SYNTHESIS OF BRANCHED-CHAIN α-AMINOACETAMIDES AS POSSIBLE HYPNOTICS

by

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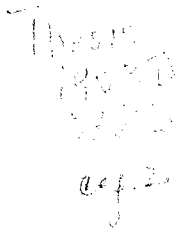

SYNTHESIS OF BRANCHED-CHAIN α-AMINOACETAMIDES AS POSSIBLE HYPNOTICS

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INTRODUCTION

In the field of hypnotics and sedatives, there has been considerable interest in amides and ureides of branched-chain aliphatic acids. Some of these compounds have been found to possess hypnotic activity coupled with low toxicity and others to possess activity as antispasmodics, anticonvulsants and analgesics. Activity seems to be linked to substitutions on the α-carbon atom of the acid; groups used in this position have been halogen, alkyl, aryl, mercapto, and in one case amino.

It was felt that α-aminodialkylacetamides and the corresponding ureides might well have hypnotic activity because of similarity of their structures to those of known hypnotics. Such compounds would be particularly interesting because they are amino acids and can be substituted with groups which are themselves known to have activity.

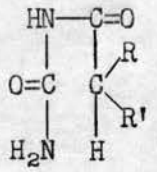
The purpose of this work was to synthesize a series of α-disubstituted α-phthalimido- and α-benzamidoacetamides as possible hypnotics and antispasmodics. Also, with the great current interest in amino acids and their derivatives, it was felt that this work would add to the knowledge of preparative methods and chemical and physical properties of such branched-chain amino acids since as a class they are practically unknown.

HISTORICAL

The development of hypnotics and sedatives has largely taken place in the last fifty years, beginning with the introduction of Barbital by Emil Fischer and von Mering. Before this the main central depressants in use were opium, cannabis, ethanol, chloral hydrate, paraldehyde, urethane, and inorganic bromides. The origin of the first three is lost in antiquity and the rest were discovered to be hypnotics during the period from 1850 to 1900. The success of Barbital, and a little later of Phenobarbital, resulted in an awakening of interest in this type of compound, and has led to the production of more than 1200 derivatives of barbituric acid alone. Because of their structural similarity to barbituric acid, many other classes of compounds have been made and tested for hypnotic activity. Among them are oxazolones, homophthalimides, hydantoins, acylureas, amides, and thiazolidones, to name only a few.

This paper will be concerned only with acyclic amides and ureides and will not undertake to review the entire field of hypnotics and sedatives. For reviews on hypnotics the reader is referred to Traubmann (58), Kindler (35), Prescott (48), Verwey (60), Lespagnol (38), Rice (50) and Dumont (23).

Isolated pharmacological studies on acetamide and a few of its derivatives and on the relation of structure to activity of substituted acetamides and acylureas led to intensified work in this field. A ureide, i.e., an acylurea, can be considered an acyclic barbituric acid minus one carbon atom.



In their properties these compounds appear to stand intermediate between the comparatively powerful barbiturates and the milder bromides.

Among the first of the amides and ureides to appear were Bromural (α-bromoisovalerylurea) and Adaline (α-bromo-γ-ethylbutyrylurea). Bromural is found in the German patent literature of 1906 (29) and along with Adaline still finds wide use. The toxicity of these compounds is considerably lower than that of the barbiturates. Overdosage, however, can be very dangerous, causing a poisoning characterized by pronounced excitation and atony of the cardiac muscles. Such a case of poisoning caused by 15 g. of Adaline was reported by Kirchberg (37).

Fourneau and Florence (25) became interested in compounds of this class and undertook a study of the influence of the position of the halogen atom on hypnotic activity of amides. There were four major points in their findings. 1. There is a parallelism between the partition coefficient (between water and olive oil) of the compound and its hypnotic activity. This applies only to compounds of the same series. 2. Displacement of the bromine atom from the alpha to the beta position decreases the activity. 3. Increase in number of bromine atoms decreases the activity. 4. Bromine in the alpha position does not necessarily bestow hypnotic properties. With reference to partition coefficients, it is interesting to note that Fruh (28) determined such constants for urethane, chloral hydrate, Sulfonal, Trional, ether, Neuronal, Bromural, Adaline, and Barbital to see if there is any relationship between such coefficients and hypnotic activity, and was forced to the conclusion that there is none.

In continuance of their interest in these compounds, Fourneau and Florence (26) studied the effect of chain branching and found that hypnotic activity increases with increased branching. They found that the ureides increased in activity in the order α-bromovaleric, α-bromoisovaleric, α-bromomethylethylacetic and bromopivalic. This effect of chain branching on hypnotic activity was confirmed by Weil, Langiert and Kassur (63). Fourneau and Billeter (27) made a number of substituted amides of glycidic acid and found them to be active as hypnotics.

Lumiere and Perrin (44) also ran some pharmacological studies on substituted acetamides at this time to discover whether there is a maximum hypnotic effect connected with certain alkyl groups. They stated that almost all known hypnotics are compounds belonging to the aliphatic series and containing alkyl groups linked to a quaternary carbon atom. They found the following order of increasing activity of N-acetyl-α,α-disubstituted acetamides: benzylethyl, ethylisobutyl, diallyl, dipropyl, and diethyl.

An extensive study of the hypnotic action of ureides and amides derived from substituted acrylic acids was made by Lott and Christiansen (42). Among these were many α-phenylacrylic acids and one heterocyclic compound, β-(2-furan)acrylic acid. Biological results on rats indicated that the whole series is characterized by low absorbability and that the ureides undergo rapid intestinal hydrolysis. Toxicity and hypnotic activity are, therefore, rather low, but the furan ring induces much more toxicity than the benzene ring, and confers no characteristic hypnotic action. These same authors (43) also discovered what they called a new type of hypnotic in N-(β-oxopropyl)diethylacetamide, which has a minimum effective hypnotic dose for rats of slightly less than 0.0016 g. per kg. of body weight. This is much lower than the dose of any currently used hypnotics.

A contribution to the ever-challenging topic, physiological activity

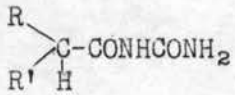
vs. chemical structure, was made by Shonle (52), who, after reviewing considerable historical evidence, came to the conclusion that the ideal hypnotic could not yet be sketched and that work must still be done in an empirical fashion. He stated that if there were any group which could be called the hypnophore, it was the alkyl group, the hypnotic effect of which can be varied by attachment to different polar groups.

A study in the field of barbiturates led Volwiler and Tabern (61) to expand their findings into the amide and ureide areas. They had found that barbiturates containing secondary pentyl groups in addition to ethyl or allyl possess unique properties of intense hypnotic action coupled with rapid recovery. They therefore prepared a series of acetylureas, acetamides, and the bromo analogs containing the 1-methylbutyl and other similar secondary alkyl groups to see if the properties mentioned would be retained. They found that activity varied as much as fivefold, and toxicity even more widely. The ethyl-1-methylbutylacetylurea and amide seemed to have the best properties. They also noticed that in the N-alkyl series of these compounds, when the N-substituent group was methyl, prolonged mild sedation occurred. When it was larger, excitement rather than sedation resulted. It was of interest that some of these compounds showed great analgesic action without any corresponding deep hypnotic effect.

The striking change from an hypnotic to a toxic material by a change of alkyl groups was shown by Bass (3), who synthesized and tested a number of α-bromo-tert-butyl-substituted amides. He found that at a level of 0.6 g. per kg., N-methyl-α-bromo-α-tert-butylacetamide and N,N-dimethyl-α-bromo-α-tert-butylacetamide produced a prolonged hypnosis in rabbits when given by mouth. A long list of similar compounds with varying alkyl groups was found to be inactive but α-bromo-α-tert-butylacetamide and its N-ethyl and N,N-diethyl derivatives at a level of 1 g. per kg.

produced convulsions or death.

In order to improve the existing knowledge of the effect of the acyl groups on hypnotic activity of acylureas, Blicke and Centolella (43) synthesized 23 disubstituted acylureas of the type



They were surprised to find that none of these compounds was effective to any great degree when injected intraperitoneally into white rats. Some of the corresponding amides (14), however, proved to be strong hypnotics which was also surprising since most of the acetamides which have been described as having hypnotic activity have been trisubstituted derivatives such as diethylallylacetamide. Those compounds shown to be very effective hypnotics were α,α-diethylacetamide, α-ethyl-α-butylthioacetamide, N-methyl-α-ethyl-α-butylacetamide, α-ethyl-α-pentylacetamide, α-ethyl-α-hexylacetamide, and N-β-hydroxyethylethyl-β'-cyclohexylethylacetamide. Especially interesting is the relatively high activity of diethylthioacetamide, which was the strongest of the group. The work was continued by Blicke and Zienty (15), who prepared a number of disubstituted acetamides in which one group was alkyl or arylalkyl and the other alkoxyalkyl or aryloxyalkyl, such as ethyl(phenylethyl)acetamide and ethyl-β-methoxyethylacetamide. Nine of these compounds were found to be active.

