THE PREPARATION OF SIMPLE PEPTIDE ANALOGS AS ANTIVIRAL AGENTS

By

JOHN JAMES HEFFERREN

A Thesis Submitted in Partial Fulfillment

of the Requirements For the Degree

of

DOCTOR OF PHILOSOPHY

at the

UNIVERSITY OF WISCONSIN

1953

ACKNOWLEDGEMENT

The author wishes to express sincere appreciation to Dr. William O. Foye for suggesting this study and for his guidance in directing it to completion.

Gratitude is also expressed to the American Foundation for Pharmaceutical Education for financial assistance to the author during this work.

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HISTORICAL

Since the discovery of the virus some fifty years ago, there has been a steadily increasing interest in these organisms, especially by those concerned with pathological organisms. With the increased incidence of poliomyelitis, there has been a concerted effort to develop therapeutic means of curbing the disease-producing viruses. Up to the present time, the most successful means of attacking viral diseases has been the utilization of biological products such as small-pox vaccine. Unfortunately, the number of viral diseases which submit to this type of therapy is rather limited.

The virus is an organism which is an obligatory intracellular parasite, smaller than bacteria and consisting of nucleoprotein (1). Viruses have a diameter of 10 to 300 mu. and are usually spherical or elipsoidal in shape. They have little or no self-sufficient enzyme system, so that they necessarily compete with the host cell for their development (1-2).

Due to their natural characteristics, the isolation and chemical identification of viruses has been very difficult. Animal viruses thus far studied differ from the plant

viruses in that they are not crystallizable, possess a lipid fraction and in some cases have a carbohydrate fraction which is not accounted for by the nucleoprotein (3). The influenza virus is one of those viruses characterized by an extremely high carbohydrate content (4). As might be expected from their relatively high protein content, viruses are attacked by specific proteolytic enzymes; however, there has not been found an enzyme which is generally applicable (2) for the treatment of viral diseases.

The elemental body of the virus consists of about 5.7% desoxyribonucleic acid and 5.6% lipid material (3). Copper and a flavin-adenine-dinucleotide have been demonstrated to be integral parts of the elemental body, suggesting that an incomplete respiratory mechanism may be present (3). Biotin has been shown to be present in purified preparations of the vaccinia virus (5).

The synthesis of the virus particle must involve catalysts provided by the virus or host, or both. Studies with known analytical procedures have thus far failed to indicate the possession by isolated viruses of enzymatic abilities, or at best revealed very restricted ones (6). The virus organism may possess partial enzymatic systems that must be complimented and completed by the host factors, or the virus may be completed devoid of independent metabolism and the enzymes required for reproduction, and as a result be completely dependent on the host cells (6).

Luria (7-8) postulated that viruses, upon entering host

cells, do not reproduce themselves by growth and division, but that they disintegrate into component parts of specific chemical configurations. These configurations after being replicated are recombined in some unknown way into complete, infectious virus particles. In contrast to this view of reproduction, there are many workers in the field who support the normal manner of reproduction, that is growth and division (6).

Between the main body of bacteria and viruses lie two fairly distinct groups of organisms, namely the rickettsiae and the viruses of the psittacosis group (9). The generic name rickettsiae is applied to a group of very small gram-negative coccobacillary organisms which are the etiologic agents of the typhus fevers, spotted fever, and related fevers (3). The rickettsiae are intracellular parasites, but they are anthropoid-transmitted and show antigenic cross reactions with proteus bacilli (2). The psittacosis with a diameter up to 300 mu. are larger than the smallest bacteria. The psittacosis viruses are to some extent antigenically related to each other and seem to have a definite life-cycle within the cytoplasm of animal cells (2).

The various viral disease have been classified into five general classes according to their general effects on the host (3):

1. Dermatropic - variola, vaccinia, trachoma and the pox diseases which are characterized by skin lesions.

2. Neurotropic - rabies, poliomyelitis, and encephalitis which cause various nerve lesions.
3. Viscerotropic - those viruses which attack abdominal or thoracic visera or produce signs indicative of generalized infection such as yellow fever.
4. Pneumonotropic - those viruses lodging in the lungs such as the influenza virus.
5. Pantropic - those viruses effecting a wide variety of tissues.

Chemotherapy

Previous to 1937, the outlook for satisfactory chemotherapeutic therapy of rickettisal and virus infections was not very promising. However, with the development of the sulfonamides there has been intensive investigation and progress in the development of chemical compounds useful in the chemotherapy of viral diseases (6).

Since the virus exists within the cell and is dependent upon the cell, the chemotherapeutic treatment of viral diseases meets with difficulties not encountered in bacterial disease. The virus multiplies within the cell until the cell becomes degenerate and breaks down, releasing the virus to attack adjacent cells. This is the general form which the virus-caused disease takes, but unfortunately the clinical symptoms of the disease are not shown until the virus multiplication has progressed to an advanced stage. With the metabolism of the host cell and virus so closely related,

the killing of the virus will ordinarily necessitate the killing of the host cell (2).

The virus may be attacked before it reaches the susceptible cells or while it is adherent to the surface of the cell (7). When only a small group of cells are concerned with the viral infection, these cells can be destroyed with the virus, as is the case in treatment of warts with podophyllin (1). When the viral infection is secondary to a bacterial infection, the use of a bacteriocidal agent to kill the bacteria will set up the virus for destruction by the natural protective mechanisms of the body (1).

Andrewes (2) developed a set of principles which may be of value in judging possible antiviral agents and explaining their mechanisms of action.

1. By presenting to the host cell chemical substances bearing a resemblance to essential metabolites, there may be developed a specific inhibition of essential metabolites necessary for the propagation of the virus. An example of this is the p-aminobenzoic acid inhibition by sulfa drugs in the treatment of psittacosis diseases.

2. The presentation to the host cells of foreign substances which are capable of being built up irreversibly into the structure of the virus. Thus if the amino group of an amidine compound was used for a source of nitrogen in the synthesis of nucleic acids, the pathogenic character of the virus may be lost.

3. By the use of chemical agents capable of combining with metabolites essential for reproduction or with enzymes which control the synthetic processes, the overall function of the virus may be disrupted. The combination of the arsenicals with the sulfhydryl groups of certain enzymes would illustrate this type of reaction (8).

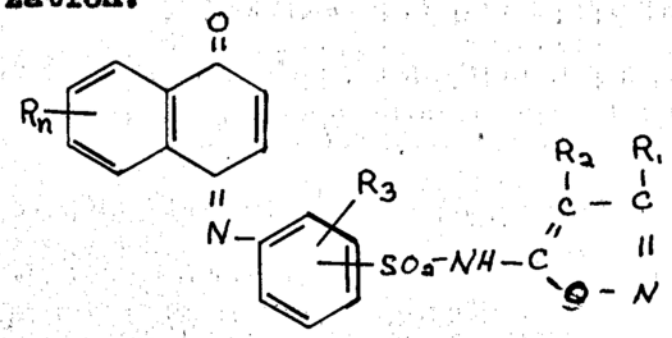
Sulfonamides

The use of the sulfonamide drugs in chemotherapy was developed many years ago and is today still a field of active research. The sulfonamides as a group have been of little value in the treatment of rickettsial diseases; however, they are effective against the psittacosis group of viruses (6). This psittacosis group of viruses contains such viruses as the lymphogranuloma venereum, psittacosis and ornithosis viruses, trachoma, inclusion conjunctivitis or inclusion urethritis, mouse pneumonitis and cat pneumonitis (2).

It appears that the sulfa drugs actually stimulate the growth of rickettsiae. p-Aminobenzoic acid, which is a sulfonamide inhibitor in other viral and bacterial diseases, has been shown to be active against rickettsial infections in mice and eggs (10). Morgan (11, 12) found that p-aminobenzoic acid competively antagonized the chemical action of sulfadiazine against the 6BC strain of psittacosis virus in chick embryos at a ratio of one part p-aminobenzoic acid to five hundred parts of sulfadiazine. Reversal of sulfonamides action was also observed by a fixed dose of pteroylglutamic

acid by the virus (11, 12). Thus there is quite a contrasting difference in effect between rickettsiae and psittacosis virus with respect to antagnosism of p-aminobenzoic acid and sulfonamides.

Hultquist (13) prepared some p-hydroxybenzene sulfonamidothiadiazoles which were claimed to exhibit some inhibitory action against poliomyelitis and equine encephalitis. In the work of Steiger (14) the thiadiazole radical was substituted with an isoxazole radical. In this series of compounds the isoxazole radical was combined with a naphthoquinoneimine structure which included a sulfonic acid group for solubilization.



R = H or SO_2 radical
n = 1, 2, 3
R_{1-2} = H or lower alkyl
R_3 = H or lower alkyl

Darvisul (N-(2-thiazolyl)-phenol-4-sulfonamide) was reported to protect mice against Columbia SK. virus (15); however, several workers have had difficulty substantiating these results (16-18).

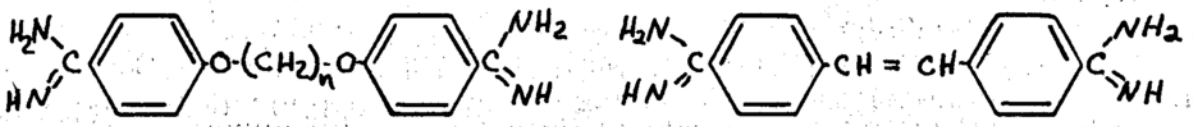
The closely related p-sulfonamidobenzamidine and p-sulfonamidobenzamidoxime were shown to possess definite antiviral activity (19). In an effort to modify the struc-

ture of the sulfoamidines Andrewes (19) prepared a series of derivatives with substitution on the amidine, sulfonamide groups, and on the ring. Alkyl substitution on the amidine and on the sulfonamide nitrogen led to complete suppression of activity in all cases except that of N-methylsulfonamidobenzamidine, but even in this case the activity was significantly reduced. The introduction of aromatic rings such as phenyl, p-sulfonamidophenyl and p-sulfondimethylamidophenyl also led to suppressed activity.

Andrewes (19) suggested the possibility that the relatively small molecular size sulfa drug may gain entrance to the parasite organism during reproduction at which time there is a high degree of protein synthesis. The non-sulfonamide portion of the molecule could then combine in a vital structure which could no longer function owing to the foreign nature of the sulfonamide group. This idea is not inconsistent with the p-aminobenzoic acid reversal of the sulfonamide drugs in specific viral diseases. In addition since the free amino group appears to be necessary for sulfonamide activity, it is difficult to imagine that this group is essential for mere salt formation (19).

Amidines

Propamidine and pentamidine exhibit antiviral activity against influenza viruses, mumps, feline pneumonitis, and meningopneumonitis in the allantoic sac of chick embryos (20-21). The related stilbamidine inhibited only mumps virus.



n = 3 Propamidine
n = 5 Pentamidine

Stilbamidine

Mumps virus was inhibited in tissue cultures of pentamidine and stilbamidine in concentrations of 0.5 - 5.0 γ/ml. added at the same time as the virus (21). Inhibition of influenza A and B was demonstrated with 5- 20 γ/ml. of pentamidine (21).

Partial inhibition of the growth of the PR8 strain of influenza virus was demonstrated with hexamidine (4-4'-diamidinodiphenoxyhexane) in the allantoic sac of chick embryos (22). With the injection of 0.25 mg. of hexamidine within five hours after the inoculation of the virus the mean hemagglutination titer was reduced by 5 - 10 fold (22).

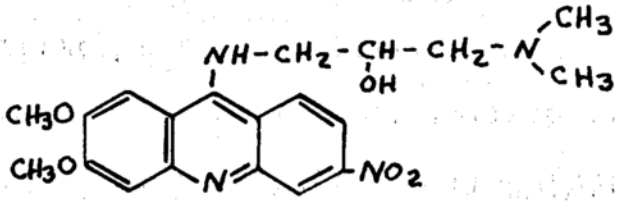
Antibiotics

The wide spectrum antibiotics such as aureomycin, terramycin and chloroamphenicol possess general activity against the larger viruses, but they are not effective against the small pathogenic viruses (23-26). Penicillin is inhibitory to all of the viruses of the psittacosis-lymphogranuloma group, but it is not clinically useful against lymphogranuloma venereum and trachoma (27, 28). In vitro tests show that chloroamphenicol was highly active against rickettsiae and viruses included in the psittacosis-lymphogranuloma group (29).

Clinical results derived from the use of antibiotics in the treatment of influenza are believed to be a result of their effect on secondary bacterial infections and not due to their action on the virus itself (30). Aureomycin and terramycin given both subcutaneously and orally failed to increase the resistance of mice and rats to the acute toxicity of influenza A virus (30).

Acridines

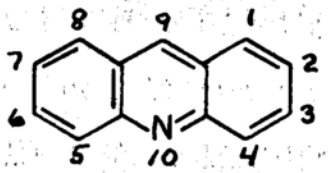
A number of acridines have been developed in recent years which inhibit and delay the growth of rickettsiae and certain viruses in eggs and mice (6). The most promising member of this group of compounds is nitroakridin 3582 (6-7-dimethoxy-3-nitro-9-(3'-diethylamino-2'-hydroxypropyl) acridine) has been shown to



inhibit or delay the growth of influenza A and B in chick embryos when injected at the same time as the virus (31, 32). Nitroakridin 3582 was shown to be ineffective in the treatment of experimental influenza A in mice, while nitroakridin and its arsenical salt, rutenol, were quite effective against rickettsiae and other viruses (33).

Acriflavin and proflavin and the related acridine quinacrine have been shown to possess some activity against

psittacosis in chick embryos and mice; however, their activity is less than nitroakridin (34-36).



Acriflavin - 3-6-diamino-10-methyl
Proflavin - 3-6-diamino
Quinacrine - 3-chloro-7-methoxy-9(1-methyl-4-diethylamino butylamino

3-Nitro-9-aminoacridine was found to be more active than nitroakridin against R. mooseri in chick embryos but more toxic than the above acridines (35).

Atabrine and other chloroacridines closely related chemically to the above mentioned nitroacridines except for the substitution of a chloro group for a nitro had insignificant activity against psittacosis (36). This indicates that a nitro group at position three or amino groups at three and six positions of the acridine ring is essential for this type of antiviral activity (35).

Rasmussen (32) suggested that the inhibition of viral growth in embryonic eggs by nitroakridin 3582 was a secondary effect to its action on the embryonic egg tissue. Since nitroakridin exhibits very low in vitro activity, virucidal effects at the concentrations used in the allantoic sac tend to preclude a direct action on the virus in the allantoic fluid. It is thought that the virus does not grow on the nitroakridin treated egg tissue, because the susceptible tissue cells have already been killed by the inhibiting drug. This explanation is supported by the observation that the

chorioallantoic membranes of eggs receiving nitroakridin 3582 were atrophic and weighed about half as much as normal (37).

This action on embryonic membranes is probably not entirely one of simple non-specific cell toxicity, since only certain compounds are effective while other closely related drugs of equal toxicity have no effect (37). Nitroakridin-like compounds tend to become firmly bound to tissues and this firmness of combination may be a factor in determining the effect by producing a local virustatic concentration higher than would be attainable with systemic administration of the drug (37).

It is not possible to completely exclude the possibility that nitroakridin may poison a specific metabolic system or systems which are required by the host cells and virus. Another possible mechanism is the direct interference with some synthetic mechanism peculiar to the virus (32).

Nitro Compounds

With the observation that certain nitroacridines showed antiviral activity while the corresponding chloro compounds had little or no antiviral activity, the antiviral activity of less complicated aromatic nitro compounds was investigated. Derivatives of nitrobenzoic acid and 5-nitro-2-furaldehyde were shown to possess inhibitory activity against the Chlamydozaceae both in chick embryos and mice (38). These compounds are related to the antibiotic chloramphenicol and

EFFECT OF NITROFURANS ON VIRAL RESPIRATORY INFECTIONS IN MICE (38).

VIRUS	SUBSTITUENT R*	DAILY DOSE mg.	NO. OF MICE	LESION SCORE % CHANGE	LUNG WT.(g) % CHANGE
Cat pneumonitis	(I) CH = N-NH-C(=O)-C(=O)-NH_2	3	22	-35	-35
	(II) CH = N-N(H)-$CONH_2$ CH_2-CH_2OH	2	15	-56	-40
Mouse pneumonitis	(I) CH = N-NH-C(=O)-C(=O)-NH_2	3	27	-54	-36
Lymphogranuloma venereum	(I) C = N-NH-C(=O)-C(=O)-NH_2	3	27	-88	-39
	(IV) $CH(CO_2CH_3)_2$	2	26	-51	-28
Meningo-pneumonitis	(I) C = N-NH-C(=O)-C(=O)-NH_2	3	36	-70	-30
		2	16	-41	-21
	(III) C = N-NH-C(=O)-NH_2	3	31	-42	-30
	(II) C = N-N(CH_2CH_2OH)$CONH_2$	2	20	-35	-24
	(IV) $CH(CO_2CH_3)_2$	2	20	-16	-11

*All are derivatives of 5-nitro-2-furaldehyde NO_2

-R where R represents the aldehyde group combined as semioxamazone (I) 2- -hydroxy-ethyl-semicarbazone (II), semicarbazone (III), or diacetate (IV).

EFFECT OF SUBSTITUTED NITROBENZENES ON VIRAL RESPIRATORY INFECTIONS IN MICE (38).

VIRUS	SUBSTITUENT R*	DAILY DOSE mg.	NO. OF MICE	LESION SCORE % CHANGE #	LUNG WT.(g) % CHANGE #
Cat Pneumonitis	COONa	10	43	-29	-14
	$CONH_2$	5	18	-40	-23
	$C(NH)NH_2$	2	40	-25	- 9
	SO_2NH_2	4	23	-52	-27
	(Sulfanilamide)	10	14	-11	- 1
	Chloramphenicol	2	16	- 5	18
	"	10	16	-36	-19
Mouse pneumonitis	COONa	10	25	14	10
	$CONH_2$	5	20	13	-14
	$C(NH)NH_2$	2	53	-13	2
	SO_2NH_2	4	21	-65	-35
	(Sulfanilamide)	10	13	-65	-37
	Chloramphenicol	2	16	-11	-24
Lymphogranuloma venereum	COONa	7.5	27	- 3	- 2
	$CONH_2$	5	16	-10	-14
	Chloramphenicol	2	16	-41	-41
Meningopneumonitis	COONa	7.5	26	- 6	- 6
	$CONH_2$	5	33	-19	- 3
	SO_2NH_2	3	31	-16	- 8
	Chloramphenicol	2	16	-28	- 9
	"	5	16	-50	-35

* Substituents in para position to nitro group:

chloramphenicol R = $CH(OH)CH(NH_2COCHCl_2)CH_2OH$

% change = 100 (-1 T/C).

T = treated mice; C = control mice.

in the case of furacin(5-nitro-2-furaldehyde semicarbazone) possess equal activity against psittacosis virus (38).

Furacin has been shown to inhibit the oxidation of glucose and pyruvate in bacteria (39, 40), while the corresponding non-nitro analog did not (41). Furacin is capable of inhibiting a large number of enzymes, especially sulfhydryl-enzymes and dehydrogenases in susceptible strains of E. coli (41). In susceptible strains of E. coli,Furacin has been shown to inhibit malic, succinic, lactic and ∝-glycerophosphate dehydrogenases while formic dehydrogenase was not inhibited (42).

Fitzpatrick (43) observed that a D.D.T. analog, 1-1-1-tricloro-2-2-bis-p-nitrophenylethane, was active against murine typhus. In 1945 this compound was tested by Kikuth in Germany and was reported to be more active than methylene blue against murine typhus while being less toxic. Toxicity studies indicate that mice will tolerate 5 g/kg of body weight dose of the nitro D.D.T. analog, whereas 0.4 mg/mouse /day of D.D.T. is toxic (43). About four times the amount of nitro D.D.T. analog are required to produce the same effect as p-aminobenzoic acid on murine typhus; however, the nitro compounds appears to be a better therapeutic drug (43).

In all the previously discussed nitro compounds which exhibit antiviral activity, the only common chemical characteristic is that the aromatic ring system is conjugated with a nitro group.

The nitro compounds are related to p-aminobenzoic acid and may be reduced in the body to corresponding amino compound. In an effort to disprove that the p-nitrobenzoic acid derivatives did not owe their activity to the reduction in the body to p-aminobenzoic acid, Hamilton (44) showed that PAB was inactive against psittacosis virus. On the other hand, PAB does inhibit the activity of rickettsiae (7).

Aldehyde Derivatives

Various aromatic aldehyde thiosemicarbazones have been shown to be highly active against a variety of viruses (45-47). Eaton (48) reported that p-nitro and p-acetylaminobenzaldehyde thiosemicarbazones as well as 5-nitrofuraldehyde semicarbazone suppressed the production of pulmonary lesions in cotton rats innoculated intranasally with a virus of primary atypical pneumonia. Isatin and 5-nitro-2-thenaldehyde thiosemicarbazone protected mice against Williamsport virus, a strain of variola-vaccinia (49).

When glucose or cyclohexane was substituted for the benzene ring in various thiosemicarbazone derivatives, the activity against vaccinia virus was negligible (47). Substitution of oxygen for sulfur in the thiosemicarbazide moiety also caused loss of activity. Thiosemicarbazide itself had little if any effect on the inhibition of vaccinia virus (47).

