SYNTHESIS OF CERTAIN AMINOOXY COMPOUNDS

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CHAPTER I

INTRODUCTION

The research described herein is concerned with the synthesis of certain organic compounds which have the aminooxy grouping and are related in structure to the naturally occurring amines, putrescine, spermidine and spermine. Several aminooxy compounds have been synthesized and studied biologically [e.g., 6-oxydihydrouracil (13, 18), canaline (15) and the hydroxylamino derivative of pyridoxal (27)]. The 6-oxydihydrouracil proved to be a highly effective competitive antagonist of uracil in bacteria (18). Pyridoxal kinase is responsible for the conversion of the three forms of vitamin B_6 to their corresponding phosphates. Condensation products formed from pyridoxal and hydroxylamine or O-substituted hydroxlamines are extremely potent inhibitors of the pyridoxal kinase of all organisms tested (19).

Putrescine, spermidine and spermine are aliphatic polyamines that are widely distributed in biological material (see Table I) and have been known for many years. Structurally, spermidine and spermine can be considered to be derivatives of 1,4-diaminobutane (putrescine).

$$NH_2CH_2CH_2CH_2CH_2NH_2$$

Putrescine 1,4-diaminobutane

TABLE I

THE DISTRIBUTION AND CONCENTRATION OF SPERMINE (6)

Origin	Milligrams
semen (man)	260
semen (bull)	0
pancreas (ox)	25-30
liver (ox)	16
kidney (ox)	15
ovary (cow)	14
muscle (calf)	12
muscle (ox)	2
spleen (ox)	11
lung (ox)	7
brain (ox)	7
testis (bull)	6
thymus (calf)	5
adrenal (ox)	5
thyroid (ox)	3
blood (ox) whole	0
blood defibrated	0
milk (cow)	0
egg (hen)	0
yeast (lebedeff)	10
yeast (distiller)	7
yeast (marmite)	40
yeast (bakers)	0

$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$

Spermidine [N-3-amino-propylbutane-1,4 diamine]

$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$

Spermine [N,N'-bis-(3-aminopropyl) butane-1,4-diamine]

Spermine was first observed and described by Van Leeuwenhoek in 1678 as the crystalline phosphate (16). The specific roles these amines play in living organisms have not yet been fully elucidated. The possible physiological importance of these amines is best indicated by studies on their activity as growth factors for certain micro-organisms and by recent

studies on their relationship to nucleic acids and to problems of membrane stability (10). They produce vivid mating colors in certain fish and induce chromosomal abberrations in plants (10). Potent inhibitory activity of spermine and spermidine has been observed against neoplastic cells in vitro in the presence of calf serum (1). These and earlier studies have led to the finding that the inhibitory effect of spermine in bacterial and mammalian cell culture systems is dependent upon the presence of the enzyme spermine oxidase (1, 9). It has been shown that spermine exhibits an inhibitory effect on spontaneous mouse tumors in vivo (2), and on the Yoshida sarcoma in vitro (20). Tokyoka reported that spermine was present in the serum of cancer patients (26). Kosaki reported that spermine was found to be a component of the phospholipid "malignolipin," allegedly present in human neoplasma (14). Spermine and spermidine may be of further interest as complexing agents for deoxyribonucleic acids (21, 12) and as stabilizers for ribosomes (4, 3). It has been reported that putrescine may control differential transcription of the phage genome during morphogenesis (23). Putrescine inhibits the binding of yeast tyrosyl transfer ribonucleic acid to yeast ribosomes with uridylyl-(3';5')-adenylyl-(3';5')-uridine (25) and acts as a growth-promoting substance for the saw-toothed grain beetle, Oryzaephilus surinamensis (5). The biosynthesis of

spermidine is given in Scheme 1. Data on the biosynthesis of spermine have not been presented but it is assumed that the mechanism is similar to that of spermidine (24).

Spermine and its derivatives have been synthesized by several investigators (11, 7, 22, 8). Dudley, Rosenheim and Starling synthesized spermine by the process given in Scheme 2. Israel, Samour and Modest synthesized spermine and its analogs by the process given in Scheme 3.

Due to uncertainties concerning the mechanism of action and even the biological roles of the naturally occurring polyamines, it was of interest to study synthetic compounds. The analogs of putrescine, spermidine and spermine containing the aminooxy grouping in lieu of an aminomethylene grouping had not been previously synthesized. The synthesis and biological study of such analogs might be of utility in shedding light on the unknowns concerning the biological roles of the naturally occurring polyamines.

