

THE SYNTHESIS OF CANALINE, CANAVANINE AND
RELATED COMPOUNDS

by

DAVID DOLPH NYBERG

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1956

APPROVED:

Redacted for Privacy

~~_____~~
Professor of Chemistry

In Charge of Major

Redacted for Privacy

~~_____~~
Chairman of Department of Chemistry

Redacted for Privacy

~~_____~~
Chairman of School Graduate Committee

Redacted for Privacy

~~_____~~
Dean of Graduate School

Date thesis is presented May 25, 1956

Typed by Norma Becker

ACKNOWLEDGEMENT

The author wishes to express his gratitude to his advisor, Dr. Bert E. Christensen, for his helpful assistance and patient guidance during the past four years of graduate study.

TABLE OF CONTENTS

INTRODUCTION	PART I	
A Brief Review of the Discovery, Occurrence, Isolation and Properties of Canavanine and Canaline. . .		1
INTRODUCTION	PART II	
Methods of Preparation of O-Substituted Hydroxylamines and Hydroxyguanidines.		8
INTRODUCTION	PART III	
Earlier Work on the Synthetic Canavanine Problem. .		14
DISCUSSION	PART I	
A New Synthesis of Canaline and Canavanine.		16
DISCUSSION	PART II	
The Attempted Synthesis of 2-Amino-3-aminoöxypropanoic Acid (Lower Homologue of Canaline). . . .		20
DISCUSSION	PART III	
The Bromination of δ -Valerolactone.		23
EXPERIMENTAL		25
SUMMARY		45
BIBLIOGRAPHY		46

LIST OF FIGURES

Figure 1 - Reaction Scheme.	19
Figure 2 - Reaction Scheme.	22

LIST OF TABLES

Table 1 - Infra-red Data.	35
-----------------------------------	----

THE SYNTHESIS OF CANALINE, CANAVANINE AND RELATED COMPOUNDS

INTRODUCTION PART I

A Brief Review of the Discovery, Occurrence, Isolation and Properties of Canavanine and Canaline

During the course of a study of the mechanism of urea formation in pig liver, in 1929, Kitagawa (21) discovered a hitherto unknown amino acid in Jack bean meal from which the enzyme urease was being prepared. The amino acid was shown to possess the constitutional formula, $\text{NH}_2\text{C}(=\text{NH})-\text{NHCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$, and given the name canavanine. On treatment with the enzyme arginase (10; 28; 29; 30), urea and another amino acid, canaline, $\text{NH}_2\text{OCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$, is formed. The behavior of canavanine and canaline are very similar to those of their corresponding structural analogs, arginine and ornithine.

Hydroxyguanidine, the characteristic structural unit of canavanine, has been synthesized from hydroxylamine and cyanamide by Pratorius-Seidler (40) and, more recently, by Adams, Kaiser and Peters (1). The basicity of this compound is much less than that of the parent compound, guanidine.

Canavanine occurs in the non-protein fraction of Jack bean (Canavalia ensiformis) and Canavalia lineata according to Kitagawa (26, p.23). Damodoran and Narayanan (10)

report its occurrence in seeds of Canavalia obtusifolia while its presence in other plants has been observed by Shibuya and Makizumi (44).

Directions for the preparation of canavanine in yields of up to 2.8 percent from the fat-free meal of Jack bean are described by Kitagawa (26, pp.24-25) and also Gulland and Morris (17, p.764).

The fact that canavanine was the first and, to the writer's knowledge, the only derivative of hydroxyguanidine to be found in nature has focused attention upon this compound.

However, the main interest in canavanine stems from the fact that it has been shown to be a competitive antagonist of arginine in many organisms. This is not surprising in view of the close structural relationships between the two amino acids. However, it is not the purpose of this dissertation to go into a discussion of the biological aspects of canavanine and canaline.

One paper regarding the biological activity of canavanine is of special interest however; this is the work by K. S. Pilcher et al. (38) on the "Inhibition of Multiplication of Lee Influenza Virus by Canavanine". This investigation of virus inhibition provided the stimulus for the work recorded in this thesis and it was for such virus inhibition studies that some of the

synthetic compounds described herein were prepared.

Canavanine is very soluble in water and insoluble in alcohol and ether; melting points observed for the free amino acid were 182-4°C and 184°C and specific rotation values reported are $[\alpha]_D^{17} = +8.0$ and $[\alpha]_D^{20} = +7.9$ (17, p.765; 26, p.25). The work of Cadden (8) and Armstrong (5) indicates that canavanine and, consequently, canaline possess the L-configuration.

Canavanine is a much weaker base than arginine. For example, according to Borek and Clarke (7, p.483), canavanine gives pK values (acidic formulation) of $pK_1 (-COOH) = 2.50$, $pK_2 -ONHC(=NH)NH_2 = 7.40$, and $pK_3 (\alpha NH_2) = 9.25$. A calculated isoelectric point from these data using the expression $pI = \frac{1}{2}(pK_m + pK_{m+1})$, where m equals the maximum charge the molecule can possess in strong acid solution, shows the isoelectric point to be 8.32. On the other hand, the isoelectric point of arginine is 10.8 (11). Tomiyama (46) gives almost identical dissociation data.

The greatly reduced basicity of canavanine is due of course to the oxygen atom in the hydroxyguanidine structural unit. In fact, this guanidinoöxy group in canavanine is a weaker base than ammonia. This reduced basicity makes it easier to obtain canavanine in the free crystalline form since no danger of carbonate formation from

atmospheric CO_2 exists, as with some other basic amino acids (48).

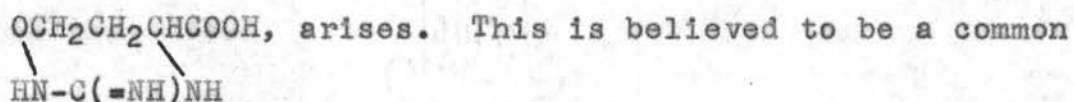
Canavanine is precipitated by many of the same reagents as arginine and occurs in the arginine fraction as isolated by Kessel-Kutschers method from Jack bean extract (26, p.25).

Canavanine gives a ninhydrin reaction, a red color when heated with ferric chloride, and as a characteristic color gives a stable ruby color with "irradiated" nitroprusside (exposed to air and sunlight) in neutral solution (22). Archibald (4) used nitopruesside treated with hydrogen peroxide followed by irradiation to devise a quantitative colorimetric method for canavanine. Fearon (13) prepared a slightly different test solution from nitroprusside and ammonia followed by exposure to air and light; the latter test was used in the study described here.

Canavanine and other compounds possessing the guanidinoxy group do not give Sakaguchi's reaction or the diacetyl reaction indicative of ordinary guanidine derivatives (26, p.26). Furthermore, oxidation with barium permanganate gives no guanidine as in the case of arginine (26, p.29). However, canavanine is hydrolyzed by acid and is less stable than arginine in this respect. Gulland and Morris (17, p.765) obtained ammonia, guanidine and α -amino- γ -butyrolactone by treatment with hot hydrobromic acid.

On the other hand, canavanine is more stable to alkaline hydrolysis than arginine. For example, when arginine is boiled with 50 percent sodium hydroxide for six hours, one-half of its total nitrogen is liberated as ammonia. In the case of canavanine, only 15-20 percent of its total nitrogen is liberated by the same treatment. On boiling with five percent baryta solution for two hours arginine is converted into ornithine and urea, while canavanine scarcely yields urea even after seven hours of this treatment (26, p.28).

On heating canavanine in neutral solutions, ammonia is lost and a cyclic compound called desamino-canavanine,



impurity in many canavanine preparations. According to Kitagawa (27), boiling of canavanine with 10 percent hydrochloric acid will remove this contaminant. Nitrous acid liberated two of the four nitrogen atoms very easily and a third with more difficulty. Only the alpha-amino group combines with formaldehyde (26, p.26). Many other derivatives of canavanine have been prepared and their properties are reported in the literature (17; 21; 22; 26, p.26).

The paper chromatography of canavanine from two different solvent systems has been described (35) and

its chromatographic behavior in two other systems has been determined in this laboratory.

