THE SYNTHESIS OF DIHYDROSPHINGOSINE

BY

JANET LOIS CROSBY

B.S., Iowa State College, 1945

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1948

URBANA, ILLINOIS

UNIVERSITY OF ILLINOIS

THE GRADUATE COLLEGE

August 5, 1948

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY JANET LOIS CROSBY

ENTITLED THE SYNTHESIS OF DIHYDROSPHINGOSINE

BE ACCEPTED* AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY

H. E. Carter
In Charge of Thesis

Roger Adams
Head of Department

Recommendation concurred in†

Carl S. Marvel
H. R. Snyder
J. R. Bloomquist
L. E. Miller

Committee on Final Examination†

* Subject to successful final examination in the case of the doctorate.
† Required for doctor's degree but not for master's.

3M—1-48—37560

ACKNOWLEDGMENT

The author wishes to thank Dr. H. E. Carter for suggesting this problem and for his advice and encouragement throughout the course of the investigation.

She also wishes to express her appreciation to David J. Wallace and Melvin I. Kohan for their aid in performing the catalytic hydrogenations reported, and to Theta Spoor and Howard Clark and his associates for the microanalyses reported.

TABLE OF CONTENTS

I. INTRODUCTION

Nerve tissue is unique in that over fifty per cent of its dry weight is composed of fatty substances. The sphingolipids, which are derivatives of the complex bases sphingosine and dihydrosphingosine, make up a large portion of these lipids. The structure of sphingosine and dihydrosphingosine have recently been established by degradation studies. The present work was undertaken in order to verify their structure by synthesis.

II. HISTORICAL AND THEORETICAL

A. Sphingosine and dihydrosphingosine

1. Occurrence and properties

Sphingosine and dihydrosphingosine are complex aliphatic bases found in the hydrolysates of certain lipids of nerve tissue. The name sphingolipids (1) has been proposed for this group of substances, which includes principally the cerebrosides, sphingomyelins, gangliosides, and perhaps other less well known types. Since extended discussions of the occurrence, properties, and structure of these lipids have been presented by Glick (2), Norris (3), Phillips (4), Rockwell (5), and Nalbandov (6), only a brief review concerning the bases themselves will be given here.

Sphingosine was first isolated by Thudichum (7) from hydrolysates of phrenosin and sphingomyelin. It is a waxy white solid, very difficultly crystallizable, melting at 83.5° C. It is insoluble in water and soluble in most organic solvents. On standing at room temperature for several days it decomposes slowly to liberate ammonia. When mixed with sugar and sulfuric acid, sphingosine gives a purple color.

Dihydrosphingosine was prepared by Levene (8) in 1912 by catalytic hydrogenation of sphingosine, but it was only recently that the reduced base was found in natural materials. In 1941 Lesuk and Anderson (9) reported the isolation of dihydrosphingosine from the cerebrosides of tapeworm larvae. Subsequently Carter and Norris (10,3,11) demonstrated the presence of considerable amounts of dihydrosphingosine in the hydrolysates of brain and spinal cord

sphingolipids. The proportion of dihydrosphingosine in the spinal cord (10-13 per cent) is several times greater than that in brain (3-4 per cent). Since most of the earlier workers used cerebrosides from brain, the low content of reduced base would account for the fact that it escaped detection. Also, since neither free sphingosine nor its sulfate is easy to purify or identify, sphingosine has usually been characterized as the triacetyl derivative. Unfortunately triacetylsphingosine and triacetyldihydrosphingosine melt at almost the same temperature and give very little depression of melting point on mixing. Optical activity measurements readily differentiate between the two compounds since they rotate in different directions, but such determinations were not generally made by the earlier workers in this field. Therefore, it seems likely that some of the products previously reported may have been mixtures of sphingosine and dihydrosphingosine.

Complete lists of the derivatives of sphingosine and dihydrosphingosine have been compiled by Glick (2), Norris (3), Phillips (4), and Rockwell (5).

2. Isolation

For the isolation of sphingosine and dihydrosphingosine probably the most satisfactory method of hydrolysis is that developed by Klenk (12). The cerebroside or sphingolipid mixture is refluxed for 5 or 6 hours with 25 volumes of 10 per cent (by weight) sulfuric acid in methanol. The hydrolysate is cooled, and the precipitated fatty acids and their esters are removed by filtration. The filtrate is extracted repeatedly with petroleum ether to remove any

acids and esters which remain in solution. The petroleum ether which has dissolved in the methanol is removed by distillation under reduced pressure, and the methanol solution is concentrated to about one-third its original volume. Methanolic potassium hydroxide solution is added until the solution is alkaline, and the precipitated potassium sulfate is removed. The filtrate is acidified with acetic acid and concentrated to a small volume. To this, water is added, and the solution is made alkaline with aqueous potassium hydroxide. Sphingosine, dihydrosphingosine, and O-methylsphingosine precipitate and are extracted with ether. The ether extract is washed well with water to remove any methyl alcohol, is dried with sodium sulfate, and evaporated to dryness to give a mixture of the crude bases.

The crude bases have usually been separated by fractional crystallization of the sulfates. The sulfate of dihydrosphingosine is practically insoluble in methanol at room temperature, while that of sphingosine is moderately soluble. However, it was found in this laboratory (11) that such a procedure does not give a clean separation. In order to overcome this difficulty, Malbandov (6) worked out a procedure which combines the fractionation of the sulfates, as used by Morris (3), with fractional crystallization of the p-hydroxyazobenzene-p'-sulfonates. By this method it is possible to obtain a good separation of sphingosine and dihydrosphingosine. Another base, O-methylsphingosine, is also obtained.

3. Proof of structure

When Thudicum (13) isolated sphingosine from the hydrolysis products of phrenosin, he reported that it was a base of apparent

formula $C_{17}H_{35}NO_2$, and described a number of its salts. Wörner and Thierfelder (14) reported, in 1900, that sphingosine absorbed bromine, indicating unsaturation in the molecule. In 1913 Levene and Jacobs (15) characterized sphingosine as an unsaturated mono-aminodihydroxy alcohol. They prepared a triacetyl derivative (m.p. 102-103° C.) and reduced sphingosine to dihydrosphingosine, using colloidal palladium as a catalyst. In the same year Thomas and Thierfelder (16) also obtained triacetylsphingosine (m.p. 100-102°C).

Lapworth (17) and Levene and West (18,19) oxidized sphingosine at the double bond with chromic acid and isolated an acid which appeared to be n-tridecanoic acid. Elementary analyses and neutral equivalents agreed well with the theoretical, although the melting points did not. Levene and West also oxidized dihydrosphingosine, under the same conditions, and obtained an acid melting at 60-61° C., which they identified as n-pentadecanoic acid. These results would place the double bond between carbon atoms 4 and 5 in a straight chain C_{17} molecule, with two hydroxyl groups and an amino group on the first 3 carbon atoms.

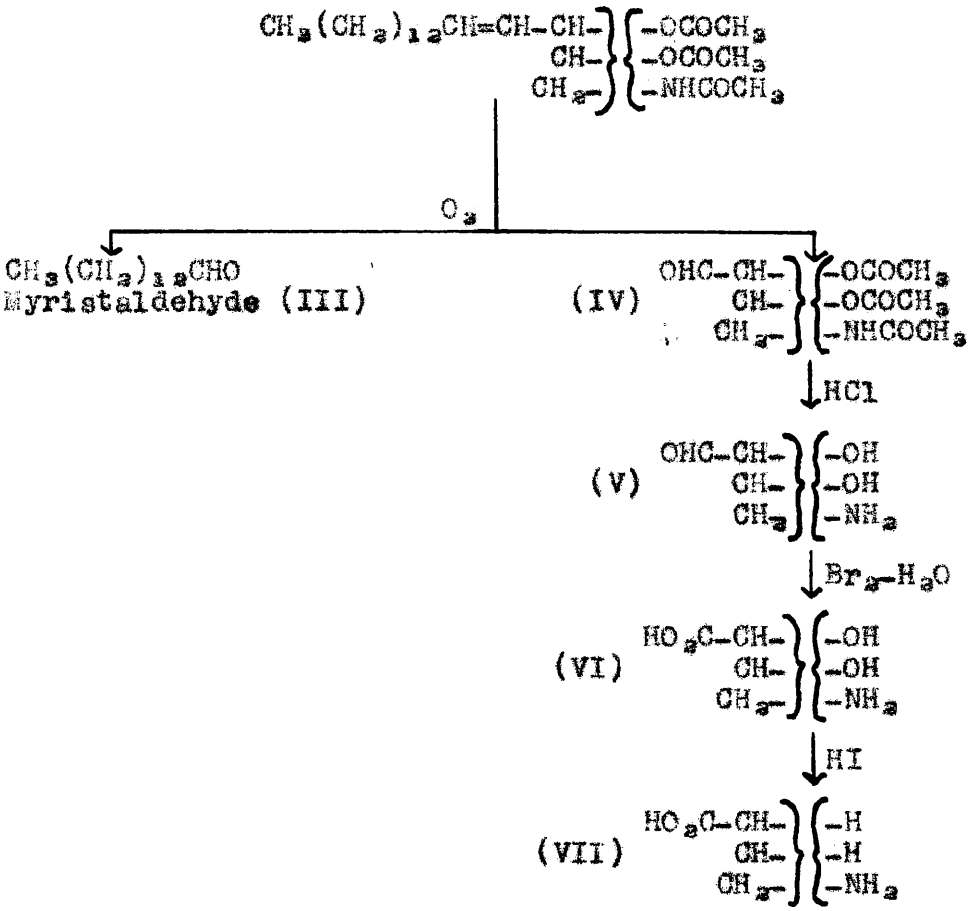
Levene and West (19) also investigated the products obtained from sphingosine by ozonolysis. The nitrogen-containing fragment was not isolated directly as the aminotetrose, but oxidized further with nitric acid. A small amount of the acid thus produced was isolated as the calcium salt, and tentatively identified as the salt of meso-tartaric acid. Although not enough of the compound was isolated for complete identification, Levene considered the evidence sufficient to support straight chain 2,3-dihydroxy structures for sphingosine (I) and dihydrosphingosine (II).

$CH_3(CH_2)_{11}CH=CHCHOHCHOHCH_2NH_2$ (I)

$CH_3(CH_2)_{13}CHOHCHOHCH_2NH_2$ (II)

In 1929, Klenk and his co-workers (20,13) repeated the oxidation studies of Lapworth and Levene, since they considered the occurrence of an odd numbered carbon chain in a natural product unlikely. The acid from sphingosine, on careful purification, was shown to be myristic acid, not tridecanoic acid. Similarly, dihydrosphingosine yielded plamitic acid instead of pentadecanoic acid. Ozonolysis of triacetylsphingosine also gave rise to myristaldehyde and myristic acid. Thus sphingosine must have an eighteen carbon chain in order to leave room for three functional groups beyond the double bond. Reinvestigation of the analytical data for sphingosine and its derivatives showed that they fitted the C_{18} structure better than, or as well as, the C_{17}.

Klenk and his co-workers also attempted to identify the nitrogenous fragment from the ozonolysis to triacetylsphingosine. They converted the aminotetrose to the acid and reduced the hydroxyl groups, leaving an aminobutyric acid, by the following series of reactions:



Compound VI melted at 135° C. and had a specific rotation of $[\alpha]_D^{16°} = -33.45°$. Compound VII decomposed at 280-285° C. Since α-aminobutyric acid decomposes at 285° C., and the β- and γ-isomers both decompose below 200° C., it was concluded that Compound VII was α-aminobutyric acid. Therefore Klenk proposed the 1,3-dihydroxy-3-amino structure for sphingosine (VIII).

$$CH_3(CH_2)_{11}CH=CHCH(NH_2)CHOHCH_2OH \qquad \text{(VIII)}$$

Niemann and Nichols (21), in 1942, described the preparation of d-threo-α-amino-β,γ-dihydroxy-n-butyric acid and its diastereoisomer, d-erythro-α-amino-β,γ-dihydroxy-n-butyric acid. The

specific rotations were $[\alpha]_D^{20^\circ} = -13.7^\circ$ and $[\alpha]_D^{20^\circ} = +16.0^\circ$, respectively. Since neither of these resembles the value obtained by Klenk for Compound VI, these authors concluded that Klenk's formula for sphingosine must be discarded.

Recently, Carter and co-workers (2,3,22,33) in this laboratory have presented evidence which seems to definitely establish the structure of sphingosine as the third possibility--that is, a 1,3-dihydroxy-2-amino compound. The fact that the functional groups are on the three terminal carbons was confirmed by the preparation from N-benzoyldihydrosphingosine of a nicely crystalline benzylidene derivative by treatment with benzaldehyde and zinc chloride. This reaction is characteristic only of 1,2- and 1,3-glycols. That the hydroxyls are not adjacent was shown by the fact that N-benzoyldihydrosphingosine was not attacked by periodic acid, a reagent which oxidizes 1,2-glycols and α-amino alcohols but does not attack an N-acyl-1,3-amino alcohol. Oxidation of dihydrosphingosine with periodic acid yielded equivalent amounts of palmitaldehyde, formaldehyde, formic acid, and ammonia, giving independent evidence for the existence of a C_{18} chain in sphingosine. Furthermore, catalytic reduction of triacetylsphingosine resulted in the formation of acetic acid, which is characteristic of an acetoxy group in an allylic position.

Thus, the structures for sphingosine and dihydrosphingosine have been established as (IX) and (X) respectively.

$$CH_3(CH_2)_{12}CH{=}CHCHOHCH(NH_2)CH_2OH \qquad (IX)$$

$$CH_3(CH_2)_{14}CHOHCH(NH_2)CH_2OH \qquad (X)$$

B. Proposed methods of synthesis

A simple method for the synthesis of 1,3-dihydroxy-2-amino-octadecane, one of the isomers of which is dihydrosphingosine, appeared to be the reduction of the corresponding α-amino-β-hydroxy-stearic ester.

$$CH_3(CH_2)_{14}\underset{OH}{CH}-\underset{NH_2}{CH}-CO_2R' \longrightarrow CH_3(CH_2)_{14}\underset{OH}{CH}-\underset{NH_2}{CH}-CH_2OH$$

1. Reduction of α-amino-β-hydroxy esters

Levene and his co-workers found that the reduction of α-amino esters, using either copper chromite (24) or Raney nickel (25,26) as the catalyst, gave good yields of the corresponding amino alcohols. When Raney nickel was used no racemization of optically active α-amino esters was noted. With large quantities of catalyst and hydrogen pressures of 2200 pounds the reduction proceeded smoothly at temperatures of 40-70° C. and very good yields were obtained.