To determine the feasibility of the organic synthesis of aminooxy analogs of putrescine, spermidine and spermine, it was decided to attempt first the synthesis of 3-aminooxypropylamine (analog of putrescine). This compound would then be employed as starting material in the synthesis of aminooxy analogs of spermidine and spermine (e.g., $H_2NO(CH_2)_3NH(CH_2)_3NH_2$ and $H_2N(CH_2)_3NHO(CH_2)_3NH_2$ respectively). The experimental methodology employed and the results obtained in this investigation are given in the following two chapters.

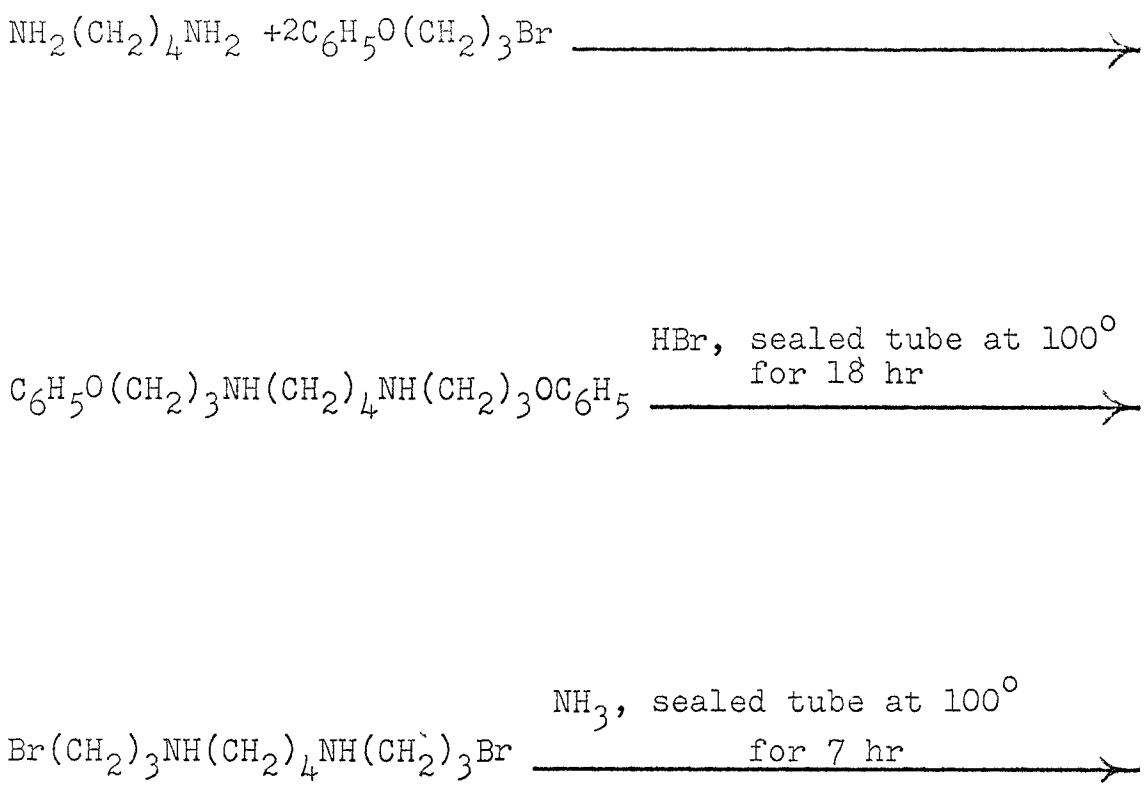
Adenosine triphosphate + Methionine $\xrightarrow{-PPi,\ -Pi}$

S-Adenosylmethionine $\xrightarrow{-CO_2}$

"Decarboxylated Adenosylmethionine" $\xrightarrow[-\ \text{Methylthioadenosine}]{+\ 1,4\text{-Diaminobutane}}$

Spermidine

Scheme 1. The biosynthesis of spermidine in *Escherichia coli* (17).