EFFECT OF VACCINIA INFECTION WITH THIOSEMICARBAZONES (50)

COMPOUND	% CONC. IN DIET	PROPORTION OF MICE SURVIVING					
		TREATED			UNTREATED		
		10^2	10^3	10^4	10^2	10^3	10^4
Benzaldehyde T.S.	0.04	4/6	2/6	5/5	0/5	1/6	1/5
P-Nitrobenzaldehyde T.S.	0.04	6/14	8/11	10/12	1/11	2/12	4/12
O-Methoxybenzaldehyde T.S.	0.06	9/12	9/12	11/12	0/12	0/12	7/12
Picolinaldehyde T.S.	0.03	0/6	1/6	0/4	0/5	1/6	3/6
Isonicotionaldehyde T.S.	0.08	6/6	6/6	5/6	3/6	1/6	5/6
Nicotinaldehyde T.S.	0.06	1/8	1/4	1/6	0/5	0/6	0/3
5-Bromo-2-thenaldehyde T.S.	0.02	4/6	2/6	5/5	0/5	1/6	1/5
3-Methyl-2-thenaldehyde T.S.	0.08	3/6	3/6	5/6	0/5	1/6	1/6
3-Thenaldehyde T.S.	0.04	6/6	6/24	12/24	4/23	2/23	7/23
Acetophenone T.S.	0.04	0/5	0/6	2/6	0/5	0/6	0/6
Pyruvic T.S.	0.10	0/6	0/6	3/6	0/6	1/6	2/6

T.S. - thiosemicarbazone derivative of the aldehyde

Since the study on these compounds with BCG tuberculosis testing, there appears to be no simple correlation for BCG in vitro activity and activity for vaccinia virus in eggs (47). In addition there was no simple correlation between antituberculosis activity in mice and antivaccinia activity in eggs. Three of the four anti-tuberculosis compounds tested were inactive against vaccinia virus (47). Sulfathiazole, 1-1(p-p'-

sulfonyldianilino)di(3-phenyl-1-3-propanedisulfonic acid), tetra sodium salt (Suphetron) and p-aminosalicylic acid were inactive, while p-p'-diaminodiphenyl sulfone showed slight activity (47).

The para-substituted benazldehyde thiosemicarbazones produce little or no protection against vaccinia infection in mice. Active antitubercular agents such as p-ethylsulfonyl, p-acetylamino, p-amino and p-dimethylaminobenzaldehyde thiosemicarbazones were disappointing in their action against vaccinia in mice (51). However, Eaton (48) showed that para substituted benzaldehyde thiosemicarbazones were active against experimental pneumonia in cotton rats.

NITRO COMPOUNDS AND ALDEHYDE DERIVATIVES AGAINST EXPERIMENTAL PNEUMONIA IN COTTON RATS (48).

COMPOUND	DAILY DOSEAGE mg/100 g. BODY WT.	NO. OF ANIMALS WITH PULMONARY LESIONS OF					AVER. L.S.	INFECTION RATIO
		2	1	½		0		
NFV	1.0	0	0	1	2	9	1.7	1/12
Control	0.0	1	4	1	2	3	12.7	6/11
NBI	6.5	0	0	2	0	4	3.3	2/6
"	10.0	0	0	1	3	5	2.8	1/9
ABI	8.0	0	1	1	1	4	5	2/7
ABII	20.0	0	0	0	0	8	0	0/8
"	5.0	0	1	0	0	7	2.5	1/8
NBII	8.0	0	1	1	3	6	4.1	2/11
NFII	8.0	0	0	1	0	6	1.4	1/7

L.S. - Lesion scores representing % of pulmonary consolidation.
NBI - p-nitrobenzaldehyde semicarbazone.
NBII - p-nitrobenzaldehyde thiosemicarbazone.
ABI - p-aminobenzaldehyde thiosemicarbazone.
ABII - p-acetylaminobenzaldehyde thiosemicarbazone.
NFI - 5-Nitro-2-furaldehyde-2-B-hydroxyethylsemicarbazone.
NFII - 5-Nitro-2-furaldehyde-4-(diethylaminopropyl)semicarbazone.

Thompson (51) made some generalizations as to the prerequisites for optimum activity in the carbonyl derivative series.

1. Thiosemicarbazone radical (= N-NH-CS-NH_2) seems to be essential for greatest activity.
2. Semicarbazones of benzaldehyde, 2-thenaldehyde, cinchoninaldehyde and isatin are devoid of activity.
3. Presence of aldehyde linkage to the thiosemicarbazide moiety contributes to the activity, but it is not essential.
4. Substitution on the aldehyde carbon atom diminishes or abolishes the activity.
5. Substitution on the ring portion of the molecule produces the same diminishing effect on the activity with the exception of p-nitro and p-methoxybenzaldehyde and 5-chloro, 5-bromo and 3-methyl-2-thenaldehyde thiosemicarbazones.

Antimetabolic Compounds (amino acid analogs, purines, benzimidazoles and uracils)

There has been considerable interest in the development of metabolite analogs which would inhibit the metabolic processes essential to the virus. The sulfonic acid derivatives of amino acids (52), benzimidazoles (53) and 2-6-diaminopurines (50) have been shown to possess antiviral activity.

Cysteic acid, dl-ethionine, N-dichloroacetyl- α-(p-nitrophenyl)glycine, α-amino-B-phenylethane sulfonic acid

and β-(2-thenyl)alanine were shown to have activity against the poliomyelitis virus in tissue cultures (52). These analogs of either aspartic, methionine or phenylalanine indicate that these amino acids at least may be essential to the synthesis of virus. The α-amino-β-phenylethane sulfonic acid is thought to be a non-competitive inhibitor of phenylalanine and probably acts upon some enzyme system which is not specific for one given amino acid (51). This is of interest since those compounds known to interfere with the synthesis of nucleic acids were found to inhibit virus propagation (52).

α-Amino-p-methoxyphenylmethane sulfonic acid in doses of 8 mg./embryonic egg completely inhibited the growth of the influenza virus. Under these same conditions the LD_{50} for the aminosulfonic acid was 30 mg/egg (54). Under special conditions α-amino-β-phenylethane sulfonic acid afforded some protection to mice infected with WS strain of influenza A virus (54). The aminosulfonic acids have also been shown to inhibit the propagation of a bacteriophage of B. coli (55).

Since the α-aminosulfonic acids are relatively unstable compounds, it was believed that possibly one of the degradation products may be responsible for the antiviral activity. Anisaldehyde, which would result from the decomposition of α-amino-p-methoxyphenylmethane sulfonic acid, was tested and found to be void of antiviral activity (54). Ammonium sulfate and sodium bisulfite were also found to be

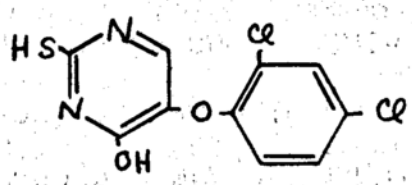
ineffective against viruses in the concentrations equivalent to those of the aminosulfonic acid used (53). This indicated that the activity of the aminosulfonic acids was not due to decomposition products but due to some inherent action of the original compounds (54).

Benzimidazole has a definite inhibition effect on the multiplication of vaccinia virus in chick embryonic tissue, while various triazolylpyrimidines were inactive (56). In concentrations of 5×10^{-6}, 2-6-diaminopurine decreased viral growth, while 2-aminopurine was inactive (56). The halogenated purines exhibit some virostatic activity, which in the case of 2-6-dichloro-7-methylpurine has approximately the same activity as 2-6-diaminopurine (56).

Since 2-6-diaminopurine is incorporated into the nucleic acid of a rat as guanine (57), it is reasonable to assume that inhibition of multiplication of vaccinia virus may be due to the interference with the nucleic acid metabolism. The activity of 2-6-diaminopurine may be due to inhibition of the growth of the vaccinia virus either by interference with the formation of viral nucleoprotein or by injury to the chick embryonic tissue (58). Since 2-6-diaminopurine has been shown to injure embryonic tissue and exerts little or no in vivo activity, the direct action of the purine on the tissue of the substrate appears to be the preferred mechanism of action (56). Here again there are compounds such as 8-azaguanine(5-amino-7-hydroxy-1-v-triazolo(d)pyrimidine) which are toxic to the embryonic tissues but do not

inhibit viral growth (56).

Thiouracil and substituted uracils were found to inhibit the growth of psittacosis virus strain 6BC (58). Thompson (59) found that a series of substituted phenoxyuracil derivatives possessed antiviral activity against vaccinia virus in chick embryonic tissues when given approximately the same time as the virus. The most active compound of this series was 5(2-4-dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine (59).



The phenoxyuracil series was found to be a rather specific chemical structure for activity, since modification of the substituents of the pyrimidine nucleus led to loss or considerable diminution of activity (59). Some variation in the benzene nucleus, however, was allowed. Electron attracting groups such as chlorine and bromine in the para position of the benzine ring increased the activity, while electron donor groups such as alkyl and alkoxy in the para position decreased activity.

Wooley (60) found that a series of dichlorophenoxy-acetic and propionic acids increased the survival time of mice infected with Lansing strain of poliomyelitis.

Amides of 5-aminouracil inhibit the multiplication of vaccinia in tissue culture (61-62). In correlation with the

inhibition of Lactobacillus casei, Thompson (58) predicted that chloroacetamidouracils or amides with pKa's near that of chloroacetic acid would be active antiviral compounds. The predicted activity was lower than the actual experimental values received, since all chloro and bromo acylamides and dichloroamides including chloroacetamide showed antiviral activity (61). The 5-chloroacetamidouracil, 5-dichloroacetamidouracil and ∝-chloro-p-nitroacetanilide are related to chloreamphenicol. It is possible that the virucidal activity resides in the dichloroacetamide linkage which the chloroamides have in common with chloreamphenicol (62).

Polysaccharides

Horsfall and McCarthy demonstrated that certain polysaccharides either of bacterial or non-bacterial origin can modify the course of infection with pneumonia virus (PVM) of mice and inhibit the multiplication of the virus in the mouse lung (63). A few micrograms of polysaccharide by the intranasal route caused inhibition when given some days before or after the inoculation of the virus (63). By giving 25-50 mg. of apple pectin intro-allantoically, Green (64) showed that the multiplication of influenza virus A(PRA) in the allantoic sac of the chick embryo was inhibited.

The capsular polysaccharide derived from type-specific strains of Friedlander bacilli cause a decrease in the susceptibility of the chick embryo to the infection with mumps virus (65). Inhibition of virus multiplication in the

THE INHIBITION OF LANSING POLIOMYELITIS VIRUS (54).

COMPOUND	Mg/ml.	NEGATIVE LOG OF LD_{50} AVER. TREAT.	AVER. UNT.	AVERAGE CONTROL MINUS AVERAGE TREATMENT
Aminoacid Analogs				
Cysteic acid	2.5	1.1	2.3	1.2
dl-Ethionine	3.0	0.5	2.1	1.6
N-dichloroacetyl-α-(p-nitrophenyl) glycine	2.5	0.5	2.5	2.0
α-Amino-β-phenyl-ethanesulfonic acid	2.5	0.1	2.3	2.2
β-(2-Thenyl)-alanine	2.5	0.6	2.5	1.9
Nucleic acid synthesis compounds				
Adenine, guanine, uracil (each)	1.0	0.5	2.2	1.7
Benzimidazole	2.5	0.1	2.3	2.2
2-6-Diaminopurine	2.75	0.3	2.5	2.2
Proflavin	0.07	0.3	2.5	2.2
Miscellaneous				
Glycerol Monacetin	5.1	0.3	2.5	2.2
Phagopedin sigma	2.5	0.0	2.7	2.7
dl-Homobiotin	2.5	0.9	2.0	1.1
Desoxypyridoxine	0.25	1.6	2.3	0.7
4-(Imidazolidone 2)caproic acid	2.5	1.4	2.0	0.6
6-methyltryptophan	0.6	1.7	2.3	0.6

allantoic sac of chick embryos with the appropriate polysaccharide given before or as late as four days after the innoculation of the virus was demonstrated when the polysaccharide was injected either into the allantoic or yoke sac of chick embryos (65). In contrast, multiplication of influenza A and B as well as Newcastle's disease viruses was not inhibited by the polysaccharide which was effective against mumps and PVM viruses (65).

Mumps and PVM in the chick embryo and the mouse multiply at relatively slow rates in comparison to influenza A and B and Newcastle's viruses (65). Correlation between the speed of multiplication does not seem justified in that the polysaccharide is not effective against the rapidly multiplying virus regardless of the length of time of injection of the polysaccharide before inoculation with the virus. The interference of the polysaccharide appears to be associated with the metabolic activity of the host cells and is not due to any direct action on the virus (65).

In addition to the compounds previously discussed, there have been numerous chemical compounds which have been shown to possess some antiviral activity. Since it is beyond the scope of this treatment to discuss these drugs in detail, they will be listed in a non-specific order.

Dyes	
Trypan Red	66
Congo Red	66
Janus Green	7
Merodiencein	8
Malachite Green	9
Metallic Compounds	
p-Arsenobenzamide	70
p-Chloromercuribenzoate	1
Mercuric Chloride	2
Mercurochrome	3
Miscellaneous	
Betaine	4
Choline	4
Methoxinine	5
Malonitrile	6
Quinine	7
Hexamidine	8
Salicylic acid	9
Ethyl-N-methylcarbamate	80
γ-Butyrolactone	81
Sulfur mustard (bis(β-chloroethyl) sulfide)	82
N-(β-chloroethyl)-dibenzylamine hydrochloride	2

3(1-4-dihydro-3-hydroxy-4-oxonaphthylidine-amino-N-(β -hydroxyethylamino)-ethyl benzamide	3
Antihistamines	4
Tocopherol esters	5
Lysine polypeptides	6

BIBLIOGRAPHY

1. Findlay, G. M., J. Pharm. & Pharmacol. 3, 193 (1951).
2. Andrewes, C. H. and King, H., Brit. Med. Bull. 4, 272 (1946).
3. Burrows, W., Bacteriology, 1949, W. B. Saunders Co., Philadelphia.
4. Taylor, A. R., J. Biol. Chem. 153, 675 (1944).
5. Rivers, T. M., Viral & Rickettsial Infections of Man, 1952, J. B. Lippincott Co., Philadelphia.
6. Sumner, J. B., The Enzymes, I pt. I, p. 116, 1950, Academic Press, New York.
7. Luria, S. E., Bacteriophage, an essay on virus reproduction in Viruses 1950, California Institute of Technology, Pasadena, California.
8. Luria, S. E., Fed. Proc. 10 528 (1951).
9. Andrewes, C. H. et al. Lancet 1, 177 (1944).
10. Snyder, J. C. et al., Virus & Rickettsial Diseases of Man, 1948, J. B. Lippincott, New York.
11. Morgan, H. R., Proc. Soc. Exptl. Biol. Med. 67, 29 (1948).
12. Morgan, H. R., J. Exptl. Med. 88, 285 (1948).
13. Hultquist, M. E., U. S. Patent 2,525,320.
14. Steiger, M., U. S. Patent, 2,555,614.
15. Sanders, M. et al., Texas Rep. on Biol. & Med. 6, 385 (1948).
16. Lo Grippo, G. A., et al., Proc. Soc. Exptl. Biol. Med. 70, 528 (1949).
17. Cox, H. R., et al., Proc. Soc. Exptl. Biol. Med. 70, 530 (1949).
18. Weil, M. L. and Warren, J., Proc. Soc. Exptl. Biol. Med. 70, 534 (1949).
19. Andrewes, C. H., et al., Proc. Royal Soc. (B), 133, 20 (1946).

20. Eaton, M. D. et al. J. Immunol, 68, 1 (1952).

21. Eaton, M. D. et al., J. Immunol, 68, 321 (1952).

22. McClelland, L. & van Rooyen, C. E., Canad. J. Res. (E) 27, 177 (1949).

23. Strax, S. and Wright, L. T., N. Y. State J. Med. 49, 1797 (1949).

24. Smadel, J. E. and Jackson, E. B., Science 106, 418 (1947).

25. Smith, R. M. et al., J. Bact. 55, 425 (1948).

26. Angistein, et al., Ann. N. Y. Acad. Sci, 51, 306 (1948).

27. Bryer, M. S. et al., J. Amer. Med. Assoc. 138, 117 (1948).

28. Dowling, H. F. et al., Ann. N. Y. Acad. Sci. 51, 214 (1948).

29. Pyran, M. J., et al. Ann. J. Trop. Med. 30, 677 (1950).

30. Kass, E. D., et al., Proc. Soc. Exptl. Biol. Med. 75, 520 (1950).

31. Green, R. H., et al., Publ. Hlth. Rep. Wash. 61, 1401 (1946).

32. Rasmussen, A. F. and Stokes, J. C., J. Immunol. 66, 237 (1951).

33. Smadel, J. E., et al., J. Immunol, 57, 155 (1947).

34. Burney, Fed. Proc. 7, 303 (1948).

35. Andrewes, C. H., et al., Brit. J. Pharmacol, 1, 15 (1946).

36. Eaton, M. D., et al., Proc. Soc. Exptl. Biol. Med. 66, 141 (1947).

37. Eaton, M. D., Ann. Rev. Microbiol, 4, 223 (1950).

38. Eaton, M. D., et al., Proc. Soc. Exptl. Biol. Med. 71, 501 (1949).

39. Dodd, M. C., J. Pharm. Exptl. Therap. 86, 311 (1946).

40. Green, M. N., Arch. Biochem., 19, 397 (1948).

41. Heath, E. C., et al., Bact. Proc. 142 (1950).

42. Green, M. N., et al., Proc. Soc. Exptl. Biol. Med. 76, 152 (1951).

43. Fitzpatrick, F. K., Proc. Soc. Exptl. Biol. Med. 70, 90 (1949).

44. Hamilton, H. L., Proc. Soc. Exptl. Biol. Med. 59, 220 (1945).

45. Hamre, D., et al., Proc. Soc. Exptl. Biol. Med. 73, 275 (1950).

46. Hamre, D., et al., J. Bact. 61, 127 (1951).

47. Hamre, D., et al., J. Immunol, 67, 305 (1951).

48. Eaton, M. D., et al., Proc. Soc. Exp. Biol. Med. 77, 422 (1951).

49. Minton, S. A., et al., J. Immunol, 70, 222 (1953).

50. Ackermann, W. W., Proc. Soc. Exp. Biol. Med. 80, 362 (1952).

51. Thompson, R. L., et al., J. Immunol, 70, 229 (1953).

52. Brown, G. C., J. Immunol, 69, 441 (1952).

53. Thompson, R. L., et al., J. Immunol, 55, 345 (1949).

54. Spizizen, J., J. Infect. Dis. 73, 212 (1943).

55. Gershoff, S. N., et al., Proc. Soc. Exptl. Biol. Med. 81, 484 (1952).

56. Thompson, R. L., J. Immunol, 65, 529 (1950).

57. Bendich, A. and Brown, G. B., J. Biol. Chem. 176, 1471 (1948).

58. Morgan, H. R., Proc. Soc. Exptl. Biol. Med. 81, 448 (1952)

59. Thompson, R. C., et al., J. Immunol, 67, 483 (1951).

60. Wooley, J. G., et al., J. Immunol, 68, 621 (1952).

61. Thompson, R. L. et al., Science, 110, 454 (1949).

62. Thompson, R. L., et al., Proc. Soc. Exptl. Biol. Med. 72, 169 (1949).

63. Horsfall, F. L. and McCarthy, M., J. Exptl. Med. 85, 623 (1947).

64. Green, R. H. and Wooley, D. W., J. Exptl. Med. 86, 55 (1947).

65. Ginsberg, H. S. et al., J. Exptl. Med., 87, 385 (1948).

66. Wood, H. G. and Rusoff, I. I., J. Exptl. Med., 82, 297 (1945).

67. Fleisher, M. S., J. Exptl. Path., 30, 123 (1949).

68. Francis, T. and Penttinen, K., J. Immunol., 63, 337 (1949).

69. Robbins, M. C., J. Immunol, 64, 431 (1950).

70. Eaton, M. D. et al., Proc. Soc. Exptl. Biol. Med., 71, 508 (1949).

71. Burney, T. E. and Gloub, O. J., J. Immunol, 60, 213 (1948).

72. Klein, M. and Perez, J. E., J. Immunol, 60, 349 (1948).

73. Bourke, A. R., et al., J. Immunol, 69, 75 (1951).

74. Sprunt, D. H., Proc. Soc. Exptl. Biol. Med., 51, 226 (1942).

75. Ackermann, W. W., J. Exptl. Med., 93, 337 (1951).

76. Szanto, P. B. and Felsenfeld, O., Proc. Soc. Exptl. Biol. Med., 72, 15 (1949).

77. Robbins, B. H., J. Pharmacol. Exptl. Therap., 76, 57 (1942).

78. McClelland, L. and Van Rooyen, C. E., Can. J. Research, 27, 177 (1949).

79. Fitzpatrick, F. K., Proc. Meetings Soc. Am. Bacteriologists, 1, 76 (1948).

80. Thompson, R. L. and Wilkin, M. L., Proc. Soc. Exptl. Biol. Med., 68, 434 (1948).

81. Rubin, B. A. and Giarman, N. J., Yale J. Biol. Med., 19, 1017 (1947).

82. Fong, J. and Bernal E., J. Immunol, 70, 89 (1953).

83. Jungeblut, C. W. and Schnitzer, R. J., Proc. Soc. Exptl. Biol. Med., 77, 176 (1951).

84. Amkes, J. L. et al., Amer. J. Pharm., 123, 60 (1951).

85. Hanan, R. Proc. Soc. Exptl. Biol. Med., 75, 440 (1950).

86. Rubini, J. P. et al., Proc. Soc. Exptl. Biol. Med., 76, 662 (1951).

PART II

DERIVATIVES OF α-AMINOALDEHYDES

PART II

DERIVATIVES OF ∝-AMINOALDEHYDES

INTRODUCTION

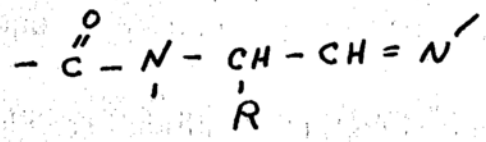
The aminoaldehydes corresponding to the naturally occurring amino acids have been suggested from time to time as possible precursors of cerain naturally occurring compounds. Neuberg (1) connected the formation of pyrazine and its homologs during the process of fermentation with the reduction of amino acids to aminoaldehydes. In 1941 Delbruck (2) postulated a theory for the biogensis of protein whereby aminoaldehydes formed a Schiff's base type polymer which in turn could be hydrated and dehydrogenated to peptide chains.