The acylureas and acetamides show the fine margin of difference which divides hypnotics and antispasmodics. Junkman (32) tested tributylacetamide for antispasmodic action and found that it not only had marked activity but was fairly nontoxic. Later, this same author (33) tested a group of alkylacetamides and compared their antispasmodic activity to that of papaverine taken as 80. He found diethylbutyl to be 15, dipropyl 0.85,

dipropylbutyl 75, dibutylpropyl 87, tributyl 100, dipentyl 150, and tripentyl 100. Diphenylglycolamide and N-benzyldiphenylglycolamide were also found to have weak antispasmodic activity by Lespagnol (39).

Nineteen amides with highly varied substituent groups were tested as anticonvulsants by Long (41) for the treatment of petit mal as measured by antimetrazol activity. A number of the compounds tested were found to have considerable activity.

A few substituted acylthioureas have also been tested for hypnotic activity by Moore and Crossley (46). The acyl radicals were derived from straight-chain acids such as acetic, propionic, and valeric, and the acylthioureas prepared were found to be relatively inactive as hypnotics. Such inactivity is not surprising since most of the successful hypnotics in the amide and ureide series contain a branched-chain acyl group.

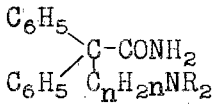
Bergmann and Haskelburg (12) became interested in the effect of chlorine on physiological activity of various compounds and prepared some chloro derivatives of acylamides and -ureas. They found that an increase in number of chlorine atoms increased the hypnotic activity, trichloroacetamide being a powerful hypnotic. They also found that the mono- and trichloro derivatives were usually more toxic than the dichloro derivatives, a fact which they could not explain.

In 1938 Stoughton (56) surveyed the literature dealing with simple, open-chain diacylureas, finding only a few scattered references. Many of the papers are patents, giving meager details as to the chemistry and hypnotic activity of the compounds prepared. He synthesized a series of these compounds derived from normal aliphatic acids by reacting ureas with the appropriate anhydride to give symmetrical diacylureas or by reacting monoureides with an acyl halide in the presence of a catalyst to give the unsymmetrical compounds. It was found that the diacylureas containing

8 - 10 carbon atoms in the molecule were active hypnotics when administered intravenously but that they had a very short anesthesia time of one or two minutes. However, the anesthesia produced, in contradistinction to that of many barbiturates, was characterized by marked analgesia and absence of excitement. In another paper Stoughton and co-workers (57) extended this work to the branched-chain aliphatic diacylureas. Again many of these compounds were active hypnotics but they still exhibited an activity of short duration. They were also characterized by producing marked analgesia and absence of excitement.

An excellent hypnotic and anticonvulsant for use in the treatment of epilepsy has been discovered in 5,5-diphenylhydantoin. The fact that α,α-diphenyl-α-aminoacetamide is a possible product in the hydrolysis of 5,5-diphenylhydantoin led Billman and Hidy (10) to prepare the amide and a number of its derivatives. A literature search revealed that compounds of this type had never been tested for pharmacological activity but these investigators felt that activity was possible, as numerous amides as well as derivatives of amino acids were active spasmolytics. The amides were prepared from α-chlorodiphenylacetyl chloride and the appropriate amine. The unsubstituted amide and the one made from p-phenetidine showed anticonvulsant activity and the amide showed considerable antispasmodic activity. Billman and Hidy (11) later synthesized a number of derivatives of α,α-diphenyl-α-aminoacetamide having dissimilar groups attached to the nitrogen atoms, but found that they did not differ greatly from the first series as to activity.

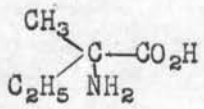
A group of compounds somewhat similar to those prepared in the present work were synthesized and tested by Cheney and co-workers (20). They prepared a number of basic amides of the general structure



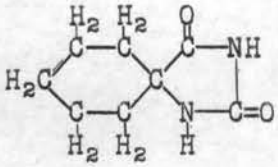
Many of the compounds of this series proved to be strong hypnotics. It is also interesting to note, in the light of the present work, that some of these amides could not be crystallized and had to be tested in the crude form.

SCHEME OF SYNTHESIS

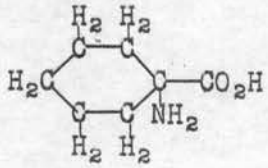
The aminoacetic acids prepared in this series were chosen so that different alkyl and aryl groups would be represented in the alpha position. The choice was limited by the availability of the ketones used as starting material. The compounds are named as derivatives of aminoacetic acid or aminoacetamide in order to throw emphasis on the change in substituents in the alpha position. Thus α-methyl-α-aminobutyric acid



is named as α-methyl-α-ethyl-α-aminoacetic acid in order to emphasize the two groups, methyl and ethyl. In the case of the compounds prepared from cyclohexanone as starting material, the cyclohexyl portion is named either as cyclohexyl or pentamethylene as in the following:



Cyclohexylspirohydantoin



Pentamethyleneaminoacetic acid

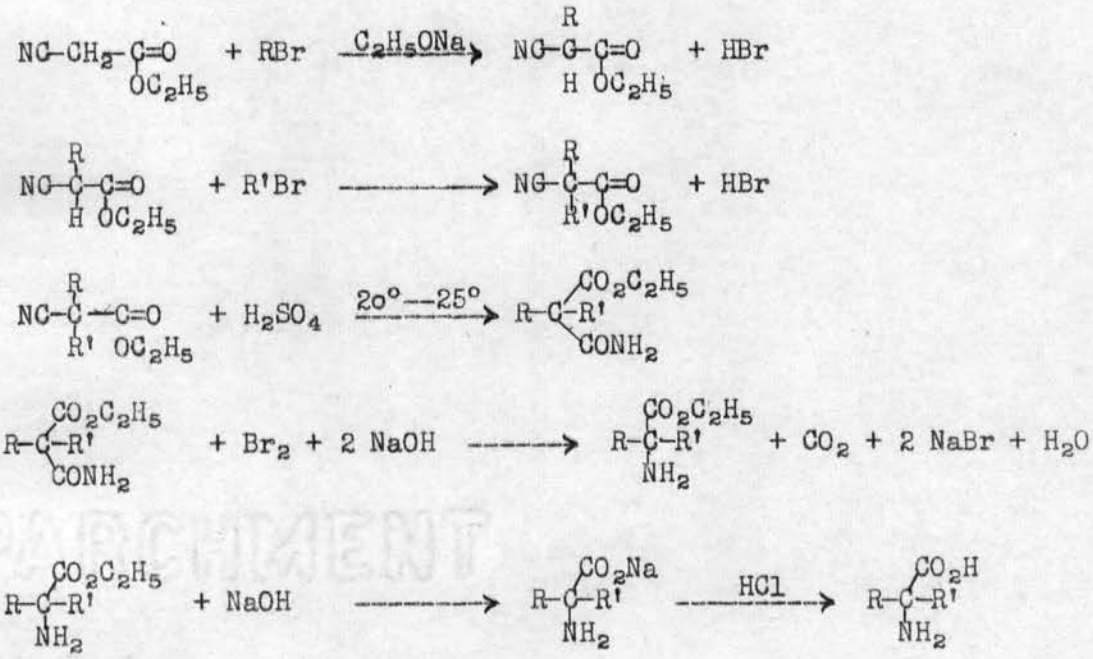
In each case the no. 1 carbon atom in the cyclohexyl ring corresponds to the no. 5 carbon of the hydantoin ring or the alpha carbon of the acetic acid.

The list of aminoacetic acids prepared is as follows: α,α-dimethyl; α-methyl-α-ethyl; α,α-diethyl; α-methyl-α-isobutyl; α-methyl-α-n-pentyl; α-ethyl-α-n-butyl; α-methyl-α-phenyl; α-ethyl-α-phenyl; α,α-diphenyl; α,α-diisopropyl; α,α-diisobutyl; and α,α-pentamethylene.