Scheme 2. Organic synthesis of spermine (7)

$$NH_2(CH_2)_XNH_2 + CH_2{=}CHCN \longrightarrow NH_2(CH_2)_XNHCH_2CH_2CN$$

$$NH_2(CH_2)_XNHCH_2CH_2CN \xrightarrow[25\ ,\ 3\text{-}4\ atm]{H_2\text{-}Ni,\ alc\ NH_3} NH_2(CH_2)_XNHCH_2CH_2CH_2NH_2$$

$$NH_2(CH_2)_4NH_2 + 2\ CH_2{=}CHCN \longrightarrow NC(CH_2)_2NH(CH_2)_4NH(CH_2)_2CN$$

$$NC(CH_2)_2NH(CH_2)_4NH)CH_2)_2CN \xrightarrow[25\ ,\ 3\text{-}4\ atm]{H_2\text{-}Ni,\ alc\ NH_3}$$

$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
(Spermine)

(X = 2,3,4,5,6,9,10,12)

Scheme 3. Organic synthesis of spermine and certain of its analogs (11)

CHAPTER II

EXPERIMENTAL

A Thomas-Hoover capillary melting point apparatus was employed for all melting point determinations, and the melting points reported are uncorrected. Infrared spectra were determined with a Perkin-Elmer 237 grating spectrophotometer. The mass spectra data were obtained with a Hitachi-Perkin-Elmer RMU-6-E mass spectrometer. The gas chromatographic data were obtained with a Loenco model 2400 series graphamatic gas chromatograph. Some of the benzohydrozamic acid and N-(3-bromopropyl)-phthalimide employed in the synthetic work were obtained from Aldrich Chemical Company, Incorporated, Milwaukee, Wisconsin.

Organic Syntheses

Attempted synthesis of 3-bromopropyl benzohydroxamate (I).--Benzohydroxamic acid (5.07 g, 0.037 mole) was added to sodium (0.86 g, 0.037 g-atom.) in 100 ml of dry ethanol. 1,3-Dibromopropane (15.0 g, 0.074 mole) was added dropwise at room temperature over a one hour period. This mixture was refluxed for twenty-four hours, concentrated to a small volume and filtered. The filtrate was concentrated to a viscous oil, then washed in 25 ml of petroleum ether to

remove the excess 1,3-dibromopropane. The oil could not be characterized by elemental, infrared or mass spectral analyses. All attempts to purify this oil were unsuccessful. The oil gave a positive test for a halogen (Beilstein test). A fraction which boiled at 115° at 3 mm was obtained upon distillation. The distillate gave a positive Baeyer's test for unsaturation but a negative Beilstein test.

Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.79; H, 6.21; N, 7.91. Found: C, 68.11; H, 6.52; N, 7.74. The mass spectral data for the distillate are given in Table II.

Allyl benzohydroxamate (II).--Benzohydroxamic acid (17.3 g, 0.126 mole) was added to 150 ml of water containing KOH (7.06 g, 0.126 mole). This reaction mixture was stirred for 0.5 hour, followed by a dropwise addition of allyl chloride (9.9 g, 0.129 mole) over a 0.5 hour period. After refluxing for twenty-five hours, the mixture was cooled and the resulting solid was removed by filtration. This solid was washed by suspending it in 100 ml of n-hexane and stirring for three hours. After filtering and drying, the product weighed 10.1 g, mp 59-62 .

Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.79; H, 6.21; N, 7.91. Found: C, 67.77; H, 6.07; N, 7.57; mass spectrum (70eV) m/c (rel intensity) 177(85), 121(15), 106(80), 105(100), 78(10), 77(100), 65(10), 51(75), 41(75), 39(30).

3-Aminooxy propane hydrobromide (III).--Allylbenzohydroxamate (3 g), was dissolved in 100 ml of hexane and a

small amount of dibenzoyl peroxide was added. Dry HBr gas was passed through this mixture with stirring for a one hour period, during which time an oily material separated from solution. The upper layer was decanted and 100 ml of absolute ethanol was added to the viscous residue. After stirring for 0.5 hour, 100 ml of anhydrous ether was added, the resulting solid was filtered and dried over phosphorous pentoxide. The solid weighed 0.91 g, mp 108-9°, and was water soluble.

Anal. Calcd for $C_{10}H_{12}NO_2Br$: C, 46.51; H, 4.65; n, 5.42. Found: C, 23.78; H, 5.25; N, 8.70. Elemental analysis indicates the predominant product to be $\bar{B}r\overset{+}{N}H_3OCH_2CH{=}CH_2$, C, 23.37; H, 5.19; N, 9.09.