Canaline is prepared by the enzymatic method (arginase) from canavanine in about 75 percent yield (26, p.31). The amino acid is very soluble in water and only slightly so in alcohol. It melts at 214°C with decomposition and is levorotatory having a specific rotation of $[\alpha]_{\text{D}}^{21} = -8.3^{\circ}$ (26, p.32). As mentioned previously, the amino acid possesses the L-configuration. The following pK values have been reported: $\text{pK}_1 = (-\text{COOH}) = 2.4$, $\text{pK}_2 (-\text{ONH}_2) = 4.3$, and $\text{pK}_3 (\alpha \text{NH}_2) = 9.20$ (7, p.483). The calculated isoelectric point is 6.75. Therefore the amino acid is, in effect, neutral in contrast to a close structural analogue lysine which is definitely basic possessing an isoelectric point of 9.47 (11).

Canaline does not reduce Fehling's solution or ammoniacal silver nitrate solution on boiling. This is indicative of the O-substituted function of the compound. N-substituted products of hydroxylamine readily reduce Fehling's solution (23; 26, p.32). Canaline gives an orange-red color in Jaffe's reaction (alkaline pictrate) which serves as a characteristic reaction according to Kitagawa (23; 26, p.32). It gives a typical ninhydrin and a red color with ferric chloride when heated (26, p.32).

Only alpha amino nitrogen is determined by the formal method or Van Slyke's method (23; 26, p.35). In general,

O-substituted derivatives of hydroxylamine are found to be converted to hydroxy compounds and ammonia on reduction.

Canaline forms derivatives of the same type as canavanine generally; their properties are adequately described in the literature (22; 26, p.33). One of these is of enough interest to describe briefly however. This is γ -ethylidencanaline, $\text{CH}_3\text{C} = \text{NOCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$, which can be considered as an O-substituted oxime. Borek and Clarke (7) attempted without success to prepare canaline by taking advantage of a similar oxime formation to protect the aminoxy group, but were forced to abandon this route to the synthesis of canaline.

INTRODUCTION PART II

Methods of Preparation of O-Substituted
Hydroxylamines and Hydroxyguanidines

The direct acylation or alkylation of hydroxylamine results in N-substitution (15, p.964). Consequently, some indirect method is necessary in order to prepare O-substituted hydroxylamines.

A common procedure involves the alkylation of oximes followed by acid hydrolysis. Since the alkylation of oximes under certain conditions gives both an O-substituted product and an isomeric N-substitution product (nitron), an isomeric separation must be made. Fortunately, this can be accomplished by the use of ether or petroleum ether; the O-substituted compound is readily soluble in these solvents in contrast to the isomeric N-substituted product (16, p.515; 43; 45, p.161).

Borek and Clarke (7) condensed the sodium salt of acetone oxime (42) with alkyl halides in the presence of a non-polar media, such as toluene in which the sodium salt is insoluble. The formation of isomers was excluded; apparently because extensive dissociation of the salt to give ions of the type $(\text{CH}_3)_2\text{C}=\text{N}-\text{O}^\ominus$ and $(\text{CH}_3)_2\text{C}^\ominus-\text{N}=\text{O}$ did not occur. The latter ion might be responsible for nitron formation.

However, in this procedure there are the disadvantages

inherent in a heterogeneous system. In fact, Fuller and King (15, p.964) abandoned the use of benzophenone oxime as a starting material because of low yields and somewhat uncertain structure.

The author, in an initial study of this method, prepared acetone O-ethyloxime by reacting the sodium salt of acetone oxime with ethyl bromide in the presence of xylene. The reaction proceeded smoothly giving a yield of 37 percent.

A serious problem frequently arises during the hydrolysis of the O-substituted oximes. The acidic hydrolysis of oximes is an equilibrium reaction and this sometimes makes it difficult to take the hydrolysis to completion. This is especially true if the oxime is volatile in steam; if the oxime is not steam volatile, the aldehyde or ketone portion can be easily removed by steam distillation during hydrolysis leaving the mineral acid salt of the O-substituted hydroxylamine behind. Such is the case in the preparation of carboxymethoxylamine hemihydrochloride (aminoöxyacetic acid hydrochloride) from acetone carboxymethoxime (3).

However, the writer found that four successive hydrolytic operations on acetone O-ethyl oxime with dilute hydrochloric acid yielded only 37 percent of the theoretical O-ethylhydroxylamine hydrochloride product. In this case it was not possible to steam distill the

acetone out at the same time hydrolysis was occurring because the oxime itself was too volatile.

It is of interest, however, to note that this reversibility of oxime hydrolysis was utilized to keep the isopropylidene group on acetone O-[2,2-(diethoxy)-ethyl]-oxime when it was hydrolyzed to give an aldehyde by conducting the hydrolysis in the presence of a large excess of acetone.

Another method of preparing O-substituted hydroxylamines consists in alkylating benzhydroxamic acid followed by a hydrolysis. Kitagawa (25) used benzhydroxamic acid with alcoholic potash and α -benzoylamino- γ -iodobutyric acid ethyl ester to prepare canaline by this method. Other aminoöxy acids were also prepared by Kitagawa using this same procedure although no yields were ever reported. Later Fuller and King (15, p.964) butylated potassium benzhydroxamate with butyl bromide in alcohol in the presence of potassium carbonate obtaining a mixture of two different alkylated products; the mono- and debutylated benzhydroxamic esters. They concluded that the method might prove suitable for simple alkoxyamines, but was not satisfactory for alkylenedioxydiamines in which they were particularly interested. Because of lack of knowledge regarding yields and possible questions regarding structures, this method was not adopted in this investigation.

Other preparations of O-substituted hydroxylamines are reported in the literature. For example, see Fuller and King (15, p.964); Andrewes, King and Walker (2, pp.43-45); and Truitt, Long and Mattison (47). These methods involve a variety of procedures and reagents and in almost all the cases offer no advantageous features. An exception, not applicable to the work described herein, is the method involving the methylation of potassium disulfonate to prepare O-methylhydroxylamine in 90 percent yield (2, pp. 43-45).

For the synthesis of canaline, the most useful procedure appeared to be that of Jones (20) and Hecker (18) which Fuller and King (15, pp.965-968) have employed extensively. The procedure is carried out by alkylating hydroxyurethane in alcoholic potassium hydroxide followed by alkaline or acidic hydrolysis of the resulting alkoxyurethanes.

The author used this method both in this and other work (37). Excellent yields (70-90 percent) of the alkoxyurethanes are usually obtained and these, in turn, give 40-60 percent yields of the O-substituted hydroxylamine. The procedures are simple and the products easily recovered in all the cases studied.

One of the nicest features of this procedure is the fact that both alkaline and acidic hydrolysis are possible and, in both cases, the hydrolysis is irreversible under

the conditions used. For example, consider the compound, 5-[2-(carbethoxyaminoxy)-ethyl]-hydantoin, which was prepared as an intermediate in the synthesis of canaline and canavanine. By alkaline hydrolysis in 13.5 percent aqueous barium hydroxide both the carbethoxy and hydantoin portions were hydrolytically attacked to give canaline. In contrast, acid hydrolysis with 48 percent hydrobromic acid causes hydrolysis of only the carbethoxy group and maintenance of the hydantoin ring.

In comparison to the oxime method, no isomers have been reported in the literature by workers using this method and the homogenous reaction medium simplifies observing the progress of the reaction via the precipitation of the potassium halide side product.

Three methods which have been used successfully to prepare the guanidine derivatives of the O-substituted hydroxylamines are described in the literature. These include the action of cyanamide (15, pp.966-968), S-methylisothiourea sulfate (7; 15, pp.966-968; 37) and O-methylisourea hydrochloride (24; 26, pp.29-31) on the hydroxylamines. Both S-methylisothiourea sulfate and O-methylisourea hydrochloride were used successfully in this work. Since the preparations (32) of cyanamide and O-methylisourea hydrochloride are involved and tedious, the writer preferred the use of the sulfur containing reagent

because of the ease of its preparation and purification.

This was prepared from dimethyl sulfate and thiourea by the method of Arndt (6) in excellent yield.