In order to discover whether α-amino-β-hydroxy esters could be similarly reduced model compounds were studied in this laboratory. Norris (3,27) subjected the methyl esters of DL-threonine and DL-allothreonine to hydrogenation in the presence of large quantities of Raney nickel (2-3 g. per g. of compound). The reductions were carried out under a hydrogen pressure of 2000 p.s.i. and the temperature raised to 90-95° C. within 15 minutes. Reduction was almost instantaneous and appeared to be complete after the first 30 minutes. The products were viscous oils which readily yielded crystalline oxalates and tribenzoyl derivatives. The tribenzoyl derivative obtained from the reduction of allothreonine (1,3-dibenzoxy-2-

benzamido-n-butane) melted sharply at 155-156° C. and there was no indication of the presence of a second isomer. Apparently little or no epimerization occurred during the esterification and reduction of allothreonine. In the case of threonine, the bulk of the tribenzoyl derivative, after repeated recrystallizations from aqueous methanol and from petroleum ether, softened at 137° C. and melted completely at 134° C. After another treatment with benzoyl chloride a small amount of compound was isolated which melted at 144-147° C. Since both materials gave the correct analytical data for the tribenzoyl derivative, it would appear that epimerization (extent undetermined) had occurred in the reduction or esterification of threonine.

Another model compound, methyl α-aminostearate, was studied by Rockwell (5,37). When this ester was reduced with Raney nickel catalyst under 1500 p.s.i. of hydrogen at 110° C., it rapidly absorbed the theoretical amount of hydrogen and a 70 per cent yield of 1-hydroxy-2-amino-octadecane was obtained.

The results obtained with these model compounds are encouraging as regards the synthesis of dihydrosphingosine. The reduction of the four diastereoisomers of methyl α-amino-β-hydroxystearate should yield the four isomeric 1,3-dihydroxy-2-amino-octadecanes, one of which is dihydrosphingosine. Such a conversion would not only provide the synthesis but would also furnish information as to the stereochemical configuration of the base. The smooth reduction of the long chain ester is particularly encouraging.

2. Synthesis of α-amino-β-hydroxy acids

The synthesis of dihydrosphingosine, according to the methods

described in B. 1., requires the synthesis of α-amino-β-hydroxystearic acid and/or ester. Several methods, discussed below, presented themselves.

a. From α,β-unsaturated acids

The addition of hypobromous acid or one of its derivatives to 2,3-octadecenoic acid and subsequent replacement of the bromine by an amino group appeared to be a profitable method of approach.

$$C_{15}H_{31}CH{=}CHCO_2H \xrightarrow{(R)\ HOBr} C_{15}H_{31}\underset{\underset{(R)}{OH}}{CH}\text{--}\underset{Br}{CHCO_2H} \xrightarrow{NH_3} C_{15}H_{31}\underset{\underset{(R)}{OH}}{CH}\text{--}\underset{NH_2}{CHCO_2H}$$

This type of reaction offers a very attractive method of preparing the desired compound, since, in introducing the asymmetric carbon atoms simultaneously by addition to a double bond, only one racemic form should be produced. The uni-directional nature of the addition of hypohalites to α,β-unsaturated acids has been demonstrated by Reed and Andrews (115). For example, the addition of hypobromous acid to cinnamic acid produced only one of the two possible pairs of diastereoisomers.

(1). Synthesis of 2,3-octadecenoic acid

The required 2,3-octadecenoic acid has been prepared in this laboratory in two different ways. Norris (3) condensed palmitaldehyde with malonic acid in pyridine containing a trace of piperidine. Rockwell (5) prepared the unsaturated acid from stearic acid by the method of Ponzio (29). Stearic acid was brominated, the bromine replaced by iodine, and the α-iodo acid was dehydrohalo-

genated by the use of concentrated alcoholic alkali. A by-product of this reaction is the α-hydroxy acid.

(3). Synthesis of α-bromo-β-hydroxy (alkoxy)-stearic acid

One possible method of arriving at the desired α-bromo-β-hydroxy (alkoxy)-stearic acid is the mercuration-bromination reaction used by Carter and co-workers (38) for the synthesis of threonine and serine. This type of synthesis involves the following steps:

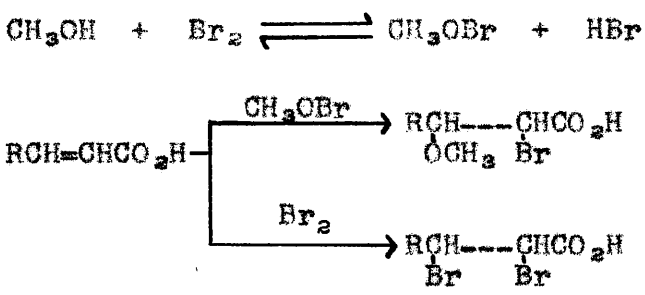
$$RCH{=}CHCO_2H \xrightarrow[CH_3OH]{Hg(OAc)_2} \underset{OCH_3}{\overset{}{R}}CH\text{----}\underset{HgOAc}{\overset{}{C}}H\text{----}CO_2H \xrightarrow{KBr}$$

$$\underset{OCH_3}{\overset{}{R}}CH\text{----}\underset{HgBr}{\overset{}{C}}HCO_2K \xrightarrow{KBr\ Br_2} \underset{OCH_3}{\overset{}{R}}CH\text{----}\underset{Br}{\overset{}{C}}HCO_2K \longrightarrow$$

$$\underset{OCH_3}{\overset{}{R}}CH\text{----}\underset{Br}{\overset{}{C}}HCO_2H$$

This method was tried on 2,3-octadecenoic acid by Norris (3) in this laboratory. Mercuric acetate was found to add readily to the unsaturated acid in methanol to give good yields of α-acetomercuri-β-methoxystearic acid. However, the acetomercuri-addition complex was so insoluble that the bromination with aqueous potassium bromide gave unsatisfactory results.

The addition of methyl hypobromite is another possible route to the desired acid. The reactions are as follows:



The competition between the last two reactions is an important feature of this method.

Conant and Jackson (30) have reported that cinnamic acid when treated with methanol and bromine yielded both α-bromo-β-methoxycinnamic acid and dibromocinnamic acid. Only one form of the bromomethoxy acid was isolated. The yield was fairly good since, when R is aromatic, the addition of bromine is relatively slow. Carter and co-workers (31) in this laboratory, however, found that in the case of crotonic acid the major product was the dibromo acid. Therefore, in order to make the reaction practical, methods of shifting the methanol-bromine equilibrium in favor of methyl hypobromite were tried. Sodium acetate (32) and silver nitrate (33) had been previously used for this purpose. Sodium acetate was found to increase the addition of methyl hypobromite only slightly in the case of crotonic acid. However, in the presence of silver nitrate excellent yields of α-bromo-β-methoxy-n-butyric acid were obtained. Only one racemic form--that corresponding to DL-allothreonine--could be isolated.

The synthesis of α-bromo-β-methoxystearic acid from 2,3-octadecenoic acid was investigated by Rockwell (5). The use of bromine and silver nitrate in methanol gave only about 50 per cent of the theoretical amount of bromomethoxy acid. The use of N,N-dibromo-

benzenesulfonamide in methanol was also tried. This latter reagent was found to be easier to handle, since the solid dibromobenzenesulfonamide could be added in several portions over a period of time, maintaining a more constant supply of reagent than in the bromine-silver nitrate method. However, the yield was still only about 50 per cent, and the product was apparently identical with that obtained by the other method.

Acylhypohalites have been prepared by Bockmuller and Hoffmann (34) by the action of a halogen on the silver salt of a low molecular weight aliphatic acid.

$$RCO_2Ag + X_2 \longrightarrow RCO_2X + AgX$$

These acylhypohalites were found to add readily to carbon-carbon double bonds. For example, silver butyrate was mixed with an ice-cold solution of bromine in carbon tetrachloride, and the mixture shaken until the bromine color disappeared. The solution of butyryl hypobromite was then filtered by suction into cold cyclohexene. The carbon tetrachloride was removed and the residue fractionally distilled at 14 mm. The butyrate of 2-bromo-cyclohexanol-1 was obtained. Some dibromocyclohexane was also isolated. The latter was due to the formation of hydrogen bromide which reacted with the acyl hypobromite yielding bromine. Ushakov, Chistov, and Zelinskii (35), using an analogous procedure were able to isolate a 32 per cent yield of the acetate of 2-bromocyclohexanol-1. The reaction of acyl hypohalites with α,β-unsaturated acids has not been explored.

The addition of free hypobromous acid to 2,3-octadecenoic acid would be the most direct route to the desired bromohydroxy acid.

This method requires a good means for preparing the hypobromous acid. One standard method is the reaction of bromine and yellow mercuric oxide (36,37). This produces an aqueous solution of hypobromous acid supposedly free from bromine. Another method is the addition of bromine to an ice-cold aqueous solution of potassium or sodium hydroxide (38,39), and subsequent acidification in the presence of the unsaturated compound to liberate the hypobromous acid. The reaction of bromine and silver salts is another method of preparing hypobromous acid. Shilov and Kanyaev (40) have reported the preparation of a bromine-free hypobromite solution from dilute bromine-water and silver phosphate. Carter (41) used the reaction of bromine and silver nitrate to form hypobromous acid for addition to crotonic acid. This procedure has the disadvantage that nitric acid is formed.

All of the above methods have the disadvantage, as far as use with 2,3-octadecenoic acid is concerned, of requiring an aqueous medium. In the case of the potassium hydroxide-bromine method the salt of the acid might be soluble enough so that reaction could take place. The behavior of oleic acid might be used as a model for this reaction.

The bromohydrin of oleic acid has apparently never been reported. However, the chlorohydrins of oleic and elaidic acids have been made. Nicolet and Poulter (42) used a 2 per cent solution of the acid containing 4 per cent of potassium carbonate. This solution was cooled below 10° C. and stirred while chlorine was passed in until the iodine number was less than 1.0. Any excess hypochlorous acid was destroyed with sodium thiosulfate and the solution

acidified. Oleic acid chlorohydrin was a viscous oil, while elaidic acid yielded a semi-solid which could not be crystallized.

Ellis (43) used dilute sodium hydroxide solutions of the acids and added dilute sodium hypochlorite solution (1 1/2 moles per mole of acid). Carbon dioxide was then passed in for a period of 12 hours. The chlorohydrins were not purified but converted to the epoxide with sodium ethylate. Atherton and Hilditch (44) used an analogous procedure but isolated the chlorohydrins. Oleic acid yielded a solid product; elaidic, a semi-solid.

In the past few years t-butyl hypochlorite has been found to add readily to carbon-carbon double bonds. The reagent has been prepared by the reaction of chlorine with t-butanol in the presence of sodium hydroxide (45, 46) or calcium carbonate (47). Since it is susceptible to photochemical decomposition, which may occur with explosive violence, the reagent must be carefully handled. In the presence of methanol, t-butyl hypochlorite has been found to react with olefins (46) to form the chloromethoxy compound:

$$>C{=}C< \;+\; t\text{-}C_4H_9OCl \;+\; CH_3OH \longrightarrow >\underset{Cl}{C}-\underset{OCH_3}{C}< \;+\; t\text{-}C_4H_9OH$$

In the presence of acetic acid the acetate was formed.

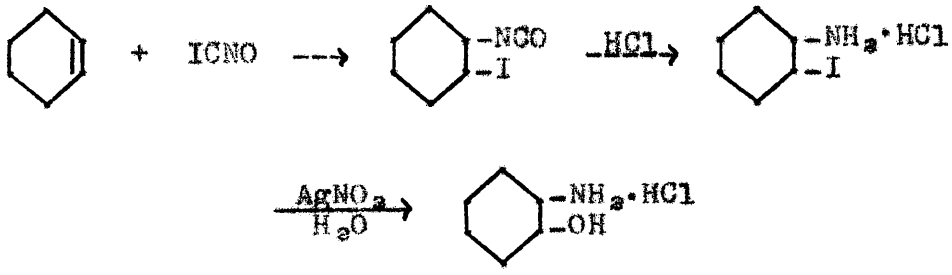
Styrene chlorohydrin (47) has been prepared by the use of t-butyl hypochlorite in the presence of acetic acid and water. Isoprene (48) reacted with the reagent, in methanol or acetic acid, to give the 1,2- and 1,4-addition products.

Cinnamic acid (49) reacted with t-butyl hypochlorite in methanol to give a 34 per cent yield of methyl α-chloro-β-methoxycinnamate and 1 per cent of the chloromethoxy acid.

Some work of Birckenbach on pseudohalogens also appears to offer a possible route to the aminohydroxy acid. When silver isocyanate was allowed to react with iodine at low temperatures, the pseudohalogen iodocyanate was formed (50):

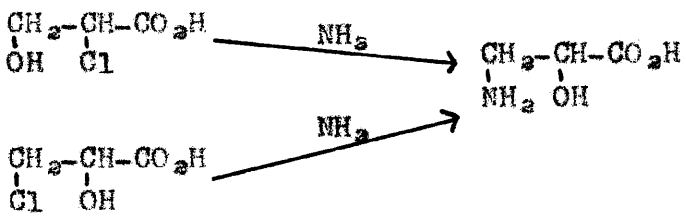
$$AgCNO + I_2 \longrightarrow ICNO + AgI$$

Iodocyanate was found to add to the double bond of cyclohexene yielding (2-iodo-cyclohexyl)-isocyanate (51). When this latter compound was refluxed for 3 hours with concentrated hydrochloric acid, (2-iodo-cyclohexyl)-amine hydrochloride (m.p. 159° C.) was isolated (52). Treatment of the amine hydrochloride with boiling aqueous silver nitrate for 1/2 hour produced (2-hydroxy-cyclohexyl)-amine hydrochloride (m.p. 175° C.).

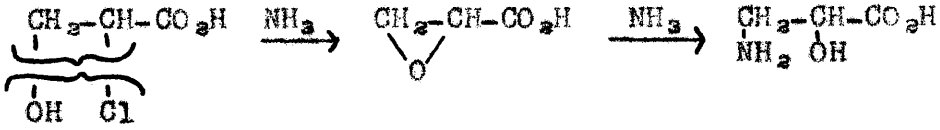


(3). Conversion of halohydrins to amino alcohols

The treatment of an α-bromo-β-hydroxy (alkoxy) acid with ammonia would be expected to lead to the replacement of the bromine by an amino group. That the reaction is not quite so simple is evidenced by the early work of Melikoff (53) and Erlenmeyer (54). Before 1900 these workers had found that α- and β-chlorolactic acid give the same aminolactic acid on treatment with ammonia, and that this amino acid is not serine but isoserine:



They also found that glycidic acid gives isoserine on treatment with ammonia. This led them to postulate that the ammonia first removed the elements of hydrochloric acid from the chlorohydrin forming the epoxide, and this latter then added ammonia to form the amino acid:

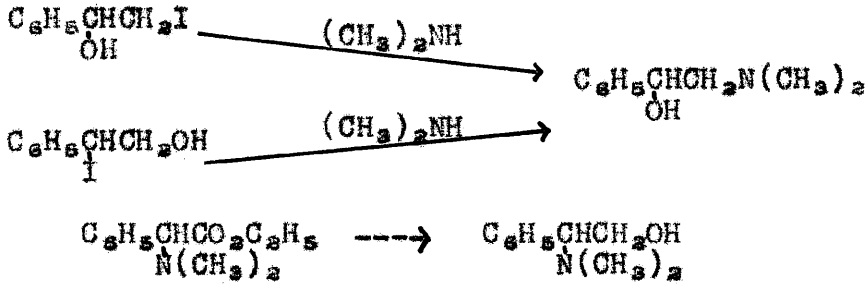


In 1908 Krassousky (55) also concluded that the epoxide was an intermediate in the formation of amino alcohols from chlorohydrins, since both the epoxide and chlorohydrin of 2-methyl butene-2 yielded the same amino alcohol. He formulated the following opinion: "In determining the structure of amino alcohols prepared from chlorohydrins, one must not base it on the position of the chlorine in the chlorohydrin, because there is formed in this reaction, as an intermediate product, the epoxide, and the structure of the amino-alcohol obtained is determined by the order of addition of the ammonia or amine to the oxide". He did not, however, isolate the epoxide intermediate.