3-Phthalimidopropyl benzohydroxamate(IV).--Sodium hydride (10.3 g of 55.9 per cent NaH in mineral oil, 0.24 mole) was added slowly to a stirring solution of 650 ml of dry ethanol. Benzohydroxamic acid (33.3 g, 0.24 mole) was added slowly, followed by the addition of 100 ml of dry benzene. This mixture was heated to 50° for two hours, cooled to room temperature, and N-(3-bromopropyl)-phthalimide (69.5g) was then added. The resulting mixture was then refluxed for forty-eight hours, cooled and filtered. The filtrate was concentrated in vacuo to a viscous oil. Upon the addition of ether to an alcoholic solution of this oil, a white solid was obtained, 51.2 g, mp 136°.

Anal. Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.93; N, 8.65. Found: C, 67.0; H, 4.87; N, 8.4.

3-Phthalimido-1-aminooxy propane hydrobromide(V).--A mixture of 10 g of (IV) and 100 ml of a 33 per cent HBr-HAc solution were refluxed for twelve hours, and then allowed to cool to room temperature. Solid material was removed by filtration, and the filtrate was concentrated *in vacuo* to a solid residue. Recrystallization from an ethanol-ether mixture yielded 0.9 g, mp 245°.

Anal. Calcd for $C_{11}H_{13}N_2O_3Br.H_2O$: C, 41.38; H, 4.70; N, 8.77. Found: C, 41.80; H, 4.34; N, 8.59; mass spectrum (70eV) m/c (rel intensity) 205(50), 188(100), 160(100), 132 (20), 130(40).

3-Aminooxy-1-aminopropane dihydrochloride(VI).--3-Phthalimidopropyl benzohydroxamate(IV), (18.3 g, 0.056 mole), was suspended in 500 ml of 6N HCl and refluxed for twelve hours; the benzoic and phthalic acids which formed were removed by filtration. The filtrate was concentrated *in vacuo*, leaving a solid residue. Recrystallization from an ethanol-ether mixture yielded 9.0g of product, mp 212°.

Anal. Calcd for $C_3H_{12}N_2OCl_2$: C, 22.10; H, 7.30; N, 17.2. Found: C, 22.30; H, 7.59; N, 17.38.

Attempted synthesis of [N,N-bis-(3-aminopropyl)-1-amino-3-aminooxy propane(VIII)].--

Method A: 3-aminooxy-1-aminopropane dihydrochloride (7.5 g, 0.046 mole) was added to 150 ml of dry ethanol

containing 15.7 g of barium hydroxide. The mixture was stirred for twelve hours at room temperature and then cooled in an ice bath. Acrylonitrile (4.87 g, 0.096 mole) was added dropwise over a seven minute period. The reaction mixture was then stirred for five hours at 15 , after which it was stirred overnight at room temperature. It was then heated in a boiling water bath for two hours and an insoluble material was removed by filtration. The filtrate was concentrated to a small volume. Two different components were isolated from the concentrated filtrate by injecting 50 ul portions into a gas chromatograph (flow rate 200 ml/min; column dimensions, 0.5" x 3.0'; column material, 25% SE-30 on Chromosorb-W, 60-80 mesh; linear program, 100-225) until 50 µl of both components were obtained. One of the components came off the column at 115° and the other at 210°. These fractions were designated VIII-A1 and VIII-A2 respectively. Fraction VIII-A1 gave a negative ninhydrin test but VIII-A2 gave a positive test.

Anal. The mass spectral data of fraction VIII-A1 are given in Table III; ir (neat) 1100 cm^{-1}(C-O-C), 2250 cm^{-1} (CN).

Anal. Calcd for $C_9H_{16}N_4O$ (VIII-A2): C, 55.10; H, 8.16; N, 28.57. Found: C, 58.08; H, 9.55; N, 22.41.

Method B: Method A was modified as follows: potassium hydroxide pellets (5.1 g, 0.091 mole) were suspended in 150 ml of anhydrous ether containing 7.5 g of (VI). The

mixture was stirred for six hours to insure complete neutralization. The addition of acrylonitrile and the working up of the reaction mixture were conducted as described in Method A. A fraction (10 µl) coming off the column at 190° was collected.