INTRODUCTION PART III

Earlier Work on the Synthetic Canavanine Problem

Kitagawa (25; 26, pp.37-39) was able to prepare L-canaline, starting with L- α -amino- γ -hydroxybutyric acid (L-homoserine). The starting material had been obtained by the hydrogenation of canaline over platinum black. The alpha amino group was protected by benzylation and, in a two step reaction, the compound α -benzoylamino- γ -iodobutyric acid ethyl ester was obtained. This was condensed with benzhydroxamic acid followed by hydrolysis with aqueous hydrochloric acid to give canaline, which was isolated as its picrate. The Kitagawa synthesis has the advantage of retaining configuration at the alpha carbon atom. Principle criticisms of Kitagawa's work are its tedious isolations and the fact that no yields were given in his directions. This leaves doubt as to the practicality of some of his procedures.

Borek and Clarke(7) attempted several methods of synthesis but were unable to prepare canaline by any of the procedures used. Their starting material was acetone β -bromoethoxime, prepared from the sodium salt of acetone oxime and ethylene bromide. They were able to prepare several aminoöxy compounds; however, the closest to the structure of canaline was γ -carboxypropoxy-amine (4-aminoöxybutanoic acid).

L-Canavanine was regenerated from L-canaline by Kitagawa (24; 26, pp.29-31) in 1936 by an extremely tedious method in which no yields were reported. Canaline was dibenzoylated and then carefully hydrolyzed to α -monobenzoyl canaline. This was reacted with O-methylisourea followed by hydrolysis to give canavanine which was isolated as its flavianate.

The fact that canaline and canavanine had been synthesized only once, and then from starting material prepared by hydrogenation of canaline itself, suggested the value of developing a practical synthesis. The results of this project are reported in the following pages.

DISCUSSION PART I

A New Synthesis of Canaline and Canavanine

The starting material for the synthesis of canaline was γ -butyrolactone (I) (see Figure 1) which was generously donated by General Aniline and Film Corporation. The advantages in starting with this material lie in its ready availability from commercial sources and the presence of a preformed four carbon straight-chained system which lends itself readily to the reactions involved. This lactone was brominated according to the directions of Livak et al. (34, p.2219) to give an 89 percent yield of α -bromo- γ -butyrolactone (II). This was treated with 28 percent aqueous ammonia to give α -amino- γ -butyrolactone hydrobromide (III) in 68 percent yield (39). Opening the lactone ring according to Fischer and Blumenthal (14) gave an 84 percent yield of DL-2-amino-4-hydroxybutanoic acid (DL-homoserine) (IV).

Livak (34, pp.2219-2220) prepared 5-(2-bromoethyl)-hydantoin (V) from homoserine in 51 percent yield. The average yield obtained in this laboratory from this material was only 42 percent. However, it was possible to prepare this compound directly from the lactone (III) in 39 percent yield. This resulted in a small increase in the total yield of this hydantoin from γ -butyrolactone.

The bromo compound was easily converted to 5-(2-iodoethyl)-hydantoin (IX) in 76 percent yield using sodium iodide in acetone.

Treatment of 5-(bromoethyl)-hydantoin with hydroxyurethane (15, p.965) in alcoholic potassium hydroxide gave a 55 percent yield of 5-[2-(carbethoxyaminoöxy)-ethyl]-hydantoin (VI). It was found that a ratio of hydroxyurethane to 5-(2-bromoethyl)-hydantoin of 2:1 gave the best results. A 1:1 ratio resulted in only an 11 percent yield and a 3:1 ratio gave no increase in yield (51 percent). Almost identical results were obtained using the iodo hydantoin. The use of potassium ethoxide in anhydrous ethanol was tried using a 1:1 ratio; very crude material in a 26 percent yield was obtained.

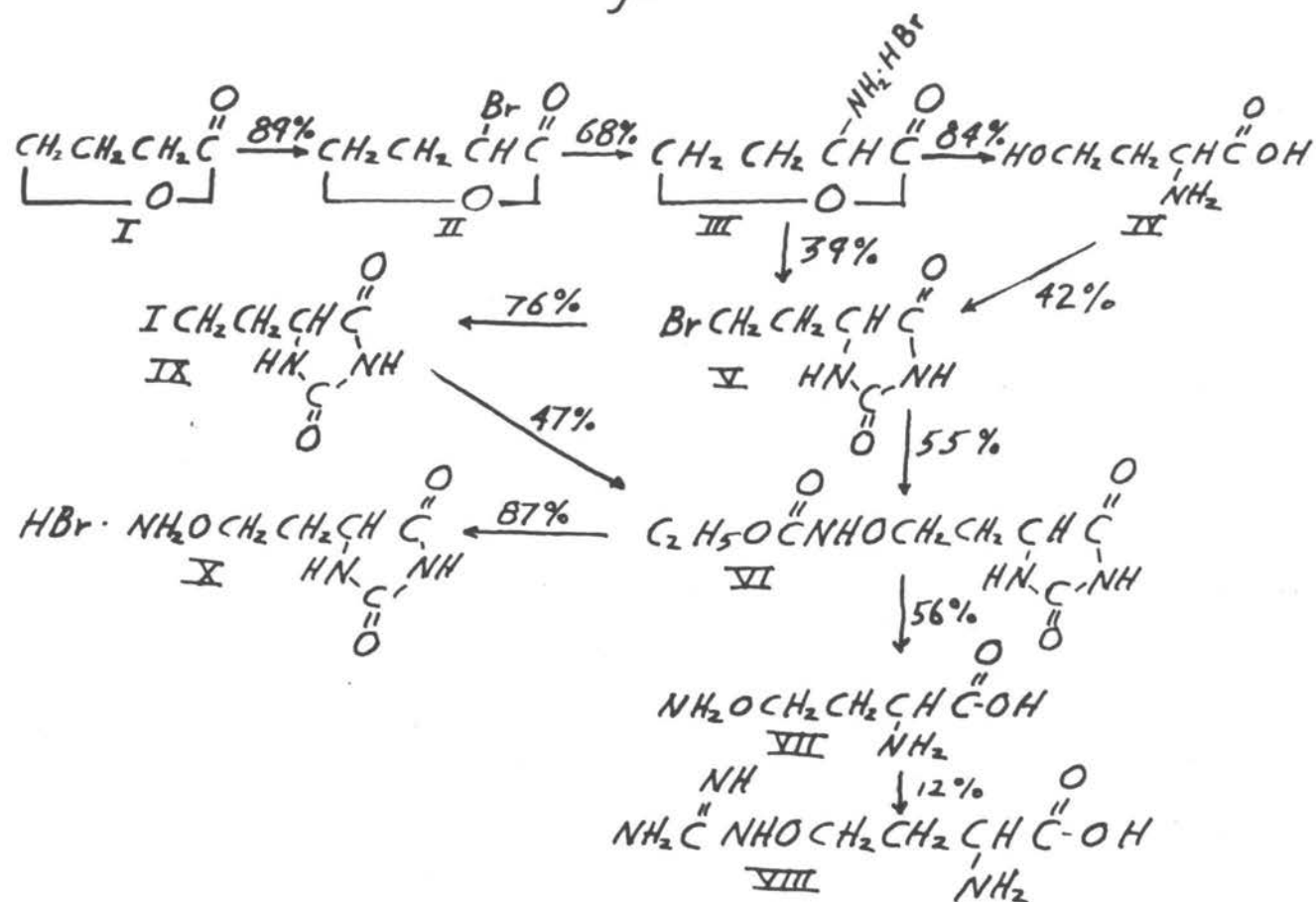
DL-canaline (VII) was obtained in 56 percent yield from 5-[2-(carbethoxyaminoöxy)-ethyl]-hydantoin by hydrolysis with 13.5 percent barium hydroxide for 12 hours. This was converted to DL-canavanine (VIII) by the use of methylisourea hydrochloride or methylisothiourea sulfate in only a 12 percent yield by using the method of Kurtz (31). The low yield in this last step was very disappointing. Various modifications in the procedure did not improve this yield.

This procedure is not applicable to the synthesis of an optically active form of canaline, due to rapid racemization of the hydantoin intermediate in alkaline solution (9).

The overall yield of canaline from γ -butyrolactone was 7 percent and that of canavanine 0.9 percent.