Fourneau (56) did isolate the epoxide in the case of the reaction of ethyl α-chloro-β-phenyl-β-hydroxypropionate with an equimolar quantity of dimethylamine. When excess dimethylamine was used the dimethylamide of α-(dimethylamino)-β-phenyl-β-hydroxypropionic

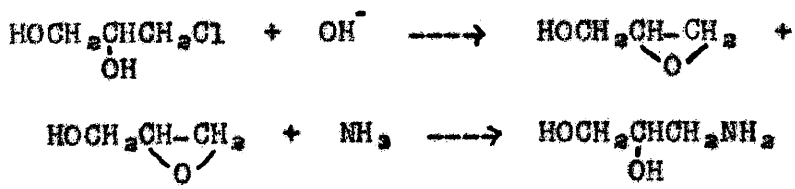
acid was formed.

To provide further proof for the epoxide intermediate Tiffeneau and Fourneau (57) prepared the two isomeric iodohydrins of styrene--the α-iodo-β-hydroxy by the reaction of hydrogen iodide on styrene oxide; the α-hydroxy-β-iodo by the action of iodine and mercuric oxide on styrene in ether solution. Both iodohydrins yielded the same amino alcohol on treatment with dimethylamine. This amino alcohol was shown to be different from the reduction product of ethyl α-phenyl-α-(dimethylamino) acetate.



These two workers (58) also demonstrated that the two isomeric chlorohydroxy-iso-butyric acids reacted with dimethylamine to form the same product--α-methyl-β-(dimethylamino)-β-hydroxypropionic acid.

Kinetic studies by Smith and Nilsson (59) gave further evidence of the stepwise nature of the conversion of halohydrins to amino alcohols. The reaction between 1-chloro-2,3-dihydroxy propane and dilute aqueous ammonia was assumed to proceed in two steps:



The first reaction was found to be the rate-determining step and its velocity was followed by determination of the chloride ion concentration. The second reaction did not interfere, not only because it proceeded at a faster rate but also because the amino alcohol formed had about the same basic strength as the ammonia consumed. The velocity constant of the first reaction was found to be 5.63-5.84 at 30° C. The velocity constant for the reaction of 1-chloro-3,3-dihydroxy propane with alkali has been found to be 6.07 at 30° C. (60). The good agreement between these values supports the corresponding mechanisms in both cases.

Thus the formation of the epoxide as an intermediate in the conversion of halohydrins to amino alcohols seems well established. Since this is true, the question arises as to whether or not amination of α-bromo-β-hydroxystearic acid will give rise to α-amino-β-hydroxystearic acid or to its isomer.

In all the examples cited above the hydroxyl group of the amino alcohol appeared on the most highly substituted carbon atom. In fact, as early as 1908 Krassousky (55) presented the rule that "in the reaction of ammonia with epoxides of asymmetric structure, the hydroxyl group occurs by preference on the carbon with the fewest hydrogens".

The cases in which we are primarily interested are the halohydrins and epoxides obtained from α,β-unsaturated acids. In the case of those from acrylic acid there seems to be no doubt that the β-amino acid is the only product formed. The published data on other members of the series tends to support the idea that the β-amino compound is the main product.

Erlenmeyer (61,62) working with cinnamic acid prepared two chlorohydrins--one an oil, the other a solid. When he treated the sodium salts of the epoxides from these two halohydrins, he obtained two different aminohydroxy acids. The oily chlorohydrin gave rise to an aminohydroxy acid melting at 220° C.; the solid chlorohydrin gave one melting at 241° C. Neither of these aminohydroxy acids was identical with an authentic sample of phenylserine.

Oesterlin (63) in 1939 treated the bromohydrin and the epoxide of cinnamic acid with ammonia and obtained an aminohydroxy acid which melted at 330° C. The methyl ester of epoxycinnamic acid yielded two aminohydroxy acids, one melting at 330° C., the other at 270-280° C. These were all shown to be the β-amino compound by oxidation studies.

In 1930 Burch (64) studied the reaction of ammonia on the chlorohydrin of crotonic acid. Because the product which he isolated gave a strong ninhydrin test but gave the theoretical amount of nitrogen in the Van Slyke analysis only after 10 minutes shaking, he concluded that it was a mixture of the α-amino-β-hydroxy and α-hydroxy-β-amino acids. However, the aminohydroxy acid obtained from acrylic acid under similar conditions also gives a strong ninhydrin test.

In contrast, unpublished work from this laboratory has shown that the formation of the α-hydroxy-β-amino acid is not generally true in this series. From the reaction of ammonia with the bromohydrin of crotonic acid only allothreonine could be isolated. The yield, however, was low and the reaction is being further studied.

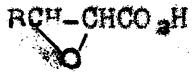
In the case of the cinnamic acid halohydrins and epoxide there

is some published evidence for the formation of the α-amino compound. Erlenmeyer (62) reported obtaining a small amount of phenylserine from the mother liquors of the amination of the solid chlorohydrin. From one experiment with epoxycinnamic acid Oesterlin (63) isolated an aminohydroxy acid whose melting point could not be raised above 334° C. Since this acid gave benzaldehyde and ammonia on oxidation, he concluded that it was phenylserine.

In this laboratory it has been possible to detect the formation of at least 35 per cent phenylserine in the amination of the bromohydrin of cinnamic acid. The structure of the product was demonstrated by conversion to β-phenyl naphthalene--a reaction given by phenylserine but not by phenylisoserine (65).

The bromohydrin of δ-phenylpentenic acid has been found to yield the α-amino-β-hydroxy acid exclusively on treatment with ammonia.

Therefore, it has been concluded that the carboxyl group exercises a labilizing effect on the α-carbon-oxygen bond in epoxy acids of the type:



If, then, the β-substituent has no strong effect, the amino group will appear in the alpha position. When R is hydrogen, the difference in the amount of substitution on the α- and β-carbons leads to the formation of the β-amino acid. When R is phenyl, the strong effect of the benzene ring causes the formation of the β-amino compound as the main product but does not prevent the appearance of some α-amino acid. When R is a methyl or a small alkyl group, the

product is the α-amino acid. Therefore, when R is a long chain alkyl group, as in the case of the stearic acid derivative, it seems likely that the major, or only, product will be the desired α-amino-β-hydroxy acid.

This problem of isomeric acids does not arise in the case of the amination of the α-bromo-β-methoxy acids. In this latter case no epoxide intermediate can be formed and the ammonia merely replaces the bromine. Also the bromine definitely appears on the alpha carbon since serine and threonine (66) have been prepared from the bromomethoxy derivative of acrylic and crotonic acids respectively. Rockwell (5) has prepared α-amino-β-methoxystearic acid by the action of ammonia on α-bromo-β-methoxystearic acid.

b. From β-keto esters

Another possible synthesis of α-amino-β-hydroxystearic acid would be the introduction of a nitrogen function on the α-carbon of β-ketostearic acid, followed by reduction of the nitrogen function to an amino group and the carbonyl function to a hydroxyl group. Two possible methods for accomplishing this will be discussed.

(1). Synthesis and reduction of α-oximino-β-keto esters

The synthesis of α-oximino-β-keto esters involves the nitrosation of the active methylene group of a β-keto ester. In general, unsubstituted β-keto esters yield the corresponding α-oximino ester, while α-monoalkyl-β-keto esters are deacylated to give simple α-oximino esters.

$$RCCH_2CO_2R' + HNO_2 \longrightarrow RC(=O)-C(=NOH)-CO_2R' + H_2O$$

$$RCCHCO_2R' + HNO_2 \longrightarrow RC(=NOH)-CO_2R' + RCO_2H$$

The large variety of reagents and reaction conditions which have been used for the introduction of the nitroso group has been thoroughly reviewed by Touster (67) and by Hartung (68). In general, inorganic or organic nitrites may be used with either acidic or basic catalysts. The proper reaction conditions must be found for each specific case.

The α-oximino acids on reduction yield the α-amino acids and this method has been used in a number of cases (69). The α-oximino-β-keto acids yield on reduction the α-amino-β-hydroxy acids.

Adkins and Reeve (70) in 1938 prepared threonine from α-oximino acetoacetic ester. However, they found it necessary to reduce the O-ethyl ether of the oxime rather than the free compound as the latter had been found to give rise to a pyrazine during reduction with Raney nickel and hydrogen. Nitrosation of ethyl acetonedicarboxylate, followed by reduction using palladium on charcoal has been used to prepare β-hydroxy glutamic acid (71). This synthesis has been repeated in this laboratory by Touster (67).

Norris (3) found that both α-oximino acetoacetic ester and its O-ethyl ether could be reduced directly to 1,3-dihydroxy-2-amino butane, using large amounts of Raney nickel. The tribenzoyl derivatives obtained were identical with that formed by the reduction product of methyl allothreonine, indicating a selective reduction of the α-oximino-β-keto esters to give configuration of allothreonine.

Norris (3) also attempted to nitrosate ethyl palmitoyl acetate (β-keto stearate) using nitrous acid and butyl nitrite. However, in no case could a nitrogen-containing product be isolated.

(2). Synthesis and reduction of α-phenylhydrazono-β-keto esters

Another nitrogen function which can be introduced on the α-carbon of a β-keto ester is the phenyl azo- or phenylhydrazono group.

The coupling of benzene diazonium salts with active methylene compounds was first reported in 1877 by V. Meyer (72) who found that acetoacetic ester in potassium hydroxide solution reacted with benzene diazonium chloride. After hydrolysis to the acid, he obtained a yellow compound melting at 154-155° C. He called it phenylazo-acetoacetic ester and assigned to it the structure:

$$CH_3COCHCO_2C_2H_5$$
$$\quad\quad N{=}NC_6H_5$$

In the following year Zublin (73) reported the synthesis of several salts of phenylazo-acetoacetic ester but was unable to cause the compound to undergo the "acid" and "ketone" cleavages common to acetoacetic ester derivatives.

In 1884 von Richter and Munzer (74) repeated the work of Meyer but added the aqueous diazonium solution to an alcoholic solution of ethyl sodioacetoacetate. They isolated the ester (m.p. 75° C.) as well as the free acid (m.p. 154-155° C.). They were also successful in effecting the "ketone" cleavage to phenylazoacetone:

$CH_3COCH_2N{=}NC_6H_5$

but could not cause the "acid" cleavage to occur.

When in 1887 Japp and Klingman (75) tried the reaction of benzene diazonium chloride with α-methylacetoacetic ester, they were surprised to find that the only product which could be isolated was α-phenylazopropionic acid. Similarly α-ethylacetoacetic ester yielded only α-phenylazobutyric acid. This led them to decide that only α-substituted acetoacetic esters would undergo "acid" cleavage after reaction with diazonium salts.

They also found that phenylazopropionic acid could be reduced with sodium amalgam to phenylhydrazopropionic acid:

$$\begin{matrix} CH_3CHCO_2H \\ | \\ NHNHC_6H_5 \end{matrix}$$

which was shown to be identical with the reduction product of the phenylhydrazone of pyruvic acid:

$$\begin{matrix} CH_3CCO_2H \\ \| \\ NNHC_6H_5 \end{matrix}$$

Meyer (76) has also reported that the condensation product of malonic ester and benzene diazonium chloride was identical with the phenylhydrazone of mesooxalic ester.

In the light of these facts and by analogy with the known reactions of nitrous acid with substituted and unsubstituted acetoacetic esters, they postulated the following mechanisms:

1. With unsubstituted acetoacetic ester, benzene diazonium salts react to form the α-phenylhydrazone:

$$CH_3CCCH_2CO_2C_2H_5 + C_6H_5N_2OH \longrightarrow CH_3COC(=NNHC_6H_5)CO_2C_2H_5 + H_2O$$

2. With α-substituted acetoacetic esters, the reaction occurs with the loss of the acetyl radical:

$$CH_3COCHCO_2C_2H_5 + C_6H_5N_2OH \longrightarrow RC(=NNHC_6H_5)CO_2C_2H_5 + RCO_2H$$

They also found that with the free acids, the reaction occurred with the loss of carbon dioxide:

$$CH_3COCHCO_2H + C_6H_5N_2OH \longrightarrow CH_3COC(R)=NNHC_6H_5 + CO_2 + H_2O$$

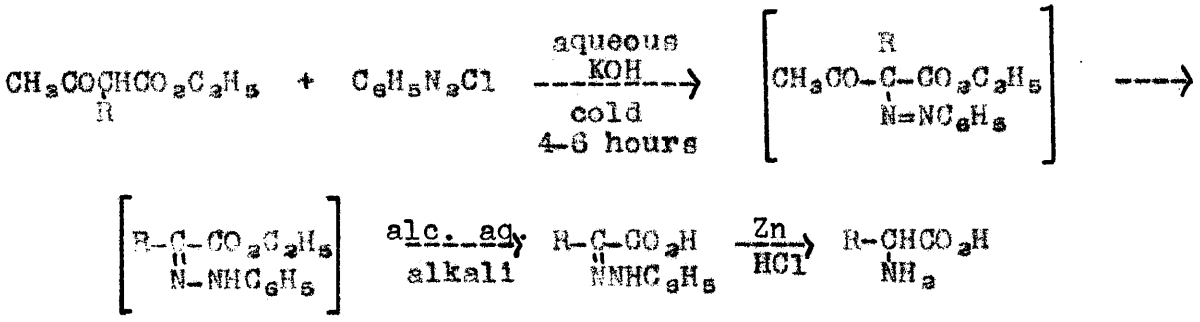
Because of the work of these two men this reaction of diazonium salts with active methylene compounds has been called the Japp-Klingman reaction.

In recent years the Japp-Klingman reaction has been utilized mainly in two fields--for the synthesis of α-amino acids and to prepare phenylhydrazones for cyclization by the Fischer method to indole derivatives.