The synthesis of amino acids and their derivatives has always presented difficulties because of the presence of both a carboxyl and an amino group in the same molecule, necessitating blocking procedures. Preparation of the branched-chain amino acids was further hindered by the fact that many of the common routes of amino acid synthesis did not give the wanted configuration. The best available methods for preparation of the present series were considered to be:

1. Reaction of α,α-disubstituted-α-haloacetyl chlorides with ammonia

2. Preparation and Hofmann degradation of alkylated cyanoacetic esters reported by Lin (40)



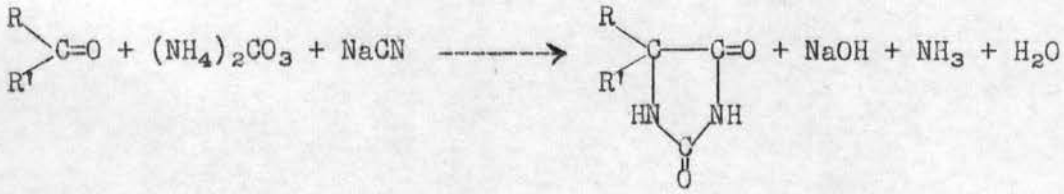
3. The preparation and hydrolysis of 5,5-disubstituted hydantoins.

In the first method above, preparation of many of the chloroacetyl chlorides would have been extremely difficult. The second method is

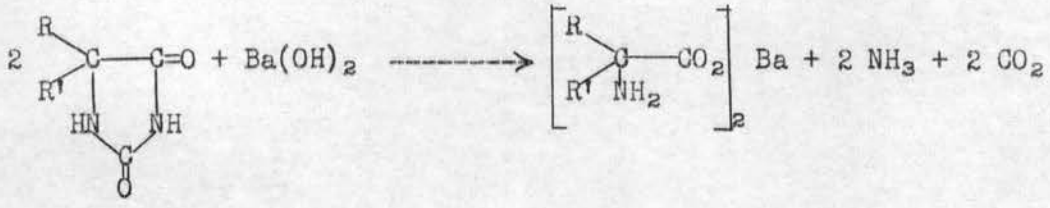
fairly long and some of the reactions rather involved. This process would have required much synthetic work to prepare one amino acid. The hydantoin method, however, was admirably suited to the preparation of the amino acids listed because synthesis of hydantoins has been well worked out by Henze (30) and the chemistry of hydantoins recently reviewed in an excellent article by Ware (62). Also the ketones necessary were commercially available in good purity and in most cases were low-priced. This method was finally chosen as the synthetic route to be used.

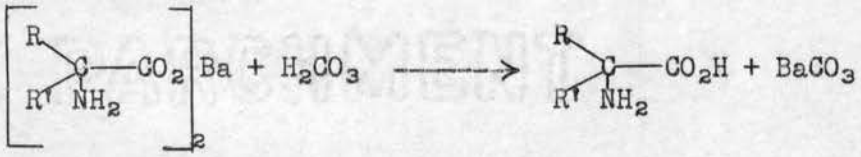
The total synthesis is comprised of five steps which will be outlined by equations and with explanation of principles where necessary.

1. Preparation of Hydantoins



2. Hydrolysis of Hydantoins to Amino Acids

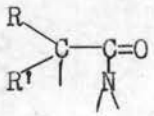




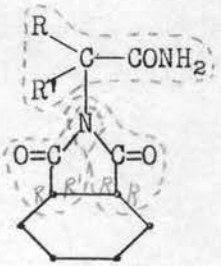
3. Phthaloylation and Benzoylation of Amino Acids

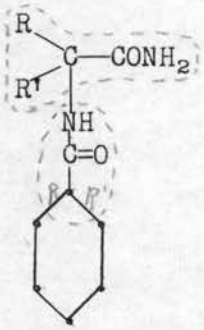
Many of the compounds which have been synthesized as prospective hypnotics have had to be discarded because they were either eliminated so quickly that only very small quantities ever got into the blood stream or were so difficult for the body to eliminate that toxicity presented too much of a problem. It was hoped that phthaloylation and benzoylation would

confer more resistance to elimination by the body, thus producing a longer-acting hypnotic. Another possible advantage was that these blocking groups might increase the hypnotic activity since it has been noticed by Doran and Shonle (21) that a large number of organic compounds exhibiting sedative action contain the following grouping



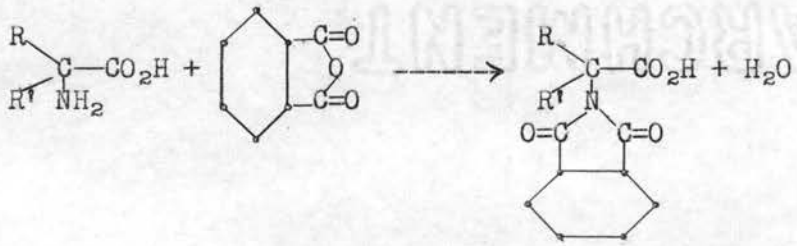
This appears in diacylureas, alkylarylhydantoins, dialkylhomophthalimides, dialkylbarbiturates, dialkyloxazolidinediones, dialkylpiperidinediones, dialkylrhodanines and dialkylthiazolidones. It is interesting to note that the present phthaloylated amino acids contain the active grouping three times and the benzoylated amino acids twice.



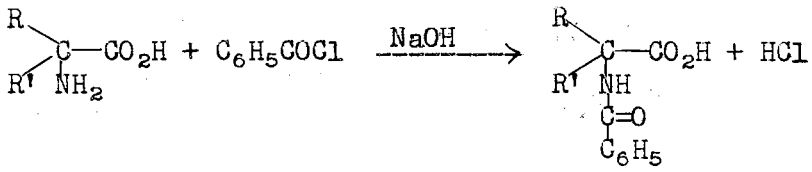


A third reason for using phthalic anhydride as a blocking group was that it can be easily removed by the use of hydrazine if so desired.

a. Phthaloylation

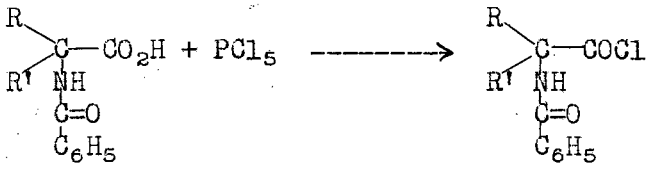


b. Benzoylation



4. Preparation of Phthalimido and Benzamidoacetyl Chlorides

These were prepared in the classical manner using phosphorus pentachloride or thionyl chloride as a reagent. The benzamido acid is used for illustration.



5. Preparation of Blocked Amino Acid Amides

6. Preparation of Acylated Amino Acid Ureides

The ureides were to be prepared by a method similar to the one used for the preparation of the amides, using urea instead of ammonia.

A method which has been used in the preparation of ureides but which has not yet been tried in the present work is

EXPERIMENTAL

I. PREPARATION OF HYDANTOINS

The series of hydantoins was prepared by a method similar to that used by Henze (29) in his extensive study of these compounds. The ketones used as starting material were commercially available and were used without further purification. Little difficulty was met in these preparations and yields were for the most part good, the exceptions being 5,5-diphenyl, 5,5-diisobutyl and 5,5-diisopropyl hydantoins. In these cases the yields were either very poor, or, as in the case of the 5,5-diphenylhydantoin, there was no yield at all, the ketone being too sluggish to react. This last hydantoin was prepared by an entirely different reaction and the preparation of it and 5,5-dimethylhydantoin will be described separately. A general reaction procedure which was used for all others will be described and followed by Table I, which gives details of the various hydantoin preparations.

A. General Procedure for Hydantoin Preparation

The solvent (water or a mixture of alcohol and water) was placed in a three-neck round-bottom flask fitted with a Hershberg stirrer and an air condenser. To this was added a 10% excess over one molar equivalent of either sodium or potassium cyanide and four molar equivalents of ammonium carbonate in the form of 1/8- to 1/4-inch cubes. The ketone, one molar equivalent, was then added and the flask heated to 60° on a water bath, with stirring, for from 2 to 6 hours. At the end of this time, the temperature

was raised to 90° for 0.5 hour to decompose any remaining ammonium carbonate, after which the mixture was poured into a beaker and allowed to cool. The hydantoin crystallized from the reaction mixture and was purified by recrystallization from a mixture of alcohol and water after treating with Norite. One recrystallization usually sufficed to give a pure compound. This reaction must be carried out in a good hood as considerable ammonia and hydrogen cyanide are given off during the formation of the hydantoin.