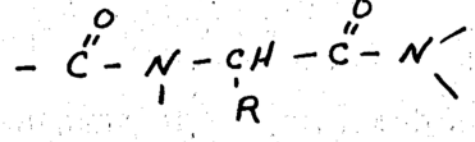
Other amino acid analogs such as cysteic acid, dl-ethionine, N-dichloroacetyl-∝-(p-nitrophenyl)glycine, ∝-amino-β-phenylethane sulfonic acid, and β-(2-thenyl) alanine have been shown to have definite antiviral activity against the poliomyelitis virus in tissue cultures (3). These amino acids analogs appear to derive their activity by inhibiting the utilization of the naturally occurring amino acids necessary to the viral synthesis.

Various aldehyde derivatives such as the semicarbazones and thiosemicarbazones have been shown to possess definite

antiviral activity (4). Although their method of action is not known, these compounds appear to require a definite chemical specificity for activity. This optimal activity is characterized by an aromatic aldehyde molecule connected to the thiosemicarbazide moiety through the aldehyde derivative (5).

The utilization of cellular material by reproducing vira suggests that analogs of ∝-amino acids or simple peptides might interfere with the normal development of viral infections. The purpose of this research is to prepare a series of substituted aminoaldehyde derivatives which are





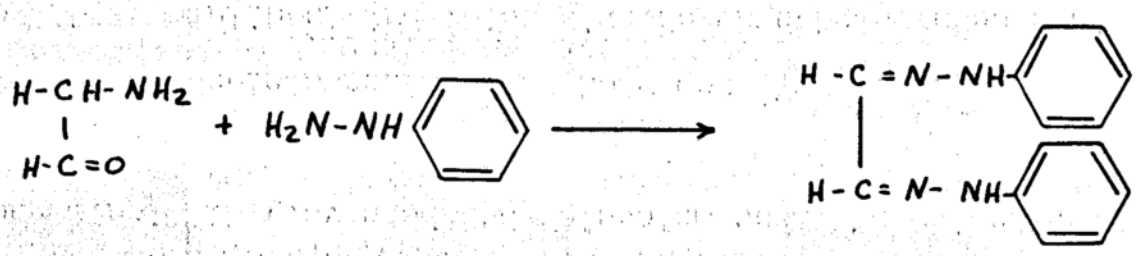
similar to simple peptide chains. These compounds will be pharmacologically evaluated as possible antiviral agents.

DISCUSSION

Many early papers describe the preparation of unsubstituted aminoaldehydes; however, due to the reactive character of the aminoaldehyde molecule the isolation and characterization of these molecules has been very difficult and often impossible.

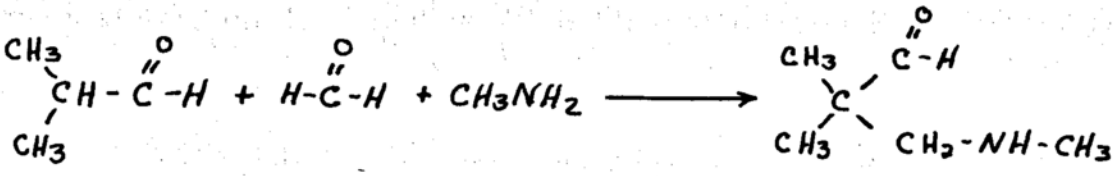
Fischer (6-7) reported that aminoacetaldehyde was stable in concentrated hydrochloric acid, but in dilute acidic or basic solutions the compound was rapidly destroyed. When

attempting to prepare the phenylhydrazone derivative of aminoacetaldehyde, Fischer (6) obtained the diphenylhydrazone of glycollic aldehyde instead of the expected product.



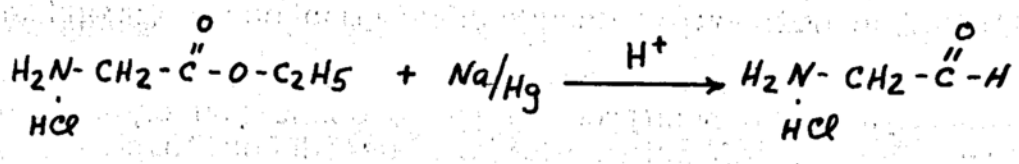
Thus under conditions of osazone formation, the aminoacetaldehyde lost ammonia and was converted to the corresponding hydroxy which was in turn oxidized to the dialdehyde.

A series of secondary and teritary aminoaldehydes was prepared via the Mannich reaction (8). The reaction involved the treatment of an aldehyde containing an alpha hydrogen with formaldehyde and an amine to give a beta substituted



aminoaldehyde. Thus 2-2-dimethyl-3-methylaminopropionaldehyde was prepared by the reaction of formaldehyde and methyl amine with isobutyl aldehyde (8).

Fischer prepared aminoacetaldehyde in low yield by the reduction of glycine ethyl ester hydrochloride with sodium amalgam (9); a method which was independently described by Neuberg (10). Application of this method to dl-alanine and dl-phenylalanine resulted in the preparation of 2-aminopro-



pionaldehyde diethyl acetal and 2-amino-3-phenylpropionaldehyde diethyl acetal respectively (11). The unsubstituted aminoaldehydes were not isolated as such since attempts to hydrolyze the acetal led to polymerization.

Akabori (12) and Bullerwell (13) applied the sodium amalgam reduction reaction to a number of amino acids; however, the resulting aminoaldehydes were not isolated as such but used immediately in the synthesis of imidazole derivatives.

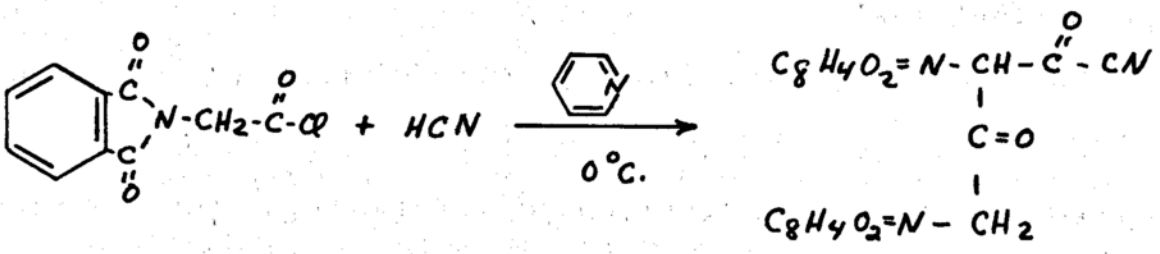
Neuberg (14) claimed the preparation of aminoacetaldehyde from 3-amino-2-hydroxypropionic acid under controlled conditions with an electric spark as a catalyst. The hydroxy acid could also be converted to aminoacetaldehyde by treatment with hydrogen peroxide and iron sulfate (15). Harries obtained the same aminoacetaldehyde by the ozonolysis of allyl amine (16).

In 1901, Wohl (17) obtained the oxalate salt of β-aminopropionaldehyde by the treatment of aminopropionacetal with oxalic acid. Attempts to isolate the free aminoaldehyde were not successful. Wohl was able to obtain the N-ethylaminopropionaldehyde by the treatment of β-chloropropionaldehyde diethyl acetal with ethyl amine followed by acid hydrolysis (18). The N-ethylaminopropionaldehyde was relatively un-

stable and on standing rapidly underwent polymerization. Mannich treated β-chlorobutyraldehyde diacetal with ammonia to form the amino acetal, but hydrolysis to the corresponding free aldehyde failed (19).

In 1922, Radde (20) made a general survey of the various known procedures and possible routes to the synthesis of aminoaldehydes. He modified the known procedures by using acylated amino compounds in an effort to prevent polymerization and thereby facilitate the isolation of the aldehydes.

In an effort to prepare phthalimidopropionyl nitrile, Radde (20) reacted phthalylglycyl chloride with hydrocyanic acid in the presence of pyridine. The nitrile could be converted to the keto acid which could then be made to lose carbon dioxide to give the desired phthalimidoacetaldehyde. However, instead of the expected simple nitrile, phthalylglycyl chloride condensed with itself to give a diketonitrile.

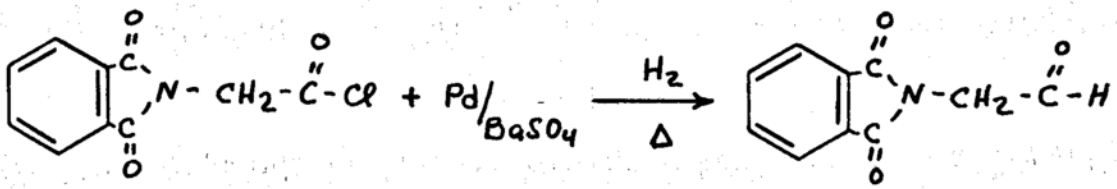


A phthalimidonitrile was obtained by treatment of phthalylglycyl amide with dehydrating agents such as thionyl chloride or phosphorous pentachloride. Attempts to reduce the nitrile to the corresponding aldehyde were not successful (20). The catalytic reduction of substituted amides such as phthalimidoacetyl anilide failed to remove the anilido

group. The phthalimidoacetyl anilide was dehydrated to the imino chloride; however, reduction to the corresponding aldehyde with zinc chloride failed (20).

By using sodium amalgam reduction of the ethyl ester of phthalylglycine according to the procedure of Fischer (9), Radde (20) was able to prepare phthalimidoacetaldehyde, but the yields were not satisfactory. By the controlled reduction of phthalimidoacetyl chloride with palladium on barium sulfate catalyst according to the Rosenmund reduction procedure, Radde was able to obtain phthalimidoacetaldehyde in fair yield. Other than the method of Fischer, this Rosenmund reduction of the acid chloride was the only successful method found by Radde in his general survey of known aldehyde preparative methods or possible routes to the preparation of aminoaldehydes.

According to the procedure of Radde (20), the phthalimidoacetyl chloride was dissolved in a toluene suspension containing palladium on barium sulfate catalyst and a sulfur-quinoline poison. This reaction mixture was then carried through the usual Rosenmund reduction procedure.



After four to five hours, 80-90% of the theoretical amount of hydrogen chloride was evolved. The catalyst was then removed, and the phthalimidoacetaldehyde allowed to

crystalize. The aldehyde was recrystallized from ligroin and melted at 112-114°, but no yield was recorded. The phenylhydrazone, oxime, and semicarbazone derivatives were prepared in the usual manner.

Using the same general procedure, Radde (20), prepared 2-phthalimidopropionaldehyde from 2-phthalimidopropionyl chloride . The semicarbazone derivative of the aldehyde was prepared, but the phenylhydrazone and oxime derivatives could not be obtained as solid derivatives.

In this paper Radde stated that the Rosenmund reduction of phthalimidoacid chlorides was limited to those aldehydes where the phthalimido grouping was in the alpha position to the aldehydo function. In this laboratory this requirement was found not to be necessary in that 3-phthalimidopropionaldehyde was prepared by the reduction of 3-phthalimidopropionyl chloride. However, it is possible that as the chain length between the acid chloride function and the phthalimido group is increased, the difficulty of the reduction reaction may also increase.

In a recent paper, Balenovic (21) discussed the preparation of optically active phthalimidoaldehydes corresponding to the naturally occurring amino acids. Optically active phthalyl-S-benzylcystine and phthalyl-O-methyltyrosine in addition to the phthalylglycine and ∝-alanine were converted to the corresponding aldehydes by the same procedure utilized by Radde (20).

After blocking the aldehyde group of the phthalylamino-

aldehyde as the diethyl acetal, Balenovic (21) was able to remove the phthalyl group by hydrazinolysis. Thus he obtained optically active aminoaldehydes as their diethyl acetals. The corresponding thioacetals were prepared in an analogous manner from the phthalylaminoaldehydes. With cystine and tyrosine, the reactive mercapto and phenolic hydroxy groups respectively had to be blocked before the phthalyl derivatives could be converted to the aldehyde.

In an effort to find a more generally applicable method of preparing aminoaldehydes, a number of possible routes to the aminoaldehydes were investigated in this laboratory. The chief interest was in the development of a preparative method whereby a wide variety of aldehydes could be prepared.

The controlled oxidation of aminoalcohols was first tried as a possible route to the aminoaldehydes. Ethanol amine was treated with benzoyl chloride and sodium carbonate in benzene according to the procedure of Billman (22) to give N-benzoylethanol amine in good yield. The N-benzoylethanol amine was then treated with a mixture of chromic and sulfuric acids in acetic anhydride and glacial acetic acid. By carefully maintaining the temperature between 0-10°, it was hoped that the alcohol would be converted to the aldehyde which would then be rapidly tied up as the diacetate before further oxidation to the acid could occur. Unfortunately, the alcohol was acetylated before the oxidation to the desired aldehyde could occur, thus O-acetyl-N-benzoylethanol amine was obtained instead of the desired

hippuryl aldehyde diacetate.

N-Benzoylethanol amine was also treated with potassium dichromate under acidic conditions in an effort to effect the controlled oxidation to the aldehyde. Even though numerous attempts to control the temperature and the time of reaction were made, the isolated reaction product was either the corresponding N-benzoylated acid, hippuric acid, or the unreacted starting material.

Dehydrogenation of an amino alcohol to the corresponding aldehyde with copper could not be used due to the high boiling points of the N-acetylated aminoalcohols. No method of blocking the amino group of the aminoalcohol could be found which would permit the use of the dehydrogenation reaction.

Another possible route involved the use of the Stephen reaction in the reduction of a nitrile to the corresponding aldehyde. Potassium phthalimide was heated with ethylene dibromide to form phthalimidoethylene bromide (23) which was then to be converted to the nitrile with sodium cyanide in methyl alcohol. The nitrile was not isolated but was immediately treated with the anhydrous stannous chloride in absolute ether to form the imino chloride. Hydrolysis of the imino chloride with dilute hydrochloric acid failed to give the expected phthalimidopropionaldehyde.

A shorter but a rather limited route due to the difficulty in obtaining the starting materials was the reaction between

potassium phthalimide and chlorodimethyl acetal. Under the usual conditions of the reaction of potassium phthalimide with a halide, the chlorodimethyl acetal underwent decomposition. Milder conditions and the use of a solvent failed to give the desired phthalimidoacetaldehyde dimethyl acetal. According to the modified procedure of Ing and Manske (24), phthalimide was heated with a mixture of chlorodimethyl acetal and potassium carbonate, but here again the expected product could not be isolated.

The Rosenmund reduction of benzoylglycine, hippuric acid, was also tried as a possible route to aminoaldehydes. Hippuric acid was treated with phosphorous pentachloride at room temperature to give hippuryl chloride in good yield (25). The hippuryl chloride could then be treated with palladium catalyst and hydrogen according to the usual Rosenmund reduction procedure; however, a suitable solvent could not be obtained. The usual hydrocarbon solvents used in the Rosenmund reaction such as toluene, xylene, and benzene failed due to their inability to dissolve the hippuryl chloride. Other inert solvents such as dioxane exhibited the same lack of solubilizing power.

The reduction of hippuryl chloride was carried out in the polar basic solvent pyridine. A very definite defect in the use of pyridine was that the progress of the reaction could not be followed as is normally done with the Rosenmund reduction, since the basic solvent would readily react with any hydrogen chloride formed during the reaction. The

hippuryl chloride was dissolved in pyridine and heated at reflux with palladium catalyst and sulfur-quinoline poison according to the usual Rosenmund procedure. At the end of four hours, the reaction was stopped and isolation of any aldehyde formed attempted. Various methods of working up the reaction mixture failed to produce any of the desired hippuryl aldehyde, so either the basic solvent at reflux conditions was too severe to permit the isolation of the aminoaldehyde formed or the reduction actually did not take place.

With the failure of other methods to produce the desired aminoaldehydes, the Rosenmund reduction of the phthalimido acid chlorides used by Radde was finally utilized to prepare a series of phthalimidoaldehydes. These phthalimidoaldehydes were then used to prepare various nitrogen aldehyde derivatives.

Instead of discussing the individual preparations of phthalimidoaldehydes, it would probably be better to describe in some detail the general method and reagents used in the conversion of acid chlorides to aldehydes originally developed by Rosenmund and Zetzsche (25). A very complete review of this reaction was written recently by Mossettig and Mozingo (27).

The Rosenmund reduction depends upon differentiation between the speed of replacement of halogen by hydrogen and that of hydrogenation of the resulting aldehyde. The method involves the addition of a small amount of poisoning agent

containing sulfur, which does not seriously inhibit the desired reduction of the acid chloride, but effectively stops the hydrogenation of the aldehyde. The acid chloride

$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}Cl \;+\; Pd/BaSO_4 \xrightarrow[\Delta]{H_2} R\text{-}\overset{O}{\overset{\|}{C}}\text{-}H \;+\; HCl\uparrow$$

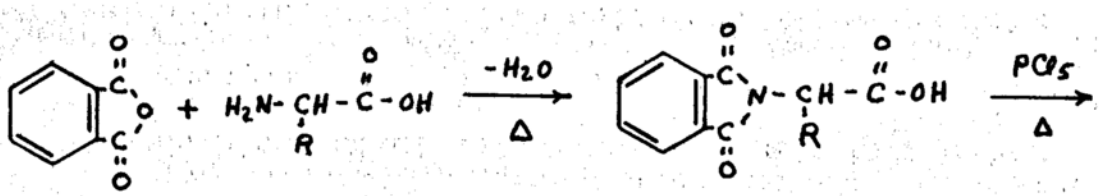
is dissolved in a hydrocarbon solvent such as xylene, toluene, or benzene and a stream of hydrogen passed through the reaction mixture. The exit gas is passed into standard base, thereby the course of the reaction can be followed by the amount of hydrogen chloride absorbed.

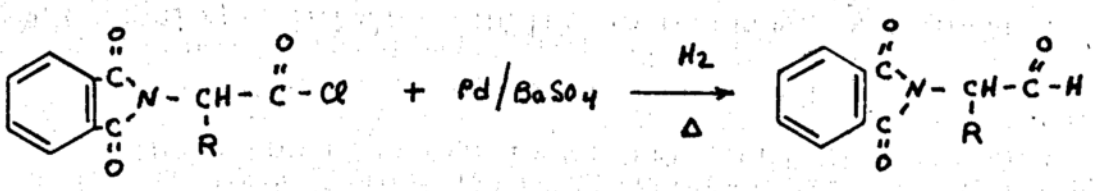
The success of this method is dependent upon the resistance of the aldehyde to reduction under the conditions which permit its formation from the acid chloride (27). Since aldehydes in the presence of hydrogen and a suitable catalyst may readily be reduced to the corresponding alcohol or hydrocarbon, the presence of sulfur containing poisons are necessary for optimal yields of the aldehydes. It has been reported that the deciding factor in the acid chloride reduction is the temperature. The optimal temperature is that point where the evolution of hydrogen chloride is effectively maintained (28).

Just as the presence of an accurate amount of catalyst is essential for optimum yields of aldehyde, the presence of an unknown amount of impurities will affect the ultimate outcome of the reaction. The presence of impurities, especially those containing sulfur, have a definite effect on the con-

version of an acid chloride and as a consequence must be carefully avoided. The presence of phosphorous and mercury also are detrimental to the reduction reaction.

The preparation of the various phthalimidoaldehydes and their derivatives was accomplished according to the same general procedure as that utilized by Radde (20). Phthalic anhydride was heated with the dl-amino acid to form the phthalimido acid which was then converted to the corresponding





acid chloride by heating with phosphorous pentachloride. The phthalimidoacid chloride was reduced to the corresponding aldehyde by heating at reflux in toluene with palladium on barium sulfate catalyst and a sulfur-quinoline poison.

The solvent chosen in the preparation of aminoaldehydes was the same as that used by Radde in his work (20). Reagent grade toluene was refluxed over sodium metal for four hours and then distilled from ground glass equipment. The re-distilled toluene was then stored in a tightly sealed bottle

over sodium. In general ten parts toluene were used with every part of the acid chloride used.

According to the general Rosenmund reduction procedure, one part 5% palladium on barium sulfate catalyst is used with every five to ten parts of the acid chloride (27). When one part catalyst was used with five parts of the phthalimido acid chloride, the reduction seemed to occur at a slow rate, and the purification of the reaction product was more difficult due to the presence of impurities. In general, it was found that one part catalyst to two or three parts of the acid chloride gave the best results.

The 5% palladium on barium sulfate catalyst was prepared according to the procedure of Mozingo (29). Palladium chloride was reduced with formaldehyde in basic solution to give a precipitate of palladium metal on very fine particles of barium sulfate.

The reaction was carried out by dissolving the phthalimido-acid chloride in toluene at room temperature. To this solution was added the palladium catalyst and the sulfur-quinoline poison, and the reaction mixture thoroughly flushed with hydrogen. The reaction mixture was then heated to reflux, after which the rapid stirring was started.

The evolution of hydrogen chloride in the first hour and a half was fairly rapid, after which there was a steady decrease in the rate of evolution. The time of the reaction varied between three and six hours at which time 80-90% of the theoretical amount of hydrogen chloride had been evolved.

Toward the end of the reaction time, the evolution of hydrogen chloride was very slow, so that it was often expedient to stop the reaction before the exit gas was completely free of hydrogen chloride. When the reaction was allowed to proceed longer than five hours, considerable decomposition seemed to occur.

The reduction was carried out in a thoroughly cleaned and dried ground-glass equipment. Wherever rubber stoppers were used, they were thoroughly washed with acetone before using to remove the sulfur-containing compounds on the surface of the rubber. A three-neck ground-glass round-bottom flask equipped with a mercury-seal stirrer was carefully adjusted, so that contamination of the reaction mixture with mercury was avoided. The stirrer was nickel-chromium alloy Hershberg type, which was capable of withstanding long periods of high speed stirring.

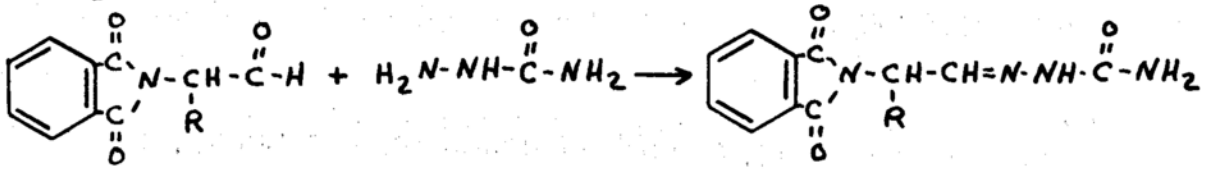
The reflux condenser used in the reduction reaction was attached to a system whereby the exit gas could readily be titrated with standard base. This was accomplished very simply by placing the tube from the condenser beneath the surface of about 400 cc. distilled water contained in a 500 cc. Erlenmeyer flask. In this way the evolved hydrogen chloride dissolved in the water and subsequently could be neutralized by the standard base. The titration of the evolved hydrogen chloride was accomplished by adding a few cc. of standard 1 N sodium hydroxide with phenolphthalein

indicator to the distilled water before the reaction was started. Then as the base was neutralized, varying amounts were added depending on the stage of the reaction.

