetate or oxalate salts even when ammonium acetate was present, the liberated carboxylic acid being eluted with only a H_2O wash. A strongly acidic sulfonic acid resin was required to convert sodium salts to the free acids; regeneration of 9-hydroxynonanoic acid from its sodium salt was only half complete with a carboxylic resin.

Mixtures of ANAM and ANAC could be separated from each other by passage through a carboxylic acid resin (NH_4^+ form), which removed only the highly basic ANAM and allowed ANAC to pass through. The ANAM then could be recovered from the resin with a dilute NH_3 wash. When ANAM was mixed with ammonium carboxylates, including that of ANAC, it could be separated by passage through a strongly basic, quaternary-type resin (OH^- form), which removed ANAC and other carboxylic acids from solution and allowed ANAM to pass through. ANAC and the other acids then could be recovered with a 5% $HCOOH/H_2O$ wash. The ANAC could be separated from the other acids by passage over a weakly acid resin (H^+ form), which retained only ANAC.

Simultaneous reductive amination-ammonolysis of soybean aldehyde oils gives a complex mixture of H_2O-soluble products. The mixture includes ANAM, ANAC, glycerol, ammonium acetate, and such other ammonium salts as pelargonate, azelate, and azelamate. These products could be separated by either scheme in Figure 1. Voogt (22) described a somewhat similar system using a sulfonic acid resin to separate mono-, di-, and trilauryl amines from nonbasic components.

A weakly basic resin was unable to liberate ANAM from its acetate or oxalates, although it did convert the monooxalate to the hemioxalate. Such a resin, however, was useful for regenerating ANAC from its mineral acid salts and also for separating ANAC from water-soluble carboxylic acids, e.g. azelaic.

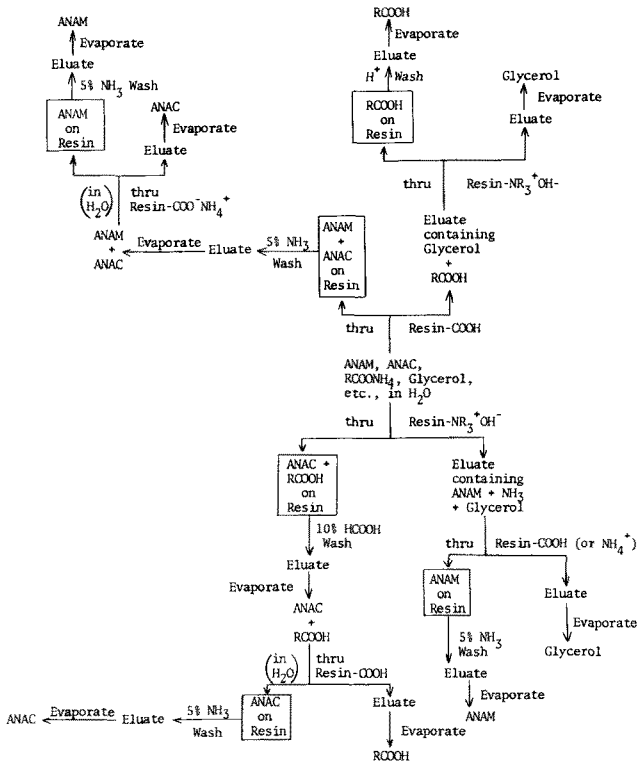

FIG. 1. Isolation of 9-aminononanamide (ANAM) and 9-aminononanoic acid (ANAC) by ion exchange.

ACKNOWLEDGMENTS

Preliminary gas liquid chromatographic studies with trifluoroacetic anhydride were conducted by D.J. Moore. Technical assistance was provided by K.J. Abbey and R.O. Adlof, and analytical work was provided by B.R. Heaton, K.A. Jones, and C.E. McGrew.

REFERENCES

1. Miller, W.R., E.H. Pryde, R.A. Awl, W.L. Kohlhase, and D.J. Moore, Ind. Eng. Chem. Prod. Res. Develop. 10:442 (1971).
2. Kohlhase, W.L., E.H. Pryde, and J.C. Cowan, JAOCS 47:183 (1970) (See "Results and Discussion" for error corrections.)
3. Anders, E.E., E.H. Pryde, and J.C. Cowan, Ibid. 42:824 (1965).
4. Kohlhase, W.L., E.H. Pryde, and J.C. Cowan, Ibid. 48:265 (1971).
5. Dal Nogare, S., and R.S. Juvet, Jr., "Gas-Liquid Chromatography," Interscience Publishers, New York, N.Y., 1965, p. 256.
6. Gehrke, C.W., and D.L. Stalling, Separ. Sci. 2:101 (1967).
7. Gehrke, C.W., H. Nakamoto, and R.W. Zumwalt, J. Chromatogr. 45:24 (1969).
8. Supelco, "Chromatography/Lipids," Supelco, 5:1 (February 1971).
9. Freedman, B., JAOCS 47:305 (1970).
10. Barney, J.E., II, S.R. Harvey, and T.S. Hermann, J. Chromatogr. 45:82 (1969).
11. Pryde, E.H., D.E. Anders, H.M. Teeter, and J.C. Cowan, JAOCS 40:497 (1963).
12. Fieser, L.F., and M. Fieser, "Reagents for Organic Synthesis," John Wiley & Sons, New York, N.Y., 1967, p. 1221.
13. Darbre, A., and K. Blau, Biochim. Biophys. Acta 126:591 (1966).
14. Bourne, E.J., M. Stacey, J.C. Tatlow, and R. Worrall, J. Chem. Soc. 2006 (1954).
15. Bergstrom, K., and J. Gürtler, Acta Chem. Scand. 25:175 (1971).
16. Mori, S., M. Furusawa, and T. Takeuchi, Anal. Chem. 42:959 (1970).
17. Shukla, P., and G. Narain, J. Prakt. Chem. 38:289 (1968).
18. Casselman, A.A., and R.A.B. Bannard, J. Chromatogr. 28:462 (1967).
19. Gütlbauer, F., Ibid. 45:104 (1969).
20. Mori, S., and T. Takeuchi, Ibid. 47:224 (1970).
21. Haworth, C., and J.G. Heathcote, Ibid. 41:380 (1969).
22. Voogt, I.P., Fette Seifen Anstrichm. 68:825 (1966).

[Received November 23, 1973]