TABLE II

MASS SPECTRUM OF ALLYL BENZOHYDROXAMATE (II) OBTAINED FROM THE CONDENSATION OF BENZOHYDROZAMIC ACID AND 1,3-DIBROMOPROPANE (IONIZATION VOLTAGE 70 eV)

Mass/charge	Intensity relative to $C_6H_5C{=}O$	Ion
177	10	$C_6H_5CO\overset{+}{N}HOCH_2CH{=}CH_2$
106	18	$C_6H_5C\overset{+}{O}H$
105	100	$C_6H_5\overset{+}{C}O$
78	18	$C_6H_6^{+\cdot}$
77	100	$C_6H_5^+$
51	75	$H_2C{=}CHC{=}C^+$
41	20	$^+CH_2CH{=}CH_2$

TABLE III

THE MASS SPECTRUM OF VIII-A1

(IONIZATION VOLTAGE OF 70 eV)

Mass/charge	Intensity relative to $\overset{+}{C}H{-}OH$	Ion
99[a]	12	$CH_3CH_2{-}\overset{+\cdot}{O}CH_2CH_2CN$
84	15	$^+CH_2OCH_2CH_2CN$
59	80	$CH_3CH_2O\overset{+}{C}H_2$

TABLE III--Continued

THE MASS SPECTRUM OF VIII-A1

(IONIZATION VOLTAGE OF 70 eV)

Mass/charge	Intensity relative to $\overset{+}{C}H-OH$	Ion
54	80	$+CH_2CH_2CN$
45	20	$CH_2{=}\overset{+}{C}HOH$
31[b]	100	$CH_2\overset{+}{O}H$
29	55	$CH_3\overset{+}{C}H_2$

a Parent peak

b Base peak

TABLE IV

MASS SPECTRUM OF VIII-B

(IONIZATION VOLTAGE OF 70 eV)

Mass/charge	Intensity relative to $\cdot CH_2\overset{+}{N}H_2-CH_2CH_2CN$	Ion
128[a]	10	$HOCH_2CH_2CH_2\overset{\cdot+}{N}HCH_2CH_2CN$
97	40	$CH_2{=}CH_2\overset{+}{N}H_2CH_2CH_2CN$
89	60	$HOCH_2CH_2CH_2\overset{+}{N}H_2CH_2\cdot$
84[b]	100	$\cdot CH_2\overset{+}{N}H_2-CH_2CH_2CN$
57	60	$\cdot CH_2CH_2\overset{H}{N}-\overset{+}{C}H_2$
45	95	$HOCH_2\overset{+}{C}H_2$
31	95	$HO\overset{+}{C}H_2$

a Apparent Parent peak

b Base peak

Anal. Calcd for $C_9H_{16}N_4O$: C, 55.10; H, 8.16; N, 28.57. Found: C, 45.97; H, 9.74; N, 18.36; mass spectral data are given in Table IV.

CHAPTER III

RESULTS AND DISCUSSION

Several methods were investigated in an effort to synthesize the aminooxy analog of putrescine, spermidine and spermine. It was decided that the aminooxy analog of putrescine, $NH_2O(CH_2)_3NH_2$, should first be made, then used to make analogs of spermidine and spermine $NH_2O(CH_2)_3NH(CH_2)_3NH_2$ and $NH_2(CH_2)_3NHO(CH_2)_3NH(CH_2)_3NH_2$ respectively. The methods that seemed the most desirable for the synthesis of the latter two analogs were similar to those described in Schemes 2 and 3. It was later decided that the method shown in Scheme 2 was questionable because of the probability that the second reaction (using HBr, in a sealed tube at 100° for eighteen hours) would cleave the aminooxy linkage. Later it was found that the aminooxy group was relatively stable in acidic solutions. A synthetic sequence similar to the reactions of Scheme 3 was actually studied during the course of this investigation.

Two methods were studied for the synthesis of the putrescine analog 3-aminooxy-1-aminopropane dihydrochloride (VI). Scheme 4 summarizes these two methods. Compound I of Method A was not isolated; the reaction (as described in the experimental section) led to fractions that could never be purified or characterized. An attempt was then made to distill this fraction to rid it of impurities, but this led

to the formation of an olefin (i.e., $C_6H_5CONHOCH_2CH=CH_2$). An indication of this was provided by the fact that the product did not give a positive Baeyer test, but after distillation it did. Also, the mass spectral data on this fraction were found to be the same as those of allyl benzohydroxamate (II) synthesized by another procedure. Furthermore, the carbon, hydrogen and nitrogen analysis is as follows: C, 68.11; H, 6.52; N, 7.74. The calculated value for $C_6H_5CONHOCH_2CH=CH_2$ is C, 67.79; H, 6.21; N, 7.91. It was reasoned that compound I could still be synthesized if one could hydrobrominate the olefin in the presence of a peroxide. However, the data obtained indicated that in the crude allyl benzohydroxamate, the acyl oxygen bond was cleaved when dry HBr was bubbled through a hexane solution of this olefin in the presence of dibenzoyl peroxide. The carbon, hydrogen and nitrogen analysis of the isolated material was as follows: C, 23.78; H, 5.25; N, 8.70. The calculated value for $CH_2{=}CHCH_2O\overset{+}{N}H_3\overset{-}{B}r$ (III) is C, 23.37; H, 5.19; N, 9.09. This water soluble substance gave a positive test for unsaturation.