B. Preparation of 5,5-Dimethylhydantoin

Because of the ready availability of acetone cyanohydrin, it was used as a starting material for the synthesis of this hydantoin.

Acetone cyanohydrin (85 g.) was dissolved in 100 ml. of water and to this was added 150 g. of ammonium carbonate. The reaction mixture was heated, with stirring, at 60° for 2 hours and then the temperature was raised to 90° for 0.5 hour. On cooling, the hydantoin crystallized as white needles which were pure enough to use without further crystallization. M.p. 173°; literature m.p. 173-4° (4).

C. Preparation of 5,5-Diphenylhydantoin

Two attempts to obtain this hydantoin using the general method failed and it was finally prepared according to the procedure given by Sikdar and Ghosh (53).

Sodium hydroxide (45 g.) was dissolved in 288 ml. of water and 180 ml. of 95% alcohol added. The mixture was cooled to room temperature and 90 g. of benzil added. After 10 minutes, 45 g. of urea was added with good stirring and the mixture was refluxed on a steam bath for 1.5 hours. After cooling the solution was poured into twice its volume of water which threw out a small

quantity of fine yellow needles. These were removed by filtration and the filtrate was acidified with concentrated hydrochloric acid to a pH of 2.5, which precipitated the hydantoin. This was then isolated by filtration and crystallized from 95% alcohol giving a yield of 65 g. (60%). M.p. 286°; literature m.p. 286° (53)

TABLE I

Preparation of Hydantoins

KETONE, g.	ALKALI CYANIDE, g.	$(NH_4)_2CO_3$, g.	SOLVENT, ml.alc.+ ml. H_2O	REACT. TIME, hrs.	YIELD, g.	%	M.P., °C	LIT. M.P., °C	
METHYL ETHYL 72	NaCN, 50	156	50+200	3	79	55	144-45	145-46	(18)
DIETHYL 172	KCN, 180	400	300+300	4.5	200	64	163	165	(5)
METHYL ISOBUTYL 200	NaCN, 140	400	300+300	4.5	220	64	145	148	(30)
METHYL n-PENTYL 228	KCN, 180	400	300+300	4.5	200	54	101	102	(30)
ETHYL n-BUTYL 228	KCN, 180	400	400+400	4.5	220	60	122-23		(28)
METHYL PHENYL 120	NaCN, 60	200	200+500	6	100	52	194-95	197	(1)
ETHYL PHENYL 134	NaCN, 60	200	200+500	6	80	38	198	199	(49)
CYCLO-HEXYL 98	NaCN, 60	200	200+500	6	150	90	215	215	(19)
DIISO-PROPYL 228	KCN, 180	400	600+400	4.5	73	20	205	207	(30)
DIISO-BUTYL 284	KCN,	400	600+400	5	50	12	147-48		(44)

II. HYDROLYSIS OF HYDANTOINS TO AMINOACETIC ACIDS

A. Hydrolysis of Methylethylhydantoin Using 60% Sulfuric Acid

This method of hydrolyzing hydantoins was described by Bucherer (18). Methylethylhydantoin (79 g.) was refluxed for 8 hours with 250 ml. of 60% sulfuric acid. The mixture was cooled and brought to a pH of 6 with barium hydroxide. The precipitated barium sulphate was filtered out, a tedious process, and the filtrate reduced in volume by vacuum evaporation until crystallization started. Abour 200 ml. of acetone were then added which precipitated a mass of needle-like crystals, which were filtered out and dried. Crude yield 72 g. The product was found to contain considerable inorganic material which interfered with subsequent reactions. This method was also tried on dimethyl- and methylphenylhydantoins but was so long and gave such poor and unreproducible results that it was abandoned.

B. Hydrolysis of Dimethylhydantoin with Barium Hydroxide

This method was used by Wheeler and Hoffman in preparing a number of natural amino acids (64).

Barium hydroxide octahydrate (300 g.) was dissolved in 600 ml. of hot water, 60 g. of dimethylhydantoin added, and the solution refluxed for 30 hours. The hot solution was filtered to remove barium carbonate and carbon dioxide was bubbled into the hot filtrate until a pH of about 7 was reached. The precipitated carbonate was again filtered off and the filtrate reduced in volume to about 100 ml. Alcohol (300 ml.) was added to cause crystallization of the amino acid. The product was filtered off and dried: yield, 33 g.

C. Hydrolysis of Diethylhydantoin with Barium Hydroxide at 15 lbs./sq. in.

A reference to the hydrolysis of hydantoins using barium

hydroxide under pressure was found in the recent literature. Nadeau (47) used this method to prepare methoxy derivatives of alanine and suggested a temperature of 160°. Since the only autoclave available at first had a 15 lbs./sq. in. limit, it was used and a longer reaction time was provided.

Barium hydroxide (450 g.) and 72 g. of diethylhydantoin were dissolved in 1 liter of boiling water and placed in the autoclave in an open beaker for 10.5 hours. The reaction mixture was then worked up as described above, giving a yield of 50 g. of amino acid.

A number of reactions were run using this method with fairly good results. A reaction time of 15 to 20 hours was found to be best in most cases.

D. General Procedure for Hydrolysis of Hydantoins with Barium Hydroxide at 50 to 60 lbs./sq. in.

The method found to give the best results in the hydrolysis of hydantoins to amino acids was found to be a 50 - 60 lbs. pressure hydrolysis using barium hydroxide. A small autoclave was made from a 24-inch length of ordinary 6-inch steel pipe fitted with a head made from flat plate and a 6-inch collar. The head had three openings which were used for a gauge, a safety valve and a discharge valve. A lead ring was used as a seal. The autoclave was heated with two separate windings of #20 nichrome wire, each wire drawing 7 amperes at 110 volts. A metal tripod was placed in the pipe and water added to just below the tripod top. An open 2-liter pyrex bottle was used as a container for the reaction mixture.

The barium hydroxide (1.5 molar equivalents) was dissolved in hot water and the hydantoin added with stirring until all was

dissolved. The solution was then poured into the bottle and placed in the autoclave, which had been previously heated. Timing of the reaction was started after a pressure of 50 lbs./sq. in. had been reached. When the reaction was over the autoclave was cooled and the accumulated ammonia gas allowed to escape slowly. The reaction mixture was filtered hot to remove the barium carbonate formed, and carbon dioxide was bubbled through the hot filtrate until a pH of about 7 was reached. Care had to be taken at this point, because in some cases the amino acids precipitated with the barium carbonate. This was found to be particularly true in the case of such acids as α-methyl-α-n-pentyl-α-aminoacetic, α-ethyl-α-n-butyl-α-aminoacetic, and α-ethyl-α-phenyl-α-aminoacetic, and was responsible for the low yields obtained. The precipitated barium carbonate was filtered off and the clear filtrate reduced in volume by vacuum evaporation on a steam bath until crystallization was well started. After cooling, the amino acid was obtained by filtration and the filtrate again reduced in volume and so on until all the amino acid had been isolated. Detailed information on various hydrolyses is given in Table II.

E. Hydrolysis of Diphenylhydantoin with 60% Sulfuric Acid

The diphenylhydantoin hydrolysis with barium hydroxide under 60 lb./sq. in. was tried twice with no success. This was probably due partially to the insolubility of the hydantoin in the basic solution. It was decided to try the 60% sulfuric acid method to see if any hydrolysis could be effected.

Diphenylhydantoin (60 g.) was suspended in 250 ml. of 60% sulfuric acid and refluxed for 8 hours. At the end of this time some of the hydantoin had gone into solution and the reaction mixture had become pink. The undissolved hydantoin was removed by filtration

and the filtrate neutralized with sodium hydroxide which caused the α,α-diphenyl-α-aminoacetic acid to precipitate. It was filtered and dried: yield, 30 g. (55%)

TABLE II

Hydrolysis of Hydantoins to Amino Acids

5,5-DISUBSTITUTED HYDANTOIN	g.	$Ba(OH)_2 8H_2O$, g.	H_2O, ml.	PRESSURE, lb./sq.in.	TIME, hrs.	YIELD, g.	YIELD, %	REF.
DIMETHYL	75	225	1200	50-60	1	47	80	(18)
METHYLETHYL	71	455	1200	50-60	1	40	70	(18)
DIETHYL	72	455	1000	15	10	50	67	(23)
METHISOBUTYL	84	472	1000	15	15	66	93	(2)
METHYL-n-PENTYL	92	300	1200	50-60	1	40	51	
ETHYL-n-BUTYL	92	400	1200	50-60	1	36	45	(6)
[1]METHYLPHENYL	126	400	1200	50-60	1	80	74	(18)
ETHYLPHENYL	110	400	1200	50-60	1	48	50	(18)
CYCLOHEXYLSPIRO	85	400	1200	50-60	2	39	55	(18)
DIISOPROPYL	60	200	1000	50-60	1	0	0	(2)
DIISOBUTYL	40	100	480	50-60	1	4	11	(2)

[1]Analysis: Calculated for $C_8H_{17}NO_2$: C, 59.92; H, 10.82; N, 8.91
Found: C, 60.30; H, 10.61; N, 8.54.