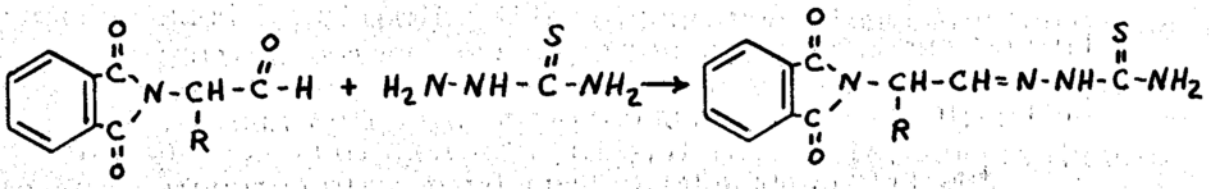
The isolation of the phthalimidoaldehyde was usually accomplished by allowing the toluene reaction solution to cool overnight in the ice box. Further crystallization could be obtained by addition of Skelly C to the toluene mother liquid. In some cases the crystallization of the aldehyde was much more difficult. Where the resulting aldehyde was a very low melting solid, crystallization was achieved by using varying mixtures of benzene and Skelly solvents.

In general as the molecular weight increased, the difficulty of crystallization also increased. The aliphatic aldehyde such as 2-phthalimido-3-methylbutyraldehyde and 2-phthalimidoisocaproaldehyde could not be obtained as crystalline solids. However, their aldehyde derivatives were obtained as fine crystalline solids. The phthalimidoaldehydes did not form sodium bisulfite derivatives and cleavage of the other aldehyde derivatives was not a practical method of obtaining the free aldehyde.

The semicarbazone of the phthalimidoaldehydes were prepared in the usual manner. The aldehyde was dissolved in an aqueous-alcoholic solution and heated at reflux with semi-



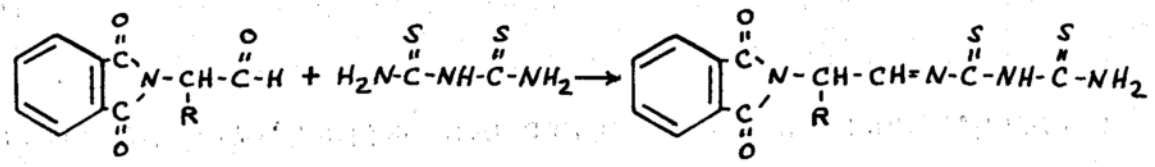
carbazide hydrochloride and sodium acetate for about one to two hours. The semicarbazone was then recrystallized from alcohol and water. The thiosemicarbazones were prepared in an analogous manner. The aldehyde was heated at reflux with



a molar quantity of thiosemicarbazide and one drop of glacial acetic acid to increase the rate of reaction (30). The thiosemicarbazones were obtained as crystalline solids which crystallized from a mixture of alcohol and water.

With 2-phthalimido-3-butyraldehyde and 2-phthalimido-isocaproaldehyde, the reaction product obtained from the reduction was a crude oil. This oil was used as such in the preparation of the thiosemicarbazone and semicarbazone derivatives.

The condensation of dithiobiuret with the phthalimidoaldehydes was effected in an analogous manner to that used by Foye and Hefferren (31) in the preparation of benzaldithiobiurets. The dithiobiuret was dissolved in the least amount of boiling glacial acetic acid. To this was added the phthalimidoaldehydes, and the mixture heated at reflux for about thirteen hours. The addition of a small amount of freshly prepared anhydrous zinc chloride to the reaction mixture seemed to favor the condensation reaction.



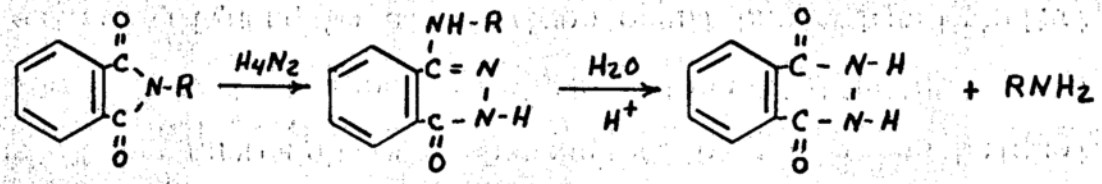
After allowing the reaction to proceed at reflux for thirteen hours, the reaction mixture was allowed to stand at room temperature for a couple of days for crystallization. The precipitated product was washed thoroughly with anhydrous ether to remove some of the occluded zinc chloride and then crystallized from glacial acetic acid. This crystallization seemed to take place best when the crystallization mixture was allowed to freeze and then slowly warm up to room temperature. After recrystallization the dithiobiuret condensation product was obtained in yields from 15-25% usually as microcrystalline products. The percentage nitrogen was determined by semimicro Kjeldahl method.

The phenylhydrazone of phthalimidoacetaldehyde was prepared in the usual manner and recrystallized from a mixture of ethyl alcohol and glacial acetic acid. The orange needles of the phenylhydrazone melted at 165-166° which agreed with the value recorded by Radde (20).

The same phenylhydrazone of phthalimidoacetaldehyde was obtained when the cleavage of the phthalyl ring was attempted according to the procedure of Schumann and Boissnnas (32). Phthalimidoacetaldehyde was heated at reflux with tributyl amine and three equivalents of phenylhydrazine in 96% ethyl alcohol for two hours. The solution was then concentrated to give a bright orange precipitate melting at 163-165°. A mixed melting point showed no depression with phthalimidoacetaldehyde phenylhydrazone, indicating phenylhydrazone formation but no cleavage. Identical results were

obtained when larger quantities of phenylhydrazine and longer periods of reflux were used.

Another more generally used method for phthalyl cleavage is that of hydrazinolysis. The original work was done by Ing in 1926 (24), but since then there have been numerous variations which are useful in a variety of situations (33-35).



The general procedure is to heat the phthalyl derivative with hydrazine in alcohol at reflux for a couple of hours. The alcohol is then removed, and the residue is heated for a short time with dilute acid.

According to the general procedure of Ing (24), the phthalylacetaldehyde phenylhydrazone was heated for two hours with hydrazine hydrate in an alcoholic solution. The alcohol was then removed, and the deep red residue heated with 2 N hydrochloric acid for about 15 minutes. During this period of heating, there seemed to be considerable decomposition. The treatment with hydrazine apparently accomplished the phthalyl cleavage, but the acid decomposition of the residue seemed to effect the cleavage of the aldehyde derivative. In order to avoid this cleavage of the aldehyde derivative, milder methods of acidification will have to be developed. There is little doubt, that after a suffi-

cient number of trials these conditions could be developed, if they are so desired.

Another possibility for the difficulty in addition to the lability to acid is the interchange of aldehyde derivatives. Thus the phenylhydrazone would be formed first and then a partial exchange might occur to give the phthalimidoaldehyde hydrazine derivative.

Phthalylmethionine was prepared and treated with phosphorous pentachloride in the usual manner. The finely ground mixture of phthalylmethionine and phosphorous pentachloride was vigorously shaken at room temperature, whereby there was a rapid evolution of hydrogen chloride. When this initial evolution of hydrogen chloride had slowed, the reaction mixture was heated on a steam bath for forty-five minutes. During this period of heating, there was an apparent decomposition occurring as evidenced by the reaction mixture turning deep red. Attempts to crystallize the acid chloride from this deep red viscous mass were unsuccessful.

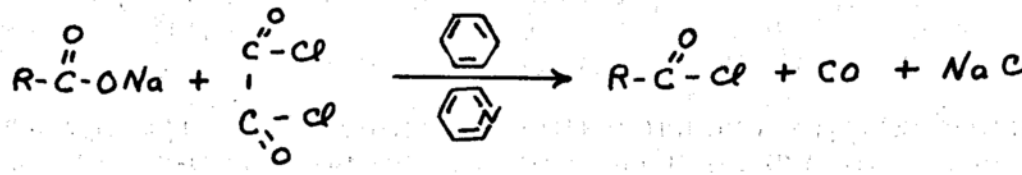
Phosphorous pentachloride was used under milder conditions, but here again there appeared to be some decomposition. In an effort to obtain a more easily isolatable product, the crude acid chloride was reduced according to the usual Rosenmund procedure. However, on completion of the reduction reaction only a very small amount of hydrogen chloride was evolved. The reduction mixture gave a negative aldehyde test with 2, 4-dinitrophehylhydrazine aldehyde reagent, indicating that little or no reduction to the alde-

hyde had occurred.

The use of thionyl chloride in the formation of the acid chloride was tried, but the results were similar to those obtained with phosphorous pentachloride; i.e., the acid chloride reaction mixture gradually turned deep red as heat was applied or the mixture was allowed to stand at room temperature.

A mild procedure for the preparation of acid chlorides developed by Adams (36) involves the treatment of the sodium salt of the acid with oxalyl chloride. This general reaction was applied to the steroid field by Wilds (37), who was able to form the acid chloride of a labile acid under very mild conditions.

Essentially the method consists of making the sodium salt by dissolving the acid in 0.1 N sodium hydroxide and lyopholizing the solution of the sodium salt. The sodium salt is then dried at 100° and 2 mm. of mercury pressure for ten hours. The sodium salt is suspended in dry benzene with a few drops of dry pyridine, and the suspension cooled to zero degrees. Redistilled oxalyl chloride is then added with the immediate evolution of carbon monoxide indicating



the start of the reaction. The reaction is allowed to proceed at reduced temperatures until the evolution of the carbon monoxide gas ceases. The solvents are then removed

under reduced pressure, and the acid chloride obtained in good yields. The reaction is very mild; however, it is very dependent on completely anhydrous conditions.

This reaction was repeated using phthalylmethionine as the acid. The sodium salt was prepared in the usual manner and freeze dried. The sodium salt was then allowed to react with oxalyl chloride under the conditions described by Wilds (37). After removal of the solvents, the acid chloride was obtained as a very light yellow oil which was used as such for conversion to the aldehyde.

The crude acid chloride, obtained from the oxalyl chloride reaction, was dissolved in toluene and reduced with palladium in the usual manner. When the reaction system was set up, hydrogen was passed through while the solution was refluxing freely. The evolution of hydrogen chloride was very slow. With the possibility that the sulfur atom contained in the phthalylmethionine molecule was present in too large a quantity, a rerun was made where the amount of the palladium catalyst was increased to one part catalyst to every one and a half parts acid chloride. In addition no sulfur-quinoline poison was added to the reaction system. In spite of the fact that the reaction was allowed to proceed for as long as twelve hours, the evolution of hydrogen chloride was approximately 30% of the theoretical value. It was also impossible to obtain a positive aldehyde test from the toluene reaction mixture, indicating that any aldehyde which may have been formed dur-

ing the reaction was not present at the time the reaction was stopped.

As has been discussed previously, the Rosenmund reduction of acid chlorides to aldehydes is accomplished by controlling the activity of a palladium on barium sulfate catalyst by adding a small amount of sulfur poison or regulator. The reduction of a sulfur-containing compound may be accomplished, but is very difficult even under ideal circumstances (27). In the conversion of phthalylmethionine acid chloride to the corresponding aldehyde, there is present a sulfur-containing molecule in addition to a very reactive acid chloride which may readily undergo an internal reaction. This internal reaction was indicated by the relative lability of the acid chloride to heating in the presence of reagents such as phosphorous pentachloride and thionyl chloride. In view of these things, it is probable that the phthalylmethionine acid chloride was destroyed before conversion to the corresponding aldehyde could be accomplished.

Billman (35) prepared phthalylvaline by heating equal molar amounts of phthalic anhydride with dl-valine for fifteen minutes at 180°. After recrystallization from a mixture of ethyl alcohol and water, the phthalylvaline was obtained in 54% yield and melted at 101.5-102°.

Finely ground phthalic anhydride and dl-valine were heated for forty-five minutes at 130-150°, followed by fifteen additional minutes at 180° to complete the reaction.

The reaction was well stirred to prevent local overheating, and all the sublimed phthalic anhydride collecting on the sides of the flask was scraped back down into the reaction mixture. The reaction mixture was allowed to cool, and recrystallization from water, alcohol, and mixtures of the two attempted without success.

Since the phthalylvaline precipitated from varying mixtures of water and alcohol as an oil, it appeared that some impurity was preventing crystallization. Thus the product obtained from heating phthalic anhydride and dl-valine was sublimed under reduced pressure and then extracted with water to remove the unreacted phthalic anhydride and dl-valine respectively. Attempts to crystallize this purified product were equally unsuccessful as the previous case in obtaining a crystalline product.

King and Kidd (33) developed a method of preparing phthalylglutamic acid which was claimed to give a purer condensation product than that obtained by just heating to phthalic anhydride and dl-glutamic acid according to the procedure of Billman (38). This method consisted in heating the phthalic anhydride and dl-glutamic acid in dry pyridine at reflux for two hours. The pyridine was then removed under reduced pressure, and the residue heated with acetic anhydride for a few minutes. After removal of the acetic anhydride under reduced pressure, the phthalylglutamic anhydride was obtained in 74% yield. The phthalylglutamic anhydride was then readily converted to the free acid by warming with

water.

Thus according to an analogous procedure, phthalic anhydride was refluxed with dl-valine in pyridine for two hours. The pyridine was removed under reduced pressure, and the residue heated with acetic anhydride for a few minutes. Removal of the acetic anhydride gave a white semi-solid product very much similar to that obtained from the Billman procedure. Attempts to crystallize the product were not successful.

In an effort to find a recrystallization solvent for phthalylvaline, it was found that the compound was readily soluble in anhydrous ether. This presented itself as a satisfactory means of purification, since both phthalic anhydride and dl-valine exhibit only limited ether solubility.

Thus the phthalylvaline was prepared according to the ordinary procedure used for the other phthalyl amino acids. The finely ground mixture of the phthalic anhydride and dl-valine were heated for forty-five minutes at 130-150°, followed by fifteen minutes at 180°. The residue was allowed to cool, and the phthalylvaline extracted from the unreacted starting materials with anhydrous ether. Attempts to further purify the phthalylvaline by recrystallization were not successful. The phthalylvaline prepared via the ether extraction method was obtained in 85% yield and melted at 100-102° which agreed with the value reported by Billman (38).

EXPERIMENTAL

I. Catalyst and Regulator.

A. 5% Palladium on Barium Sulfate (29).

A solution of 8.2 g. (0.046 mole) of palladium chloride was dissolved in 20 cc. of concentrated hydrochloric acid and 50 cc. of distilled water by heating on a steam bath for about two hours. To a rapidly stirred solution of 126.2 g. (0.4 mole) of reagent grade barium hydroxide octahydrate in 1.2 liters of water heated to 80° was added all at once 120 cc. (0.36 mole) of 6 N. sulfuric acid. More 6 N. sulfuric acid was added to make the suspension just acid to litmus.

To this hot suspension of barium sulfate was added the hot solution of palladium chloride and 8 cc. of 37% aqueous formaldehyde solution. The suspension was made slightly alkaline to litmus with 30% sodium hydroxide solution, just causing the reduction of the palladium chloride to metallic palladium which precipitated on the finely divided particles of barium sulfate. The black suspension was stirred for an additional five minutes after the addition of the base and then allowed to settle. The clear supernatant liquid was decanted and replaced with distilled water, and the catalyst resuspended. The catalyst was washed by decantation in this manner ten times. After the final de-

cantation, the catalyst was collected on a 90 mm. sinter glass funnel and washed with 250 cc. of distilled water in five portions. The funnel and cake of catalyst were then placed in an oven at 80° for thorough drying. After powdering 95 g. of the 5% palladium on barium sulfate catalyst was obtained.

B. Quinoline-Sulfur Poison (39).

In a round bottom flask, 6 g. of finely ground sulfur was refluxed with 6 g. of freshly distilled quinoline. After cooling the mixture was diluted with purified xylene to 70 cc. This solution contained 0.1 g. of regulator per cc. and could be used as such or diluted to appropriate concentrations.

II. Phthalimidoacetaldehyde and Derivatives.

A. dl-Phthalylglycine.

In a beaker, 14.8 g. (0.01 mole) of phthalic anhydride and 6.1 g. (0.1 mole) of dl-glycine were finely ground and intimately mixed. The mixture was then slowly heated with stirring until the temperature gradually rose to 130-150°, where it was held constant for about thirty minutes. During this time of heating, there was a vigorous evolution of steam and sublimination of phthalic anhydride. The sublimined phthalic anhydride collecting on the sides of the beaker was scraped down into the reaction mixture.

After heating at 130-150° for thirty minutes, the tem-

perature was raised to approximately 180° for fifteen minutes. The reaction mixture was then allowed to harden slowly, after which it was recrystallized from hot water. After recrystallization 16.6 g. (83%) of phthalylglycine was obtained as needles which melted at 194-196°. The melting point agreed with the recorded value (40).

B. Phthalimidoacetyl Chloride.

In a Claisen flask, 7.4 g. (0.037 mole) of Phthalylglycine and 7.7 g. (0.037 mole) of phosphorous pentachloride were vigorously shaken for about fifteen minutes at room temperature, during which time there was a rapid evolution of hydrogen chloride. After the evolution of hydrogen chloride ceased, the reaction mixture was heated on a steam bath for forty-five minutes. With heating, the mixture turned to a light yellow liquid. The phosphorous oxychlorides were removed under reduced pressure with slight heating. The residue was recrystallized from Skelly E to give 7.2 g. (92%) of the phthalylacetyl chloride in form of needles. The m.p., 82-83° agreed with the reported value (41).

C. Phthalimidoacetaldehyde.

In the carefully dried apparatus previously described, 23.5 g. (0.11 mole) of phthalimidoacetyl chloride was dissolved in about 100 cc. of purified toluene. To this was added 12.2 g. of palladium catalyst and 1.2 cc. of sulfur-quinoline poison. The reaction system was thoroughly flushed with hydrogen, and then heated to reflux temperature. After

the solvent was vigorously refluxing, the stirring was started. The evolved gasses were passed through distilled water containing a known amount of standard base.

In the first hour and a half, the reaction progressed rapidly and then proceeded at a slowly decreasing rate. At the end of four hours, 85% of the theoretical amount of hydrogen chloride was evolved. At this point, Norite was added to the reaction mixture in order to facilitate the removal of the spent catalyst. The reaction mixture was filtered and placed in the ice box overnight for crystallization. The addition of a little Skelly E aided the further precipitation of the product from the mother liquid. The phthalimidoacetaldehyde was recrystallized from a mixture of benzene and Skelly solvents. After recrystallization, 15.7 g. (76%) of phthalimidoacetaldehyde was obtained. The m.p., 112-114° agreed with the recorded value (20).

D. Phthalimidoacetaldehyde phenylhydrazone

In a 125 cc. Erlenmeyer flask, 2.5 g. (0.018 mole) of phenylhydrazine hydrochloride was dissolved in 30 cc. of water with 3.75 g. of sodium acetate. The mixture was decolorized with charcoal, and 3.4 g. (0.018 mole) of phthalimidoacetaldehyde in 35 cc. of an aqueous-alcoholic solution was added. The resulting yellow suspension was vigorously shaken and heated for half an hour, after which it was set aside for crystallization. After recrystallization from ethyl alcohol-glacial acetic acid mixture, 3.3 g.

(60%) of phthalimidoacetaldehyde phenylhydrazone was obtained as needles melting at 163-165° which agreed with the reported value.

E. Phthalimidoacetaldehyde semicarbazone.

In a 125 cc. Erlenmeyer flask, 3.4 g. (0.018 mole) of phthalimidoacetaldehyde was dissolved in approximately 30 cc. of ethyl alcohol, and then water was added until there resulted a slight cloudiness. To this solution was added 2.1 g. (0.019 mole) of semicarbazide hydrochloride and 3.2 g. of sodium acetate. The mixture was heated on a steam-bath for one hour, during which time a voluminous precipitate formed. After recrystallization from a mixture of ethyl alcohol and water, 3.8 g. (85%) of the semicarbazone was obtained as needles which melted at 224-226°. The reported value is 233-244° (20).

F. Phthalimidoacetaldehyde thiosemicarbazone.

In a 125 cc. Erlenmeyer flask, 3.2 g. (0.017 mole) of phthalylimidoacetaldehyde was dissolved in about 70 cc. of an aqueous-alcoholic mixture. To this was added 1.6 g. (0.018 mole) of thiosemicarbazide, and the resulting mixture heated on a steam-bath for one hour. After about fifteen minutes of heating, a voluminous white precipitate appeared. The reaction mixture was cooled overnight, and the white precipitate removed by suction filtration. After recrystallization from an aqueous-alcoholic mixture, 3.5 g. (81%) of the phthalimidoacetaldehyde thiosemicarbazone was obtained

melting at 215-216°.

Anal. - Calcd. for $C_{11}H_{10}N_4O_2S$: N. 21.36

Found: N. 21.92

G. Phthalimidoethylidine dithiobiuret.

In a 250 cc. round-bottom flask, 3.4 g. (0.025 mole) of dithiobiuret was dissolved in the minimum amount of boiling glacial acetic acid. To this was added 5.6 g. (0.03 mole) of phthalimidoacetaldehyde and approximately 0.5 g. of freshly prepared anhydrous zinc chloride, and the resulting mixture was heated at reflux for thirteen hours. With heating, the reaction mixture gradually took on a dark red appearance.

After heating at reflux for thirteen hours, the reaction mixture was reduced to two-thirds its original volume by heating and directing a current of air over the solution. The red solution was allowed to stand at room temperature for two or more days in order to achieve complete precipitation. The yellow precipitate was then removed by suction filtration, washed with anhydrous ether, and recrystallized from glacial acetic acid. After recrystallization, 0.6 g. (15%) of the phthalimidoethylidine dithiobiuret was obtained as a microcrystalline product melting at 250-251°.