The amino acid work has been carried out in Russia by Feofilaktov and his co-workers. In 1939 after studying the reaction of nitrous acid with various butyrolactone derivatives (78), Feofilaktov turned his attention to the reaction of benzene diazonium chloride on these same compounds (79). He found that when α-acetobutyrolactone reacted with benzene diazonium chloride in basic solution at 0-3° C., the acetyl group was cleaved and the phenylhydrazone of α-ketobutyrolactone was formed in 90-96 per cent yield. α-Carboxybutyrolactone produced the same product.

Reduction of this phenylhydrazone with tin and hydrochloric acid produced α-amino-γ-hydroxybutyric acid. However, the α-carbethoxy derivative was stable to cleavage and yielded α-phenylazo-α-carbethoxybutyrolactone. The α-cyanobutyrolactone, also, resisted cleavage and α-phenylazo-α-cyanobutyrolactone was formed (80).

Feofilaktov then developed the method into a convenient synthesis for α-amino acids from α-alkylacetoacetic esters (81). The reactions are as follows:



The condensation products were red-brown oils. Whether or not they still contained the acetyl group was not determined, as no attempt at purification was reported. The cleavage may have occurred either in the cold aqueous condensation medium or later during the hydrolysis of the ester group. Since the acetyl group of α-acetobutyrolactone was lost during the condensation (79), Feofilaktov decided that a similar cleavage occurred in the cold alkaline condensation medium (82). Using this method on the appropriate alkylacetoacetic esters he synthesized isoleucine (82,86), leucine (83,86), phenylalanine (83,86), alanine (84,86), norleucine (87,90), valine (85,86, 88), and tyrosine (89, 90). The yields obtained were good, varying from 40-75 per cent for the conversion of the alkylacetoacetic ester to the α-phenylhydrazono acid. The reductions gave nearly

quantitative yields.

It was found that alkylmalonic esters also would condense with benzene diazonium chloride (91,92):

$$RCH(CO_2C_2H_5)_2 + C_6H_5N_2Cl \longrightarrow \left[\begin{matrix} & CO_2C_2H_5 \\ R-C- & N=NC_6H_5 \\ & CO_2C_2H_5 \end{matrix}\right] \longrightarrow HC(=NNHC_6H_5)CO_2H \longrightarrow RCH(NH_2)CO_2H$$

In this case, by analogy to α-carbethoxybutyrolactone (79), Feofilaktov decided that the cleavage did not occur until the ester was saponified.

The fact that phenylhydrazones cyclize to indole derivatives when heated in the presence of acids has also stimulated work on the Japp-Klingman reaction. By means of this reaction many phenylhydrazones are readily available which would otherwise be very difficult to obtain. The accompanying table is a survey of the compounds which have been used to make phenylhydrazones for cyclization to indoles.

3. Synthesis of 2-amino-3-hydroxypropionaldehyde

The methods of synthesis applicable to dihydrosphingosine are of no value in the case of sphingosine due to the presence of the double bond between carbons 4 and 5. The most feasible method would seem to be to synthesize a 3-carbon unit containing a nitrogen function between two oxygen functions and then lengthen the chain by means of a suitable condensation reaction. An ideal 3-carbon unit would be 2-amino-3-hydroxypropionaldehyde. This aldehyde could be condensed with the appropriate acetylenic Grignard reagent and the triple bond selectively reduced to a double bond:

TABLE I

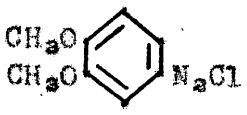
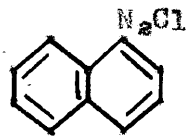
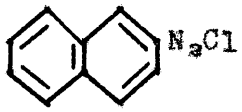
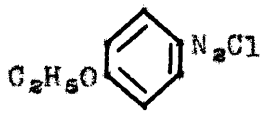
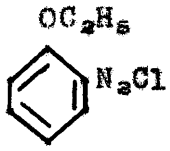
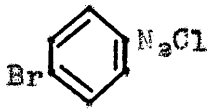
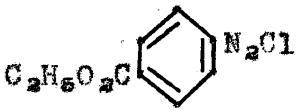
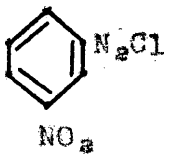
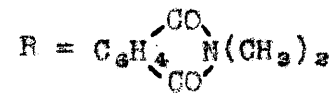
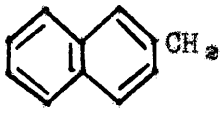
Active methyline compound	Diazonium salt	Reference
1. Acetoacetic esters: $CH_3COCHCO_2C_2H_5$ (R)		
a. R = CH_3, C_2H_5, $C_6H_5CH_2$		93
b. R = CH_3, C_2H_5, n-C_4H_9, $C_6H_5CH_2$		94
c. R = CH_3, C_2H_5, n-C_4H_9, $C_6H_5CH_2$		94
d. R = CH_3, C_2H_5, n-C_4H_9, $C_6H_5CH_2$		94
e. R = CH_3, C_2H_5, n-C_4H_9, $C_6H_5CH_2$		94
f. R = CH_3, C_2H_5, n-C_4H_9, $C_6H_5CH_2$		94

TABLE I (2)

Active methyline compound	Diazonium salt	Reference
g. R = CH_3, C_2H_5, $\underline{n}$-C_4H_9, $C_6H_5CH_2$		94
h. R = CH_3, C_2H_5, $\underline{n}$-C_4H_9, $C_6H_5CH_2$		94
i. R = CH_3, C_2H_5, $\underline{n}$-C_3H_7, $\underline{n}$-C_4H_9, $C_4H_9CO_2H$, $C_6H_5CH_2$		95
j. R = $C_6H_5CH_2$	$C_6H_5N_2Cl$	96
k. R = 	$C_6H_5N_2Cl$	96
l. R = 	$C_6H_5N_2Cl$	97
m. R = $(C_2H_5)_2NCH_2CH_2$	$C_6H_5N_2Cl$	98

2. <u>Acetoacetic acids</u>:

a. R = $C_6H_5CH_2$	$C_6H_5N_2Cl$	96

TABLE I (3)

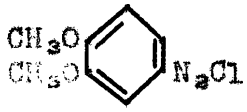
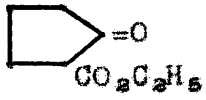
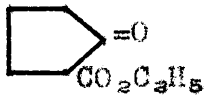
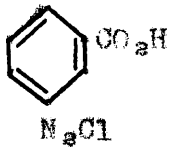
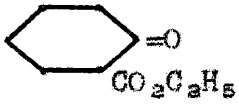
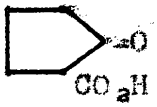
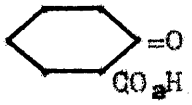
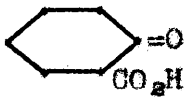
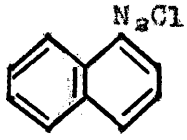
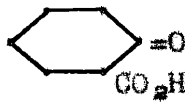
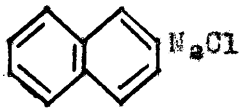
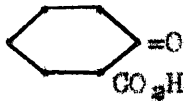
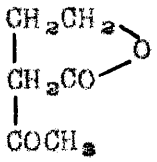
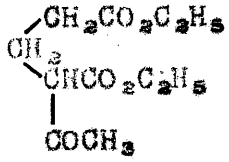
Active methyline compound	Diazonium salt	Reference
b. R = CH_3, C_2H_5, $C_6H_5CH_2$		93
3. <u>Cyclic keto esters</u>:		
a. 	$C_6H_5N_2Cl$	96, 99
b. 		100
c. 	$C_6H_5N_2Cl$	93, 101, 102
4. <u>Cyclic keto acids</u>:		
a. 	$C_6H_5N_2Cl$	103
b. 	$C_6H_5N_2Cl$	103
c. 		103

TABLE I (4)

Active methyline compound	Diazonium salt	Reference
d. 		103
e. 		103

5. Others:

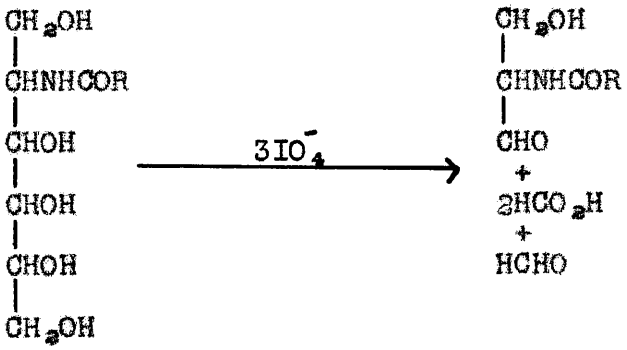
a. 	$C_6H_5N_2Cl$	104
b. 	$C_6H_5N_2Cl$	105

$$RC{\equiv}CMgBr + \underset{OH}{CH_2}\text{-}\underset{NH_2}{CH}\text{-}CHO \longrightarrow RC{\equiv}C\text{-}\underset{OH}{CH}\text{-}\underset{NH_2}{CH}\text{-}\underset{OH}{CH_2}$$

$$\longrightarrow RCH{=}CH\text{-}\underset{OH}{CH}\text{-}\underset{NH_2}{CH}\text{-}\underset{OH}{CH_2}$$

(R = $C_{13}H_{27}$ for sphingosine)

The naturally occurring amino-sugar, D-glucosamine, seemed a favorable starting place since it already contains an amino group between two carbon-bound oxygens. Periodate oxidation of an N-acyl-D-glucosamine should yield the desired aldehyde:



The amino group must be covered in this reaction since periodate will oxidize 1,2-amino-alcohols but not N-acylamino-alcohols.

The reduction of the aldehyde group of D-glucosamine could be accomplished either before or after acylation of the amino group. D-Glucosaminol hydrochloride has been prepared by the reduction of D-glucosamine hydrochloride with Raney nickel and hydrogen for 12 hours at 110° C. (106). It has also been prepared by low pressure (35 p.s.i.) hydrogenation using the Adams catalyst (107). D-Glucosaminol hydrochloride melts at 157-158° C. (107) and has an optical rotation of $[\alpha]_D^{16°} = -2.4°$ (in 20 per cent hydrochloric acid). Acylation of D-glucosaminol has not been reported.

N-Acetyl-D-glucosamine was first prepared in 1898 by Breuer (108) by prolonged action of acetic anhydride on D-glucosamine. More recent methods (109,110) have employed acetic anhydride and silver acetate in absolute methanol. The product so obtained melts with decomposition at 196° C. and has an optical rotation of $[\alpha]_D^{18^\circ} = +41.2^\circ$ (in water).

N-Acetyl-D-glucosamine has been reduced to N-acetyl-D-glucosaminol using either Raney nickel and hydrogen at 90° C. (106) or the Adams catalyst and 35 p.s.i. of hydrogen pressure (107).

N-Benzoyl-D-glucosamine has been prepared by Bergman (111). He first prepared tetra-acetyl-D-glucosamine from the free sugar. After benzoylation to N-benzoyl-tetra-acetyl-D-glucosamine, the acetyl groups were removed leaving the N-benzoyl derivative. Since this long process seemed unnecessary, the direct reaction of benzoyl chloride on D-glucosamine in sodium bicarbonate solution was tried in this laboratory (112). The product obtained melted at 203-204° C. with decomposition, and analyzed correctly for nitrogen. The reduction of N-benzoyl-D-glucosamine has not been reported.

A number of α-halo-acyl halides have been found to condense with D-glucosamine in cold alkaline solution. In this way N-chloroacetyl-D-glucosamine (113), N-(α-bromopropionyl)-D-glucosamine (113, 114), N-(α-bromo-<u>iso</u>-hexoyl)-D-glucosamine (113,114), and N-(α-bromolauroyl)-D-glucosamine (114) have been prepared.

III. DISCUSSION OF RESULTS

A. Syntheses from β-keto esters

1. Ethyl palmitoylacetoacetate

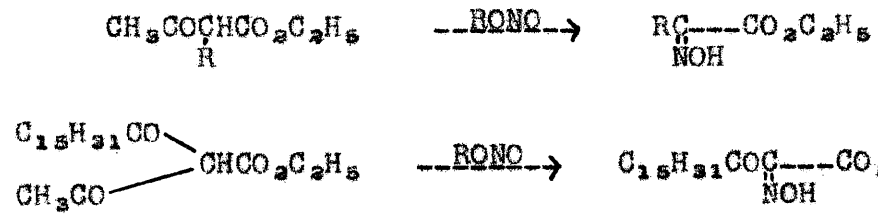
Ethyl palmitoylacetoacetate (116) was prepared by the condensation of palmitoyl chloride (117) with sodioacetoacetate. This reaction gave yields of only 50 per cent. Since the product is low melting (35.5-36.5° C.), care must be taken during recrystallization to prevent the product from separating from solution above its melting point. Slow cooling of a dilute solution gives a nice crystalline product.

2. Ethyl palmitoylacetate

Ethyl palmitoylacetoacetate has been converted to ethyl palmitoylacetate (ethyl β-ketostearate) by treatment with dilute aqueous sodium hydroxide (116,3). The yield in this cleavage reaction is also about 50 per cent and the melting point of the product (37-38° C.) is very close to that of ethyl palmitoylacetoacetate. Therefore, the introduction of an α-nitrogen function was carried out on ethyl palmitoylacetoacetate.

3. Reaction with alkyl nitrites

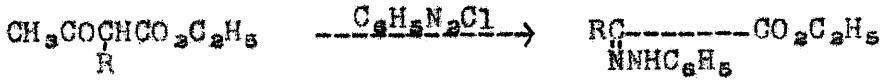
Norris (3) was unable to effect the nitrosation of ethyl palmitoylacetate with either nitrous acid or butyl nitrite. However, ethyl palmitoylacetoacetate might react with alkyl nitrites with loss of the acetyl group. This reaction is analogous to that occurring with α-alkylacetoacetic esters.



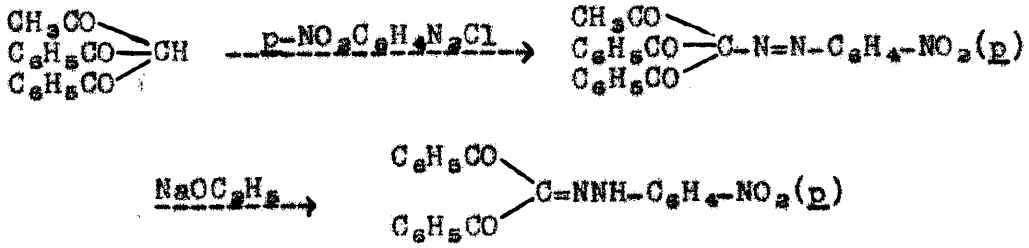
Butyl nitrite with both acidic and basic catalysts was tried, but nitrogen could not be introduced into the molecule.