It was found that 5-[2-(carbethoxyaminoöxy)-ethyl]-hydantoin could be hydrolyzed with 48 percent hydrobromic acid to 5-[2-(aminoöxy)-ethyl]-hydantoin (X). This material was treated with methylisothiourea sulfate in an endeavor to introduce the guanidinoöxy group. The product from this operation, without characterization, was immediately treated with 13.5 percent barium hydroxide for 12 hours and the hydrolysate worked up in a manner similar to that of canavanine. Only canaline monoflavianate was isolated however. It was not possible to tell from this result whether the aminoöxy hydantoin had been guanylated or not. Such a compound may have been degraded, under the alkaline conditions used in the hydrolysis, to the aminoöxy compound again. It had been hoped that such a guanidinoöxy compound would be stable, under such alkaline conditions, in view of the known alkaline stability of canavanine (26, p.28).

Figure 1



DISCUSSION PART II

The Attempted Synthesis of 2-Amino-3-Aminoöxypropanoic Acid (Lower Homologue of Canaline)

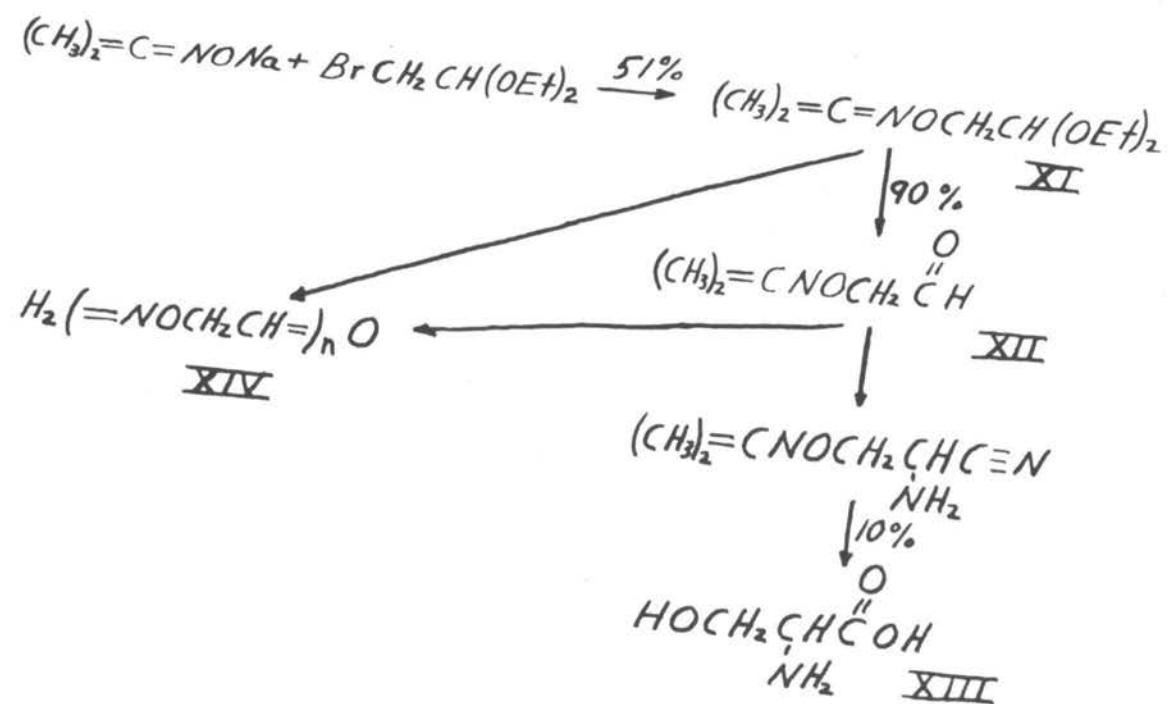
One of the objectives of this study was the synthesis of the lower homologues of canaline and canavanine for reasons of biological testing. For this purpose the Strecker reaction was employed to make the amino acid, and the oxime method to introduce the aminoöxy group. The acetal, acetone O-[2,2(diethoxy)ethyl]-oxime (XI) (See Figure 2), was prepared in 51 percent yield from the sodium salt of acetone oxime and bromoacetal in dry toluene. (An attempt to substitute commercially available chloroacetal for bromoacetal was unsuccessful). This acetal was hydrolyzed with one percent hydrochloric acid in the presence of a large excess of acetone. This liberated the aldehyde group but retained the oxime linkage to give crude acetone O-formylmethyloxime (XII). On the basis of analytical data it appears that acid hydrolysis in the absence of acetone caused a hydrolytic cleavage of the substituted oxime to initially form 2-aminoöxy-acetaldehyde, $\text{NH}_2\text{OCH}_2\text{CHO}$, which polymerized in a head to toe fashion to give a polyoxime, $\text{H}_2(=\text{NOCH}_2\text{CH}=\text{O})_n\text{O}$.

Having obtained the crude aldehyde (acetone O-formylmethyloxime) (XII) in good yield, a Strecker reaction was run in an attempt to produce the desired amino acid. As is

well known, a strong mineral acid is used to hydrolyze the alpha-amino nitrile intermediate in this synthesis. Since only serine (XIII) was isolated from the reaction, it was concluded that the acid hydrolysis resulted in the degradation of the aminoöxy group to the hydroxyl group. Such a result was not completely surprising since Sidgewick (45, p.161) mentions that O-ethylhydroxylamine is hydrolyzed by hydrochloric acid at 150° to ethyl chloride and hydroxylamine.

An attempt to utilize a hydantoin intermediate according to the method of Holland and Naylor (19) to make the amino acid was also unsuccessful.

Figure 2:



DISCUSSION PART III

The Bromination of δ -Valerolactone

The successful utilization of γ -butyrolactone in the synthesis of canaline and canavanine suggested the analogous use of δ -valerolactone for the synthesis of the higher homologues of these amino acids. This method would first involve the bromination of δ -valerolactone to give α -bromo- δ -valerolactone. In order to prepare this material, a sample of polymeric δ -valerolactone (m.p. 54-55°C) was obtained from Carbide and Carbon Chemicals Co. This compound was treated with bromine in the same manner as Livak *et al.* (34, p.2219) brominated γ -butyrolactone. The brominated reaction product was distilled under 4-8 mm. of pressure to give a very dark viscous oil which on analysis appeared to be a mixture of 2,5-dibromopentanoic acid and α -bromo- δ -valerolactone. This result was disappointing since only the α -bromo- δ -valerolactone was desired. It was believed that complete cyclization to the lactone would have occurred on the vacuum distillation of the reaction mixture since Livak *et al.* obtained only α -bromo- γ -butyrolactone in the bromination of γ -butyrolactones, and in as much as Linstead and Rydon (33, p.583) found that by heating 5-iodopentanoic acid sodium salt under vacuum, a 38 percent yield of δ -valerolactone could be obtained.

In order to identify more definitely the bromination product of δ -valerolactone, it was refluxed with water for several hours. The initial two-phase system was now a homogeneous solution which on ether extraction gave a good yield of very crude α -hydroxy- δ -valerolactone. This material was identified by the conversion of a small aliquot to the phenylhydrazide of 2,5-dihydroxypentanoic acid which has been reported in the literature (41, p.654).

Before a practical route to the higher homologue of canaline can be achieved by this method, it will be necessary either to devise a method of separating the bromination product into its constituent parts or to modify the reaction conditions in such a way as to obtain only α -bromo- δ -valerolactone. Unfortunately, there was not sufficient time available to this investigator to pursue this study.

EXPERIMENTAL

 α -Amino- γ -butyrolactone hydrobromide (III):

This compound was synthesized according to Plieninger (39, p.267) from α -bromo- γ -butyrolactone (II) prepared from γ -butyrolactone (I) by Livak's (34, p.2219) procedure. Slightly modified and more detailed directions than those of Plieninger's are presented here.

α -Bromo- γ -butyrolactone (II) (200 g., 1.21 moles) was dissolved in 1.2 liters of 28 percent aqueous ammonia and allowed to stand for 24-48 hours at room temperature. Barium hydroxide octahydrate (473 g., 1.5 moles) was added with stirring and the resultant slurry boiled for 4-1/2 hours to remove excess ammonia. A small amount of ammonia was still evident at the end of this time but this was due probably to some decomposition. The slurry was then acidified very slowly with a solution of 84.5 ml. of concentrated sulfuric acid dissolved in 600 ml. of water; extensive foaming occurred when the acid was added too rapidly. After removing barium sulfate, the solution was evaporated to dryness in vacuo. The residue was triturated with 200 ml. of absolute alcohol and filtered to remove a dark colored impurity. An additional treatment with 50 ml. of absolute alcohol removed a further small amount. The stout, tan colored crystals were air dried with an aspirator. Yield: 150 g. (68 percent) which

melted at 220-23°C with slight sintering at 207°C
(Plieninger obtained a melting point of 218°C).