III. PREPARATION OF α-PHTHALIMIDOACETIC ACIDS

The first method tried in the preparation of α-phthalimido acids (N-phthaloyl derivatives of amino acids) was that of Sheehan (51), who fused the dry amino acids with phthalic anhydride at 185° for 0.5 hr. When difficulty in isolating and crystallizing the products occurred, attempts were made to modify the procedure by the use of solvents but this met with little success. The only investigators who used any solvent in this reaction were King and Kidd (35), who refluxed the amino acid and phthalic anhydride in pyridine, removed the solvent, and heated again with acetic anhydride. This method evidently gave rise first to the phthalamic acid, which was then cyclized to the phthaloyl derivative by the acetic anhydride. This method was also tried but no products could be isolated. Sheehan's method, with a slight modification, was finally adopted as being the best. A general procedure based on this method will be outlined in addition to other methods used.

A. Preparation of α,α-Diethyl-α-Phthalimidoacetic Acid

Phthalic anhydride (14.8 g.) and α,α-diethyl-α-aminoacetic acid (13.1 g.) were suspended in 150 ml. of tetralin and heated to 200° for 0.5 hr. The suspension was cooled to 100° and filtered, the filtrate was chilled and the phthaloyl derivative crystallized. The crystals were filtered out, washed with heptane and dried. Yield 9 g. (34%), M.p. 161-2°. Neutral equivalent: calc., 261; found, 258.

B. Preparation of α-Methyl-α-isobutyl-α-phthalimidoacetic Acid

Phthalic anhydride (3.7 g.) and α-methyl-α-isobutyl-α-aminoacetic acid (3.6 g.) were dissolved in 50 ml. of diethylcarbitol and heated to 180° in a metal bath. A downward condenser was attached to the

reaction flask and water and the carbitol were allowed to distill slowly over a period of 0.75 hr. until about 10 ml. had been collected. The flask was cooled and its contents poured into 300 ml. of water. A small amount of gummy material was precipitated but no crystalline material of any kind could be isolated, even on long standing.

In another trial of the reaction, the experiment was tried using toluene as part of the solvent.

Phthalic anhydride (7.4 g.) and α-methyl-α-isobutyl-α-amino-acetic acid (7.2 g.) were suspended in 200 ml. of a mixture of 50% toluene and 50% diethylcarbitol. The reaction flask was fitted with a condenser and a water trap. After refluxing for 8 hours, 1.25 ml. of water were collected. Since 0.9 ml. of water is theoretical, there must have been some water present in either the solvent or the aminoacetic acid. The solvents were removed by vacuum distillation and the residue was dissolved in saturated sodium bicarbonate solution. This solution was extracted with ether. The second ether extract upon evaporation left a red, gummy substance which crystallized on standing overnight. The crystals had a thin coat of gummy material covering them. After drying for 2 weeks they were recrystallized from dilute methanol at considerable loss in yield. M.p. 162-4°. Neutral equivalent: calc., 275; found, 278. Yield: 1.5 g. (11%).

C. Preparation of α,α-Dimethyl-α-Phthalimidoacetic Acid

It was felt that this reaction might proceed better if a solvent was used in which both the amino acid and the phthalic anhydride were soluble. Accordingly, phthalic anhydride (7.4 g.) and α,α-dimethyl-α-aminoacetic acid (5.1 g.) were dissolved in 100 ml. of glacial acetic acid and refluxed for 8 hours. When the

acetic acid was removed by vacuum distillation, the residue set to a white solid on cooling. It was dissolved in 100 ml. of boiling water and allowed to crystallize and the crystals were filtered out. Yield 4.5 g. 38%. M.p. 148-50°; literature m.p. 152-3° (8).

D. Attempted Preparation of α,α-Dimethyl-α-Phthalimidoacetic Acid Using Pyridine.

This method is described by King and Kidd (35), who used it to prepare the N-phthaloyl derivative of glutamic acid.

α,α-Dimethyl-α-aminoacetic acid (5.1 g.) and phthalic anhydride (7.4 g.) were suspended in 100 ml. of pyridine and refluxed for 2 hours. Both solids dissolved after about 0.25 hr. heating. The pyridine was removed by vacuum distillation and 50 ml. of acetic anhydride were added to the syrupy mass. This solution was then refluxed for 10 minutes and the acetic anhydride removed by vacuum distillation. A gummy residue was left which could not be crystallized.

E. General Procedure

The reaction of natural amino acids such as alanine, glycine, and phenylalanine with phthalic anhydride goes very smoothly and the authors who have used this method report high yields of products of good purity. However, the branched-chain acids such as those derived from the hydantoin hydrolyses previously described evidently present quite a different problem. The biggest difficulty encountered is in the crystallization of the substituted acids, most of them being gums or glasses to start with, and showing a very definite tendency to oil out of solution - a tendency that persists through as many as eight attempts at crystallization. After more than a year's work on this problem it was discovered that the phthaloylated acids could be made to crystallize by vigorous boiling of the gum

or glass in a large amount of water. This procedure evidently removed impurities which were hindering crystal formation. It did not work in every instance, however; some of the substituted acids either could not be crystallized or formed gummy crystals that could not be purified.

The general procedure used was based on reports by Sheehan (51) and Billman and Harting (8), the major change being the mode of mixing the ingredients for fusion.

The phthalic anhydride was placed in a round-bottom flask and heated to about 160° on a metal bath until a clear melt was obtained. One molar equivalent of the amino acid was then introduced in portions and stirred in with a stirring rod until a homogeneous solution was obtained. In this way acids which were very fluffy came into contact with the hot, melted anhydride and solution occurred. The melt was then raised to a temperature of 180 - 210° and kept there for 0.25 - 0.75 hr. A slight frothing which gradually subsided showed that a reaction was taking place. In cases where this was not apparent, poor yields were usually obtained. The reaction mixture was cooled to 100° and 200 ml. of hot water added. The oily reaction product was well mixed by shaking and poured into about 500 ml. of water in a beaker. This was then brought to a rolling boil for 5 minutes, during which time the oil or gum often set solid. The suspension was allowed to cool and the operation repeated if the derivative still showed signs of being gummy. If it had set to a solid, it was filtered out and crystallized from a mixture of methanol and water.

Of the various amino acids tried, α-ethyl-α-n-butyl-α-amino-acetic acid gave uncrystallizable products when reacted with

phthalic anhydride. α,α-Pentamethyleneaminoacetic acid gave no reaction with the anhydride and was recovered unchanged from the reaction mixture. α-Ethyl-α-phenyl-α-aminoacetic acid and α,α-diphenyl-α-aminoacetic acid did not react in the usual way and a description of their behavior will follow Table III.

Table III gives the details of the various reactions tried, neutral equivalent of the products, etc.