4. Reaction with benzene diazonium chloride

The reaction of benzene diazonium chloride with α-alkylacetoacetic esters to give phenylhydrazones of alkylacetic esters has been described above.



The reaction of diazonium salts with acylacetoacetic esters has not been reported. However, Dimroth (118) found that acetyldibenzoylmethane reacted with p-nitrobenzene diazonium chloride to form p-nitrophenylazo-acetyldibenzoylmethane (m.p. 110.5° C.) in "quantitative" yield. When the azo compound was treated with sodium ethoxide, the acetyl group was preferentially cleaved yielding the p-nitrophenylhydrazone of dibenzoylmethane.



Exactly analogous reactions were obtained with p-bromobenzene

diazonium chloride (119). Therefore, it seemed possible that the reaction of benzene diazonium chloride with ethyl palmitoylaceto-acetate would yield the α-phenylhydrazone of ethyl palmitoylacetate (ethyl β-ketostearate).

Two general methods have been used for the condensation of benzene diazonium salts with active methylene compounds. In the first method the diazonium solution is added to a cold basic solution of the active methylene compound (93,94,95,96,97,102,103,104,105,118, 119). In the second method a solution of benzene diazonium hydroxide is prepared by adding the diazonium salt solution to a cold solution of potassium or sodium hydroxide. The active methylene compound is then added (79,80,81,82,83,84,85,86,87,88,89,90,91,92,100). Since ethyl palmitoylacetoacetate was found to be insoluble in aqueous or aqueous-alcoholic solution of potassium hydroxide, the second method proved to be more convenient. Since the ester is a solid, it was dissolved in the minimum amount of ether and the solution was added to a strongly basic aqueous solution of benzene diazonium hydroxide.

Most of the red-brown product of the condensation reaction could be extracted directly from the basic solution with ether. However, more complete extraction was obtained when the reaction mixture was first made strongly acid with hydrochloric acid. The oily or semi-solid residue from the ether extracts could be crystallized from absolute ethanol. A yellow crystalline material (m.p. 47.5-48.5° C.) which analyzed correctly for ethyl α-phenylhydrazono- β-ketostearate

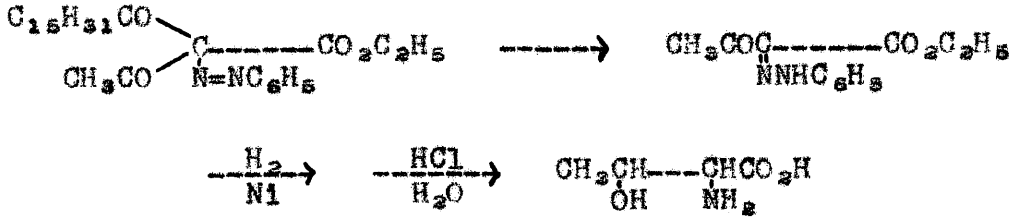
was obtained. The yield of crystalline material was quite low--38 per cent. The recrystallization procedure was tedious since the product tended to form a solvated mass which was difficult to filter. Repeated cooling furnished further crops of crystals.

The low yield of crystalline phenylhydrazone might be due to cleavage of the palmitoyl group rather than the acetyl group, to lack of any cleavage, or to the presence of uncrystallizable, polymorphic forms of the phenylhydrazone. Attempts to estimate quantitatively the amount of acetic acid in the aqueous mother liquor from the condensation reaction were unsuccessful. Feofilaktov (79-93) found that although the phenylhydrazones obtained from the condensation of α-alkylacetoacetic esters and benzene diazonium salts were red-brown oils which could not be crystallized, good yields of the α-phenylhydrazono acids were obtained on hydrolysis. Moreover, Lions and his co-workers (93,94,95,103,104), who prepared a large number of phenylhydrazones by this method, obtained only two crystalline products. All of the phenylhydrazones, however, gave good yields of the corresponding indole derivatives on treatment with hot acid. Therefore, the crude red-brown oil obtained from ethyl palmitoylacetoacetate and benzene diazonium chloride was reduced without purification.

5. α-Amino-β-hydroxystearic acid

The reduction of the crude phenylhydrazone was carried out under 2000 lbs. of hydrogen pressure using Raney nickel catalyst. Hydrogen was absorbed at 75-85° C. The temperature was then raised to 110-120° C. to complete the reduction. In one case the compound absorbed between 70 and 100 per cent of the theoretical amount of hydrogen.

The aminohydroxy ester was not isolated as such but hydrolyzed to the acid. A soapy brown material was obtained from which only 20-23 per cent of the theoretical amount (based on the ethyl palmitoylacetoacetate used) of α-amino-β-hydroxystearic acid hydrochloride could be isolated by recrystallization from glacial acetic acid. The glacial acetic acid solution was always very dark in color and contained materials which were never fully identified. Since the palmitoyl group might have been cleaved instead of the acetyl group, an attempt was made to identify α-amino-β-hydroxybutyric acid in the hydrolysis, but without success.



6. Methyl α-amino-β-hydroxystearate

Methyl α-amino-β-hydroxystearate hydrochloride was prepared from the acid by the action of dry hydrogen chloride in methanol. The white crystalline compound so obtained melted over a range (105-118° C., capillary). This was to be expected since the method of synthesis used should lead to the formation of a mixture of the two possible pairs of diastereoisomers. However, microanalysis showed that the ester hydrochloride had the proper empirical composition. The free ester was also prepared.

7. N-Benzoyl derivatives

Since attempts to separate the isomers by fractional

crystallization of the ester hydrochloride or the free ester were unsuccessful, attention was turned to the N-benzoyl derivatives. α-Aminostearic acid was used as a model compound. The technique developed by Carter and Stevens (130) for the benzoylation of α-amino acids failed due to the insolubility of the long chain amino acid in 0.5 N aqueous potassium hydroxide. The use of 50 per cent methanolic base made it possible to isolate about 50 per cent of the theoretical amount of the N-benzoyl derivative in the case of α-aminostearic acid, but results were still unsatisfactory with the aminohydroxy acid.

Benzoylation of methyl α-aminostearate in pyridine gave good yields (85 per cent) of the N-benzoyl ester (m.p. 83-84° C.). This compound was hydrolyzed to benzoyl-α-aminostearic acid with 0.5 N potassium hydroxide in 90 per cent methanol. A 90 per cent yield of a nicely crystalline compound (m.p. 116-116.5° C.) was obtained. Both compounds gave correct analytical results.

The action of benzoyl chloride in pyridine on methyl α-amino-β-hydroxystearate yielded a yellow solid (86 per cent of the theoretical amount) which could not be recrystallized satisfactorily. Hydrolysis to the acid yielded a cream colored solid (70 per cent--based on the original ester hydrochloride) which melted over a wide range (60-160° C.) suggesting that a mixture of isomers was present.

The N-benzoyl acid could be separated into two fractions on the basis of benzene solubility. The faction easily soluble in benzene carried with it most of the color of the original product. This soluble fraction (m.p. 60-65° C.) could be recrystallized from methanol. The colored impurities, however, continued to separate with

the product and analysis showed only one-half of the theoretical amount of nitrogen. The benzene insoluble fraction was a white crystalline material melting from 155-165° C. Most of it dissolved in absolute ethanol and recrystallized on the addition of a small amount of benzene. The recrystallized material melted from 165-170° C. and analyzed correctly.

8. Tribenzoyl-1,3-dihydroxy-2-amino-octadecane

A small amount of the original methyl α-amino-β-hydroxystearate was reduced with Raney nickel and hydrogen. Benzoylation of the product in pyridine yielded a white material which could be recrystallized from absolute ethanol. The crystalline solid melted at 139-143° C. and analyzed correctly. This product represents one of the racemic forms of 1,3-dihydroxy-2-amino-octadecane, one of the four optical isomers of which is dihydrosphingosine.

B. Addition of hypobromous acid to α,β-unsaturated acids

The addition of hypobromous acid or one of its derivatives to 2,3-octadecenoic acid, followed by amination would be an ideal route to α-amino-β-hydroxystearic acid, since only one of the two possible pairs of diastereoisomers would be formed.

$$C_{15}H_{31}CH{=}CHCO_2H \xrightarrow{HOBr} C_{15}H_{31}\underset{OH}{CH}\text{-}\underset{Br}{CH}\text{-}CO_2H \xrightarrow{NH_3} C_{15}H_{31}\underset{OH}{CH}\text{-}\underset{NH_2}{CH}\text{-}CO_2H$$

Oleic and crotonic acids were used as model compounds for the exploration of various methods of preparation and addition of hypobromous acid.

A standard method for the preparation of hypobromous acid

solutions is the reaction of bromine with yellow mercuric oxide (36, 37). It was found that a 70 per cent yield of hypobromous acid could be obtained if the reaction mixture was kept cold (0° C.). The cold solution did not decompose appreciably over a period of 1 hour. The concentration of hypobromite was determined by allowing the sample to react with sodium arsenite and titrating the excess arsenite with iodine (121).

Other methods of preparing hypobromite are the addition of bromine to basic (potassium hydroxide or sodium carbonate) or acidic (acetic acid-sodium acetate) solutions of the unsaturated compound. In the case of the basic media, the solution must be slowly acidified after the addition of the bromine to liberate free hypobromous acid. The reaction of potassium bromide and potassium bromate in acid solution has also been used to generate hypobromous acid (122). The results obtained by these various methods are summarized below:

Unsaturated acid	Source of HOBr	% Yield[1]	N.E. of product[2]
Oleic acid	Br_2-Na_2CO_3	80	426
Oleic acid	Br_2-HOAc-NaOAc	97	356
Crotonic acid	Br_2-HgO	52	332
Crotonic acid	Br_2-Na_2CO_3	69	186
Crotonic acid	Br_2-KOH	34	196
Crotonic acid	Br_2-HOAc	47	217
Crotonic acid	KBr-$KBrO_3$	91	228

[1]Based on the calculated amount of bromohydroxy acid.

[2]Bromohydroxyoleic acid--379; dibromo-oleic acid--443; bromohydroxy-crotonic acid--183; dibromocrotonic acid--246.

These results indicate that in several cases a large portion of the product must have been the dibromo compound.

All of the products obtained were yellow to brown oils. No attempts were made to purify the oils as such. Several of the products from crotonic acid were aminated with concentrated ammonium hydroxide. No appreciable amount of α-amino-β-hydroxybutyric acid was ever isolated. This would indicate that either the addition products were mostly the dibromo compound, as suggested by the neutral equivalents, or that the amination procedure needed improvement.

All of the procedures so far described have the disadvantage, as far as oleic acid (or 3,3-octadecenoic acid) is concerned, that an aqueous medium is used. Oleic acid was not soluble in either the carbonate or the acetic acid reaction mixture.

The use of acetyl hypobromite would overcome this disadvantage since it is prepared by the action of bromine in carbon tetrachloride on silver acetate. With crotonic acid acetyl hypobromite gave a 56 per cent yield of an oil having a neutral equivalent of 233 (calculated--225). When this product was aminated, a small amount of aminohydroxy acid was isolated. This was proved to be allothreonine by conversion to the N-benzoyl derivative. With oleic acid, the product was a yellow oil of very high neutral equivalent (calculated--421; found--616). It is possible that acetyl hypobromite in the presence of the free acid yields peroxides which would cause the formation of high molecular weight products. Esters would probably be more suitable compounds for reaction with acyl hypohalites.

C. Syntheses from D-glucosamine

An attempt was made to synthesize 2-amino-3-hydroxypropionalde-

hyde which in the form of an appropriate derivative might be condensed with an acetylenic Grignard reagent to yield a sphingosine derivative.

N-Benzoyl-D-glucosamine was prepared by reaction of benzoyl chloride with D-glucosamine hydrochloride suspended in sodium bicarbonate solution. Early reactions gave fair yields (67-74 per cent) of the crude N-benzoyl derivative. Large losses occurred, however, when recrystallization was attempted. Later reactions failed to give good yields and no reason for this failure could be ascertained. N-Benzoyl-D-glucosamine was reduced with Raney nickel and hydrogen to N-benzoyl-D-glucosaminol. This latter compound resisted all efforts at recrystallization and was isolated by lyophilization.

N-Acetyl-D-glucosamine was prepared from D-glucosamine hydrochloride by the action of silver acetate and acetic anhydride in dry methanol. Yields were around 50 per cent, and large losses accompanied recrystallization. Reduction of N-acetyl-D-glucosamine with Raney nickel and hydrogen produced solutions which no longer gave a Benedict's test, but it was never found possible to isolate N-acetyl-D-glucosaminol.

Periodate oxidation rate studies showed that N-benzoyl-D-glucosaminol absorbed close to the theoretical 3 moles of periodate in less than 15 minutes. N-Benzoyl-D-glucosamine absorbed 3 moles of periodate only after 36 hours; N-acetyl-D-glucosamine absorbed 3 moles after 24 hours. Periodate oxidation of N-benzoyl-D-glucosaminol on a preparative scale yielded two products which gave positive Tollen's tests. The first of these melted at 136-137° C. and contained 4.69 per cent nitrogen. The calculated value for 3-amino-3-

hydroxypropionaldehyde is 7.35 per cent. The second product melted at 144-147° C. and contained 6.23 per cent nitrogen. Since the yields were very poor, the method was abandoned.

IV. EXPERIMENTAL

A. Syntheses from β-keto esters

1. Palmitoyl chloride

In a 5-liter round-bottomed 3-necked flask, fitted with a mercury-sealed mechanical stirrer, a dropping funnel, and a reflux condenser connected to a gas trap, were placed 500 g. (1.99 moles) of Eastman palmitic acid, melting at 61-62° C. The flask was warmed on a water bath until the acid melted and could be stirred. Then 357 g. (3 moles) of thionyl chloride were added slowly from the dropping funnel. The mixture was refluxed for 2 hours after the addition was completed. The excess thionyl chloride was removed by distillation under reduced pressure (water pump) at the temperature of boiling water. The palmitoyl chloride was then distilled under reduced pressure (oil pump). Since palmitoyl chloride tends to decompose at its boiling point, the heating bath (oil or Wood's metal) was heated to 200° C. before the flask was immersed in it. The water-white palmitoyl chloride (438 g.--80 per cent of the theoretical amount) distilled at 163-165° C./2 mm.