5-(2-Bromoethyl)-hydantoin (V):

α -Amino- γ -butyrolactone hydrobromide (III) (27.3 g., 0.15 mole) was dissolved in 90 ml. of water and anhydrous potassium carbonate (10.4 g., 0.075 mole) added with stirring. The slightly alkaline solution was then heated on the steam bath for a few minutes and then a solution of potassium cyanate (13.0 g., 0.16 mole) in 50 ml. of water was added. The final solution was heated on the steam bath for two hours. The rest of the procedure was carried out according to Livak et al. (34, p.2219) who used DL-homoserine (IV) for this preparation. The solution was treated with 100 ml. of 48 percent hydrobromic acid and heated for an additional two hours on the steam bath. The solution was evaporated to dryness in vacuo and the residue digested with 150 ml. of hot acetone and filtered. The potassium bromide residue was washed until white with hot acetone. The acetone filtrate was evaporated and the residue heated for two hours on the steam bath with another 100 ml. of 48 percent hydrobromic acid. After evaporating in vacuo, the residue was dissolved in 75 ml. of hot water, filtered and cooled. The crude crystalline product was removed and immediately recrystallized from 70 ml. of

water. Yield: 9.5- 12.1 g. (31-39 percent) which melted at 139.5-40.0°C. A mixed melting point with the same product prepared from DL-homoserine showed no depression.

5-(2-Iodoethyl)-hydantoin (IX):

5-(2-Bromoethyl)-hydantoin (V) (2.07 gms, 0.010 mole) and sodium iodide (1.80 g, 0.012 mole) were dissolved in 30 ml. of acetone and the solution refluxed for one-half hour. An additional 20 ml. of acetone was added, the solution cooled to room temperature, and potassium bromide removed by filtration. The filtrate was evaporated to remove acetone and the residue taken up in 35 ml. of hot water, filtered and cooled to precipitate the product as tan colored tiny crystals. Yield: 1.93 g. (76 percent), m.p. 168.0-169.5°C. Two recrystallizations from water gave tiny white scales melting at 172.5-173.0°C.

Analysis: Calculated for $C_5H_7IN_2O_2$: C, 23.6; H, 2.8

Found: C, 23.2; H, 2.7

5-[2-(Carbethoxyaminooxy)-ethyl]-hydantoin (VI):

To an alcoholic solution of potassium hydroxide made from 3.96 g. (0.06 mole) of 85 percent potassium hydroxide and 60 ml. of absolute alcohol was added 6.21 g. (0.03 mole) of 5-(2-bromoethyl)-hydantoin (V) and a solution of 6.30 g. (0.06 mole) of hydroxyurethane (15, p.965) in

40 ml. of absolute alcohol. On warming, a light brown solution was obtained which was refluxed for three hours, cooled and filtered to remove potassium bromide. The filtrate was evaporated to dryness and the sirupy residue dissolved in 30 ml. of water, neutralized with dilute hydrochloric acid and placed in the refrigerator. The next day a crop of white needles (2.88 g., m.p. 151.5-153°C) was removed. The mother liquor was evaporated on the steam bath to one-half of its original volume and exhaustively extracted with ether. The ether extract was evaporated to one-fourth of its original volume and cooled to give an additional 0.76 g. (m.p. 150-3°C) of product. Yield: 3.64 g. (52 percent). Recrystallization from water gave colorless needles melting at 152-3°C.

Analysis: Calculated for $C_8H_{13}N_3O_5$: C, 41.6; H, 5.7

Found: C, 41.3; H, 5.8

Experiments with 5-(2-iodoethyl)-hydantoin did not improve the yield nor did decreasing the ratio of 5-(2-bromoethyl)-hydantoin to hydroxyurethane from 1:2 to 1:3; a 1:1 ratio resulted in only an 11 percent yield of a very crude product.

5-[2-(Aminoxy)-ethyl]-hydantoin (X):

5-[2-(Carbethoxyaminoxy)-ethyl]-hydantoin (2.31 g., 0.01 mole) was refluxed in 4.2 ml. of 48 percent hydrobromic acid for two hours. The brown hydrolysate was

was immediately evaporated to dryness in vacuo and the residue thoroughly dried by adding a little absolute alcohol and evaporating once again. The residue from this operation was triturated with a small amount of absolute alcohol and finally placed in the refrigerator. Brown colored crystals were then recovered and washed with ether. Yield; 2.09 g. (87 percent). A recrystallization from 95 percent ethyl alcohol gave tan colored crystals, m.p. 167-168°C with decomposition.

Analysis: Calculated for $C_5H_{10}O_3N_3Br$: C, 25.0; H, 4.2

Found: C, 24.7; H, 4.3

DL-Canaline (DL-2-amino-4-aminooxybutanoic acid) (VII):

5-[2-(carbethoxyaminooxy)-ethyl]-hydantoin (2.31 g., 0.01 mole) and barium hydroxide octahydrate (18.15 g.) were refluxed in 55 ml. of water for 12 hours. The white solid residue was removed and the filter cake extracted with 25 ml. of boiling water and finally washed with 25 ml. of hot water. The combined filtrate and washings were treated with 5.7 g. of ammonium carbonate by heating and stirring, the barium carbonate removed and washed with hot water, and the filtrate and washings evaporated to dryness in vacuo. Attempts to bring about crystallization of the material obtained at this stage were unsuccessful. Picric acid (5.4 g., 0.02 mole) was added with 100 ml. of water

and the mixture was heated to effect solution, filtered and allowed to crystallize at room temperature. Tiny yellow needles of DL-Canaline dipicrate were obtained, removed and washed with a little water. Yield: 3.38 g. (57 percent) melting at 189.5-91.5°C with slight sintering at 187.5°C. Recrystallization from water gave crystals melting at 190.5-91.0°C.

Analysis: Calculated for $C_{16}H_{16}N_8O_{17}$: C, 32.45; H, 2.7

Found: C, 32.45; H, 2.9

In order to prepare free canaline, the dipicrate (14.6 g., 0.0247 mole) was decomposed with 100 ml. of hot 10 percent sulfuric acid. After cooling in a refrigerator overnight, the picric acid was removed and washed with a little ice water. In order to remove all traces of picric acid from the filtrate and washings, they were combined and extracted exhaustively with ether. The now colorless solution was diluted to 450 ml. and treated with barium hydroxide octahydrate to quantitatively remove sulfate ion. The barium sulfate was removed and washed with a little hot water. The filtrate and washings were evaporated to near dryness in vacuo and 20 ml. of isopropyl alcohol added to the residue followed by refrigeration. Yield: 3.18 g. (96 percent from picrate) of white crystals melting at 198-201°C with decomposition. Two recrystallizations from aqueous isopropyl alcohol gave white needles, m.p. 195-98°C with decomposition.

A paper chromatogram using the butanol-acetic acid-water (4:1:5) system gave an R_f value of 0.59 and indicated only slight traces of impurity. The synthetic product gave a positive ninhydrin test and an orange-red color with alkaline picrate (Jaffe's test) which is characteristic of canaline.

Analysis: Calculated for $C_4H_{10}N_2O_3$: C, 35.8; H, 7.5

Found: C, 35.9; H, 7.8

DL-Canaline monoflavianate:

Prepared from equimolar quantities of canaline and flavianic acid in water. Obtained yellow crystals which melted at 201-202.5°C. Recrystallization from water raised the melting point to 207-208°C.