TABLE III

Preparation of α-Phthalimidoacetic Acids

α-AMINOACETIC ACID	g.	PHTHALIC ANHYDRIDE, g.	TEMP., °C	TIME, min.	YIELD, g.	YIELD, %
DIMETHYL	10.3	14.8	175	30	18	76
METHYLETHYL	13.1	14.8	180	15	14	54
DIETHYL	13.3	14.8	210	15	15	57
METHYLISOBUTYL	28.6	29.6	185	45	38	70
METHYL-n-PENTYL	4.0	3.7	185	15	Oil	
ETHYL-n-BUTYL	8.0	7.4	180	30	"	
METHYLPHENYL	8.5	7.4	190	30	7	47
ETHYLPHENYL	9.0	7.4	200	15	Diketopiperazine formation	
PENTAMETHYLENE	4.3	14.8	180	30	No reaction	
DIPHENYL	11.3	7.4	200	15	Decarboxylation occurred	

PHTHALIMIDO DISUBSTITUTED ACETIC ACID	NEUTRAL EQUIVALENT CALC.	NEUTRAL EQUIVALENT FOUND	M.P. °C	LIT. M.P.	
DIMETHYL	233	230	152-53	152-53	(8)
METHYLETHYL	247	253	137-38	139-40	(8)
DIETHYL	261	258	163-64		
METHYLISOBUTYL	275	278	162-64		
METHYLPHENYL	299	304	186-87		

F. Attempted Preparation of α-Ethyl-α-Phenyl-α-Phthalimidoacetic Acid

Phthalic anhydride (7.4 g.) was heated to 160° until a clear melt was formed. α-Ethyl-α-phenyl-α-aminoacetic acid was added in portions with stirring. Instead of becoming a clear melt, the mixture set to a fairly stiff gel and no bubbling occurred unless the temperature was raised at least to 210°. Since decarboxylation is more likely to occur at high temperatures the gel was heated at 200° for 15 minutes, cooled to 100° and treated with water, and the whole suspension was decanted into a beaker. The gel solidified in the hot water and was filtered out. It was found to be insoluble in alcohol, 2 N sodium hydroxide, and concentrated hydrochloric acid, but crystallized from glacial acetic acid and analyzed. Analysis proved this compound to be 2,5-diphenyl-2,5-diethyldiketopiperazine.

Analysis: Calculated for $C_{20}H_{22}N_2O_2$: C, 74.50; H, 6.84; N, 8.70.

Found: C, 74.13; H, 7.05; N, 8.54.

G. Attempted Preparation of α,α-Diphenyl-α-Phthalimidoacetic Acid

Phthalic anhydride (7.4 g.) was heated to a temperature of 160°. until a clear melt was obtained and α,α-diphenyl-α-aminoacetic acid (11.3 g.) was added in portions. It was necessary to raise the temperature to 200° before solution occurred and at this temperature a violent frothing started, this arousing the suspicion that decarboxylation was taking place. After 0.25 hr. at 200° the melt was cooled to 100°, treated with water and boiled therein. The reaction mass, which set to a solid, was isolated by filtration and crystallized once from butyl alcohol and twice from methanol and water. Analysis showed that the acid had actually decarboxylated to form the amine, which of course appeared as the phthaloyl derivative. M.p. 220°; literature m.p. 225° (59).

Analysis: Calculated for $C_{21}H_{15}NO_2$: C, 80.51; H, 4.49; N, 4.86.

Found: C, 79.85; H, 5.07; N, 4.69.

IV. PREPARATION OF ACID CHLORIDES AND AMIDES OF α-PHTHALIMIDOACETIC ACIDS

At the time when these reactions were first attempted difficulty was being experienced in purifying the phthalimido acids. When thionyl chloride was used to replace the hydroxyl group, it was found that unless the starting acid was pure, discoloration occurred and poor yields of amide were obtained. Since the classical method of forming acid chlorides of substituted amino acids is by the use of phosphorus pentachloride, this reagent was ultimately used almost exclusively. It gave much better results than did the thionyl chloride.

The amides were first formed in a solvent such as benzene by bubbling ammonia through a cold solution of the acid chloride. Precipitation of ammonium chloride caused so much clogging of the inlet tube and undesirable thickening of the reaction mixture that this method was abandoned in favor of adding a dioxane solution of acid chloride to a cold concentrated ammonium hydroxide solution.

A. Preparation of α-Methyl-α-Ethyl-α-Phthalimidoacetamide

The crystallized α-methyl-α-ethyl-α-phthalimidoacetic acid (10 g.) was suspended in thionyl chloride (30 ml.) and benzene (50 ml.) and heated at 50° for 2 hours. The solvent and excess thionyl chloride were removed by vacuum distillation and 50 ml. of fresh benzene was added. This soltuion was cooled in an ice bath and ammonia bubbled in until the solution was saturated. A white mass of amide and ammonium chloride was formed during the reaction and considerable heat was evolved. The precipitated material isolated by filtration, washed with water, and crystallized from

a mixture of methanol and water. Yield 7.2 g. (72%). M.p. 208°.

Analysis: Calculated for $C_{13}H_{14}N_2O_3$: C, 63.41; H, 5.69; N, 11.30.

Found: C, 63.27; H, 5.76; N, 11.25.

B. Preparation of α,α-Dimethyl-α-Phthalimidoacetamide

α,α-Dimethyl-α-phthalimidoacetic acid (11 g.) and phosphorus pentachloride (10 g.) were suspended in 100 ml. of dry benzene and refluxed for 1 hour during which time the acid went into solution. The solvent was removed by vacuum distillation from a steam bath, leaving a syrupy residue. Benzene (75 ml.) was added and the solution cooled in an ice bath while ammonia gas was bubbled through it until it was saturated. The white precipitate formed was filtered out, washed with alcohol, water, alcohol and finally with ether. It was then crystallized from glacial acetic acid. Yield 6.5 g. (65%). M.p. 258-60°.

Analysis: Calculated for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.17; N, 12.07.

Found: C, 62.43; H, 5.28; N, 11.91.

This experiment was repeated using the same amount of α,α-dimethyl-α-phthalimidoacetic acid as above with an excess of thionyl chloride instead of phosphorus pentachloride. Yield 5 g. (45%). M.p. 258-60°.

C. Preparation of α,α-Diethyl-α-Phthalimidoacetamide

α,αDiethyl-α-Phthalimidoacetic acid (13.1 g.) and phosphorus pentachloride (10.35 g.) were suspended in 100 ml. of dry benzene and refluxed on the steam bath for 0.5 hr. The benzene was removed by vacuum distillation and 75 ml. of fresh benzene added. Ammonia was bubbled through this cold solution until it was saturated and the white precipitate formed was removed by filtration and washed with water. The precipitate was crystallized from acetone and water.

M.p. 190°. Yield 8 g. (61%).

Analysis: Calculated for $C_{14}H_{16}N_2O_3$: C, 64.61; H, 6.15; N, 10.76.
Found: C, 64.63; H, 6.26; N, 10.88.

D. Attempted Preparation of α-Ethyl-α-n-Butyl-α-Phthalimidoacetamide

Since it was found to be impossible to crystallize the N-phthaloyl derivative of α-ethyl-α-n-butyl-α-aminoacetic acid, an attempt was made to prepare the amide without purifying the acid. The glassy reaction product from the fusion of phthalic anhydride and α-ethyl-α-n-butyl-α-aminoacetic acid (8 g.) was dissolved in dry dioxane, treated with phosphorus pentachloride, and heated on a steam bath for 0.5 hr. Most of the dioxane was removed by vacuum distillation and the residue poured slowly into an excess of cold concentrated ammonium hydroxide solution. A gummy solid which could not be crystallized was precipitated.

E. Attempted Preparation of α-Methyl-α-Pentyl-α-Phthalimidoacetamide

Because of the same difficulty in purifying the N-phthaloyl derivative of this acid, an attempt was made using the glassy reaction product from the fusion of phthalic anhydride and α-methyl-α-n-pentyl-α-aminoacetic acid. The procedure was the same as described in part D, and the amide was precipitated in the form of a gum which could not be purified.

F. Preparation of α-Methyl-α-Phenyl-α-Phthalimidoacetamide

A number of preparations were tried using the partially purified phthalimidoacetic acid as this acid was very difficult to crystallize. Results were poor and gave amides that could not be crystallized easily. The products from several runs were combined, yielding about 20 g., and attempts made to purify the material. Finally, about 0.5 g. of a crystalline substance was isolated. That

this material was impure was shown by the fact that it melted over a range of 15°, therefore this particular synthesis was temporarily abandoned as being impractical.

Later, when techniques had been developed further, this experiment was tried again. The reaction between phthalic anhydride and α-methyl-α-phenyl-α-aminoacetic acid, according to the general procedure, gave a crystalline acid that melted at 189°. (Previous m.p. 187°). This acid was not the expected phthalimido acid but was phthalic acid, m.p. 190-91°. as shown by subsequent formation of the amide. When the acid chloride was dripped into cold concentrated ammonium hydroxide, according to the general procedure, no amide precipitated. A white crystalline material was finally isolated by removing some of the ammonia from the solution by evaporation. These crystals were recrystallized from glacial acetic acid and water and melted at 222°. Literature m.p. 219-20° for phthalamide. (7).