2. Ethyl palmitoylacetoacetate

In a 5-liter round-bottomed 3-necked flask, fitted with a mercury-sealed mechanical stirrer, a reflux condenser equipped with a drying tube, and a dropping funnel, were placed 3 liters of anhydrous ether and 23 g. (1 mole) of powdered sodium. Two hundred and sixty grams (2 moles) of ethyl acetoacetate (b.p. 89-90° C./25 mm.) were added slowly from the dropping funnel. The mixture was allowed

to stir over-night to obtain complete reaction. A slight yellow color developed. Two hundred and seventy-five grams (1 mole) of palmitoyl chloride were added over a period of 1 hour. The ethyl sodioacetoacetate went into solution, and a turbidity due to sodium chloride appeared. The mixture was refluxed for 1 hour to complete the reaction. The ether solution was washed twice with water and dried over anhydrous sodium sulfate. The ether was removed by distillation under reduced pressure and the oil solidified by cooling in ice. The product was recrystallized twice from 95 per cent ethanol, giving 190 g. (50 per cent of the theoretical amount) of a white solid melting at 35.5-36° C.[1]

$C_{21}H_{40}O_3$	Calculated:	C 71.74 H 10.87
(368)	Found:	C 71.68 H 11.10

3. Ethyl α-phenylhydrazono-β-ketostearate

In a 2-liter 3-necked round-bottomed flask, equipped with a mechanical stirrer, was placed a solution of 34 g. (0.6 moles) of potassium hydroxide in 500 ml. of water. The flask was immersed in an ice-salt bath. When the temperature of the solution reached 0-3° C., a cold (0-3° C.) solution of benzene diazonium chloride, prepared from 9.3 g. (0.1 moles) of aniline, 35 ml. of concentrated hydrochloric acid, 7 g. (0.1 moles) of sodium nitrite, and 500 ml. of water, was added with stirring. The solution was colored yellow but remained clear. Through a dropping funnel was added a solution

[1] All melting points, unless otherwise indicated, were taken on a Kofler microblock.

of 36.8 g. (0.1 moles) of ethyl palmitoylacetoacetate dissolved in 300 ml. of ether. The addition required 30-45 minutes. The reaction mixture turned orange and cloudy during the addition. The reaction mixture was kept cold and was stirred for 3-4 hours during which time the color gradually turned from orange to dark red-brown. The mixture was then allowed to warm up to room temperature, made acid with concentrated hydrochloric acid, and extracted with ether. The first extract was dark red-brown; the second, orange; the third, light yellow. The remaining water solution was light red, but no more color could be extracted. The ether extracts were washed with dilute hydrochloric acid and with water, combined, and dried over anhydrous sodium sulfate. The ether was removed by distillation under reduced pressure and the residual brown oil dried under vacuum. After several days a semi-solid red-brown mass weighing a little more than the theoretical amount (41.3 g.) was obtained.

This semi-solid could be recrystallized from absolute ethanol but only with difficulty. In one case it was possible to isolate a 38 per cent yield of yellow crystalline material melting at 47.5-48.5° C.

$C_{36}H_{42}O_3N_2$	Calculated:	C 73.56	H 9.77	N 6.50
(430)	Found:	C 73.10	H 9.56	N 6.42

(ash from C,H--0.3%)

4. α-Amino-β-hydroxystearic acid hydrochloride

Because of the difficulties encountered in recrystallizing the phenylhydrazone of ethyl β-ketostearate, the crude red-brown oil obtained from the condensation reaction was reduced without

purification. The oil was dissolved in absolute ethanol and reduced in the presence of Raney nickel at hydrogen pressures of 1500-2000 p.s.i. Hydrogen was absorbed first at 75-85° C. The temperature was then raised to 110-120° C. to complete the reduction. From 70-100 per cent of the theoretical amount of hydrogen (based on the amount of ethyl palmitoylacetoacetate used) was absorbed.

The catalyst was separated by filtration and the alcohol removed by distillation under reduced pressure. The residue, a dark brown oil, was refluxed for 2 hours with 400 ml. of 6 N hydrochloric acid. A dark brown soapy material was obtained on cooling. The aqueous layer was dark brown in color and contained aniline. The soapy solid was recrystallized from glacial acetic acid yielding 7.0-8.2 g. (20-23 per cent of the theoretical amount based on the ethyl -ketostearate used) of a slightly purple crystalline solid melting at 180-190° C.

5. Methyl α-amino-β-hydroxystearate hydrochloride

In a 500-ml. round-bottomed 3-necked flask, equipped with a reflux condenser with drying tube and a gas-inlet tube, were placed 7.57 g. of α-amino-β-hydroxystearic acid hydrochloride suspended in 200 ml. of anhydrous methanol. Hydrogen chloride gas was run in rapidly without cooling. After about 1/2 hour the solution had warmed to reflux temperature and all the solid material had dissolved. The solution was then refluxed for 2 hours, while hydrogen chloride was run in slowly. The hot solution was filtered, cooled in ice, and the precipitated hydrochloride removed by filtration. The filtrate was evaporated to dryness under reduced pressure.

The residue, combined with the precipitate, was 7.4 g. (94 per cent of the theoretical amount) of a slightly purple powder. After two recrystallizations from ethyl acetate the melting point was 118-119° C. (capillary).

$C_{19}H_{40}O_3NCl$	Calculated:	C 62.40	H 10.95	N 3.83
(365.5)	Found:	C 62.46	H 11.11	N 3.98

6. Methyl α-amino-β-hydroxystearate

In a separatory funnel were placed 0.365 g. (0.001 moles) of methyl α-amino-β-hydroxystearate hydrochloride suspended in 115 ml. of 0.0088 N aqueous sodium hydroxide. The mixture was shaken for several minutes. The cloudy solution thus obtained was extracted 3 times with ether. The ether extracts were washed with water, combined, and dried over anhydrous magnesium sulfate. The ether was removed under reduced pressure. The residue was 0.30 g. (91 per cent of the theoretical amount) of a white powder. After recrystallization from methanol and water the melting point was 55.5-56.6° C. (capillary).

7. Methyl benzoyl-α-aminostearate

To an ice-cold solution of 1 g. (0.003 moles) of methyl α-aminostearate dissolved in 20 ml. of anhydrous pyridine was added 0.5 ml. (0.0035 moles) of benzoyl chloride. The mixture was allowed to stand at room temperature for 3 hours. The solution was cooled and water (3 ml.) added. It was then poured on cracked ice and hydrochloric acid (20 ml.) in a separatory funnel. The white precipitate which formed was extracted with ether. The ether solution

was washed with dilute hydrochloric acid, dilute sodium bicarbonate, and water, and dried over anhydrous sodium sulfate. The ether was removed by distillation under reduced pressure. The dried product was 1.11 g. (85 per cent of the theoretical amount) of a white crystalline material. After two recrystallizations from petroleum ether (b.p. 90-100° C.) the product melted at 83-84° C.

$C_{26}H_{43}O_3N$	Calculated:	C 74.82	H 10.31	N 3.36
(417)	Found:	C 74.23	H 10.38	N 3.34

(Ash from C,H--1.13%)

8. Benzoyl-α-aminostearic acid

One gram (0.0024 moles) of methyl benzoyl-α-aminostearate was dissolved in 50 ml. of 0.5 N potassium hydroxide in 90 per cent methanol. The solution was allowed to stand at room temperature for 36 hours. Hydrochloric acid was added until the solution was acid to congo red. The white precipitate was separated by filtration and dried. After two recrystallizations from methanol 0.87 g. (90 per cent of the theoretical amount) of a white crystalline material (m.p. 116-116.5) was obtained.

$C_{25}H_{41}O_3N$	Calculated:	C 74.44	H 10.17	N 3.47
(403)	Found:	C 74.36	H 9.95	N 3.43

9. α-Benzoylamino-β-hydroxystearic acid

To a cold solution of 7.4 g. (0.0202 moles) of methyl α-amino-β-hydroxystearate hydrochloride in 75 ml. of dry pyridine were added 2.4 ml. (0.0203 moles) of benzoyl chloride. The solution was

allowed to stand at room temperature for 5 hours. The solution was cooled and 5 ml. of water added. The solution was poured on ice and 75 ml. of concentrated hydrochloric acid. The solid material formed was removed by filtration. The dried product was 7.54 g. (86 per cent of the theoretical amount) of a brownish solid. It would not recrystallize satisfactorily from petroleum ether (b.p. 36-42° C.), hexane, or methanol.

The impure product was suspended in 500 ml. of 0.5 N. potassium hydroxide in 90 per cent methanol. The reaction mixture was allowed to stand at room temperature for 24 hours. Not all of the solid dissolved. The brownish solid was removed by filtration. The solution was diluted with 300 ml. of water, and concentrated hydrochloric acid added until the pH was less than 2. The white precipitate which formed was removed by filtration and dried.

The brownish material which had not dissolved in the methanolic base was treated with another 500 ml. of 0.5 N potassium hydroxide in 90 per cent methanol. Since the solid still did not all dissolve after 18 hours at room temperature, the mixture was warmed to 60-75° C. for 1 hour. It was filtered warm and worked up as before. The combined precipitates were 6.0 g. (70 per cent of the theoretical amount based on methyl α-amino-β-hydroxystearate hydrochloride) of a cream colored crystalline solid (m.p. 60-160° C.).

The crude α-benzoylamino-β-hydroxystearic acid was separated into two fractions of the basis of benzene solubility. The benzene soluble fraction (3.88 g.), which contained most of the color of the original product, was recrystallized from methanol. The recrystallized material melted at 60-65° C., and was still cream colored.

$C_{25}H_{41}O_4N$	Calculated:	N 3.34
(419)	Found:	N 1.66

The benzene insoluble fraction (2.13 g.) was a white crystalline material. On treatment with hot absolute ethanol all except 0.22 g. dissolved. When a small amount of benzene was added and the solution cooled, white crystals (m.p. 165-170° C.) separated.

$C_{25}H_{41}O_4N$	Calculated:	C 71.50	H 9.76	N 3.34
(419)	Found:	C 71.41	H 9.54	N 3.56

10. Tribenzoyl-1,3-dihydroxy-2-amino-octadecane

A suspension of 0.85 g. (0.0026 moles) of methyl α-amino-β-hydroxystearate in 30 ml. of anhydrous methanol was reduced with Raney nickel (2-3 g. per g. of compound) and hydrogen (3000 p.s.i.) at 110° C. After 1 hour it had absorbed approximately the theoretical amount of hydrogen. The catalyst was removed by filtration and the solution evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml. of dry pyridine. The solution was cooled, and 1.2 ml. (0.0104 moles) of benzoyl chloride added. The solution was allowed to stand at room temperature for 3 hours. The solution was cooled, and 3 ml. of water added. The solution was poured onto ice and 25 ml. of concentrated hydrochloric acid. A waxy solid separated, was removed by filtration, and dried. This product (1.06 g.--68 per cent of the theoretical amount) could be recrystallized from ethanol or benzene. After 2 recrystallizations from benzene the melting point was 116-121° C.

$C_{39}H_{61}O_8N$	Calculated:	C 76.31	H 8.38	N 2.28
(614)	Found:	C 74.61	H 9.32	N 2.98

Since these values could result from a mixture of di- and tribenzoylated products, the solid was dissolved in 30 ml. of dry pyridine and rebenzoylated with 1 ml. of benzoyl chloride. Since the product did not separate cleanly when the solution was poured on ice and hydrochloric acid, the suspension was extracted twice with ether. The extracts were washed with water, combined, and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure. The residue was 1.13 g. (73.5 per cent of the theoretical amount) of a white solid. After 2 recrystallizations from absolute ethanol the melting point was 139-143° C.

$C_{39}H_{61}O_8N$	Calculated:	C 76.31	H 8.38	N 2.28
(614)	Found:	C 76.51	H 8.29	N 2.43

B. Addition of hypobromous acid to α,β-unsaturated acids

1. Determination of hypobromite

The sample of hypobromite solution was added to 35 ml. of 0.1 N sodium arsenite and 75 ml. of 5 per cent sodium bicarbonate in a 125-ml. Erlenmeyer flask. At least 2 ml. of 0.1 N arsenite should remain in excess. After 5 minutes 10 drops of a freshly prepared 1 per cent starch solution were added and 2-3 ml. of 3 N acetic acid were added dropwise with vigorous stirring until carbon dioxide was evolved. The excess arsenite was titrated with 0.5 N iodine solution until a permanent light blue color appeared.

3. Stability of hypobromite solutions

Eight grams of yellow mercuric oxide were suspended in 100 ml. of water. One milliliter (0.03 moles) of bromine was added. The mixture was stirred until all the drops of bromine had reacted. This required about 5 minutes. The solution was filtered and 2 ml. aliquots removed at intervals and titrated as described above.

In the first experiment the solution was maintained at room temperature; in the second, it was kept cold in ice and the precipitate washed with 50 ml. of cold water. The results are summarized below.

Time (min.)	g. HOBr/100 ml.
Experiment 1	
10	1.396
25	1.349
60	1.180
Experiment 2	
10	1.363
30	1.355
60	1.337

The calculated amount of hypobromite was 1.94 g. per 100 ml.

Therefore by keeping the solution cold and washing the precipitate a 71 per cent yield of hypobromous acid may be obtained after 10 minutes and the cold solution is fairly stable for 1 hour.

3. Addition of hypobromous acid to oleic acid

a. Sodium carbonate method

A solution of sodium hypobromite was prepared by adding 10 ml. (0.3 moles) of bromine dropwise to an ice-cold solution of 57 g. (0.525 moles) of sodium carbonate in 600 ml. of water. This solution was added with stirring to 56.4 g. (0.3 moles) of oleic acid suspended in a solution of 28 g. (0.2 moles) of potassium carbonate in 400 ml. of water. The mixture was stirred for 1 hour at 0° C. Concentrated hydrochloric acid was added dropwise over several hours until a permanent bromine color formed (117 ml. required). During the addition of the acid a solid material separated, which for the most part redissolved. At the end of the addition the reaction mixture consisted of a brown solution and a brown semi-solid mass which would not filter.

The solution was decanted and extracted twice with ether. The ether solution was decolorized by shaking with dilute sodium bisulfite solution, washed with water, and dried over anhydrous sodium sulfate. When the ether was removed by distillation under reduced pressure, the residue was negligible and was discarded.

The semi-solid mass was dissolved in ether, and the ether solution washed and dried as above. When the ether was removed, the residue was a yellow-brown oil. After drying under reduced pressure for 24 hours it weighed 80.4 g. (80 per cent of the theoretical amount of bromohydrin).

Neutral equivalent

Calculated:	(bromohydroxyoleic acid)	379
	(dibromoleic acid)	442
Found:		435, 437

b. Acetic acid method

To a vigorously stirred suspension of 56.4 g. (0.2 moles) of oleic acid in 200 ml. of 90 per cent acetic acid were added dropwise 10 ml. (0.3 moles) of bromine. The addition required 2 1/2 hours. The mixture was stirred over-night. The reaction mixture then consisted of two layers which were separated and worked up separately.

The upper layer was yellow-green in color, and a white cloudy suspension was formed when water was added. This suspension was extracted with ether. The ether solution was washed with dilute sodium bisulfite solution and with water, dried over anhydrous sodium sulfate, and the ether removed under reduced pressure. A brown residue (3.2 g.) which smelled like oleic acid was obtained.