Analysis: Calculated for $C_{14}H_{16}N_4O_{11}S$: C, 37.5; H, 3.6

Found: C, 37.6; H, 3.5

DL-Canavanine (DL-2-amino-4-guanidinoöxybutanoic acid) (VIII):

Canaline (VII) (0.67 g., 0.005 mole) was converted to its copper complex with 0.72 g. of cupric carbonate or 0.44 g. of cupric oxide by boiling the reactants in water for 10 minutes and then filtering. The deep blue solution was evaporated to 7-8 ml. on the steam bath. After cooling in an ice bath, methylisourea hydrochloride (32) (1.1 g.,

0.01 mole) and 5.0 ml. of 2N sodium hydroxide were added. The solution was allowed to sit at room temperature for two weeks. At the end of this time, it was noted that crystallization of a purple-pink material had occurred and the pH of the solution had decreased from approximately 10 to 8. The solution was acidified to Congo Red with dilute hydrochloric acid (purple-pink crystals dissolved), treated with hydrogen sulfide and filtered. The filtrate was brought to boiling to remove hydrogen sulfide. (At this point Kitagawa (26) refluxed his synthetic product for 20 hours with 10 percent hydrochloric acid to remove a contaminant. Similar treatment of this product gave no improvement in yield or quality.

Flavianic acid dihydrate (1.75 g.) dissolved in a little warm water was added and the solution placed in the refrigerator. The next day yellow crystals of a crude canavanine flavianate were collected (1.90 g.). This was immediately recrystallized from water to remove a high-melting contaminant of unknown constitution. Yield: 1.22 g., m.p. 200-200°C with decomposition. Satisfactory analytical data for this compound were not obtained but the results suggest the material to be a monoflavianate.

This flavianate without further characterization was dissolved in 40 ml. of hot water and treated with 1.13 g. of barium hydroxide octahydrate dissolved in boiling water. On cooling, the precipitated barium flavianate was

removed and washed twice with hot water. The still yellow filtrate was treated with sulfuric acid to quantitatively remove barium and finally charcoaled to remove the last traces of flavianic acid. The now colorless solution was evaporated to dryness in vacuo and to the residue was added 20 ml. of absolute alcohol. White to slightly gray crystals separated which melted at 180-84°C with decomposition on rapid heating. An authentic sample of L-canavanine prepared from Jack bean meal gave a melting point of 180-182°C with decomposition on fast heating and a mixed melting point showed no depression of temperature. Yield: 0.09-0.11 g. (10-12 percent).

The synthetic material gives a positive ninhydrin test and a positive canavanine test with amidine-pentacyanoferrate reagent (13).

Comparative paper chromatograms from two different solvent systems of the synthetic and natural products as well as mixed material gave identical R_f values within each system. The R_f values obtained from the butanol-acetic acid-water (4:1:5) system were 0.054 and 0.077 in two different runs while the phenol-water (80 percent phenol) system gave an R_f of 0.47.

Analysis: Calculated for $C_5H_{12}N_4O_3$: C, 34.1; H, 6.9; N, 31.8

Found: C, 32.9; H, 7.1; N, 31.2

In order to compare synthetic DL-canavanine to the natural product, the infra-red absorption spectra of these materials were obtained. A Perkin-Elmer Model 112 C instrument fitted with a sodium chloride prism and employing the double pass monochromator principle was used to obtain the data. The sample was prepared as a nujol mull and pressed between two sodium chloride windows.

It was rather surprising to the author to find distinct differences between the two absorption spectras. However, similar results have been obtained with at least five other amino acids. The optically active and DL-forms of cystine, valine, leucine, phenylalanine, and alanine give different absorption data. Wright (49; 50) attributes this to the fact that the DL-forms as obtained by crystallization from solution are compounds and not mixtures. In contrast to this, Wright has shown that mechanical mixtures of D- and L-amino acids absorb in the same identical manner as the pure D- or L-amino acids.

In Table 1 is recorded the data obtained for convenience of comparison. The approximate absorption intensity is given by the symbols (s)-strong; (m)-medium and (w)-weak.

TABLE 1

WAVE NUMBERS AND INTENSITIES OF INFRA-RED
ABSORPTION BANDS

<u>L-Canavanine</u>		<u>DL-Canavanine</u>		Remarks
Wave No. (cm ⁻¹)	Inten- sity	Wave No. (cm ⁻¹)	Inten- sity	
1641	s	1627.5	s	These absorptions occur as individual peaks in one broad band.
1606	s	1599	s	
1580	s	1570	s	
1559	s	1527	s	
1545	s			
1416	m	1406	m	
1378	w	1378	m	
1362	m	1364	m	
		1338	w	
		1318	m	
1263	w	1250	w	
1235	m	1233	w	
1181	w	1173	m	
1159	m	1120	m	
1096	s	1077	s	
1069	m	1051	w	
1027	s	1022	m	
918	s	1000	w	
875	m	957	m	
792	m	928	m	
		878	m	
		836	m	

Acetone O-[2,2-(diethoxy)ethyl]-oxime XI:

The sodium salt of acetone oxime (0.4 mole) was prepared in the same manner as was done previously in the preparation of acetone O-ethyloxime. The dried material was made into a slurry with 160 ml. of dry toluene and placed aside in a beaker temporarily. To the same flask (1000 ml., 3-necked) used in the preparation of the oxime salt were fitted a stirrer and reflux condenser with a drying tube. Bromoacetal (36) (160 g., 0.81 mole) and toluene (40 ml.) were introduced and heated by means of an oil bath at 135°C. The slurry of sodium acetone oxime was added in small portions over one hour and the subsequent mixture stirred and heated for 24 hours. After cooling, the reaction mixture was filtered to remove the insoluble solids. The brown filtrate was distilled through a 42 cm. vigreux column and the fraction boiling above 92°C/20 mm. collected. The lower boiling portion was distilled again and additional material boiling above 92°C/20 mm. collected. Approximately 40-50 g. was accumulated in this manner. Two more distillations gave 22-38 g. (29-51 percent), b.p. 40-45°C/1.5 mm., 98-99°C/20 mm., $n_D^{25} = 1.4259$ of a colorless oil.

Analysis: Calculated for $C_9H_{19}NO_3$: C, 57.1; H, 10.1

Found: C, 56.5; H, 9.9

The unreacted bromoacetal can be recovered from the lower boiling distillates with little trouble.

The acid hydrolysis of acetone
0-[2,2-(diethoxy)-ethyl]-oxime (XI):

A. Hydrolysis with aqueous hydrochloric acid in the presence of excess acetone:

In a solution of 35 ml. of acetone and 19 ml. of 1 percent hydrochloric acid were dissolved 4 g. (0.0212 mole) of acetone 0-[2,2-(diethoxy)-ethyl]-oxime (XI). After refluxing for one hour, the solution was cooled in an ice bath and then neutralized with anhydrous potassium carbonate to pH 7.5-8.5. The acetone was removed by evaporation in vacuo at temperatures not over 45°C. The residue was chilled in an ice bath and extracted with three to four portions of ether. Anhydrous sodium sulfate was used as a salting-out agent to insure complete extraction. The ether extracts were dried with anhydrous sodium sulfate. After evaporation the ether, 1.3-2.5 g. of a slightly yellow oil was obtained. This crude product decomposed even on vacuum distillation and it was not possible to obtain pure material. However, the oil gave the typical tests expected for an aldehyde (Schiff's and Tollen's tests) and is probably crude acetone 0-formyl-methyloxime (XII). Attempts to prepare several aldehyde derivatives were all unsuccessful. Treatment with dilute aqueous hydrochloric acid gave a white insoluble precipitate similar in its thermal properties to the polymeric material described in part B.

B. Hydrolysis with aqueous hydrochloric acid in the absence of acetone:

When the acetal, acetone O-[2,2-(diethoxy)-ethyl]-oxime (XI), was hydrolyzed in 1:1 or 2:1 one percent aqueous hydrochloric acid-95 percent ethanol in the absence of acetone by refluxing for 15-60 minutes, a cream colored to white gummy insoluble material was deposited. On drying over P_2O_5 in vacuo, it became more friable and amorphous. Two grams of the acetal gave about 0.55 g. of this material. The sample analyzed below melted over the range 90-190°C with decomposition and gave positive Schiff's and Tollen's tests.

Analysis: Calculated for $C_2H_3NO(=NOCH_2CH=)$:
C, 42.4; H, 5.26
Found: C, 42.9; H, 5.9

This analysis suggests the material to be polymeric in nature and composed of the units $=NOCH_2CH=$ since the carbon and hydrogen content of possible monomeric products are much higher than that found for the material isolated.