Since the synthesis of putrescine by the method shown in Method A, Scheme 4, was unsuccessful, another method was considered. In this method, Scheme 4, Method B, the synthesis of the aminooxy analog of putrescine was successful and is described in the experimental section. The evidence that the acyl oxygen bond was cleaved in the reaction of allyl benzohydrozamate with HBr in hexane was very interesting;

consequently, the idea of selective cleavage of the acyl oxygen bond came to mind. The reactions shown in Scheme 5 were to be employed to synthesize aminooxy analogs of spermidine. However, the first reaction in Method A did not lead to the desired cleavage. A selective acyl oxygen bond cleavage was tried in HBr-chloroform but the results were the same (i.e., no reaction). The possibility also existed that hydrazinolysis of compound IV would give rise to 3-aminopropylbenzohydrosamate, $C_6H_5ONHOCH_2CH_2CH_2NH_2$, which could be used to synthesize an aminooxy analog of spermidine as shown in Scheme 5, Method B. It was found, however, that the hydrazinolysis of IV led only to the formation of $\bar{C}l\overset{+}{N}H_3OCH_2CH_2CH_2\overset{+}{N}H_3\bar{C}l$.

At this time it was thought to modify the first reaction in Scheme 5, Method A, by using a mixture of HBr and acetic acid (33% HBr). A water soluble material was obtained, indicating a cleavage product was formed. The carbon, hydrogen and nitrogen analysis indicated the formation of a compound in which the benzoyl grouping was cleaved (compound V). Further proof of the structure of the isolated product was obtained by a mass spectral determination. N-(3-Bromopropyl)-phthalimide gave the following mass spectral data: (70eV) m/c (rel intensity) 270(30), 268(30), 204(30), 188(80), 160 (100), 77(33), 76(35). Benzohydroxamic acid gave the following mass spectral data: (70eV) m/c (real intensity) 137(15), 121(35), 105(100), 77(85). The mass spectral data of III are

as follows: (70eV) m/c (rel intensity) 205(50), 188(100), 160(100), 132(20), 130(40). No peak for compound V was observed at 105 and only a small one at 77 mass units; this was taken as evidence that the benzoyl grouping was absent. Large peaks at 188(100) and 160(100) indicated that the $C_6H_4C_2O_2CH_2CH_2CH_2$- grouping was present. Selective cleavage of the benzoyl grouping by the above process to make analogs of spermidine was never used because only small amounts of V could be obtained from the reaction mixture.

The next method considered in making analogs of spermidine and spermine is described in Scheme 3 except the aminooxy analog of putrescine (VI) was to be employed. As described in the experimental section (VIII-A, Method A), when acrylonitrile was added to compound VI in ethanol with barium hydroxide, only undesirable products were obtained. Fraction VIII-A1 mass spectral data gave the same base peak as diethyl ether and its fragmentation pattern was very similar. Furthermore, this fraction had the same infrared spectrum as the following compound: $CH_3CH_2OCH_2CH_2CN$. From these data it is believed that fraction VIII-A1 is the above compound. It appears obvious that such a compound resulted from a reaction between the acrylonitrile and the ethanol solvent.

Some of the possible compounds that fraction VIII-A2 could be are given in Table V. The carbon, hydrogen and nitrogen analysis of fraction VIII-A2 agrees more closely

METHOD A

$C_6H_5CONHOH + BrCH_2CH_2CH_2\text{-}Br \xrightarrow{Na,\ C_2H_5OH}$

$C_6H_5CONHOCH_2CH_2CH_2\text{-}Br \xrightarrow{C_6H_4C_2O_2NK}$

(I)

$C_6H_5CONHO(CH_2)_3NO_2C_2C_6H_4 \xrightarrow{(aq)\ HCl}$

(V)

$\bar{C}l\overset{+}{N}H_3OCH_2CH_2CH_2\overset{+}{N}H_3Cl$

(VI)

METHOD B

$C_6H_5CONHOH + C_6H_4C_2O_2NCH_2CH_2CH_2Br \xrightarrow{NaH,\ C_2H_5OH\text{-}C_6H_6}$