The lower layer was brown and oily. When water was added an oil separated. This oil was removed by extraction with ether. The ether solution was washed and dried as above. The ether was removed under reduced pressure and the oil dried in a desiccator. After 48 hours it weighed 74.0 g. (97 per cent of the theoretical amount of bromohydrin).

Neutral equivalent

Calculated:	(bromohydroxyoleic acid)	379
Found:		355, 358

4. Addition of hypobromous acid to crotonic acid

a. Mercuric oxide method

A solution of hypobromous acid was prepared by adding 9 ml. (0.1 moles + 60 per cent excess) of bromine to an ice-cold suspension of 45 g. of yellow mercuric oxide in 200 ml. of water. This solution of hypobromous acid was filtered into a cold solution of 8.6 g. (0.1 moles) of crotonic acid in 100 ml. of water. A slight bromine color developed. The solution was extracted 3 times with ether. The ether extracts were washed with dilute sodium bisulfite solution and with water, combined, and dried over sodium sulfate. When the ether was removed under reduced pressure, the residue was 9.6 g. (52 per cent of the theoretical amount of bromohydrin) of a brown oil.

Neutral equivalent

Calculated:	(bromohydroxycrotonic acid)	183
	(dibromocrotonic acid)	246
Found:		232

The aqueous mother liquor was extracted twice more with ether. The extracts were washed and dried as before, and the ether removed. The residue was 2.5 g. of an oil which partially solidified after several days in a vacuum desiccator.

Neutral equivalent

Found: 192

b. Sodium carbonate method

A solution of sodium hypobromite was prepared by adding 5 ml. (0.1 moles) of bromine to an ice-cold solution of 30 g. (0.3 moles) of sodium carbonate in 150 ml. of water. This solution was added to a solution of 8.6 g. (0.1 moles) of crotonic acid and 15 g. (0.1 moles) of potassium carbonate in 150 ml. of water. Dry ice was added to the solution. At intervals 2-ml. aliquots of the reaction mixture was titrated for hypobromite by the method previously described. When the titration values for the sample were equal to those of the blank, addition of carbon dioxide was discontinued, and the reaction mixture allowed to stand over-night. Concentrated hydrochloric acid was added until the pH was less than 2, and the solution extracted 3 times with ether. The extracts were washed with dilute sodium bisulfite solution and with water, combined, and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure and the residue dried in a vacuum desiccator. The product was 13.6 g. (69 per cent of the theoretical amount) of a light brown oil.

Neutral equivalent

Calculated:	(bromohydroxycrotonic acid)	183
Found:		186

c. Potassium hydroxide method

A solution of potassium hypobromite was prepared by adding 7.5 ml. (0.15 moles) of bromine to a cold solution of 42.0 g. (0.75 moles) of potassium hydroxide in 350 ml. of water. This yellow

solution was added to a cold solution of 8.6 g. (0.1 moles) of crotonic acid in 250 ml. of water. Solid carbon dioxide was added until titration of a 3-ml. aliquot showed that no more hypobromite was present. The slightly yellow solution was allowed to stand overnight. The then colorless solution was made strongly acid (pH less than 3) with concentrated hydrochloric acid. It was extracted 6 times with ether. The extracts were washed with dilute sodium bisulfite solution and with water, combined, and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure. The residue was 3.3 g. (34 per cent of the theoretical amount) of a yellow-brown oil.

Neutral equivalent

Calculated:	(bromohydroxycrotonic acid)	183
Found:		196

d. Acetic acid method

To a solution of 8.6 g. (0.1 moles) of crotonic acid and 27.3 g. (0.3 moles) of sodium acetate in 300 ml. of 90 per cent acetic acid were added dropwise 5 ml. (0.1 moles) of bromine. The addition required 30-45 minutes. The solution was allowed to stand over-night. Concentrated hydrochloric acid was added until the pH of the solution was less than 3. A slight yellow color developed. The solution was extracted 4 times with ether. The extracts were washed with dilute sodium bisulfite solution and with water, combined, and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure. After prolonged drying under reduced pressure over potassium hydroxide the residue no longer smelled of

acetic acid. The product was 8.5 g. (47 per cent of the theoretical amount) of a light yellow oil.

Neutral equivalent

Calculated:	(bromohydroxycrotonic acid)	183
	(dibromocrotonic acid)	246
Found:		317

e. Bromide-bromate method

The hypobromite solution was prepared by mixing equal volumes of a solution containing 36 g. of potassium bromide per 100 ml. of water and a solution containing 10 g. of potassium bromate per 100 ml. of water. For 8.6 g. (0.1 moles) of crotonic acid 190 ml. of each solution was used. The crotonic acid dissolved readily in this mixture. Concentrated hydrochloric acid was added dropwise until a permanent bromine color developed. The solution was allowed to stand over-night. Since titration of a 2-ml. aliquot showed that hypobromite was still present, more hydrochloric acid was dropped in. When the titration showed that no more hypobromite was present, the solution was extracted 5 times with ether. The ether extracts were washed with dilute sodium bisulfite solution and with water, combined, and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure, and the oily residue thoroughly dried. The product was 16.7 g. (91 per cent of the theoretical amount of bromohydrin) of a light-brown oil.

Neutral equivalent

Calculated:	(bromohydroxycrotonic acid)	183
	(dibromocrotonic acid)	346
Found:		338

5. Addition of acetyl hypobromite to α,β-unsaturated acids

a. To oleic acid

A cold (0° C.) solution of 5 ml. (0.1 moles) of bromine in 500 ml. of carbon tetrachloride was added in portions with shaking to 30.4 g. (0.1 moles + 20 per cent excess) of silver acetate. The suspension was light yellow. This solution of acetyl hypobromite was filtered into a cold (0° C.) solution of 28.2 g. (0.1 moles) of oleic acid in 100 ml. of carbon tetrachloride. The color was discharged within 15 minutes. The solution was allowed to stand in ice for 1 hour. The solution was extracted with an equal volume of 3 per cent aqueous sodium bisulfite solution, washed with water, and dried over anhydrous magnesium sulfate. The carbon tetrachloride was removed under reduced pressure. After thorough drying under reduced pressure the product was 35.5 g. (85 per cent of the theoretical amount) of a yellow oil.

Neutral equivalent

Calculated:	431
Found:	609, 624

b. To crotonic acid

To 20 g. (0.1 moles + 20 per cent excess) of silver acetate was added with vigorous shaking a cold (0° C.) solution of 5 ml. (0.1 moles) of bromine in 150 ml. of carbon tetrachloride. The light-brown acetyl hypobromite solution was filtered into a cold (0° C.) solution of 8.6 g. (0.1 moles) of crotonic acid in 100 ml. of carbon tetrachloride. Most of the color was discharged. The solution was kept cold (0° C.) and stirred for 1 hour. The solution was extracted with dilute sodium bisulfite solution, washed with water, and dried over calcium chloride. The carbon tetrachloride was removed under reduced pressure. The residue was 12.7 g. (56 per cent of the theoretical amount) of a brown oil.

Neutral equivalent

Calculated:	225
Found:	233

The oil was treated with 800 ml. of concentrated ammonium hydroxide. Not all of the material dissolved. The solution was decanted from the sticky residue, placed in 2 500-ml. bottles, and kept for 10 days in a hot room (37° C.). The bottles were removed, cooled, and opened. The yellow solution was concentrated under reduced pressure to an oil. The oil was dried with acetone, and dissolved in 15 ml. of water and 150 ml. of absolute ethanol. Crystals separated on cooling, were filtered, and dried. The product was 6.6 g. (55 per cent of the theoretical amount, based on the crotonic acid used) of a white material melting with decomposition at 212-216° C.

To 2.38 g. of the aminohydroxy acid dissolved in 15 ml. of 2 N sodium hydroxide and 5 ml. of water were added 4.6 ml. of benzoyl chloride and 46 ml. of 2 N sodium hydroxide alternately in portions with shaking and cooling. The solution was acidified with 6.8 ml. of concentrated hydrochloric acid. The precipitate was removed by filtration. The solution was concentrated to a small volume and the solid removed by filtration. The combined precipitates were extracted with hot petroleum ether (b.p. 90-100° C.). The residue from this extraction was 1.85 g. (41.5 per cent of the theoretical amount) of a white crystalline material. After 3 recrystallizations from water it melted at 176-176.5° C. (capillary). There was no depression of melting point on mixing with N-benzoylallothreonine but considerable depression on mixing with N-benzoylthreonine.

C. Syntheses from D-glucosamine

1. N-Benzoyl-D-glucosamine

To 5.38 g. (0.025 moles) of D-glucosamine hydrochloride partially dissolved in a solution of 5.25 g. (0.0625 moles) of sodium bicarbonate in 25 ml. of water was added in portions 3.5 g. (0.025 moles) of benzoyl chloride. The mixture was shaken vigorously after each addition and cooled in ice. A thick white slurry was formed which made shaking difficult. The solid was collected on a filter and dried under reduced pressure over phosphorus pentoxide, giving 5.2-5.7 g. (67-74 per cent of the theoretical amount) of a white crystalline material, which melted with decomposition at 196-198° C. Recrystallization from 90 per cent methanol yielded only 3.8-3.1 g. of material, which melted with decomposition at 202-204° C.

$C_{13}H_{17}O_6N$	Calculated:	N 4.94
(283)	Found:	N 4.78, 5.01
		(micro Kjeldahl)

The optical rotation was $[\alpha]_D^{25°} = +43.0°$ (100.2 mg. in 10 ml. water).

2. N-Benzoyl-D-glucosaminol

N-Benzoyl-D-glucosamine (2.14 g.) was reduced using Raney nickel catalyst at 100° C. and 1000 pounds hydrogen pressure. After 3 hours the solution gave a negative Benedict's test. The solution was concentrated to a small volume and, after all attempts at recrystallization failed, was lyophilized. The resulting product consisted of 1.6 g. (75 per cent of the theoretical amount) of a fluffy white amorphous powder, which melted at 147-149° C.

$C_{13}H_{19}O_6N$	Calculated:	N 4.91
(285)	Found:	N 4.91, 4.91
		(micro Kjeldahl)

3. N-Acetyl-D-glucosamine

In a 500-ml. ground-glass stoppered Erlenmeyer flask were placed 10 g. (0.0465 moles) of D-glucosamine hydrochloride, 7.5 g. (0.045 moles) of silver acetate, 6.76 g. (0.066 moles) of acetic anhydride, and 100 ml. of anhydrous methanol. The flask was wrapped in tinfoil to exclude light and shaken mechanically for 12 hours. The mixture was then refluxed for 5 minutes and filtered while hot. The solid was washed with 50 ml. of hot water, and the washings added to the filtrate. One drop of concentrated hydrochloric acid

was added, and the solution allowed to stand in the dark for 4 hours. The precipitated silver chloride was removed by filtration, and the filtrate concentrated to dryness under reduced pressure. The residue was taken up in a hot mixture of 35 ml. of methanol and 15 ml. of water. Since nothing separated on cooling, 50 ml. of ether were added. The product, which now crystallized from the cold solution, consisted of 4.7-5.4 g. (46-53 per cent of the theoretical amount) of a white crystalline material, which melted with decomposition at 185° C. As in the case of the N-benzoyl compound large losses were sustained on recrystallization. Only about 60 per cent of the material could be recovered from the methanol-water-ether mixture. This latter material melted with decomposition at 196° C.

$C_8H_{15}O_6N$	Calculated:	N 6.33
(221)	Found:	N 6.31, 6.37
		(micro Kjeldahl)

The optical rotation was found to be $[\alpha]_D^{25°} = +41.3°$ (water). While the value reported by White (110) is $[\alpha]_D^{18°} = +40.5°$ (water).

4. Periodate oxidations

a. N-Benzoyl-D-glucosaminol

In a 200-ml. volumetric flask were placed 27.1 mg. (1×10^{-4} moles) of N-benzoyl-D-glucosaminol and 100 ml. of an 0.02 M aqueous solution of sodium metaperiodate. The solution was then diluted to the mark with methanol. Ten-milliliter aliquots were removed at intervals, and enough sodium bicarbonate (about 5-8 g.) was added to provide an excess throughout the titration.

Approximately 1 g. of potassium iodide was added, and the liberated iodine was titrated to the usual starch endpoint with 0.01 M sodium arsenite. A blank containing only 0.01 M sodium metaperiodate in 50 per cent methanol was titrated at the same time. Within 15 minutes the sample had consumed 2.88 moles of periodate per mole of compound, and this figure remained unchanged after 12 hours.

b. N-Benzoyl-D-glucosamine

An exactly analogous study was run on 36.9 mg. (1×10^{-5} moles) of N-benzoyl-D-glucosamine. The uptake of periodate was much slower in this case, reaching 2.92 moles per mole of compound after 36 hours and remaining constant at this figure.

c. N-Acetyl-D-glucosamine

An exactly analogous study was made on 30.7 mg. (1×10^{-5} moles) of N-acetyl-D-glucosamine. The uptake of periodate in this case reached 2.98 moles per mole after 24 hours and remained constant at this figure.

5. Attempted preparation of 2-(N-benzoylamino)-3-hydroxy propionaldehyde

To 1 g. (0.00325 moles) of N-benzoyl-D-glucosaminol dissolved in 15 ml. of water was added a solution of 2.35 g. (0.011 moles) of sodium metaperiodate in 15 ml. of water. The reaction mixture was placed in ice. After about 1 hour long white needles precipitated, and these were removed by filtration. This material (1.12 g.) gave a negative Tollen's test, did not melt below 300° C., and gave a

residue on ignition.

The reaction mixture was then allowed to warm up to room temperature. After one-half hour more crystals appeared, and these were removed by filtration. This product (0.31 g.) gave a positive Tollen's test, melted at 136-137° C. with decomposition, and turned yellow on standing.

$C_{10}H_{11}O_3N$	Calculated:	N 7.25
(193)	Found:	N 4.70, 4.67
		(micro Kjeldahl)

The reaction mixture was then left in the refrigerator for 24 hours. More white crystals appeared, and these were separated by filtration. This material (0.09 g.) gave a positive Tollen's test and melted at 144-147° C. with decomposition.

$C_{10}H_{11}O_3N$	Calculated:	N 7.25
(193)	Found:	N 6.35, 6.31
		(micro Kjeldahl)

V. SUMMARY

1. The α-phenylhydrazone of ethyl β-ketostearate was prepared from ethyl palmitoylacetoacetate and benzene diazonium chloride. This is the first reported application of the Japp-Klingman reaction to an acylacetoacetic ester.

2. Ethyl α-phenylhydrazono-β-ketostearate was reduced with hydrogen and Raney nickel to α-amino-β-hydroxystearic acid. This acid was characterized as the methyl ester hydrochloride.