Anal. - Calcd. for $C_{12}H_{10}N_4O_2S_2$: N 18.37

Found: N 18.10, 18.48 Aver. 18.29

III. 2-Phthalimidopropionaldehyde and Derivatives.

A. dl-α-Phthalylalanine.

In a 125-cc. beaker, 32.6 g. (0.22 mole) of finely ground phthalic anhydride was thoroughly mixed with 19.6 g. (0.22 mole) of dl-α-alanine. The mixture was gradually heated on a hot plate to 130-150° for thirty minutes. During this time there was a rapid evolution of steam and some sublimination of phthalic anhydride on the walls of the beaker. The reaction mixture was stirred and the phthalic anhydride scraped from the walls of the beaker into the reaction mixture. After this initial heating period, the temperature was increased to approximately 180° for fifteen minutes. The residue was allowed to cool and then was recrystallized from hot water. After recrystallization, 42.6 g. (94%) of the phthalylalanine was obtained as needles melting at 162-163°, which agreed with the recorded value (42).

B. 2-Phthalimidopropionyl chloride.

In a 50-cc. Claisen flask, 5.0 g. (0.023 mole) of 2-phthalimidopropionic acid was vigorously shaken with 4.8 g. (0.023 mole) of phosphorous pentachloride. After the initial rapid evolution of hydrogen chloride ceased, the mixture was heated on a steam bath for about forty-five minutes to complete the reaction. The phosphorous oxychlorides were then removed under reduced pressure with slight heating. The residue was recrystallized from Skelly E, yielding 4.6 g. (85%) of 2-phthalimidopropionyl chloride melting at 71-73°, which agreed with the recorded value (43).

C. 2-Phthalimidopropionaldehyde.

The reduction of 2-phthalimidopropionyl chloride to the corresponding aldehyde was carried out in an analogous manner to that used in the preparation of phthalimidoacetaldehyde. The general apparatus and procedure was essentially identical.

In a 250-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer, hydrogen tap, and reflux condenser with attached tubing for titration of the evolved gases, 4.6 g. (0.02 mole) of 2-phthalimidopropionyl chloride was dissolved in 40 cc. of purified toluene. To this was added 2.3 g. of palladium catalyst and 0.23 cc. of sulfur-quinoline poison. The system was flushed with hydrogen and heated to reflux. After the solvent was refluxing vigorously, the stirrer was started.

The evolution of hydrogen chloride during the first hour was rapid, but followed by a gradual decrease in the rate. After four hours of heating and stirring at reflux, there was only a minimal amount of hydrogen chloride being evolved. Norite was added to the reaction mixture, and the solution was filtered. Skelly E was added to the toluene solution which was put in the ice box overnight for crystallization. After recrystallization from a mixture of benzene and Skelly E, 1.6 g. (60%) of 2-phthalimidopropionaldehyde was obtained. The aldehyde melted at 109-111° which agreed with the recorded value (20).

D. 2-Phthalimidopropionaldehyde semicarbazone.

In a 125-cc. Erlenmeyer flask 2.6 g. (0.013 mole) of 2-phthalimidopropionaldehyde was dissolved in an aqueous-alcohol solution. To this was added 1.6 g. (0.014 mole) of semicarbazide hydrochloride and 2.4 g. of sodium acetate, and the resulting mixture was heated on a steam-bath for an hour and a half. After recrystallization from an ethyl alcohol-water mixture, 2.9 g. (85%) of the semicarbazone was obtained melting at 224-225°, which agreed with the recorded value (20).

E. 2-Phthalimidopropionaldehyde thiosemicarbazone.

In a 200-cc. round-bottom flask, 3.2 g. (0.016 mole) of 2-phthalimidopropionaldehyde was dissolved in about 50 cc. of ethyl alcohol, and water was added until a slight cloudiness appeared. To this solution was added 1.5 g. (0.016 mole) of thiosemicarbazide and one drop of glacial acetic acid. The resulting solution was heated at reflux for one hour, during which time a white precipitate began forming. After allowing the reaction mixture to stand in the ice box overnight, the white precipitate was removed by suction filtration and recrystallized from a mixture of alcohol and water. After recrystallization, 2.6 g. (60%) of the 2-phthalimidopropionaldehyde thiosemicarbazone was obtained as needles melting at 206-208°.

Anal. - Calcd. for $C_{12}H_{12}N_4O_2S$: N 20.28

Found: N 19.99

F. 2-Phthalimidopropylidine dithiobiuret.

In a 200-cc. round-bottom flask, 4.3 g. (0.032 mole) of dithiobiuret was dissolved in about 70 cc. of boiling glacial acetic acid. To this solution was added 8.2 g. (0.04 mole) of 2-phthalimidopropionaldehyde and approximately 0.5 g. of freshly prepared anhydrous zinc chloride. The reaction mixture was then allowed to react at reflux for thirteen hours. With heating the solution gradually took on a red color. After heating at reflux, the reaction mixture was allowed to stand at room temperature for at least two days in order to complete precipitation of the condensation product. The yellow precipitate was removed from the solution by suction filtration and washed with anhydrous ether. After recrystallization from glacial acetic acid, 2.0 g. (20%) of the 2-phthalimidopropylidine dithiobiuret was obtained as a microcrystalline product, melting at 251-253°.

Anal. - Calcd. for $C_{13}H_{12}N_4O_2S_2$: N 17.49

Found: N 17.29 17.16

IV. 3-Phthalimidopropionaldehyde and Derivatives.

A. dl-β-Phthalylalanine.

In a 125-cc. beaker, 22.2 g. (0.15 mole) of finely ground phthalic anhydride and 13.4 g. (0.15 mole) of dl-β-alanine were thoroughly mixed and gradually heated to about 150°. At this temperature there was a rapid evolution of steam, and some sublimination of phthalic anhydride. The sublimed phthalic anhydride was scraped from the walls of the beaker back into the reaction mixture, which was continually

stirred. After about forty-five minutes of heating at 150°, the temperature was increased to about 180° in order to complete the reaction. The liquid was allowed to solidify, and the solid residue crystallized from hot water. After recrystallization, 29.7 g. (91%) of the phthalylalanine was obtained in the form of needles melting at 150-151°. The melting point agreed with the recorded value (42).

B. 3-Phthalimidopropionyl chloride.

In a 50-cc. Claisen flask, 6.6 g. (0.03 mole) of 3-phthalimidopropionic acid was vigorously shaken with 6.25 g. (0.03 mole) of phosphorous pentachloride until the initial rapid evolution of hydrogen chloride ceased. The semi-solid mixture was then heated on a steam bath for about forty-five minutes in order to complete the reaction. The volatile phosphorous oxychlorides were removed under reduced pressure with slight heating. The residual material was then recrystallized from Skelly E to give 21 g. (91%) of the 3-phthalimidopropionyl chloride. The observed melting point of 107-108° agreed with the observed value (44).

C. 3-Phthalimidopropionaldehyde.

In a 250-cc. three-neck ground-glass round-bottom flask, equipped with a mercury-seal stirrer, hydrogen tap, and a reflux condenser with attached tubing to permit the titration of the evolved gases, 6.6 g. (0.028 mole) of 3-phthalimidopropionyl chloride was dissolved in about 60 cc. of purified toluene. To this was added 3.5 g. of palladium catalyst and

0.3 cc. of sulfur-quinoline poison. The system was flushed with hydrogen and heated to reflux temperature at which point stirring was started.

The evolution of hydrogen chloride in the first hour was relatively rapid after which it gradually decreased. After four hours 90% of the theoretical amount of hydrogen chloride evolved. Norite was then added to the reaction mixture in order to facilitate the recovery of the catalyst, and the solution filtered. The toluene solution was diluted with Skelly E and placed in the ice box for crystallization. After recrystallization from a benzene-Skelly E mixture, 4.0 g. (70%) of the 3-phthalimidopropionaldehyde was obtained. The aldehyde melted at 115-117°.

Anal. - Calcd. for $C_{11}H_9NO_3$: C 65.01 H 4.46

Found: C 65.21 H 4.79

D. 3-Phthalimidopropionaldehyde semicarbazone.

In a 125-cc. Erlenmeyer flask, 3.4 g. (0.017 mole) of 3-phthalimidopropionaldehyde was dissolved in 70 cc. of an aqueous-alcoholic solution. To this was added 1.9 g. (0.017 mole) of semicarbazide hydrochloride and 2.8 g. of sodium acetate, and the resulting solution heated on a steam bath for one hour. There was an almost immediate reaction with the formation of a fine white precipitate. After recrystallization from a mixture of ethyl alcohol and water, 2.9 g. (66%) of the 3-phthalimidopropionaldehyde semicarbazone was obtained as needles melting at 219-220°.

Anal.-Calcd. for $C_{12}H_{12}N_4O_3$: N 21.53

Found: N 21.30

E. 3-Phthalimidopropionaldehyde thiosemicarbazone.

In a 125-cc. Erlenmeyer flask, 3.6 g. (0.018 mole) of 3-phthalimidopropionaldehyde was dissolved in 70 cc. of an aqueous-alcoholic solution. To this was added 1.64 g. (0.018 mole) of thiosemicarbazone and one drop of glacial acetic acid, and the resulting solution heated on a steam bath for two hours. There was an immediate reaction with the formation of a white precipitate. After recrystallization from a mixture of ethyl alcohol and water 3.5 g. (70%) of the thiosemicarbazone was obtained, melting at 211-212°.

Anal. - Calcd. for $C_{12}H_{12}N_4O_2S_2$: N 20.28

Found: N 19.67

F. 3-Phthalimidopropylidine dithiobiuret.

In a 200 cc. round-bottom flask, 4.8 g. of dithiobiuret was dissolved in the minimum amount of boiling glacial acetic acid. To this hot solution was added 10.0 g. (0.05 mole) of 3-phthalimidopropionaldehyde and about 0.3 g. of freshly prepared anhydrous zinc chloride. The reaction mixture was heated at reflux for thirteen hours, and then the volume reduced to two-thirds its original size. The red solution was then allowed to stand a few days at room temperature in order to complete crystallization. The yellow precipitate was removed by suction filtration, thoroughly washed with anhydrous ether, and recrystallized from glacial acetic acid. After recrystallization, 2.2 g. (20%) of the very light yellow 3-phthalimidopropylidine dithiobiuret was obtained as

a microcrystalline compound melting at 243-244°.

Anal. - Calcd. for $C_{13}H_{12}N_4O_2S_2$: N 17.49

Found: N 17.26, 16.94, 16.98

V. 2-Phthalimido-3-phenylpropionaldehyde and Derivatives.

A. dl-phthalylphenylalanine.

In a 50 cc. beaker, 2.9 g. (0.02 mole) of finely ground phthalic anhydride and 3.3 g. (0.02 mole) of dl-phenylalanine were thoroughly mixed and gradually heated to 130-150°. With heating the mixture liquified and rapidly gave off steam. The sublimed phthalic anhydride was scraped from the walls of the beaker back into the reaction mixture. After heating for about 30 minutes at 130-150°, the temperature of the mixture was raised to approximately 180° for fifteen minutes. The reaction mixture was then allowed to solidify. After recrystallization from a mixture of ethyl alcohol and water, 5.3 g. (90%) of phthalylphenylalanine was obtained as needles, melting at 175-177° which agreed with the recorded value (45).

B. 2-Phthalimido-3-phenylpropionyl chloride.

In a 125-cc. Claisen flask, 28.0 g. (0.095 mole) of 2-phthalimido-3-phenylpropionic acid and 19.8 g. (0.095 mole) of phosphorous pentachloride were vigorously shaken at room temperature until the initial evolution of hydrogen chloride ceased. The semisolid mixture was then heated on a steam bath for approximately thirty minutes during which time the reaction mixture became a clear yellow liquid. The volatile phosphorous oxychlorides were then removed under reduced

pressure with slight heating leaving the acid chloride as a solid residue. After recrystallization from a mixture of benzene and Skelly E, 24.0 g. (83%) of the 2-phthalimido-3-phenylpropionyl chloride was obtained as needles, melting at 132-134°, which agreed with the recorded value (45).

C. 2-Phthalimido-3-phenylpropionaldehyde.

In a 250-cc. three-neck ground-glass round-bottom flask equipped with a mercury-seal stirrer, hydrogen tap, and a reflux condenser with attached tubing permitting the titration of the evolved gases, 13.3 g. (0.042 mole) of 2-phthalimido-3-phenylpropionyl chloride was dissolved in 100 cc. of purified toluene. To this solution was added 6.7 g. of palladium catalyst and 0.6 cc. of sulfur-quinoline poison. The system was thoroughly flushed with hydrogen and then heated to reflux temperature. When the solvent was freely refluxing, stirring was started, and the evolved gases titrated with standard base.

The hydrogen chloride formed in the reaction was evolved rapidly in the first hour, after which it gradually decreased. At the end of four and a half hours, 85% of the theoretical amount of hydrogen chloride had been evolved. Norite was then added, and the hot solution filtered. The solution was diluted with Skelly E and placed in the ice box for crystallization. The 2-phthalimido-3-phenylpropionaldehyde was recrystallized from a mixture of benzene and Skelly E to give 7.2 g. (60%) of the aldehyde, melting at 219-221°.

Anal. - Calcd. for: $C_{17}H_{13}NO_3$: C 73.11 H 4.69
Found: C 73.44 H 4.91

D. 2-Phthalimido-3-phenylpropionaldehyde semicarbazone.

In a 125-cc. Erlenmeyer flask, 3.6 g. (0.013 mole) of 2-phthalimido-3-phenylpropionaldehyde was dissolved in 40 cc. of alcohol, and water added until a slight cloudiness appeared. To this solution was added 1.5 g. (0.013 mole) of semicarbazide hydrochloride and 2.2 g. of sodium acetate, and the mixture heated on a steam bath for one hour. During this period of heating, a voluminous white precipitate quickly formed. After allowing the reaction mixture to cool in the ice box overnight, the white precipitate was removed by suction filtration and recrystallized from a mixture of alcohol and water. After crystallization, 3.8 g. (88%) of the semicarbazone was obtained as needles, melting at 215-216°.

Anal. - Calcd. for: $C_{18}H_{16}N_4O_3$: N 16.66
Found: N 16.52

E. 2-Phthalimido-3-phenylpropionaldehyde thiosemicarbazone.

In a 125-cc. Erlenmeyer flask, 2.8 g. (0.01 mole) of 2-phthalimido-3-phenylpropionaldehyde was dissolved in about 30 cc. of alcohol and water added until there was a slight cloudiness. To this solution was added 0.9 g. (0.01 mole) of thiosemicarbazide and one drop of glacial acetic acid, and the mixture heated at reflux for two hours. Unlike the previous aldehyde derivatives, there was little or no precipitate formed during the heating period; however, a voluminous white precipitate was obtained on cooling. After re-

crystallization from an aqueous-alcoholic solution, 2.9 g. (85%) of the thiosemicarbazone was obtained as needles, melting at 204-205°.

Anal. - Calcd. for $C_{18}H_{16}N_4O_2S$: N 15.90

Found: N 15.13

F. 2-Phthalimido-3-phenylpropylidine dithiobiuret.

In a 200-cc. round-bottom flask, 4.0 g. (0.03 mole) of dithiobiuret was dissolved in a minimum amount of boiling glacial acetic acid. To this hot solution was added 10.1 g. (0.036 mole) of 2-phthalimido-3-phenylpropionaldehyde and about 0.3 g. of freshly prepared anhydrous zinc chloride. The reaction was then allowed to procede for thirteen hours at reflux temperature, during which time the solution gradually turned red. After heating at reflux overnight, the reaction mixture was reduced to two-thirds its original volume and allowed to stand at room temperature for at least two days. The yellow precipitate was removed by suction filtration, washed thoroughly with anhydrous ether, and recrystallized from glacial acetic acid. After recrystallization, 2.3 g. (20%) of the dithiobiuret condensation product was obtained as a microcrystalline compound, melting at 256-258°.

Anal. - Calcd. for $C_{19}H_{16}N_4O_2S_2$: N 14.13

Found: N 13.63

VI. 2-Phthalimidoisocaproaldehyde and Derivatives.

A. dl-Phthalylleucine.

In a 250-cc. beaker, 34.0 g. (0.23 mole) of finely

ground phthalic anhydride and 30.0 g. (0.23 mole) of dl-leucine were gradually heated on a hot plate to 130-150°, for thirty minutes. At this temperature, there was a rapid evolution of steam, and some sublimination of unreacted phthalic anhydride. The phthalic anhydride collecting on the walls of the beaker was scraped back into the reaction mixture. After this period of heating at 130°, the temperature was increased to 180° for fifteen minutes in order to complete the reaction. The liquid reaction mixture was then allowed to solidify and recrystallized from a mixture of alcohol and water. After recrystallization 53.1 g. (88%) of the phthalylleucine was obtained as needles, melting at 142-144° which agreed with the recorded value (46).

B. 2-Phthalimidoisocaproyl chloride.

In a 125-cc. Claisen flask, 10.0 g. (0.038 mole) of phthalylleucine was vigorously shaken at room temperature with 7.9 g. (0.038 mole) of phosphorous pentachloride. After the initial evolution of hydrogen chloride lessened, the reaction mixture was heated on a steam bath for about forty-five minutes. The volatile phosphorous oxychlorides were then removed under reduced pressure leaving a viscous yellow mass. Attempts to recrystallize the crude 2-phthalimido-isocaproyl chloride failed, so the acid chloride was used as such for the conversion to the aldehyde.

C. 2-Phthalimidoisocaproaldehyde.

In a 250-cc. three-neck ground-glass round-bottom flask

equipped with a mercury-seal stirrer, hydrogen tap, and reflux condenser with attached tubing permitting the titration of the evolved gases, the crude acid chloride obtained from 0.038 mole of phthalylleucine was dissolved in about 80 cc. of purified toluene. To this solution was added 4.0 g. of palladium catalyst had 0.4 cc. of sulfur-quinoline poison, system was thoroughly flushed with hydrogen and then heated to reflux temperature. After the solvent was refluxing freely, the stirrer was started.

After four hours, approximately 80% of the theoretical amount of hydrogen chloride was evolved. Norite was then added, and the solution filtered. Attempts to crystallize the 2-phthalimidoisocaproaldehyde were not successful, so the crude aldehyde was used in the preparation of derivatives.

D. 2-Phthalimidoisocaproaldehyde semicarbazone.

In a 125-cc. Erlenmeyer flask, 2.6 g. (0.0107 mole) of 2-phthalimidoisocaproaldehyde was dissolved in 30 cc. of ethyl alcohol, and water added until a slight cloudiness appeared. To this solution was added 1.18 g. (0.0107 mole) of semicarbazide hydrochloride and 1.8 g. of sodium acetate, and the mixture heated on a steam bath for about one hour and a half. After cooling the reaction mixture in the ice box overnight, a voluminous white precipitate of the semicarbazone was formed. Recrystallization from an aqueous-alcoholic solution gave 2.9 g. (90%) of 2-phthalimidoisocaproaldehyde

semicarbazone melting at 200-201°.

Anal. - Calcd. for $C_{15}H_{18}N_4O_3$: N 18.53
Found: N 18.28

E. 2-Phthalimidoisocaproaldehyde thiosemicarbazone.

In a 125-cc. Erlenmeyer flask, 2.5 g. (0.01 mole) of 2-phthalimidoisocaproaldehyde was dissolved in 25 cc. of alcohol, and water added until a slight cloudiness appeared. To this hot solution was added 1.0 g. (0.01 mole) of thiosemicarbazide and one drop of glacial acetic acid, and the mixture heated at reflux for two hours. At the end of this period of heating, there was little or no precipitate formed. Cooling in the ice box overnight resulted in a voluminous precipitate which was removed by suction filtration and recrystallized from a mixture of alcohol and water. After recrystallization 2.1 g. (65%) of the 2-phthalimidoisocaproaldehyde thiosemicarbazone was obtained as needles, melting at 190-191°.

Anal. - Calcd. for $C_{15}H_{18}N_4O_2S$: N 17.60
Found: N 17.27

F. 2-Phthalimidoisocaproylidine dithiobiuret.

In a 150-cc. beaker, 3.1 g. (0.03 mole) of dithiobiuret was dissolved in about 50 cc. of boiling glacial acetic acid. This solution was then heated at reflux in a 200 cc. round-bottom flask with 8.0 g. (0.033 mole) of 2-phthalimidoisocaproaldehyde and about 0.4 g. of freshly prepared anhydrous zinc chloride. With heating the solution took on a

characteristic red color. After heating about thirteen hours, the solution was reduced to approximately two-thirds its original size and allowed to stand for a couple of days at room temperature for crystallization. The yellow dithiobiuret condensation product was removed by suction filtration and thoroughly washed with anhydrous ether. After recrystallization from glacial acetic acid, 2.0 g. (18%) of the 2-phthalimidoisocaproylidine dithiobiuret was obtained as a very light yellow microcrystalline compound melting at 258-260°.

Anal. - Calcd. for $C_{16}H_{16}N_4O_2S_2$: N 15.54
Found: 15.07, 15.36, 14.86

VII. 2-Phthalimido-3-methylbutyraldehyde and Derivatives.

A. dl-Phthalylvaline.

In a 100-cc. beaker, 14.8 g. (0.10 mole) of finely ground phthalic anhydride and 11.7 g. (0.10 mole) of dl-valine were intimately mixed and gradually heated on a hot plate to between 130-150° for forty-five minutes. During this period of heating the reaction was kept well stirred, and the sublimed phthalic anhydride collecting along the sides of the beaker was scraped back down into the reaction mixture. After heating at 130-150° the temperature of the reaction mixture was elevated to approximately 180° for twenty minutes. On cooling the mixture solidified into a clear plastic-like mass from which the phthalylvaline was extracted with anhydrous ether. On removal of the ether,

20.9 g. (85%) of the phthalylvaline was obtained as a white solid mass, melting at 99-101° which agreed with the recorded value (38).