$C_6H_5CONHO(CH_2)_3NO_2C_2C_6H_4 \xrightarrow{(aq)\ HCl}$

(V)

$\bar{C}l\overset{+}{N}H_3OCH_2CH_2CH_2\overset{+}{N}H_3\bar{C}l$

(VI)

Scheme 4. Proposed methods for the organic synthesis of an aminooxy analog of putrescine

METHOD A

$C_6H_4C_2O_2NCH_2CH_2CH_2ONHCOC_6H_5 \xrightarrow{\text{HBr in hexane}}$

$C_6H_4C_2O_2NCH_2CH_2CH_2ONH_3HBr \xrightarrow{KOH,\ C_6H_4C_2O_2CH_2CH_2CH_2Br,\ C_2H_5OH}$

$C_6H_4C_2O_2N(CH_2)_3ONH(CH_2)_3O_2C_2C_6H_4 \xrightarrow{\text{aq HCl}}$

$NH_2\text{-}(CH_2)_3ONH(CH_2)_3NH_2 \cdot 3HCl$

METHOD B

$C_6H_4C_2O_2NCH_2CH_2CH_2ONHCOC_6H_5 \xrightarrow{NH_2\text{-}NH_2H_2O_3HCl}$

$C_6H_5CONHO(CH_2)_3NH_2 \cdot HCl \xrightarrow{KOH,\ C_6H_4C_2O_2CH_2CH_2CH_2Br,\ C_2H_5OH}$

$C_6H_5CONHOCH_2CH_2CH_2NHCH_2CH_2CH_2NO_2C_2C_6H_4 \xrightarrow{\text{(aq) HCl}}$

$NH_2OCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2 \cdot 3HCl$

Scheme 5. Proposed methods for the synthesis of aminooxy analogs of spermidine

TABLE V

POSSIBLE COMPOUNDS OF FRACTION VIII-A-2

Formula	Compound	MW	Calcd % C	H	N
(a) $C_6H_{13}N_3O$	$NH_2O(CH_2)_3NHCH_2CH_2CN$	143	50.34	9.09	29.37
(b) $C_9H_{16}N_4O$	$NH_2O(CH_2)_3N(CH_2CH_2CN)_2$	196	55.10	8.16	25.57
(c) $C_9H_{15}N_3O$	$NC(CH_2)_2O(CH_2)_3NHCH_2CH_2CN$	181	59.66	8.28	23.20
(d) $C_6H_{12}N_2O$	$HO(CH_2)_3NHCH_2CH_2CN$	128	56.25	9.38	21.87
(e) $C_9H_{15}N_3O$	$HO(CH_2)_3N(CH_2CH_2CN)_2$	181	59.66	8.28	23.20
(f) $C_{12}H_{18}N_4O$	$NC(CH_2)_2O(CH_2)_3N(CH_2CH_2CN)_2$	234	61.53	7.69	23.93
(5) $C_9H_{16}N_4O$	$NC-(CH_2)_2-NHO-(CH_2)_3-NH(CH_2)_2CN$	196	55.10	8.16	28.57

with the theoretical values of compounds (c), (d), and (e), of Table V, and not with the anticipated compound (g). The data definitely do not agree with any compound having the aminooxy linkage. Mass spectral data could not be obtained with this compound (VIII-A2).

When Method B (see the experimental section) was carried out, one fraction was obtained (VIII-B). The carbon, hydrogen and nitrogen analysis of this fraction is vastly different from the theoretical values of those given in Table V. However, it must be said that great difficulty was encountered in handling this viscous oil (VIII-B), and the duplication of analytical data was not obtained. The mass spectral data given in Table IV fit most closely to the probable fragmentation products of compound (d) of Table V. A delination of the mass spectral data and the assignment of the probably structure of fraction (VIII-B) follow.

There were no peaks beyond 128 mass units. The infrared spectrum showed a peak at 2250 cm^{-1}, which is characteristic of nitriles (i.e., CN linkage). Two of the possible structures for compound VIII-B are given below for comparative purposes.