3. A study was made of the benzoylation of α-aminostearic acid and α-amino-β-hydroxystearic acid under a variety of conditions. The reaction failed in aqueous solutions due to the insolubility of these long chain amino acids in aqueous alkali. Poor yields of benzoyl derivative were obtained with aqueous methanol as the solvent. A more satisfactory procedure consisted in benzoylating the esters in pyridine. Mild alkaline hydrolysis of the N-benzoyl esters yielded the corresponding N-benzoyl acids.

4. 1,3-Dihydroxy-2-amino-octadecane was obtained by reduction of methyl α-amino-β-hydroxystearate with Raney nickel and hydrogen. The aminoglycol was characterized as the tribenzoyl derivative. This product represents one of the racemic forms of 1,3-dihydroxy-2-amino-octadecane, one of the four optical isomers of which is dihydrosphingosine.

5. The addition of acetyl hypobromite to crotonic acid was found to yield a bromoacetoxy acid which on treatment with ammonia yielded DL-allothreonine.

VI. BIBLIOGRAPHY

1. Carter, H.E., Proc. 105th Meeting, Am. Chem. Soc., Detroit, Michigan, April 12-16, 1943.
2. Glick, F.J., Ph.D. Thesis, University of Illinois, 1943.
3. Norris, W.P., Ph.D. Thesis, University of Illinois, 1944.
4. Phillips, G.E., Ph.D. Thesis, University of Illinois, 1944.
5. Rockwell, H.E., Ph.D. Thesis, University of Illinois, 1946.
6. Nalbandov, O., Ph.D. Thesis, University of Illinois, 1946.
7. Thudichum, J.L.W., Ninth Ann. Rep. of Local Governm. Board, 1879-80, London, p. 143.
8. Levene, P.A., and Jacobs, W.A., J. Biol. Chem., 11, 547 (1913).
9. Lesuk, A., and Anderson, R.J., J. Biol. Chem., 139, 457 (1941).
10. Carter, H.E., and Norris, W.P., J. Biol. Chem., 145, 709 (1942).
11. Carter, H.E., Norris, W.P., Glick, F.J., Phillips, G.E., and Harris, R., J. Biol. Chem., 170, 369 (1947).
12. Klenk, E., and Diebold, W., Z. physiol. Chem., 198, 25 (1931).
13. Thudichum, J.L.W., J. prakt. Chem., 25, 19 (1882).
14. Werner, E., and Thierfelder, H., Z. physiol. Chem., 30, 543 (1900).
15. Levene, P.A., and Jacobs, W.A., J. Biol. Chem., 11, 547 (1913).
16. Thomas, K., and Thierfelder, H., Z. physiol. Chem., 77, 511 (1912).
17. Lapworth, A., J. Chem. Soc., 103, 1039 (1913).
18. Levene, P.A., and West, C.J., J. Biol. Chem., 16, 549 (1914).
19. Levene, P.A., and West, C.J., J. Biol. Chem., 18, 481 (1914).
20. Klenk, E., Z. physiol. Chem., 185, 169 (1929).
21. Niemann, C., and Nichols, P.L.,Jr., J. Biol. Chem., 143, 191 (1942).
22. Carter, H.E., Glick, F.J., Norris, W.P., and Phillips, G.E., J. Biol. Chem., 142, 449 (1942).

23. Carter, H.E., Glick, F.J., Norris, W.P., and Phillips, G.E., J. Biol. Chem. 170, 285 (1947).

24. Christman, C.C., and Levene, P.A., J. Biol. Chem., 124, 453 (1938).

25. Ovakimian, G., Christman, C.C., Kuna, M., and Levene, P.A., J. Biol. Chem., 134, 151 (1940).

26. Ovakimian, G., Kuna, M., and Levene, P.A., J. Biol. Chem., 135, 91 (1940).

27. Carter, H.E., Norris, W.P., and Rockwell, H.E., J. Biol. Chem., 170, 295 (1947).

28. West, H.D., and Carter, H.E., J. Biol. Chem., 119, 103 (1937).

29. Ponzio, Gaz. Chim. Italiana, 34 II, 77, (1904).

30. Conant, J.B., and Jackson, E.L., J. Am. Chem. Soc., 46, 1737 (1924).

31. West, H.D., Krummel, G.S., and Carter, H.E., J. Biol. Chem., 122, 605 (1938).

32. Meinel, K., Ann., 516, 231 (1935).

33. Fieser, L.F., Jacobsen, R.P., and Price, C.C., J. Am. Chem. Soc., 58, 2163 (1936).

34. Bockmuller, W., and Hoffman, F. W., Ann., 519, 165 (1935).

35. Ushakov, M.I., Chistov, V.O., and Zelinskii, N.D., Ber., 68B, 624 (1935).

36. Houben, J., Die Methoden der Organischen Chemie, 3rd edition, Liepzig, 1930, vol. 3, p. 1160.

37. King, A., Inorganic Preparations, Van Nostrand, New York, 1936, p. 65.

38. Baxter, R.A., and Spring, F.S., J. Chem. Soc., 1945, 239.

39. Aston, J.G., Newkirk, J.D., Dorsky, J., and Jenkings, D.M., J. Am. Chem. Soc., 64, 1413 (1942).

40. Shilov, E.A., and Kanyaev, N.P., Chem. Abs., 32, 6968 (1938); (J. Gen. Chem. (U.S.S.R.), 8, 445 (1938)).

41. Carter, H.E., unpublished data.

42. Nicolet, B.N., and Poulter, T.C., J. Am. Chem. Soc., 52, 1186 (1930).

43. Ellis, G.W., Biochem. J., 30, 753 (1936).

44. Atherton, D., and Hilditch, T.P., J. Chem. Soc., 1943, 207.

45. Chattaway, F.D., and Backeberg, O.G., J. Chem. Soc., 123, 3999 (1923).

46. Irwin, C.F., and Hennion, G.F., J. Am. Chem. Soc., 63, 858 (1941).

47. Hanby, W.E., and Rydon, H.N., J. Chem. Soc., 1946, 114.

48. Oroshnik, W., J. Am. Chem. Soc., 67, 1637 (1945).

49. Emling, B.L., Vogt, R.R., and Hennion, G.F., J. Am. Chem. Soc., 63, 1634 (1941).

50. Birckenbach, L., and Linhard, M., Ber., 63, 2544 (1930).

51. Birckenbach, L., and Linhard, M., Ber., 64, 961 (1931).

52. Birckenbach, L., and Linhard, M., Ber., 64, 1076 (1931).

53. Melikoff, P., Ber., 13, 1265 (1880).

54. Erlenmeyer, E., Ber., 13, 1077 (1880).

55. Krassousky, K., Compt. rend., 146, 236 (1908).

56. Fourneau, F., Bull. soc. chim. (4), 1, 549 (1907).

57. Tiffeneau, M., and Fourneau, E., Bull. soc. chim. (4), 13, 971 (1913).

58. Fourneau, E., and Tiffeneau, M., Bull. soc. chim. (4), 15, 19 (1914).

59. Smith, L., and Nilsson, T., J. prakt. Chem., 162, 63 (1943).

60. Smith, L., and Lindberg, J., Ber., 64, 515 (1931).

61. Erlenmeyer, E., Jr., Ann., 271, 137 (1892).

62. Erlenmeyer, E., Jr., Ber., 39, 791 (1906).

63. Oesterlin, M., Chem. Zentr., 1939, II, 1398.

64. Burch, W.J.N., J. Chem. Soc., 1930, 310.

65. Carter, H.E., and Van Loon, E.J., J. Am. Chem. Soc., 60, 1077 (1938).

66. Carter, H.E., Or. Syn., 20, 81, 101 (1940).

67. Touster, O., Ph.D. Thesis, University of Illinois, 1947.

68. Barry, R.H., and Hartung, W.H., J. Org. Chem., 12, 460 (1947).

69. Block, R.J., Chem. Rev., 38, 501 (1946).

70. Adkins, H., and Reeve, E.W., J. Am. Chem. Soc., 60, 1328 (1938).

71. Harington, C.R., and Randall, S.S., Biochem. J., 25, 1917 (1931).

72. Meyer, V., Ber., 10, 2075 (1877).

73. Zublin, J., Ber., 11, 1417 (1878).

74. Richter, V., and Munzer, H., Ber., 17, 1936 (1884).

75. Japp, F.R., and Klingman, F., Ber., 20, 3942 (1887).

76. Meyer, R., Chemikerzeitung, 1887, 836.

77. Japp, F.R., and Klingman, F., Ann., 247, 190 (1888).

78. Feofilaktov, V.V., and Onishchenko, A.S., Chem. Abs., 34, 378 (1940); (J. Gen. Chem. (U.S.S.R.), 9, 304 (1939)).

79. Feofilaktov, V.V., and Onishchenko, A.S., Chem. Abs., 34, 376 (1940); (J. Gen. Chem. (U.S.S.R.), 9, 314 (1939)).

80. Feofilaktov, V.V., and Onishchenko, A.S., Chem. Abs., 34, 379 (1940); (J. Gen. Chem. (U.S.S.R.), 9, 325 (1939)).

81. Feofilaktov, V.V., Compt. rend. acad. sci. U.R.S.S., 24, 755 (1939); Chem. Abs., 34, 1971 (1940).

82. Feofilaktov, V.V., J. Gen. Chem. (U.S.S.R.), 10, 247 (1940); Chem. Abs., 34, 7383 (1940).

83. Feofilaktov, V.V., and Vinogradova, E., Chem. Abs., 34, 7383 (1940); (J. Gen. Chem. (U.S.S.R.), 10, 255, (1940)).

84. Feofilaktov, V.V., and Zaitseva, V., Chem. Abs., 34, 7383 (1940); (J. Gen. Chem. (U.S.S.R.), 10, 358 (1940)).

85. Feofilaktov, V.V., and Zaitseva, V., Chem. Abs., 35, 3606 (1941); (J. Gen. Chem. (U.S.S.R.), 10, 1391 (1941)).

86. Feofilaktov, V.V., et al., Chem. Abs., 35, 3606 (1941); (Bull. acad. sci. U.R.S.S., Classe sci. chim., 1940, 259).

87. Feofilaktov, V.V., and Blanko, F., Chem. Abs., 36, 4096 (1942); (J. Gen. Chem. (U.S.S.R.), 11, 859 (1941)).

88. Feofilaktov, V.V., and Zaitseva, V., Chem. Abs., 38, 1311 (1944); (J. Gen. Chem. (U.S.S.R.), 13, 358 (1943)).

89. Feofilaktov, V.V., Zaitseva, V., and Surothina, K.I., Chem. Abs., 38, 1311 (1944); (J. Gen. Chem. (U.S.S.R.), 13, 383 (1943)).

90. Feofilaktov, V.V., et al., Chem. Abs., 37, 3347 (1943); (Bull. acad. sci. U.R.S.S., Classe sci. chim., 1941, 531).

91. Feofilaktov, V.V., and Vinogradova, E., Compt. rend. acad. sci. U.R.S.S., 24, 759 (1939); Chem. Abs., 34, 1971 (1940).

92. Feofilaktov, V.V., and Vinogradova, E., Chem. Abs., 34, 7283 (1940); (J. Gen. Chem. (U.S.S.R.), 10, 380 (1940)).

93. Lions, F., and Spruson, M.J., J. Proc. Roy. Soc. N.S. Wales, 66, 171 (1932).

94. Hughes, G.K., and Lions, F., J. Proc. Roy. Soc. N.S. Wales, 71, 475 (1938).

95. Hughes, G.K., Lions, F., and Ritchie, E., J. Proc. Roy. Soc. N.S. Wales, 72, 309 (1938).

96. Helmuth, R., Manske, F., Perkin, W.H., Jr., and Robinson, R., J. Chem. Soc., 1927, 1.

97. Sempronj, A., Gazz. Chim. Ital., 68, 363 (1938).

98. Hegedus, B., Helv. Chim. Acta, 29, 1503 (1946).

99. Kalb, L., Schweizer, F., and Schimf, G., Ber., 59, 1858 (1926).

100. Koelsch, C.F., J. Org. Chem., 8, 395 (1943).

101. Feofilaktov, V.V., and Ivanov, A., Chem. Abs., 38, 3355 (1944); (J. Gen. Chem. (U.S.S.R.), 13, 457 (1943)).

102. Jackson, R.W., and Manske, R.H., J. Am. Chem. Soc., 52, 5029 (1930).

103. Lions, F., J. Proc. Roy. Soc. N.S. Wales, 66, 516 (1932).

104. Harradence, R.H., and Lions, F., J. Proc. Roy. Soc. N.S. Wales, 72, 221 (1938).

105. King, F.E., and L'Ecuyer, P., J. Chem. Soc., 1934, 1901.

106. Karrer, P., and Meyer, J., Helv. Chim. Acta, 20, 626 (1937).

107. Levene, P.A., and Christman, C.C., J. Biol. Chem., 120, 575 (1937).

108. Breuer, R., Ber., 31, 2193 (1898).

109. Zuckerandl, F., and Messiner-Klebermass, L., Biochem. Z., 236, 19 (1931).

110. White, T., J. Chem. Soc., 1940, 438.

111. Bergman, M., and Zervas, L., Ber., 64B, 975 (1931).

112. Hinman, J.W., private communication.

113. Bertho, A., Holder, F., Meiser, W., and Huther, F., Ann., 485, 137 (1931).

114. Hopwood, A., and Weizmann, C., Proc. Chem. Soc., 28, 361 (1912).

115. Read, J., and Andrews, A.C.P., J. Chem. Soc., 1931, 1774.

116. Levene, P.A., and Haller, H.L., J. Biol. Chem., 63, 689 (1925).

117. Clark, R.H., and Bell, A., Trans. Roy. Soc., Canada (3), 27, III, 97, (1933).

118. Dimroth, O., Ber., 40, 3404 (1907).

119. Dimroth, O., Ber, 40, 4430 (1907).

120. Carter, H.E., and Stevens, C.M., J. Biol. Chem., 138, 627 (1941).

121. Farkas, L., and Lewin, M., Ind. Eng. Chem. Anal. Ed., 19, 662 (1947).

122. Vergez, G., Chimie and Industrie, 37, 659 (1935).

VITA

The author was born June 1, 1923 in Chicago, Illinois. She was graduated from Hyde Park High School, Chicago in June, 1941 and entered Iowa State College the following September. During her third year at Iowa State College she held a Lane-Wells Scholarship. She received the degree Bachelor of Science in June, 1945. From July through September, 1945 she was employed by the Sherwin-Williams Company, Kensington, Illinois as a Junior Chemist. She entered the graduate school at the University of Illinois in October, 1945, where she served as a half-time Assistant in Chemistry from October, 1945 to June, 1946. From July, 1946 to August, 1947 she held a special Research Fellowship granted to the University by the Upjohn Pharmaceutical Company. During her third year (September, 1947 to June, 1948) she received a University Fellowship. From June to August 1948 she again held an Upjohn Fellowship. She is a member of Iota Sigma Pi, Phi Kappa Phi, and Sigma Xi.