G. Preparation of α-Methyl-α-Isobutyl-α-Phthalimidoacetamide

This amide was impossible to get pure until the crystalline phthalimidoacetic acid was used as starting material.

α-Methyl-α-isobutyl-α-phthalimidoacetic acid (6 g.) and phosphorus pentachloride (6 g.) were suspended in 75 ml. of benzene and treated from then on as in part F. The amide was crystallized from methanol and water. Yield 1 g. (16%). M.p. 279-80°.

Analysis: Calculated for $C_{15}H_{18}N_2O_3$: C, 65.69; H, 6.57; N, 10.21.

Found: C, 65.64; H, 6.98; N, 10.59.

V. PREPARATION OF α-BENZAMIDO ACIDS

The preparation of the benzoyl derivatives of amino acids was first attempted using benzoyl chloride with pyridine as a solvent.

When no reaction product could be isolated from these attempts, fusion with benzoic anhydride was tried. This too was found to give unsatisfactory yields and finally a modified Schotten-Baumen reaction was used according to the directions of Steiger (54), who claimed it a general method of benzoylating amino acids. This was found to work well and gave fairly good yields.

A. Attempted Preparation of α,α-Dimethyl-α-Benzamidoacetic Acid

α,α-Dimethyl-α- aminoacetic acid (10.4 g.) and benzoyl chloride (14 g.) were dissolved in 50 ml. of pyridine and refluxed for 3 minutes. The reaction mixture was poured into ice and water but no product was precipitated.

B. Preparation of α-Methyl-α-Ethyl-α-Benzamidoacetic Acid

α-Methyl-α-ethyl-α-aminoacetic acid (11.7 g.) and benzoyl chloride (14 g.) were dissolved in 100 ml. of pyridine. The solution was refluxed for 2 hours, cooled, and poured into ice water. A red oil which settled out soon solidified and was filtered out. It was dissolved in hot water and treated with Norite, filtered out, and allowed to cool. White crystals appeared which were isolated and dried. Yield 2 g. M.p. 190°. The method was discarded because of poor yield and low melting point. (See Table IV).

C. Attempted Preparation of α,α-Dimethyl-α-Benzamidoacetic Acid with Benzoic Anhydride

α,α-Dimethyl-α-aminoacetic acid (10.3 g.) and benzoic anhydride (22.6 g.) were heated to 170° on a metal bath and kept at that temperature for 20 minutes. The reaction mixture was cooled to 100° and 200 ml. of water added. The insoluble portion, a white powder, was filtered out and dried. It was purified by dissolving in saturated sodium bicarbonate solution, treating with Norite,

filtering, and finally precipitating with hydrochloric acid. The material was recrystallized from methanol and water but the product so obtained was not pure. Neutral Equivalent: Calc: 207. Found: 188.

D. General Procedure for the Preparation of α-Benzamido Acids

The procedure used for these preparations is the one outlined by Steiger (54).

The amino acid, usually 0.1 mole, was dissolved in 100 ml. of 1 N sodium hydroxide in a three-neck, round-bottom flask fitted with a good stirrer. The flask was immersed in an ice bath and the solution cooled to 0-5°. With rapid stirring 11.6 ml. of benzoyl chloride and 50 ml. of 2 N sodium hydroxide were dropped in at such a rate that the flow of sodium hydroxide was 4.3 times as fast as that of the benzoyl chloride. The total addition time was about 0.75 hr. Stirring was continued for 0.25-0.50 hr. after the addition was complete. The solution was then treated with Norite and filtered. To the ice-cold filtrate was added slowly with stirring about 20 ml. of 5 N hydrochloric acid. The benzoylated product precipitated and was filtered out. In some cases the acid came out as a gum which usually hardened on standing. If it showed a tendency to remain gummy, it was boiled with a large amount of water. This boiling with water was later added as part of the procedure as a method of separating the product from benzoic acid.

Table IV gives detailed information on the various benzoylated compounds prepared. Yields given are of recrystallized products.

TABLE IV

Preparation of α-Benzamidoacetic Acids

α,α-DISUBSTITUTED AMINOACETIC ACIDS	MOLES	YIELD, g.	%	M.P., °C	NEUTRAL EQUIVALENT CALC.	FOUND	LIT. M.P., °C
DIMETHYL	0.2	30	72	196	207	203	193-98 (9)
METHYLETHYL	0.1	12	54	196-97	221	227	
DIETHYL	0.1	17	72	211	235	232	
METHYLISOBUTYL	0.15	20	53	179-80	249	246	
METHYL-n-PENTYL	0.1	15	57	131	264	261	
ETHYL-n-BUTYL	0.1	10.5	40	125	263	257	
METHYLPHENYL	0.1	12	44	145-46	269	263	
ETHYLPHENYL	0.083	20	85	180-81	283	280	
PENTAMETHYLENE	0.2	8	13.5	190-91	247	244	190 (18)
[1]DIPHENYL	No Reaction						

1. Benzoylation of this compound was attempted twice with no useful results. This failure is undoubtedly due to the insolubility of the sodium salt of the acid.

VI. PREPARATION OF ACID CHLORIDES AND AMIDES OF α-BENZAMIDOACETIC ACIDS

These compounds were prepared by the same process as that used in the synthesis of the phthalimido acid chlorides and amides. Although thionyl chloride was used successfully once, it failed in two attempts and phosphorus pentachloride was used exclusively after these failures.

A. Attempted Preparation of α,α-Dimethyl-α-Benzamidoacetamide

α,α-Dimethyl-α-aminoacetic acid (8.3 g.) and purified thionyl chloride (15 ml.) were mixed with 50 ml. of dry ether. After refluxing on the steam bath for 0.5 hr., very little of the acid had gone into solution and the experiment was abandoned.

B. Attempted Preparation of α-Methyl-α-Isobutyl-α-benzamidoacetamide

α-Methyl-α-isobutyl-α-aminoacetic acid (5 g.) was suspended in 50 ml. of dry benzene and 15 ml. of thionyl chloride added. The reaction mixture was refluxed on a steam bath for 0.5 hr. and the benzene removed by vacuum distillation. Dioxane (25 ml.) was added to the residue and this solution dropped into cold concentrated ammonium hydroxide solution with good stirring. A brown solid was precipitated but it could not be purified.

This reaction was tried again with a change in solvents and reagent.

α-Methyl-α-isobutyl-α-aminoacetic acid (12 g.) and phosphorus pentachloride (12 g.) were suspended in dry ether and refluxed about one hour. The ether was removed by evaporation at room temperature and 50 ml. of dry acetone added to the residue. This solution was then dripped with stirring into cold concentrated ammonium hydroxide. A white mass of sugar-like product was isolated and recrystallized from methanol and water. The suspicion that there was considerable

unreacted acid present was confirmed by dissolving the material in ether and extracting three times with 2 N sodium hydroxide. Acidification of the alkaline solution precipitated the acid. The ether layer was allowed to evaporate and about 1 g. of a white crystalline compound was left. This was recrystallized from methanol and water, isolated by filtration and dried. M.p. 114°. Two subsequent recrystallizations raised the m.p. to 118°. Analysis of the compound proved that it was the unreacted acid.

Analysis: Calculated for $C_{14}H_{19}NO_3$: C, 67.50; H, 7.64; N, 5.63
Found: C, 68.63; H, 7.92; N, 5.36.

It is evident from these results that the formation of the acetyl chloride is sluggish and requires a higher temperature than the boiling point of ether. The same conclusion can be drawn for α,α-dimethyl-α-benzamidoacetyl chloride (Part A).

C. Preparation of α-Methyl-α-n-Pentyl-α-Benzamidoacetamide

α-Methyl-α-n-pentyl-α-aminoacetic acid (7.92 g.) was suspended in 100 ml. of dry benzene and 15 ml. of thionyl chloride added. The reaction mixture was refluxed on a steam bath 0.5 hr. and treated exactly as in paragraph one of part B. A white gum that precipitated from the ammonium hydroxide solution crystallized on standing. It was filtered out and crystallized from methanol and water. Yield 4.3 g. (54%). M.p. 125°.

Analysis: Calculated for $C_{15}H_{22}N_2O_2$: C, 68.70; H, 8.39; N, 10.68.
Found: C, 68.42; H. 8.44; N, 10.35.