The attempted synthesis of 3-aminooxy-2-aminopropanoic acid via the Strecker reaction and the isolation of serine (XIII):

Material believed to be crude acetone O-formylmethyl-oxime (XII) (4.1 g., 0.0356 mole) prepared as described above was added to 7.2 g. of 10 percent methanolic ammonia. To the resulting solution was added ammonium chloride

(2.86 g., 0.0534 mole) dissolved in 17 ml. of water. The mixture was heated at 80°C for four hours. The dark red solution was cooled and then added to 50 ml. of concentrated hydrochloric acid (hood) and allowed to sit overnight. The next day, the solution was heated on the steam bath for 3-1/2 hours; additional acid (20 ml.) was added and heating continued for six hours longer. The solution was then evaporated to dryness in vacuo and the residue redissolved in water and filtered to remove a black insoluble material. The clear brown colored filtrate was passed through an IRA-400 strongly basic anionic exchange resin to remove the anions from the solution. The resin was then eluted with 2N hydrochloric acid to give an amino acid effluent which was identified by means of the ninhydrin test. The fractions containing the amino acid were evaporated to dryness and treated with silver carbonate (0.1068 equiv.) to remove chloride. After removing silver chloride, the filtrate was saturated with hydrogen sulfide and filtered again. The final solution was evaporated to dryness and 50 ml. of absolute alcohol added. The cloudy solution was placed in the refrigerator. The next day a slightly yellow crystalline material was removed and immediately recrystallized from water-isopropyl alcohol. A crystalline material, 0.46 g. (10.3 percent yield of serine), m.p. 175-275°C with decomposition was

thus obtained. The material was again recrystallized from water-isopropyl alcohol.

Analysis: Calculated for serine ($C_3H_7NO_3$): C, 34.3; H, 6.7

Calculated for 3-aminoöxy-2-aminopropanoic acid

($C_3H_8N_2O_3$): C, 30.0; H, 6.7

Found: C, 33.2; H, 6.6

A paper chromatogram using the phenol-water system with an authentic sample of serine showed the material to be predominately serine with only a trace of some other material which also gives a ninhydrin test.

Bromination of δ -valerolactone:

Polymeric δ -valerolactone (m.p. $54-55^{\circ}\text{C}$) was placed in a flask equipped with a stirrer, dropping funnel, reflux condenser with a drying tube and a thermometer. The lactone was melted followed by the addition of 0.5 ml. of phosphorous tribromide. Through the dropping funnel was introduced 12.2 ml. (38 g.) of dry bromine over one-half hour while the temperature was maintained at $70-75^{\circ}\text{C}$. The resultant mixture was stirred at this temperature for 24 hours and heated finally to 100°C for one hour. The dark bromination product was vacuum distilled (caustic trap) to give 31.2 g. of a dark viscous oil, b.p. $146-165^{\circ}\text{C}/4$ mm.

Analysis: Calculated for 2,5-dibromopentanoic acid

($\text{C}_5\text{H}_8\text{O}_2\text{Br}_2$): C, 23.1; H, 3.1

Calculated for α -bromo- δ -valerolactone

($\text{C}_5\text{H}_7\text{O}_2\text{Br}$): C, 33.5; H, 3.9

Found: C, 29.1; H, 4.1

This bromination product (10.3 g.) was refluxed with 20 ml. of water for 24 hours. The resulting solution was exhaustively extracted with ether, separated, and the ether extract dried over anhydrous sodium sulfate.

Evaporation of the ether gave a brown oil (5.16 g.) which was tentatively identified as very crude α -hydroxy- δ -valerolactone according to its analysis. This

identification was confirmed by the conversion of a small aliquot of this material to the phenyl-hydrazide of 2,5-dihydroxypentanoic acid according to the directions of Reichstein and Grussner (41, p.654). White crystals melting at 104-104.5°C were obtained. (Reichstein and Grussner reported a melting point of 106-107°C).

Analysis: Calculated for $C_{11}H_{16}O_3N_2$: C, 58.9; H, 7.2

Found: C, 58.7; H, 7.2

Acetone O-ethyloxime (Acetone ethoxime):

To a cooled solution of 15.6 g. (0.678 mole) of sodium in 300 ml. of absolute alcohol were added 50 g. (0.684 mole) of acetone oxime (42). The resulting solution was evaporated in vacuo and the solid residue dried by heating in an oil bath at 110°C under 1 mm. pressure for one hour. This dry sodium salt of acetone oxime was crushed and mixed thoroughly with 200 ml. of dry xylene in the same flask in which it was prepared. Ethyl bromide (296 g., 2.7 moles) was added and the mixture refluxed with stirring for 15-20 hours. Sodium bromide residue was then removed and washed well with xylene. The clear solution was fractionated through a 77 cm. column containing glass helixes. The fraction boiling at 88-95°C was collected to give 20-25 g. (29-37 percent) of a colorless oil. Dunstan and Goulding (12) reported a boiling point of 91.5°-92.5°C.

O-Ethylhydroxylamine Hydrochloride:

Acetone O-ethyloxime (9.24 g., 0.0915 mole) was added to a solution of 10 ml. of concentrated hydrochloric acid in 30 ml. of water. The mixture was refluxed for one hour; then 5 ml. more of concentrated hydrochloric acid and 15 ml. of water were added and refluxing continued for an additional 75 minutes. Although only one phase was

present at the end of this time, it was found that there was still unhydrolyzed acetone O-ethyloxime present. This was collected on distillation of the solution and subjected to three more treatments with hydrochloric acid. The aqueous residues containing the product were evaporated to dryness in vacuo and the crude product dissolved in 12 ml. of absolute alcohol, filtered, and finally precipitated with excess ethyl acetate (250 ml.). The product, which is quite hygroscopic, was dried over P_2O_5 in vacuo. Yield: 3.3 g. (37 percent), m.p. $125^{\circ}C$. Jones (20) reported a melting point of $127-128^{\circ}C$.

Analysis: Calculated for C_2H_8ClNO : Cl, 36.4

Found: Cl, 36.5

SUMMARY

A brief review of the discovery, occurrence, isolation and properties of canavanine and canaline has been given.

A discussion of the methods of preparation of O-substituted hydroxylamines and hydroxy-guanidines was presented along with a description of earlier work on the synthesis of canavanine.

A new synthesis of canaline by a five-step reaction scheme (Figure 1) from γ -butyrolactone in seven percent overall yield was described. Canavanine was prepared directly from canaline in 12 percent yield. New compounds prepared during this study were discussed.

An attempt to synthesize the lower homologue of canaline via a Strecker reaction (Figure 2) from the compound, acetone O-[2,2-(diethoxy)-ethyl]-oxime, resulted only in the isolation of DL-serine. A material believed to be a polyoxime appeared as a side-product in this investigation.

The bromination of δ -valerolactone gave a mixture of what is probably 2,5-dibromovaleric acid and α -bromo- δ -valerolactone. This prevented the immediate utilization of this scheme to make the higher homologue of canaline.

BIBLIOGRAPHY

1. Adams, P., D. W. Kaiser and G. A. Peters. Chemistry of dicyandiamide. IV. Reaction of acyldicyandiamides with hydroxylamine and cyanamide. *Journal of organic chemistry* 18:934-40. 1953.
2. Andrewes, C. H., Harold King and James Walker. Experimental chemotherapy of typhus. Antirickettsial action of p-sulphonamidobenzamidine and related compounds. *Proceedings of the Royal society of London* 133B:20-62. 1946.
3. Anker, H. S. and H. T. Clarke. Carboxymethoxylamine hemihydrochloride (acetic acid, aminoöxy-, hydrochloride). In *Organic syntheses. Collective vol. 3*. New York, John Wiley, 1955. pp. 172-174.
4. Archibald, Reginald M. Colorimetric determination of canavanine. *Journal of biological chemistry* 165:169-178. 1946.
5. Armstrong, Marvin D. The preparation of D- and L-Homoserine. *Journal of the American chemical society* 70:1756-1759. 1948.
6. Arndt, F. Darstellung von Methylmercaptan. *Berichte der Deutschen chemischen Gesellschaft* 54:2236-2242. 1921.
7. Borek, Ernest and H. T. Clarke, Compounds related to canaline and canavanine. *Journal of biological chemistry* 125:479-494. 1938.
8. Cadden, J. F. Spatial configuration and preparation of canavanine. *Proceedings of the Society for experimental biology and medicine* 45: 224-226. 1940.
9. Dakin, Henry Drysdale. On amino-acids. Hydroxyglutamic acid. *Biochemical journal* 13:398-429. 1919.
10. Damodaran, M. and K. G. A. Narayanan. A comparative study of arginase and canavanase. *Biochemical journal* 34:1449-1459. 1940.