B. 2-Phthalimido-3-methylbutyryl chloride.

In a 50-cc. Claisen flask, 4.9 g. (0.02 mole) of 2-phthalimido-3-methylbutyric acid was finely ground and vigorously shaken with 4.2 g. (0.02 mole) of phosphorous pentachloride. There was an immediate and rapid evolution of hydrogen chloride. After this initial evolution of gas, the semisolid mixture was heated on a steam bath for forty-five minutes which gave a clear light yellow liquid. The volatile oxychlorides were removed from the acid chloride by distillation under reduced pressure with very slight heating. Attempts to isolate the 2-phthalimido-3-methylbutyryl chloride as a crystalline solid were not successful, so the acid chloride was used as such for the reduction reaction. About 15 cc. of thiophene-free dry benzene was added twice and removed by vacuum distilled to insure a more complete removal of the volatile phosphorous oxychloride impurities.

The use of thionyl chloride as a reagent in the formation of the acid chloride was found to be equally advantageous. A three molar excess of thionyl chloride was used. Particular care had to be exercised in removing all of the excess thionyl chloride.

C. 2-Phthalimido-3-methylbutyraldehyde.

In a 250-cc. round-bottom three-neck ground-glass flask equipped with mercury-seal stirrer, hydrogen tap, and reflux condenser with attached tubing permitting the titration of the evolved gasses, the crude 2-phthalimido-3-methylbutyryl chloride obtained from 0.02 mole of the acid was dissolved in 35 cc. of purified toluene. To this solution was added 2.6 g. of palladium catalyst and 0.2 cc. of sulfur-quinoline poison. The reaction mixture was thoroughly flushed with hydrogen and then heated to reflux temperature. When the solvent was freely refluxing, the stirrer was started.

The evolution of hydrogen chloride in the first hour and a half was very rapid, after which the rate of evolution slowly decreased. At the end of four hours, the evolution of hydrogen chloride was proceeding at a very slow but constant rate. In order to avert excessive decomposition of the aldehyde already formed, the reaction was stopped. Norite was added to the toluene solution, and the palladium catalyst together with the occluded charcoal was removed by filtration. The 2-phthalimido-3-methylbutyraldehyde gave no signs to crystallizing from solvents used, so the aldehyde as a light yellow viscous oil was used as such for the preparation of derivatives.

D. 2-Phthalimido-3-methylbutyraldehyde semicarbazone.

In a 125-cc. Erlenmeyer flask, 4.3 g. (0.019 mole) of 2-phthalimido-3-methylbutyraldehyde was dissolved in about

40 cc. of hot alcohol, and water was added to the solution until a slight cloudiness appeared. To this solution was added 2.1 g. (0.019 mole) of semicarbazide hydrochloride and 3.1 g. of sodium acetate, and the mixture heated on a steam bath for ninety minutes. After cooling in the ice-box overnight, the voluminous precipitate was removed by suction filtration and recrystallized from a mixture of alcohol and water. After recrystallization 3.2 g. (60%) of the 2-phthalimido-3-methylbutyraldehyde semicarbazone was obtained in the form of needles, melting at 228-229°.

Anal. - Calcd. for $C_{14}H_{16}N_4O_3$: N 19.44

Found: N 18.67

E. 2-Phthalimido-3-methylbutyraldehyde thiosemicarbazone.

In a 125-cc. Erlenmeyer flask, 4.1 g. (0.018 mole) of 2-phthalimido-3-methylbutyraldehyde was dissolved in 60 cc. of an aqueous alcoholic solution. To this solution was added 1.6 g. (0.018 mole) of thiosemicarbazide, and the mixture heated at reflux for two hours. One drop of glacial acetic acid was added as a catalyst. After allowing the reaction to stand in the ice box overnight, the thiosemicarbazone was removed by suction filtration and recrystallized from a mixture of alcohol and water. After recrystallization 3.4 g. (64%) of the 2-phthalimido-3-methylbutyraldehyde thiosemicarbazone was obtained as gleaming white crystals, melting at 214-215°.

Anal. - Calcd. for $C_{14}H_{16}N_4O_2S$: N 18.41

Found: N 17.21

F. 2-Phthalimido-3-methylbutyrylidine dithiobiuret.

In a 150-cc. beaker, 2.4 g. (0.018 mole) of dithiobiuret was dissolved in about 40 cc. of boiling glacial acetic acid. To this hot solution was added 4.4 g. (0.022 mole) of 2-phthalimido-3-methylbutyraldehyde and about 0.3 g. of freshly prepared anhydrous zinc chloride. The mixture was heated in a 200-cc. round-bottom flask for thirteen hours, after which time the volume was reduced to two-thirds its original size. On standing for a few days, a light yellow precipitate appeared. After recrystallization from glacial acetic acid, 0.8 g. (14%) of 2-phthalimido-3-methylbutyrylidine dithiobiuret was obtained in the form of fine needles, melting at 237-239°.

Anal. - Calcd. for $C_{15}H_{16}N_4O_2S_2$: N 16.08

Found: N 15.75

BIBLIOGRAPHY

1. Neuberg, C., Ber. 41, 959 (1908).
2. Delbruck, Cold Spring Harbor Symposia Quant. Biol. 9, 122 (1941).
3. Brown, G. C., J. Immunol 69, 441 (1952).
4. Eaton, M. D., Proc. Soc. Exptl. Biol. Med. 77, 422 (1951).
5. Thompson, R. L., et al., J. Immunol. 70, 229 (1953).
6. Fischer, Emil, Ber. 26, 94 (1893).
7. Fischer, Emil, Ber. 27, 171 (1894).
8. Mannich, C. et al., Ber. 65, 378 (1932).
9. Fischer, Emil, Ber. 41, 1021 (1908).
10. Neuberg, C., Ber. 41, 959 (1908).
11. Fischer, E. and Kamataka, Ann. 365, 7 (1909).
12. Alkabori, Shiro, Ber. 66, 151 (1933).
13. Bullerwell and Lawson, J. Chem. Soc. 3030 (1951).
14. Neuberg, C. Bio. Z. 17, 277.
15. Neuberg, C. Bio. Z. 20, 532.
16. Harries, C. and Reichard, P. Ber. 37, 613 (1904).
17. Wohl, A. Ber. 34, 1917 (1901).
18. Wohl, A. Ber. 38, 4172 (1905).
19. Mannich, C. Arch. Pharm. 264, 167 (1926).
20. Radde, Erich, Ber. 55, 3174 (1922).
21. Balenovic, K. et al. J. Org. Chem. 18, 297 (1953).
22. Billman, J. H. et al. J. Amer. Chem. Soc. 65, 2455 (1943).

23. Gabriel, S. Ber. 22, 1137 (1889).

24. Ing, H. R. and Manske, F. J., J. Chem. Soc. 2348 (1926).

25. Fischer, Emil, Ber. 38, 612 (1905).

26. Rosenmund and Zetzsche, Ber. 54, 425 (1921).

27. Mosettig, E. and Mozingo, R. Organic Reactions, Vol. IV, John Wiley and Sons, N. Y., 1948.

28. Boehm, et al. Arch. Pharm. 271, 490 (1933).

29. Mozingo, R. Org. Syntheses, 26, 77 (1946).

30. Campaigne, E. et al., J. Amer. Chem. Soc. 75, 988 (1953).

31. Foye, W. O. and Hefferren, J. J., J. Amer. Pharm. Assoc. 42, 31 (1953).

32. Schumann, I. and Boissnnas, B. A. Nature 169, 154 (1952).

33. King, F. E. and Kidd, A. E., J. Chem. Soc. 3315 (1949).

34. Sheehan, J. C., J. Amer. Chem. Soc. 71, 1856 (1949).

35. Grassman, Ber. 83, 244 (1950).

36. Adams, Roger, J. Amer. Chem. Soc. 42, 605 (1920).

37. Wilds, A. et al., J. Amer. Chem. Soc. 70, 2427 (1948).

38. Billman, J. H. et al., J. Amer. Chem. Soc. 70, 1443 (1948).

39. Hershberg and Cason, Org. Syntheses, 21, 84 (1941).

40. Reese, Ann. 242, 1

41. Gabriel, S. Ber. 40, 2648 (1907).

42. Gabriel, S. Ber. 38, 634 (1905).

43. Gabriel, S. Ber. 41, 247 (1908).

44. Gabriel, S. Ber. 41, 243 (1908).

45. Pfaehler, E., Ber. 46, 1701 (1913).

46. Ulrich, A., Ber. 37, 1695 (1904).

PART III

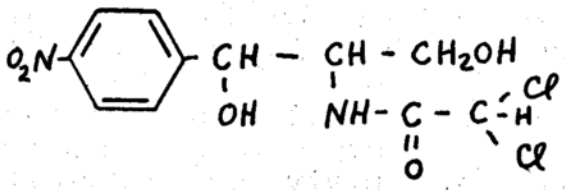
AMIDES OF 5-NITRO-2-THENOIC ACID

PART III

AMIDES OF 5-NITRO-2-THENOIC ACID

INTRODUCTION

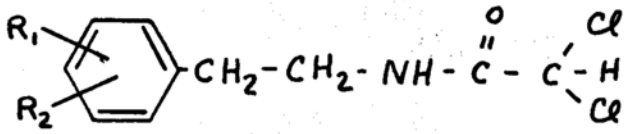
The discovery of the broad spectra antibiotic chloroamphenicol (I) and the subsequent identification of its structure has initiated many efforts to develop suitable chemotherapeutic agents (1, 2). The relatively simple



(I)

chemical structure of this naturally occurring antibiotic, which is characterized by a p-nitrophenyl and dichloroacetamide moeities, has been an extraordinary model for possible chemotherapeutic agents.

Cutler and coworkers (3) prepared a series of sulfur analogs of chloroamphenicol in which the para nitro group was replaced by alkylmercapto and sulfonyl groupings. In the work of Phillips and his coworkers (4), the dihydroxyaminopropyl side chain of chloroamphenicol was replaced with an ethylamino chain, and the benzene ring was substituted with alkyl, alkoxy, and nitro groups (II).

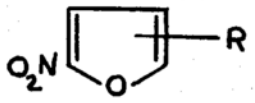


(II)

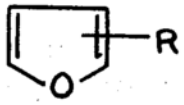
R_1 - H, alkyl, or alkoxy

R_2 - nitro or alkoxy

In the comparison of the therapeutic effect of a series of furan derivatives, Dodd and Stillman (5) found that the presence of a nitro group in the 5-position of the furan ring conferred considerable bacteriostatic activity as compared with the non-nitro derivative. The specificity of this nitro group in activating these simple furan compounds was indicated very vividly by a comparison of the nitrofurans (III) with their non-nitrated analogs (IV). The nitro analogs in all cases investigated were considerably more active.



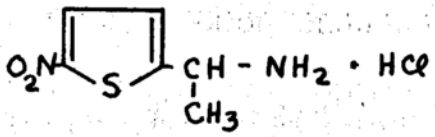
(III)



(IV)

In a study of aromatic nitro compounds, Johnson (6) found that 5-nitro-2-thiophenecarboxylic acid and the corresponding unsubstituted amide were highly active therapeutic agents against Streptococcus hemolyticus. In this activity they compared favorably with the sulfonamides. On the other hand, the analogous 5-amino-2-thiophenecarboxyamide was in-

active. Carrara (7) in a study of nitrothiophene derivatives showed that 1(5-nitro-2-thenyl)-ethylamine hydrochloride (V) was active against Shigella dysenteriae.



(V)

In 1949, Dann (8) postulated that microorganisms could be specifically arrested by a nitro compound, if there existed in the organism a minimal concentration of a monomolecular reduction product between the nitro and the amino stage, probably a HONH compound, present over a sufficient period of time. Thus according to this theory, the activity of a nitro compound would depend upon the nature of the carbon atom which carries the nitro group. Since the nitro group at an unsaturated carbon atom is reducible at a relatively negative potential, and aromatic reduction products would be stabilized by resonance, aromatic nitro compounds should exhibit therapeutic activity. This was demonstrated by the fact that nitromethane, nitroethane, and tetranitromethane do not specifically arrest the growth of microorganisms, whereas nitrothiophene and nitrofuran derivatives exhibit definite activity.

The purpose of this research project is to prepare a series of 5-nitro-2-thiophenecarboxylic acid substituted amides for pharmacological evaluation in an effort to find more active antiviral agents.

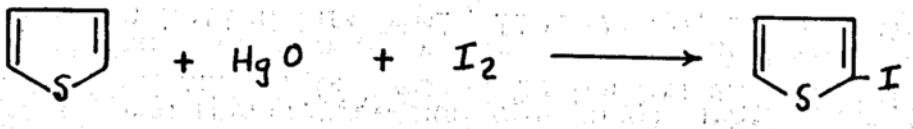
DISCUSSION

In 1932, Rinkes reported the preparation of 5-nitro-2-thiophenecarboxylic acid by the direct nitration of 2-thiophenecarboxylic acid in acetic anhydride with concentrated nitric acid (9). No yields were recorded; however, the isolation of the 4-nitro-2-thiophenecarboxylic acid as well as the 5-isomer was described in the paper. This isolation and separation of the two nitrothiophenecarboxylic acids was accomplished by the selective precipitation of their barium salts. It might be expected from this, that the reaction would be of questionable use as a preparative method for 5-nitro-2-thiophenecarboxylic acid.

Somewhat later Dann (10) described the stepwise preparation of 5-nitro-2-thiophenecarboxylic acid starting from 2-iodothiophene. The iodothiophene was nitrated with concentrated nitric acid to give 5-nitro-2-thiophene which was then converted to the corresponding nitrile by treatment with cuprous cyanide. The 5-nitro-2-cyano-thiophene was hydrolyzed with concentrated hydrochloric acid to give the desired 5-nitro-2-thiophenecarboxylic acid in an overall yield of 33% based on the 2-iodothiophene. This method was used in our laboratory for the preparation of the nitroacid.

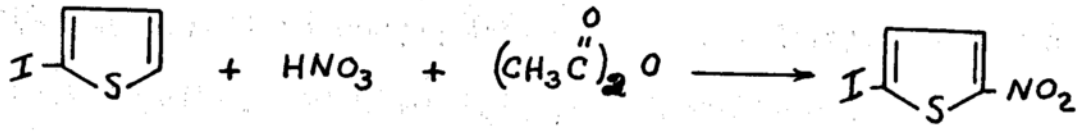
2-Iodothiophene was prepared by the iodination of thiophene according to the procedure of Minnis (11). The thiophene was dissolved in anhydrous benzene and vigorously

shaken at room temperature with yellow mercuric oxide and



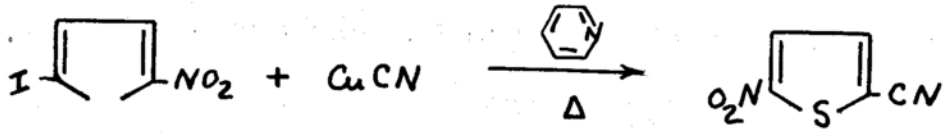
iodine. The crux of the iodination reaction rested on the vigorous shaking, for it was this shaking which allowed the thiophene to come into intimate contact with the suspended inorganic salts. After separation of the 2, 5-diiodothiophene by vacuum distillation, the 2-iodothiophene was obtained in yields between 50-60%.

5-Nitro-2-iodothiophene was prepared by the nitration of 2-iodothiophene with concentrated nitric acid in the cold according to the procedure of Dann (10). The concentrated nitric acid was added to acetic anhydride at 0° to form the



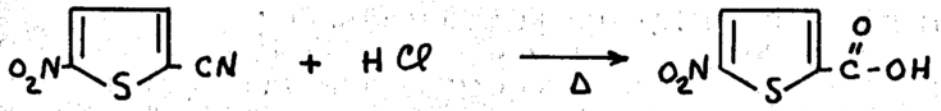
acetylnitro complex. The 2-iodothiophene was nitrated in this mixture at -10°. After recrystallization from absolute methanol, the 5-nitro-2-iodothiophene was obtained as light yellow needles in yields between 60-65%.

The conversion of the 5-nitro-2-iodothiophene to the corresponding nitrile was accomplished by treatment of the

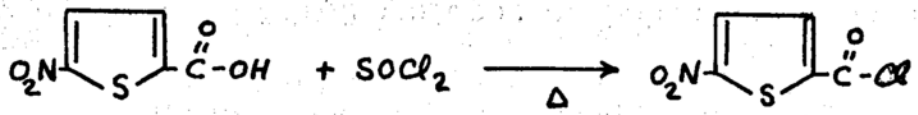


nitroiodothiophene with cuprous cyanide in pyridine. After

removal of the inorganic salts, the 5-nitro-2-cyanothiophene was obtained by vacuum distillation. The cyano derivative was immediately refluxed with concentrated hydrochloric acid for two hours. After recrystallization from hot water, the 5-nitro-2-thiophenecarboxylic acid was obtained as light yellow needles in yields varying between 40-53%, based on the 5-nitro-2-iodothiophene.



The 5-nitro-2-thiophenecarboxylic acid was converted in quantitative yield to the 5-nitro-2-thiophenecarboxylic acid chloride according to the procedure of Dann (10). The 5-nitro-2-thiophenecarboxylic acid was heated at reflux for

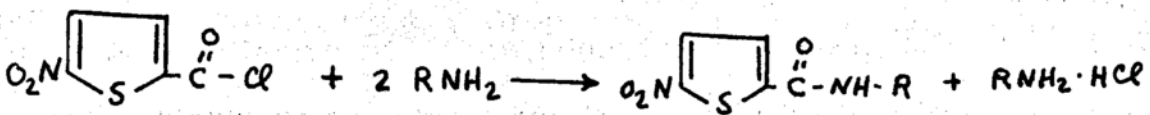


one hour with a five molar excess of thionyl chloride. The light yellow reaction mixture was poured into a large evaporating dish, and a steady stream of dry air blown over the solution, thus removing the excess thionyl chloride. The nitroacid chloride was fairly susceptible to moisture, so that the acid chloride was immediately taken up in dry benzene after removal of the thionyl chloride.

In his preparation of the 5-nitro-2-thiophenecarboxylic acid chloride, Dann (10) was able to isolate the nitroacid chloride in 99% yield. This nearly quantitative conversion

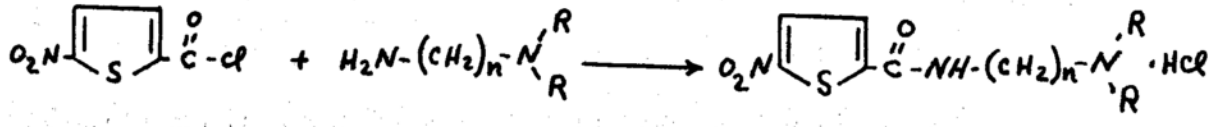
to the acid chloride from 5-nitro-2-thiophenecarboxylic acid was found to be essentially true, so that in the preparation of amide derivatives of the acid, the acid chloride was not isolated as such but was reacted immediately with the corresponding amino compound. The amino compound was used in an equivalent molar quantity based on the original 5-nitro-2-thiophenecarboxylic acid. The susceptibility of the 5-nitro-2-thiophenecarboxylic acid chloride to moisture was another factor justifying the immediate use of the acid chloride without isolation and purification. In the preparation of all the amide derivatives of 5-nitro-2-thiophenecarboxylic acid, the acid chloride was prepared in the same manner.

The method of preparation of the amides on 5-nitro-2-thiophenecarboxylic acid in this series may be divided into four general types. The first group includes the amide formation utilizing the very reactive amino compounds such as allyl amine, β-diethylaminoethylamine, piperidine, morpholine, N-ethylpiperazine, pyrollidine, and benzyl amine. When these amines were added to a benzene solution of 5-nitro-2-thiophenecarboxylic acid chloride, there was an immediate reaction with the evolution of heat and the formation of a voluminous precipitate of the amine hydrochloride. In all cases except those of β-diethylaminoethylamine and N-ethylpiperazine a second molar quantity of the amine was added to pick up the hydrogen chloride formed in the reaction. The precipitate of the amine hydrochloride was not removed



by filtration, but was dissolved by the addition of chloroform to the original benzene solution. The hydrochloride was then removed by extraction with water, just avoiding any loss of nitrothiophene amide which may be occluded on the precipitate. In addition this water extraction method presented a convenient method of removing any unreacted starting material which may have been present.

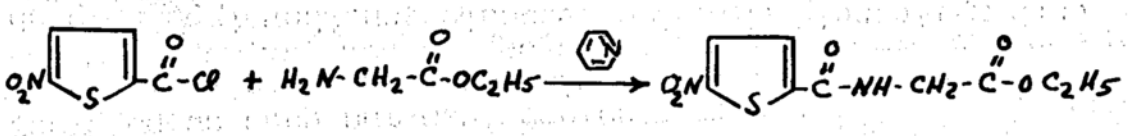
In the preparation of β-diethylaminoethylamine and N-ethylpiperazineamides, a second mole of the amine was not added to the reaction mixture to pick up the hydrogen chloride formed in the reaction. Since these amino compounds



contain two amino functions, a primary and teritary, the addition of a second mole of the amine to the reaction mixture was not necessary. In the two above cases, the 5-nitro-2-thiophenecarboxylic amide was isolated as the amine hydrochloride, since the teritary amino function took up the mole of hydrogen chloride formed in the reaction.

The reaction of an amino acid with 5-nitro-2-thiophenecarboxylic acid chloride presented another type of problem.

In the reaction of the amino acid ester hydrochloride of



glycine, the problem was in finding of the conditions which favored amide formation over that diketopiperazine formation, which is characteristic of amino acid esters.

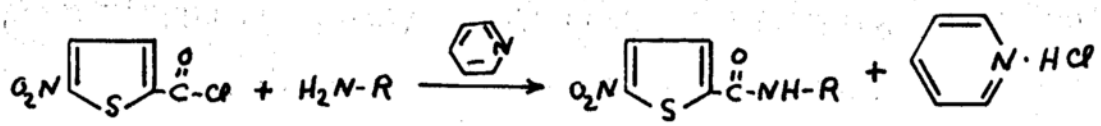
The best conditions for carrying out the reaction were found to be the addition at room temperature of glycine ethyl ester hydrochloride to a benzene solution of the nitro-acid chloride containing two equivalents of pyridine. One equivalent of base was used to release the amino acid ester hydrochloride, and one was used to pick up the hydrogen chloride formed in the reaction. After the addition of the amino acid ester hydrochloride at room temperature, the reaction mixture was gradually heated to 50° where it was held for thirty minutes. During this period of heating, the reaction mixture took on a purple cast, which apparently indicated that some diketopiperazine formation was taking place.