32	46	60	74	89	103		
NH_2O	CH_2	CH_2	CH_2	NH	CH_2	CH_2	CN
	111	97	83	69	54	40	

(b)

31	45	59	74	88			
$HOCH_2$	CH_2	CH_2	NH	CH_2	CH_2	CN	
	97	83	69	54	40		

(d)

The numbers to the right of the lines indicate the mass of the positively charged fragment to the right and the numbers to the left of the line indicate the mass of the positively charged fragment to the left. If (b) were the predominant fraction in VIII-B, the mass spectrum would have values given for most of the numbers above in (b), with the largest peak predicted at 46, 103 and 83 mass units. If (d) were the predominant fraction of (VIII-B), the mass spectrum should have values for most of the numbers given for that compound (see above), with the largest peaks being at 31, 83, 88 and 45 mass units. As shown in Table IV, this is fairly consistent with what was observed; furthermore, fractions (VIII-A2) and (VIII-B) have similar infrared spectra, although no definite statement concerning their identity can be made. The data do indicate that the aminooxy group had been cleaved by some process. Even though the synthesis of spermidine and spermine by the process given in the experimental section (Methods A and B) was not successful, it is believed that this method should be attempted again in greater detail so that a better understanding of the stability of the aminooxy linkage in various solvents and with various substrates can be gained.

In conclusion, the synthesis of the aminooxy analog (3-aminooxy-1-aminopropane) of the diamine, putrescine (1,4-diaminobutane), was accomplished in this investigation. Employing this analog of putrescine as a reactant, several methods were investigated in an effort to synthesize aminooxy

analogs of the polyamines, spermidine and spermine. Although the synthetic methods which were investigated failed to produce the desired aminooxy analogs, several new compounds were synthesized by some rather novel reaction processes.

CHAPTER BIBLIOGRAPHY

1. Alarcon, R. A., Foley, G. E., and Modest, E. J., Archives Biochemistry and Biophysic, 94 (1961), 540.

2. Boyland, E., Biochemical Journal, 35 (1941), 1283.

3. Cohen, S. S., and Liehteustein, J., Journal of Biological Chemistry, 235 (1960), 2112.

4. Colbourn, J. L., Witherspoon, B. H., and Herbst, E. J., Biochimica et Biophysica Acta, 49 (1961), 422.

5. Davis, G. F. R., Comparative Biochemistry and Physiology, 19 (3) (1966), 619.

6. Dudley, Harold, and Rosenheim, Otto, Biochemical Journal, 19 (1925), 1035.

7. Dudley, H. W., Rosenheim, O., and Starling, W. W., Biochemical Journal, 20 (1926), 1082.

8. Heininger, S. Allen, Journal of Organic Chemistry, 22 (1957), 704.

9. Hirsch, J. G., Journal of Experimental Medicine, 97 (1953), 327.

10. Hochster, R. M., and Quastel, J. H. Metabolic Inhibitors. Vol. I. Academic Press Inc., New York, N. Y., 1963.

11. Israel, Mervyn, Samour, Joan, and Modest, Edward J., Journal of Medicinal Chemistry, 7 (1964), 710.

12. Kaiser, D., Tabor, H., and Tabor, C. W., Journal of Molecular Biology, 6 (1963), 141.

13. Kornowski, H. and others, Bulletin Des Societes Chimiques de(France), (1966), 679.

14. Kosaki, T. and others, Science, 127 (1958), 1176.

15. Krtogawa, Matsunosuke, Journal of Agricultural Chemistry Society (Japan), 12 (1936), 871.

16. Leeuwenhoek, A. V., Philosophical Transactions of the Royal Society of London, 12 (1678), 1040.

17. Luck, J. Murray and Allen, Frank W. Annual Review of Biochemistry. Vol. 30, California, 1961, p. 585.

18. Masingale, Robert E., Bryant, Sarah R., and Skinner, Charles G., Journal of Medicinal Chemistry, 12 (1969), 152.

19. McCormic, Donald B., and Snell, Esmond E., Journal of Biological Chemistry, 236 (1961), 1085.

20. Miyaki, K. and others, Chemical and Pharmaceutical Bulletin (Tokyo), 8 (1960), 933.

21. Razin, S., and Rozansky, Archives Biochemistry and Biophysic, 81 (1959), 36.

22. Schultz, Harry P., Journal of the American Chemical Society, 70 (1948), 2666.

23. Shalitin, Channa, Journal of Virology, 1 (3) (1967), 569.

24. Tabor, H., Tabor, C. W., and Rosenthal, S. M., "Annual Review of Biochemistry", 30 (1961), 585.

25. Tanner, M. J. A., Biochemistry, 6 (9) (1967), 2686.

26. Tukuoka, T., Acta Scholastic Medicine Universal Kioto, 27 (1950), 241.

27. Werner, Alfred, Berichte, 26 (1893), 1567.