11. Dunn, M. S. Properties of amino acids. In Handbook of chemistry and physics. 31st ed. Cleveland, Chemical rubber publishing co. 1949. p. 1454.
12. Dunstan, Wyndham and Ernest Goulding. The action of alkyl haloids on aldoximes and ketoximes. II. Alkylated oximes and iso-oximes, and the constitution of aliphatic oximes. Journal of the Chemical society, 1901, pp. 628-641.
13. Fearon, W. R. The amidine-pentacyanoferrate reaction: a test for guanidines, urea, and thiourea. Analyst 71:562-566. 1946.
14. Fischer, Emil and Herbert Blumenthal. Synthese der α -amino- γ -oxy-buttersaure. Berichte der Deutschen chemischen Gesellschaft 40:106-113. 1907.
15. Fuller, A. T. and Harold King. Some alkoxy- and alkylenedioxydi-amines and alkoxy- and alkylendioxydi-guanidines. Journal of the Chemical society, 1947, pp. 963-969.
16. Fuson, Reynold C. Advanced organic chemistry. New York, John Wiley, 1950. 669p.
17. Gulland, J. Masson and Colin J.O.R. Morris. Canavanine. Journal of the Chemical society, 1935, pp. 763-766.
18. Hecker, Charles H. A study of some new alkyl-hydroxylamines. American chemical journal 50:444-466. 1913.
19. Holland, D. O. and J. H. C. Naylor. Experiments in the synthesis of methionine. Journal of the Chemical society, 1952, pp. 3403-3409.
20. Jones, Lauder W. On salts of nitroparaffins, and acylated derivatives of hydroxylamine. American chemical journal 20:1-51. 1898.
21. Kitagawa, Matsunosuke and Tetsuo Tomiyama. A new amino-compound in the Jack bean and a corresponding new ferment. Journal of biochemistry (Japan) 11:265-271. 1929.
22. Kitagawa, Matsunosuke and Hiroshi Yamada. Studies on a di-amino acid, canavanin. (II.) Journal of biochemistry (Japan) 16:339-349. 1932.

23. Kitagawa, Matsunosuke and Shin-ichi Monobe. Studies on a diamino acid, canavanin. III. The constitution of canalin. Journal of biochemistry (Japan) 18:333-343. 1933.
24. Kitagawa, Matsunosuke and Akishige Takani. Studies on a diamino acid, canavanin. IV. The constitution of canavanin and canalin. Journal of biochemistry (Japan) 23:181-185. 1936.
25. Kitagawa, Matsunosuke. Studies on a diamino acid, canavanin. V. The synthesis of canalin. Journal of biochemistry (Japan) 24:107-112. 1936.
26. Kitagawa, Matsunosuke. A di-amino acid, canavanin, and a mono-amino acid, canalin. Journal of biochemistry (Japan) 25:23-41. 1937.
27. Kitagawa, Matsunosuke and Jiro Tsukamoto, Studies on a di-amino acid, canavanin. VI. The formation of desamino canavanin from canavanin. Journal of biochemistry (Japan) 26:373-385. 1937.
28. Kitagawa, Matsunosuke and Sachio Eguchi. Action of arginase on canavanine and arginine. I. Identification of canavanase and arginase. Journal of the agricultural chemical society of Japan 14:525-531. 1938. (Abstracted in Chemical abstracts 32(22):9104⁶. 1938.)
29. Kitagawa, Matunosuke and Tuneki Watanabe. Action of arginase on canavanine and arginine. II. Effects of manganese and ferrous salts and cysteine on arginase. Journal of the agricultural chemical society of Japan 14:779-90. 1938. (Abstracted in Chemical abstracts 33(7):2545². 1939.)
30. Kitagawa, Matunosuke. Action of arginase on canavanine and arginine. III. Journal of the agricultural chemical society of Japan 15:267-279. 1939. (Abstracted in Chemical abstracts 34(1):119⁶. 1940.)
31. Kurtz, Alton C. Use of copper (II) ion in masking α -amino groups of amino acids. Journal of biological chemistry 180:1253-1267. 1949.

32. Kurzer, Frederick and Alexander Lawson. Methylisourea hydrochloride (Pseudourea, 2-methyl-, hydrochloride). In Organic syntheses. Vol. 34. New York, John Wiley, 1954. pp. 67-71.
33. Linstead, R. P. and H. N. Rydon. Investigations of the olefinic acids. XI. The formation of lactones from some Δ^{γ} -un-saturated acids, and an example of ring-chain (lactoenic) tautomerism. Journal of the Chemical society, 1933, pp. 580-586.
34. Livak, J. E. et al. Synthesis of dl-methionine. Journal of the American chemical society 67: 2218-2221. 1945.
35. Makisumi, Satoru. Paper chromatography of guanidine compounds. Journal of the chemical society of Japan, pure chemistry section 73:737-739. 1952. (Abstracted in Chemical abstracts 47 (12): 5848d. 1953.)
36. McElvain, S. M. and D. Kundiger. Bromoacetal (acetaldehyde, bromo-, diethyl acetal). In Organic syntheses. Collective vol. 3. New York, John Wiley, 1955. pp. 123-125.
37. Nyberg, David D. and Bert E. Christensen. The synthesis of certain O-substituted derivatives of hydroxyguanidine and hydroxybiguanide. Journal of the American chemical society 78: 781-783. 1956.
38. Pilcher, K. S. et al. Inhibition of multiplication of Lee influenza virus by canavanine. Proceedings of the Society for experimental biology and medicine 88:79-86. 1955.
39. Plieninger, Hans. Die aufspaltung des γ -butyrolactons and α -amino- γ -butyrolactons mit natriummethylmercaptid bzw-selerid. Eine synthese des methionins. Chemische Berichte 83:265-268. 1950.
40. Pratoius-Seidler, Gustav. Zur kenntniss des cyanamids. Journal fur praktische Chemie 21:129-150. 1880.
41. Reichstein, T. and A. Grussner. Polyoxy-acyl-derivate des β -alanins. Helvetica chimica acta 23:650-657. 1940.

42. Semon, Waldo L. Hydroxylamine hydrochloride and acetoxime. In Organic syntheses. Collective vol. 1. New York, John Wiley, 1941. pp. 318-321.
43. Semper, Leopold and Leo. Lichtenstodt. Uber N- and O-methylather von ketoximen. Die 4 isomeren methyl ather des p-tolylphenyl-ketoxims. Berichte der Deutschen chemischen Gesellschaft 51:928-942. 1918.
44. Shibuya, Shinichi and Satoru Makizumi. Biochemical studies on guanidine bodies. VIII. Distribution of extractable guanidine bodies in animals and plants. Journal of Japanese biochemical society 25:210-213. 1953. (Abstracted in Chemical abstracts 48(2):765e, 1954.)
45. Sidgwick, N. V., T. W. J. Taylor, and Wilson Baker. The organic chemistry of nitrogen. Rev. ed. Oxford, Clarendon Press, 1937. 590p.
46. Tomiyama, Tetuo. Some physico-chemical properties and methods of isolation and determination of certain amino acids. Journal of the Imperial fisheries institute (Japan) 33:124-207. 1939. (Abstracted in Chemical abstracts 33(20):84748.)
47. Truitt, Price, Loren M. Long, and Marjorie Mattison. A new synthesis of O-substituted hydroxylamines. Journal of the American chemical society 70: 2829. 1948.
48. Vickery, Hubert Bradford and Charles A. Cook. The preparation of crystalline ornithine. The picrates and monosulfates of ornithine. Journal of biological chemistry 94:393-399. 1931.
49. Wright, Norman. The infra-red absorption spectra of the stereoisomers of cystine. Journal of biological chemistry 120:641-646. 1937.
50. Wright, Norman. The infra-red absorption spectra of amino acids. Journal of biological chemistry 127:137-141. 1939.