After allowing the reaction mixture to slowly return to room temperature, the benzene solution was diluted to twice its volume with Skelly B causing the precipitation of the glycylamide. Recrystallization from a mixture of alcohol and water gave the 5-nitro-2-thiophenecarboxyglycylamide in 42% yield.

The general type of amide formation was that of the

nitroacid chloride with slightly basic amines such as 2-aminothiazole, 2-aminopyridine, m-bromoaniline, m-nitroaniline, and p-nitroaniline. The addition of a molar quantity of pyridine and varying periods of heating at reflux in benzene were required. Due to the relative insolubility of these amino compounds in benzene, the reaction had to be carried out as a suspension.

The relatively insoluble amino compound was added to a benzene solution of the 5-nitro-2-thiophenecarboxy acid chloride, and the well stirred mixture heated at reflux. As the reaction proceeded, the very insoluble 5-nitro-2-thiophenecarboxylic acid amide was formed and precipitated from the solution together with the pyridine hydrochloride formed in the reaction. This precipitate was thoroughly washed succes-



sively with water, dilute acid, and again with water to remove the pyridine hydrochloride and any starting material which might be present.

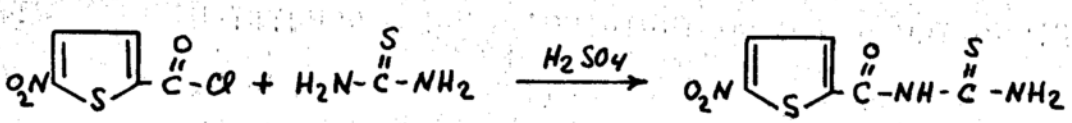
In the reaction with 2-aminopyridine, it was found that the addition of a molar quantity of pyridine or triethyl amine gave a mixture of hydrochlorides including that of N-(2'pyridino)-5-nitro-2-thiophenecarboxyamide hydrochloride. By extending the time of reaction, it was found that the reaction proceeded fairly well without the addition of a

second amine specifically to pick up the hydrogen formed in the reaction. However, it was found that even without the addition of a second amine a mixture of the amide hydrochloride and the free amide, so the hydrochloride salt present had to be neutralized with base. Recrystallization from ethyl alcohol gave the desired N-(2'-Pyridino)-5-nitro-2-thiophenecarboxyamide in good yield.

The third type of the preparation was characterized by the formation of 5-nitro-2-thiophenecarboxyureides. This was accomplished by heating a well stirred suspension of the urea compound with the nitro acid chloride in benzene with one drop of concentrated sulfuric acid. Since the amino groups of urea type molecule are only slightly basic, the addition of acid and prolonged periods of heating were necessary for amide formation.

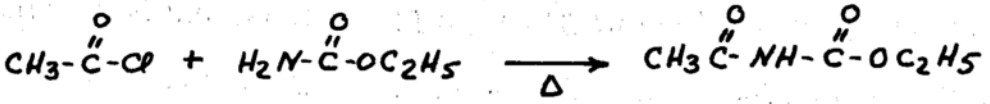
In the preparation of 5-nitro-2-thiophenecarboxythioureide, it was anticipated that the prolonged heating of the sulfur-containing compound under acidic conditions would effect the loss of sulfur, so milder conditions were first utilized. The nitroacid chloride was refluxed with thiourea in benzene with one drop of sulfuric acid for two hours; however, under these conditions only a small amount of the nitrothiophene-thioureide could be isolated. Under conditions used with some of the less basic amino compounds such as the substituted anilines; i.e., refluxing in benzene with a molar equivalent of pyridine, failed to effect the desired amide formation.

With the failure of these two milder procedures, the method analogous to the preparation of the normal ureide was utilized. The thiourea was heated at reflux with the 5-nitro-2-thiophenecarboxylic acid chloride in benzene containing one drop of concentrated sulfuric acid. The reaction mixture was

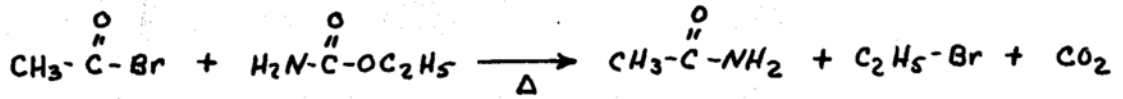


refluxed for eight hours and then allowed to stir at room temperature overnight. The 5-nitro-2-thiophenecarboxy-thioureide was recrystallized from an aqueous-alcoholic solution in 60% yield.

The preparation of 5-nitro-2-thiophenecarboxyurethan was attempted by the treatment of 5-nitro-2-thiophenecarboxylic acid chloride with urethan under a variety of conditions. In 1951, Ben-Ishai and Katchalski (12) prepared N-acylatedurethans by reacting acetyl chloride with a variety of substituted and unsubstituted urethans. The method consisted of just



heating for a few hours the urethan derivative with acetyl chloride as a reactant and solvent. Under the same conditions, acetyl bromide reacted with N,N-disubstituted, N-substituted, and substituted urethans to give a bromide, acetamide derivative, and carbon dioxide.

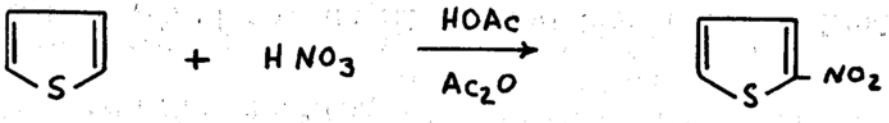


In the use of 5-nitro-2-thiophenecarboxylic acid chloride as an acylating agent, unlike acetyl chloride, the nitroacid chloride is a solid compound of relatively high melting point. Thus the simple heating of the two reactants is not feasible, and the utilization of an appropriate solvent is necessary. In addition it is necessary to contend with the fact that urethan spontaneously undergoes decomposition when heated above its melting point of approximately 50°.

With these facts in mind, ether was first tried as a solvent. The reaction was first run at room temperature with equimolar amounts of the urethan, nitroacid chloride, and pyridine dissolved in anhydrous ether at room temperature. Although the reaction mixture was allowed to stand at room temperature for as long as a week, there was no apparent reaction. Refluxing the ether solution with an excess of urethan was also tried with similar results. Benzene was tried as a solvent with an excess of the urethan to allow for that destroyed during the period of heating. In spite of refluxing the benzene solution for as long as ten hours, none of the N-acylated product could be isolated. Here is a reaction which may proceed as expected, if the appropriate reaction conditions are developed.

In another phase of this work under nitrothiophene derivatives analogous to simple peptides, 5-nitro-2-thiophene-aldehyde was the basic compound from which a variety of derivatives were to be made. Since at this time the synthesis of this aldehyde was not reported in the literature, the development of a method of preparation was undertaken.

The first possible method involved the introduction of a chloromethyl group in the free alpha position of 2-nitrothiophene. 2-Nitrothiophene was prepared by the nitration of thiophene with fuming nitric acid in a mixture of acetic anhydride and glacial acetic acid according to the procedure of Babasinian (14). In three-neck flask equipped



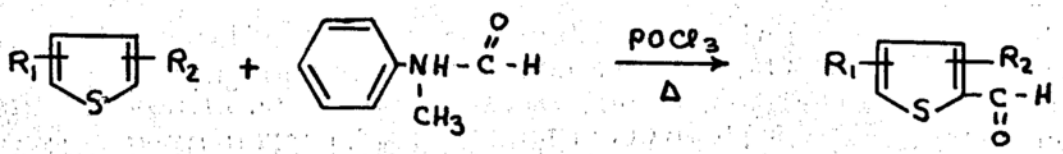
with a mechanical stirrer and cooled in an ice bath, fuming nitric acid was added to glacial acetic acid at a controlled temperature of about zero degrees. To this mixture was added thiophene in acetic anhydride. After the addition of the thiophene was completed, the reaction was allowed to stir for an additional two hours, whereupon the reaction mixture was poured on chipped ice. After recrystallization the 2-nitrothiophene was obtained in 60% yield.

The chloromethylation of 2-nitrothiophene was first tried according to a method used in the preparation of chloromethylthiophene (14). The 2-nitrothiophene was placed in a

system of formaldehyde and concentrated hydrochloric acid cooled to about zero degrees. A steady stream of dry hydrogen chloride was then passed through the solution for various periods of time from two to ten hours. After working up the reaction mixture, a nearly quantitative yield of the unreacted 2-nitrothiophene was always obtained.

A more vigorous method of chloromethylation involved the treatment of 2-nitrothiophene with paraformaldehyde in a mixture of glacial acetic acid and 85% phosphoric acid at elevated temperatures. The method simply involved dissolving the 2-nitrothiophene in the acid mixture and passing a steady stream of hydrogen chloride through the system. The temperature of the reaction was varied from 50-100°, and the time of reaction extended for as long as eighteen hours. Here again isolation of the reaction product showed that little or none of the 2-nitrothiophene starting material had been chloromethylated in the desired manner. Similar results were obtained using methylal and dry hydrogen chloride according to the procedure of Birchler (15).

The inability of 2-nitrothiophene to permit the substitution of a chloromethyl group in the free alpha position must be caused by the introduction of a nitro group, because the unsubstituted thiophene molecule readily undergoes chloromethylation at reduced temperatures. King and Nord (16) noted that their procedure of formylation with N-methylformanilide and phosphorous oxychloride failed with 2-nitro-



thiophene, whereas the same procedure was extremely useful in the preparation of a wide variety of substituted thiophene aldehydes. Thus here is further evidence of the difficulty encountered in the substitution of an aldehydo or readily convertible group in the free alpha position of 2-nitrothiophene.

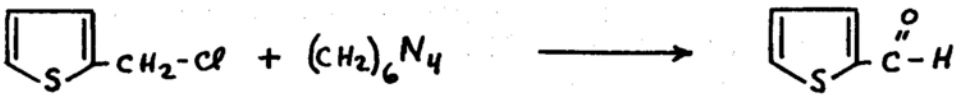
Attention was now turned to those thiophene derivatives possessing substituents which could readily be converted to an aldehyde and possessed the ability of undergoing nitration. 2-Chloromethylthiophene, prepared by the method of Blicke and Leonard (14), was nitrated with fuming nitric acid in mixture of glacial acetic acid and acetic anhydride at reduced temperatures. After allowing the nitration to proceed for an appropriate time, the reaction mixture was poured on chipped ice. A light orange viscous oil immediately appeared, which seemed to undergo decomposition on standing. Since attempts to vacuum distill or crystallize this product were not successful, the oil was used as such in the Somelet reaction.

The oil obtained from the nitration of 2-chloromethylthiophene was refluxed with hexamethylenetetramine in chloroform according to the general Somelet Reaction. Subsequent hydrolysis of the hexamethylenetetramine salt failed to give

the desired 5-nitro-2-thiophenealdehyde. Similar attempts to convert 5-nitro-2-bromomethylthiophene, obtained from the treatment of 5-nitro-2-methylthiophene with N-bromosuccinimde, to the nitrothiophenealdehyde via the Somelet reaction were unsuccessful (17).

Another method of approach was the preparation of iodothiophene which could then be converted to the desired 5-nitro-2-thiophenealdehyde via a Grignard aldehyde preparation. 2-Iodothiophene was nitrated with concentrated nitric acid in acetic anhydride in 65% yield according to the procedure of Dann (10), see p. 87. The 5-nitro-2-iodothiophene was then treated with magnesium metal according to the usual Grignard method; however, the formation of the magnesium metal complex was not accomplished. Various means of activation such as iodine and the entrainment procedure with ethyl iodide failed to initiate the reaction.

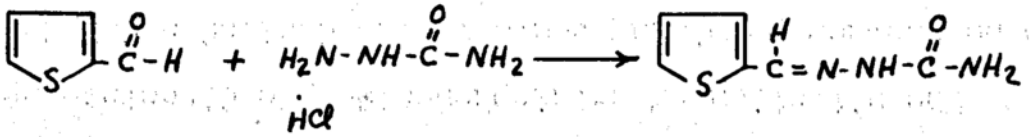
The nitration of 2-thiophenealdehyde derivatives was tried as another possible route to the nitroaldehyde. 2-Thiophenealdehyde was prepared by treatment of 2-chloromethylthiophene with hexamethylenetetramine according to the general Somelet procedure (18).



The 2-chloromethylthiophene was heated at reflux for about two hours with hexamethylenetetramine in chloroform. The white salt was then thoroughly washed with ether to remove

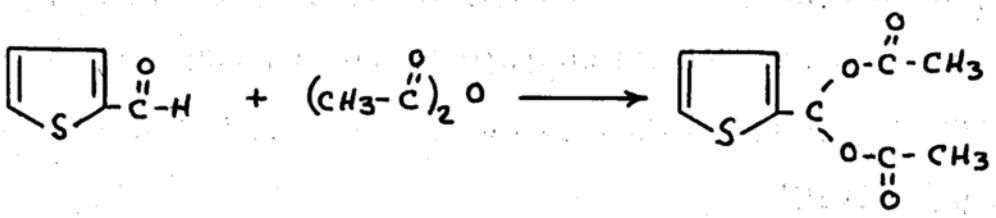
traces of impurities and destroyed by steam distillation. The distillate was acidified, and the 2-thiophenealdehyde extracted with ether. After removal of the ether, the 2-thiophenealdehyde was obtained by vacuum distillation in about 45% yield.

The 2-thiophenealdehyde was heated with semicarbazide hydrochloride and sodium acetate in an aqueous-alcoholic solution to give the corresponding 2-thiophenealdehyde semicarbazone in about 80% yield (19). The semicarbazone was then nitrated in a mixture of nitric and sulfuric acids at 0°.



The isolation and identification of the reaction product was accomplished, but elemental analysis failed to agree with the desired 5-nitro-2-thiophenealdehyde semicarbazone.

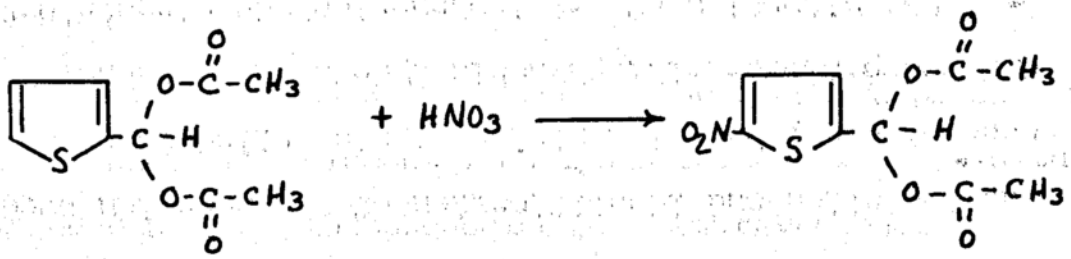
The nitration of 2-thiophenealdehyde diacetate appeared to be another promising route to the desired aldehyde. 2-Thiophenealdehyde was protected as the diacetate by treatment of the aldehyde with acetic anhydride and one drop of



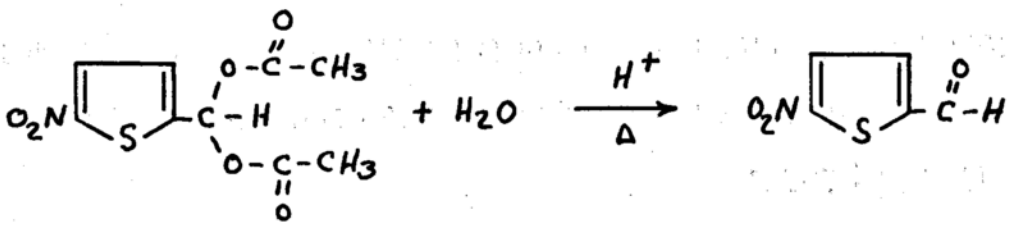
concentrated sulfuric acid as catalyst. The 2-thiophenealdehyde diacetate was then nitrated with fuming nitric acid in

acetic anhydride in the cold. The nitration product was poured on chipped ice to give the crystalline 5-nitro-2-thiophenealdehyde diacetate. While a variety of methods were being tried for the subsequent hydrolysis of the diacetate protecting group, Patrick & Emerson (20) reported the preparation of the same aldehyde by this same general method. They accomplished the hydrolysis of the 5-nitro-2-thiophenealdehyde diacetate by steam distillation in 1 N. hydrochloric acid. The nitroaldehyde was then recovered from the distillate in 76% yield.

Combes (21) prepared 5-nitro-2-thiophenealdehyde by the same procedure involving the nitration of 2-thiophene-

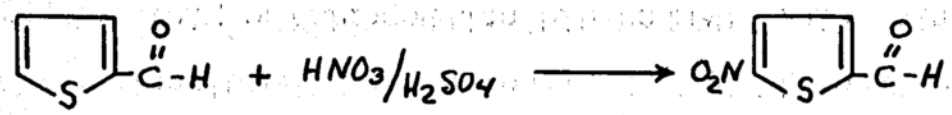


aldehyde diacetate; however, he accomplished the hydrolysis to the free aldehyde by treatment of the diacetate with 20% sulfuric acid. Combes did not report the yield of the nitro aldehyde in his preparation. Dullagahan (17) obtained the 5-nitro-2-thiophenealdehyde by the selenium dioxide oxidation



of 5-nitro-2-thenyl bromide; however, he was not able to obtain the nitro aldehyde in a crystalline form.

Another method of preparing 5-nitro-2-thiophenealdehyde was also being developed at this same time in our laboratory. It involved the direct nitration of 2-thiophenealdehyde with mixed acids. The 2-thiophenealdehyde was added to previously cooled concentrated sulfuric acid. To this well stirred mixture concentrated nitric acid was added dropwise. By dropping solid pieces of dry ice into the reaction mixture, the desired cooling was accomplished, and an inert atmosphere was obtained. After allowing the reaction to proceed for



about two hours at 0-5°, the nitration mixture was poured into a chipped ice-water mixture. The nitrated product was extracted with ether, and the ethereal extract dried over sodium sulfate. After removal of the ether, the residue was vacuum distilled to give the desired 5-nitro-2-thiophene-aldehyde in about 50% yield together with some of the unreacted 2-thiophenealdehyde.

Since the preparation of the basic 5-nitro-2-thiophene-aldehyde was reported simultaneous to the completion of this work, the proposed project of preparing derivatives of the nitroaldehyde was abandoned.

EXPERIMENTAL

I. Synthesis of 5-Nitro-2-thiophenecarboxylic Acid.

A. 2-Iodothiophene.

The 2-iodothiophene was prepared in 50-60% yield by the iodination of thiophene in benzene with mercuric oxide and iodine according to the method of Minnis (11); b.p. 46-52° (2 mm).

B. 5-Nitro-2-iodothiophene.

The 5-nitro-2-iodothiophene was prepared in 60-65% yield by the nitration of 2-iodothiophene with concentrated nitric acid in acetic anhydride according to the procedure of Dann (10); m.p. 73-74°.

C. 5-Nitro-2-thiophenecarboxylic acid.

The 5-nitro-2-iodothiophene was converted to the 5-nitro-2-cyanothiophene by treatment with cuprous cyanide and pyridine according to the method of Dann (10). The nitrile was not isolated but immediately hydrolyzed with concentrated hydrochloric acid to 5-nitro-2-thiophenecarboxylic acid in 40-53% yield based on the 5-nitro-2-iodothiophene, m.p. 155-156°.

II. Synthesis of Aliphatic 5-Nitro-2-thiophenecarboxamides.

A. N-Allyl-5-nitro-2-thiophenecarboxamide.

In a 50-cc. ground-glass round-bottom flask, 8.6 g.

(0.05 mole) of 5-nitro-2-thiophenecarboxylic acid was refluxed on a steam bath for one hour with 30.0 g. (0.25 mole) of thionyl chloride. The reaction mixture was poured into a large evaporating dish, and the excess thionyl chloride removed by blowing air over the yellow solution. The yellow 5-nitro-2-thiophenecarboxylicacid chloride was then taken up in about 50 cc. of dry benzene, and the solution cooled in an ice bath for a few minutes. To this slightly cooled solution contained in a 250-cc. Erlenmeyer flask was added dropwise and with shaking 5.7 g. (0.10 mole) of allyl amine in about 10 cc. of dry benzene. There was an immediate reaction with considerable evolution of heat and the formation of a voluminous white precipitate. The reaction was allowed to proceed at room temperature for three hours with occasional shaking.

After being allowed to stand at room temperature for three hours, the reaction mixture was diluted with about 50 cc. of chloroform which caused the precipitate to dissolve. The benzene-chloroform was thoroughly extracted with water to remove the allyl amine hydrochloride and unreacted allyl amine which may be present. The benzene-chloroform extract was then dried overnight over anhydrous sodium sulfate. After removal of the solvents, the white residue was recrystallized from a mixture of alcohol and water. After recrystallization, 9.6 g. (85%) the N-allyl-5-nitro-2-thiophenecarboxamide was obtained as fine needles which melted at 113-114°.

Anal. - Calcd. for $C_8H_8O_3N_2S$: C 45.27 H 3.90

Found: C 45.58 H 4.02

B. N-(β -Diethylaminoethyl)-5-nitro-2-thiophenecarboxamide Hydrochloride.

In the same manner as previously discussed, 6.0 g. (0.035 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux with 21.4 g. (0.18 mole) of thionyl chloride. The acid chloride obtained after removal of the excess thionyl chloride was dissolved in about 30 cc. of dry benzene. To this benzene solution of the acid chloride contained in a 250-cc. round-bottom flask equipped with a mechanical stirrer 4.1 g. (0.035 mole) of β-diethylaminoethylamine in 20 cc. of dry benzene was added with stirring. There was an immediate reaction with the evolution of heat and formation of a white precipitate. The reaction was allowed to proceed at room temperature for about three hours with occasional shaking. The solution was then filtered, and the white precipitate washed thoroughly with anhydrous ether. After recrystallization from absolute ethanol, 6.1 g. (60%) of the N-(β-diethylaminoethyl)-5-nitro-2-thiophenecarboxamide hydrochloride was obtained as short monoclinic crystals melting at 200-202°.

Anal.-Calcd. for $C_{11}H_{18}N_3O_3S$ Cl: C 42.92 H 5.89

Found: C 42.10 H 5.83

C. Ethyl-5-nitro-2-thiophenecarboxyglycylamide.

In the manner previously described, 4.2 g. (0.025 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux for one hour with 14.9 g. (0.13 mole) of thionyl chloride. After

removal of the excess thionyl chloride, the residual nitro acid chloride was dissolved in 100 cc. of dry benzene contained in a 500-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer and reflux condenser. To this benzene solution was added 3.5 g. (0.025 mole) of glycine ethyl ester hydrochloride and 3.5 g. (0.05 mole)of redistilled pyridine (dried over potassium hydroxide). The mixture was allowed to react at room temperature for about thirty minutes and then heated to about 50° for thirty minutes. During this period of heating the reaction mixture took on a deep red color. After heating at 50°, the well-stirred mixture was allowed to react at room temperature for about two hours.

The deep red benzene solution was diluted to twice its volume with Skelly B. The precipitate obtained on cooling the Skelly-benzene solution was crystallized from an aqueous-alcoholic solution. After recrystallization, 2.7 g. (42%) of the ethyl-5-nitro-2-thiopheneglycylamide was obtained which melted at 122-124°.

Anal. - Calcd. for $C_9H_{10}N_2O_5S$: C 41.85 H 3.90
Found: C 42.11 H 3.81

III. Synthesis of 5-Nitro-2-thiophenecarboxyureides.

A. 5-Nitro-2-thiophenecarboxyureide.

The 5-nitro-2-thiophene acid chloride was prepared in the same manner used previously. In a small round-bottom flask, 4.5 g. (0.026 mole) of 5-nitro-2-thiophenecarboxylic

acid was heated at reflux with 15.3 g. (0.13 mole) of thionyl chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 40 cc. of dry benzene, and the solution placed in a 250-cc. round-bottom three-neck flask equipped with a mercury-seal stirrer and reflux condenser. To this solution was added 1.6 g. (0.026 mole) of urea and one drop of concentrated sulfuric acid suspended in about 100 cc. of dry benzene.

The reaction mixture was heated at reflux and stirred for six hours, and then allowed to stir at room temperature overnight. During this period of heating, an ever-increasing amount of light grey precipitate accumulated. This precipitate was removed by suction filtration and thoroughly washed with Skelly B. After recrystallization from glacial acetic acid, 3.8 g. (67%) of the 5-nitro-2-thiophenecarboxyureide was obtained, m.p. 216-218°.

Anal. - Calcd. for $C_6H_5N_3O_4S$: C 33.49 H 2.34
Found: C 32.85 H 2.39

B. 5-Nitro-2-thiophenecarboxythioureide.

In the manner previously discussed, 2.0 g. (0.011 mole) of 5-nitro-2-thiophenecarboxylic acid was refluxed with 7.2 g. (0.055 mole) of thionyl chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 40 cc. of benzene, and the solution added to a 250-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer and reflux condenser. To this solution was added 0.9 g.(0.011 mole) of thiourea and one drop of concentrated

sulfuric acid in about 100 cc. of dry benzene, and the reaction mixture heated at reflux with stirring for eight hours.

With heating, a brown precipitate gradually separated from the orange-colored reaction mixture. This precipitate was removed by suction filtration and thoroughly washed with Skelly B. After recrystallization from ethyl alcohol and Norite, 1.8 g. (64%) the 5-nitro-2-thiophenecarboxythioureide was obtained; m.p. 205-206°.

Anal. - Calcd. for $C_6H_5N_3O_3S_2$: C 31.16 H 2.18
Found: C 31.35 H 2.17

IV. Synthesis of 5-Nitro-2-thiophenecarboxylic heterocyclic amides.

A. 5-Nitro-2-thiophenecarboxypiperidide.

In the manner as previously discussed, 2.6 g. (0.015 mole) of 5-nitro-2-thiophenecarboxylic acid and 8.9 g. (0.075 mole) of thionyl chloride were refluxed for one hour. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in 30 cc. of dry benzene. To this well stirred benzene solution was added dropwise 2.5 g. (0.03 mole) of piperidine in about 15 cc. of dry benzene. There was an immediate reaction with the evolution of heat and the formation of a flocculant precipitate.

The reaction was allowed to proceed at room temperature for about three hours with occasional shaking. The reaction mixture was then diluted with about 100 cc. of chloroform, and the resulting solution thoroughly extracted with water

to remove the piperidine hydrochloride and any unreacted piperidine present. The benzene-chloroform solution was then dried over sodium sulfate. After removal of the benzene and chloroform, the yellow residue was recrystallized from an aqueous-alcoholic solution. After recrystallization, 2.9 g. (75%) of the 5-nitro-2-thiophenecarboxypiperidide was obtained as light yellow flakes which melted at 94-95°.

Anal. - Calcd. for $C_{10}H_{12}N_2O_3S$: C 49.98 H 5.03
Found: C 50.16 H 5.07

B. 5-Nitro-2-thiophenecarboxymorpholide.

In the manner previously described, 2.25 g. (0.013 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux for one hour with 7.7 g. (0.065 mole) of thionyl chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in 50 cc. of dry benzene. To this well stirred solution was added dropwise 2.3 g. (0.026 mole) of morpholine. There was an immediate reaction with the evolution of heat and the formation of a voluminous white precipitate.

The reaction was allowed to react at room temperature for about three hours with occasional stirring, after which the suspension was diluted with about 100 cc. of chloroform. The resulting benzene-chloroform solution was then thoroughly extracted with water to remove the morpholine hydrochloride and any unreacted morpholine present. The benzene-chloroform solution was dried over sodium sulfate. After removal of the solvents, the residue was recrystallized from a mixture of

alcohol and water. The 5-nitro-2-thiophenecarboxymorpholide was obtained as light yellow needles which turned a light pink in the presence of light. After recrystallization, 2.8 g. (80%) of the morpholide was obtained; m.p. 89-90°.

Anal. - Calcd. for: $C_9H_{10}N_2O_4S$: C 44.62 H 4.16
Found: C 44.95 H 4.36

C. N-(2'-thiazoyl)-5-nitro-2-thiophenecarboxamide.

In the manner described previously, 4.5 g. (0.026 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux for one hour with 15.5 g. (0.13 mole) of thionyl chloride to give 5-nitro-2-thiophenecarboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in 50 cc. of dry benzene, and the benzene solution added to a 500-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer and a reflux condenser. To this solution was added 2.6 g. (0.026 mole) of 2-aminothiazole and 2.1 g. (0.026 mole) of redistilled pyridine in about a 150 cc. of dry benzene. The yellow colored suspension was heated at reflux for about three hours and then allowed to stir at room temperature for about an additional three hours.

With heating a yellow flocculant precipitate gradually formed. This precipitate was removed by suction filtration and thoroughly washed with distilled water to remove the pyridine hydrochloride formed in the reaction. The yellow precipitate was finally washed successively with dilute hydrochloric acid and distilled water to remove the pyridine

hydrochloride and any unreacted 2-aminothiazole, and then the residue was recrystallized from a large quantity of glacial acetic acid. After recrystallization, 5.3 g. (80%) of the N-(2'-thiazoyl)-5-nitro-2-thiophenecarboxamide was obtained as a microcrystalline product which melted above 330°.

Anal. - Calcd. for $C_8H_5N_3O_3S_2$: C 37.64 H 1.97
Found: C 38.52 H 2.17

D. N'-Ethyl-N(-5-nitro-2-thiophenecarboxy)piperazine Hydrochloride.

1. Ethylpiperazine-1-carboxylate.

N-Carbethoxypiperazine was prepared in 73% yield by treating piperazine hexahydrate with ethylchlorocarbonate under carefully controlled conditions of pH according to the method of Moore (23); b.p. 116-118° (3 mm).

2. Ethyl-4-ethylpiperazine-1-carboxylate.

The N-carbethoxypiperazine was ethylated with ethyl-p-toluenesulfonate in 46% yield according to the method of Moore (23); b.p. 85-90° (2 mm.).

3. N-Ethylpiperazine.

The N-carbethoxy group was cleaved from the piperazine ring by heating with concentrated hydrochloric acid according to the procedure of Moore (23). The N-ethylpiperazine dihydrochloride was then converted to the free N-ethylpiperazine by atmospheric distillation with dry calcium hydroxide according to the method of Moore (20). The N-ethylpiperazine was obtained in 76% overall yield and boiled at 152-155°.

4. N'-Ethyl-N(5-nitro-2-thiophenecarboxy)piperazine Hydrochloride.

In the same manner used previously, 1.8 g. (0.0105 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux with 5.95 g. (0.052 mole) of thionyl chloride to obtain 5-nitro-2-thiophenecarboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 20 cc. of dry benzene. To this solution in a 125 cc. Erlenmeyer flask, 1.2 g. (0.0105 mole) of N-ethylpiperazine in about 10 cc. of dry benzene was added slowly with shaking.

There was an immediate reaction with the evolution of heat and the formation of a voluminous white precipitate. The reaction mixture was allowed to stand at room temperature for about three hours with occasional stirring. The benzene solution was then cooled, and the white precipitate of the hydrochloride salt removed by suction filtration. The precipitate was washed with anhydrous ether and recrystallized from a mixture of absolute methyl alcohol and anhydrous ether. After recrystallization, 1.8 g. (56%) the N'-ethyl-N(5-nitro-2-thiophenecarboxy)piperazine hydrochloride was obtained as needles which melted at 262-264° (D).

Anal. - Calcd. for $C_{11}H_{16}N_3O_3ClS$: C 43.20 H 5.28
Found: C 43.46 H 5.39

E. 5-Nitro-2-thiophenecarboxypyrrolide.

In the manner previously discussed, 3.8 g. (0.022 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux with 12.0 g. (0.11 mole) of thionyl chloride to obtain 5-nitro-

2-thiophenecarboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in 50 cc. of dry benzene. To this solution contained in a 250-cc. three-neck round-bottom flask equipped with a mechanical stirrer was added dropwise with stirring 3.13 g. (0.044 mole) of pyrrolidine in about 15 cc. of dry benzene. There was an immediate reaction with the evolution of heat and the formation of a voluminous precipitate.

The mixture was allowed to stir for three hours at room temperature and then diluted with about 100 cc. of chloroform. The benzene-chloroform solution was thoroughly extracted with water and dried over anhydrous sodium sulfate. After removal of the benzene and chloroform, the residue was recrystallized from a mixture of ethyl alcohol and water. After recrystallization, 3.7 g. (75%) of the 5-nitro-2-thiophenecarboxypyrrolide was obtained; m.p. 179-180°.

Anal. - Calcd. for $C_9H_{10}N_2O_3S$: C 47.77 H 4.56
Found: C 48.10 H 4.78

F. N-(2'-pyridino)-5-nitro-2-thiophenecarboxamide.

In the manner discussed previously, 4.3 g. (0.025 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux for one hour with 13.0 g. (0.115 mole) of thionyl chloride to form the 5-nitro-2-thiophenecarboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 50 cc. of dry benzene. The benzene solution of the acid chloride was placed in a 500-cc.

three-neck round-bottom flask equipped with a mercury-seal stirrer and reflux condenser with attached calcium chloride tube. To this solution was added 6.7 g. (0.025 mole) of 2-aminopyridine and 2.0 g. (0.025 mole) of redistilled pyridine, and the mixture heated at reflux for about twelve hours.

With heating there was a gradual formation of a dirty green precipitate. This precipitate was removed by suction filtration and washed with 5% sodium carbonate and water. The residue was recrystallized from ethyl alcohol in the form of gleaming needles. After recrystallization, 4.0 g. (65%) of the N-(2'-pyridino)-5-nitro-2-thiophenecarboxamide was obtained; m.p. 198-200°.

Anal. - Calcd. for $C_{10}H_7N_3O_2S$: C 48.19 H 2.83
Found: C 48.19 H 2.65

V. Synthesis of 5-Nitro-2-thiophenecarboxycarbocyclic amides.

A. 5-Nitro-2-thiophenecarboxy-m-bromoanilide.

In the manner described previously, 3.8 g. (0.022 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux for one hour with 13.1 g. (0.11 mole) of thionyl chloride to give 5-nitro-2-thiophenecarboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 50 cc. of dry benzene. To this benzene solution in a 500-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer and reflux condenser with attached drying tube was added 3.7 g. (0.022 mole) of m-bromoaniline

and 1.7 g. (0.022 mole) of redistilled pyridine in about 100 cc. of dry benzene. The benzene solution was heated at reflux for about two hours, during which time there was a light yellow precipitate formed.

The yellow precipitate was removed by suction filtration and thoroughly washed with water to remove the pyridine hydrochloride occluded on the precipitate. The residue was then crystallized from ethyl alcohol and obtained in the form of light yellow needles. After recrystallization, 6.4 g. (89%) of the 5-nitro-2-thiophenecarboxy-m-bromoanilide was obtained; m.p. 232-234°.

Anal. - Calcd. for $C_{11}H_7N_2O_3SBr$: C 40.50 H 2.16
Found: C 40.93 H 2.10

B. 5-Nitro-2-thiophenecarboxy-m-nitroanilide.

In the manner described previously, 3.46 g. (0.02 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux with 11.9 g. (0.10 mole) of thionyl chloride to give the 5-nitro-2-thiophenecarboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 50 cc. of dry benzene. This benzene solution was placed in a 500-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer and reflux condenser, and 2.76 g. (0.02 mole) of m-nitroaniline together with 1.58 g. (0.02 mole) of redistilled pyridine in about 150 cc. of dry benzene was added. The suspension was heated at reflux for ten hours during which time there was a deep yellow precipitate formed, different from the original precipitate

of m-nitroaniline.

The yellow precipitate was removed by suction filtration and washed with water, dilute hydrochloric acid, and finally again with water. The residue was recrystallized from glacial acetic acid in the form of yellow needles. After recrystallization, 4.5 g. (80%) of the 5-nitro-2-thiophene-carboxy-m-nitroanilide was obtained; 204-205°.

Anal. - Calcd. for $C_{11}H_7N_3O_5S$: C 45.05 H 2.41

Found: C 45.07 H 2.85

C. 5-Nitro-2-thiophenecarboxy-p-nitroanilide.

In the manner previously described, 3.46 g. (0.02 mole) of 5-nitro-2-thiophene carboxylic acid was heated at reflux for one hour with 11.9 g. (0.10 mole) of thionyl chloride to give the corresponding 5-nitro-2-thiophene carboxylic acid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 50 cc. of dry benzene placed in a 500-cc. three-neck round-bottom flask equipped with a mercury-seal stirrer and reflux condenser with attached calcium chloride drying tube. To this solution was added 2.76 g. (0.02 mole) of p-nitroaniline and 1.58 g. (0.02 mole) of redistilled pyridine in about 200 cc. of dry benzene.

The resulting suspension was heated at reflux for about ten hours during which time there was a new yellow precipitate formed. This yellow precipitate was removed by suction filtration and washed with water, dilute hydrochloric acid, and again with water. The yellow residue was recrystallized

from a large quantity of glacial acetic acid in the form of needles. After recrystallization, 5.0 g. (90%) of the 5-nitro-2-thiophenecarboxy-p-nitroanilide was obtained; m.p. 274-275°.

Anal. - Calcd. for $C_{11}H_7N_3O_5S$: C 45.05 H 2.41
Found: C 45.35 H 2.65

D. 5-Nitro-2-thiophenecarboxybenzyl amide.

In the same manner as previously described, 3.5 g. (0.02 mole) of 5-nitro-2-thiophenecarboxylic acid was heated at reflux with 11.9 g. (0.02 mole) of thionyl chloride to give the corresponding 5-nitro-2-thiopheneacid chloride. After removal of the excess thionyl chloride, the nitro acid chloride was dissolved in about 40 cc. of dry benzene. To this benzene solution in an Erlenmeyer flask at room temperature was added 4.28 g. (0.04 mole) of benzyl amine in about 15 cc. of dry benzene.

There was an immediate reaction with the evolution of heat and formation of a voluminous white precipitate. The mixture was allowed to stand at room temperature for about three hours with occasional stirring. The solution was then diluted with about 100 cc. of chloroform, and the benzene-chloroform solution was thoroughly washed with water and dried over anhydrous sodium sulfate. After removal of the solvents, the residue was recrystallized from an aqueous-alcoholic mixture. After recrystallization, 3.9 g. (95%) of the 5-nitro-2-thiophenecarboxybenzylamide was obtained as needles which melted at 117-118°.

Anal. - Calcd. for $C_{12}H_{10}N_2O_3S$: C 54.95 H 3.84

Found: C 55.63 H 4.02

BIBLIOGRAPHY

1. Rebstock , M. C. et al., J. Amer. Chem. Soc. 71, 2458 (1949).
2. Controulis, J. et al., J. Amer. Chem. Soc. 71, 2463 (1949).
3. Cutler, R. A. et al., J. Amer. Chem. Soc. 74, 5475 (1952).
4. Phillips, A. R., J. Amer. Chem. Soc. 74, 6125 (1952).
5. Dodd, M. C. and Stillmann, W. B., J. Pharmacol. & Exptl. Ther. 82, 11 (1944).
6. Johnson, O. H. et al., J. Biol. Chem. 153, 37 (1944).
7. Carrara, Gino, et al., Farm. sci. e tec(Pavia) 6, 3 (1951), C. A. 45, 6691.
8. Dann, O. and Moller, E. F., Ber. 82, 76 (1949).
9. Rinkes, I. J., Rec. Trav. Chim. 51, 1134 (1932).
10. Dann, O., Ber. 76, 425 (1943).
11. Minnis, W., Org. Syntheses, Coll. Vol. II, 357.
12. Ben-Ishia and Katchalski, J. Org. Chem. 16, 1025 (1951).
13. Babasinian, V. S., Org. Syntheses, Coll. Vol. II, 466.
14. Blicke and Leonard, J. Amer. Chem. Soc. 68, 1934 (1946).
15. Birchler, Org. Syntheses, 20, 59 (1940).
16. King, W. J. and Nord, F. F., J. Org. Chem. 13, 635 (1948).
17. Dullaghan, et al., J. Amer. Chem. Soc. 74, 2676 (1952).
18. Dunn, Dittmer, and Waugh, J. Amer. Chem. Soc. 68, 2118 (1946).
19. Grischkewitsch-Trochimowski, Chem. Zent. 1912, II, 1561.
20. Patrick, T. M. Jr. and Emerson, W. S., J. Amer. Chem. Soc. 74, 1356 (1952).
21. Combes, Georges, Bull. Soc. Chim. De France, 701 (1952).
22. Moore, T. S., Boyle, M. and Thorn, V. M., J. Chem. Soc., 46 (1929).

SUMMARY

1. A number of phthalyl- α -aminoaldehydes and the corresponding semicarbazone, thiosemicarbazone, and dithiobiuret derivatives have been prepared for pharmacological evaluation as antiviral agents.

2. A number of 5-nitro-2-thiophenecarboxylic acid amide derivatives have been prepared for pharmacological evaluation as antiviral agents.

3. The following new compounds have been prepared:

Phthalimidoacetaldehyde thiosemicarbazone

Phthalimidoethylidine dithiobiuret

2-Phthalimidopropionaldehyde thiosemicarbazone

2-Phthalimidopropylidine dithiobiuret

3-Phthalimidopropionaldehyde

3-Phthalimidopropionaldehyde semicarbazone

3-Phthalimidopropionaldehyde thiosemicarbazone

3-Phthalimidopropylidine dithiobiuret

2-Phthalimido-3-phenylpropionaldehyde

2-Phthalimido-3-phenylpropionaldehyde semicarbazone

2-Phthalimido-3-phenylpropionaldehyde thiosemicarbazone

2-Phthalimido-3-phenylpropylidine dithiobiuret

2-Phthalimidoisocaproaldehyde

2-Phthalimidoisocaproaldehyde semicarbazone

2-Phthalimidoisocaproaldehyde thiosemicarbazone

2-Phthalimidoisocaprylidine dithiobiuret

2-Phthalimido-3-methylbutyraldehyde

2-Phthalimido-3-methylbutyraldehyde semicarbazone

2-Phthalimido-3-methylbutyraldehyde thiosemicarbazone

2-Phthalimido-3-methylbutylidine dithiobiuret

N-Allyl-5-nitro-2-thiophenecarboxamide

N-(β-Diethylaminoethyl)-5-nitro-2-thiophenecarboxamide hydrochloride

Ethyl-5-nitro-2-thiophenecarboxyglycylamide

5-Nitro-2-thiophenecarboxyureide

5-Nitro-2-thiophenecarboxythioureide

5-Nitro-2-thiophenecarboxypiperidide

5-Nitro-2-thiophenecarboxymorpholide

N-(2'-thiazoyl)-5-nitro-2-thiophenecarboxamide

N'-Ethyl-N-(5-nitro-2-thiophenecarboxy)piperazine hydrochloride

5-Nitro-2-thiophenecarboxypyrrolide

N-(2'-pyridino)-5-nitro-2-thiophenecarboxamide

5-Nitro-2-thiophenecarboxy-m-bromoanilide

5-Nitro-2-thiophenecarboxy-m-nitroanilide

5-Nitro-2-thiophenecarboxy-p-nitroanilide

5-Nitro-2-thiophenecarboxybenzyl amide

BIOGRAPHY

John James Hefferren

Born

Chicago, Illinois

August 12, 1928

Education

De La Salle High School, Chicago

Loyola University, B. S., Chicago, 1950.

University of Wisconsin, M. S., 1952.

Honors and Societies

American Chemical Society

American Pharmaceutical Association

Rho Chi

The Society of Sigma Xi

Fellow of the American Foundation for Pharmaceutical Education, 1950 - 1953

Lambda Chi Sigma (Honorary Chemistry Fraternity - Loyola)

Phi Sigma

APPROVED William O. Faye

DATE Sept. 14, 1953