D. General Procedure

The α-benzamido disubstituted acids were suspended in benzene, about 100 ml. per 0.1 mole of acid, and an excess of phosphorus pentachloride was added. The reaction mixture was refluxed on the

steam bath for 0.5 hr. and the benzene then removed by vacuum distillation, care being taken not to heat above 80°. About 25 ml. of dry dioxane was added to the residue and this solution dropped slowly into about 400 ml. of cold concentrated ammonium hydroxide solution. Most of the amides precipitated as solids; in one or two cases where a gum was formed it either was allowed to stand until solid (8 hours) or, if this did not work, was boiled with a large amount of water, which usually solidified the amide.

It is interesting to note that the majority of benzoylated amides melt lower than the corresponding acids, and one of them, the α-ethyl-α-n-butyl, remained an oil in spite of all attempts to crystallize it.

Table V gives the details of various preparations of the benzoylated amides.

TABLE V

Preparation of α-Benzamidoacetamides

α-BENZAMIDO-α,α DISUBSTITUTED AMINO ACID	g.	PCl_5, g.	YIELD, g.	%	M.P., °C
DIMETHYL	8.3	8.3	1.5	18	199-200
METHYLETHYL	12	12	5	41	158-59
DIETHYL	10	10	4	40	211-12
METHYLPHENYL	12	12	10	83	97-8
ETHYLPHENYL	6	6	2	33	99-100
PENTAMETHYLENE	7	7	5	71	187-88

AMIDE	ANALYSIS CALCULATED C %	H %	N %	FOUND C %	H %	N %
DIMETHYL	63.90	6.82	13.60	64.30	6.92	13.58
METHYLETHYL	64.45	7.27	12.72	65.71	7.40	12.44
DIETHYL	66.66	7.69	11.96	66.93	7.90	12.21
METHYLPHENYL	71.64	5.97	10.44	70.47	5.86	9.90
ETHYLPHENYL	72.34	6.34	9.92	70.81	6.81	9.54
PENTAMETHYLENE	68.29	7.31	11.38	66.15	7.76	11.12

VII. ATTEMPTED PREPARATION OF ACID UREIDES

Since many of the acid ureides of branched-chain aliphatic acids have hypnotic properties, it was hoped that some of the ureides of the amino acids could also be made. A number of attempted preparations were made, but no ureides could be isolated.

A. Attempted Preparation of α-Methyl-α-Ethyl-α-Phthalimidoacetic Acid Ureide

α-Methyl-α-ethyl-α-phthalimidoacetic acid (2.5 g.) was suspended in 50 ml. of dry benzene and 4 g. of phosphorus pentachloride added. The mixture was refluxed about 0.5 hr. and the benzene removed by vacuum distillation. Fresh benzene was added (50 ml.) and 0.8 g. of dry urea added to the benzene solution. The mixture was refluxed again for 0.5 hr. A small pool of liquid, found later to be water, formed in the bottom of the flask during this time. The benzene was decanted, reduced to a small volume, and cooled. A small amount of material crystallized, but it proved to be urea. No ureide could be isolated.

B. Attempted Preparation of α,α-Dimethyl-α-Phthalimidoacetic Acid Ureide

The method used was essentially that described by Stoughton (55) in the preparation of ureides of substituted acetic acids.

α,α-Dimethyl-α-phthalimidoacetic acid (18 g.) was suspended in 100 ml. of dry benzene and 14.5 g. of phosphorus pentachloride added. The mixture was refluxed for 0.5 hr. on the steam bath and the benzene removed by vacuum distillation. Fresh benzene (25 ml.) was added and this solution added dropwise to 5.46 g. of urea suspended in 100 ml. of dry benzene containing 2 drops of concentrated sulfuric acid. The mixture was refluxed for 4 hours. A small pool of liquid, which was formed in the bottom of the flask, solidified

on cooling. The benzene was decanted and evaporated by vacuum distillation to a small volume. A small amount of product was obtained which was crystallized from benzene; m.p. 154°.

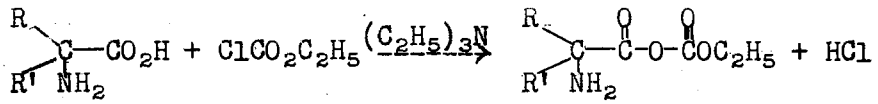
Analysis: Calculated for $C_{13}H_{13}N_3O_4$: C, 56.72; H. 4.72; N. 15.27

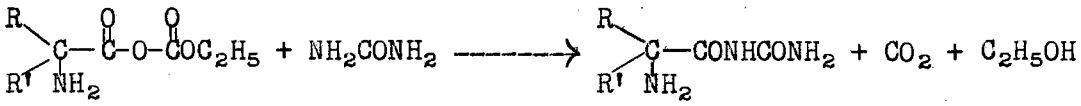
Found: C, 61.31; H, 6.35; N, 4.80.

The analysis showed that this product was probably impure α,α-dimethyl-α-phthalimidoacetic acid.

C. Attempted Preparation of α-Methyl-α-Isobutyl-α-Phthalimidoacetic Acid Ureide

An excellent method of peptide synthesis was described by Boissonas (16) and it was decided to try this method in the ureide synthesis. The postulated reaction is as follows:





α-Methyl-α-isobutyl-α-phthalimidoacetic acid (13.8 g.) was dissolved in 75 ml. of dioxane and to this was added 5 g. of triethylamine. Methyl chlorocarbonate (4.7 g.) was added and an immediate precipitate of amine hydrochloride appeared. The reaction mixture was kept at 0° during the addition and for 10 minutes thereafter. Urea (3.9 g.) was then added but after 10 minutes standing no evidence of reaction, i.e., evolution of carbon dioxide, could be noticed. The mixture was then cautiously heated in hot water. Evolution of carbon dioxide

took place immediately and the mixture separated into two layers. Careful working up of each layer produced nothing except unreacted urea, and three subsequent reactions of this type, with changes in solvents and temperature also showed failure of the urea to react. It was evident that urea is not a strong enough base to force the reaction to take place.

Another trial was made using pyridine as a catalyst. α-Methyl-α-isobutyl-α-phthalimidoacetyl chloride from 13.8 g. of the acid was prepared as previously described and dissolved in 50 ml. of benzene. Pyridine (25 ml.) was added, and then 4 g. of urea. The solution was refluxed 4 hours and the solvents were removed by vacuum distillation, leaving a gummy residue. All attempts to crystallize this product failed and the mixture was discarded.

D. Attempted Preparation of α,α-Dimethyl-α-Phthalimidoacetic Acid Ureide

This reaction was tried again because preparation of the pure amide at this time showed that the procedure for the acid chloride synthesis was satisfactory.

α,α-Dimethyl-α-phthalimidoacetyl chloride from 0.1 mole of acid was prepared and crystallized from a 50% mixture of ethyl ether and Skellysolve (60°-100°). The crystalline chloride was added to 100 ml. of pyridine and 8 g. of urea was also added. Upon the addition of the urea, heat was evolved. The solution was refluxed 1.25 hr. and the volume reduced to 30 ml. by vacuum distillation. The residue, when poured into ice water, deposited a small amount of reddish-brown precipitate but this could not be purified.

DISCUSSION

I. PREPARATION OF HYDANTOINS

The preparation of the hydantoins proceeded very well indeed. Little difficulty was met except with 5,5-diphenylhydantoin, which could not be made by the general procedure, but was easily made by the method of Sikdar and Ghosh (53). The yields of diisopropyl- and diisobutylhydantoins were very small and probably could be improved by a reaction time of 8-10 hours, instead of 4 or 5 as used.

II. HYDROLYSIS OF HYDANTOINS TO AMINO ACIDS

A number of methods of hydrolysis of hydantoins to amino acids have been used. Most of these, when done at ordinary pressure, required considerable time, no matter whether the hydrolyzing agent was 60% sulfuric acid, concentrated hydrochloric acid, or barium hydroxide. Other methods have been used but a survey of these methods showed little advantage to be gained by their use. Boyce and Robson (17) found that aqueous ammonium sulfide would hydrolyze hydantoins. It gave highest yields, however, when carried out at 180° and showed no advantage over barium hydroxide at 140°-160°. There is also a report in the patent literature by Moffett (45) of continuous hydrolysis of hydantoins in a tubular autoclave using sodium hydroxide as the hydrolyzing agent at a preferred temperature of 200°-300°. This method had the disadvantage that water-soluble aminoacids formed would be likely to be contaminated with sodium salts formed upon neutralization of the hydroxide. This method might,

however, have worked well in the case of such hydantoins as ethylphenyl and methylphenyl where the amino acid formed was not very soluble in water and could be freed of the salt by crystallization.

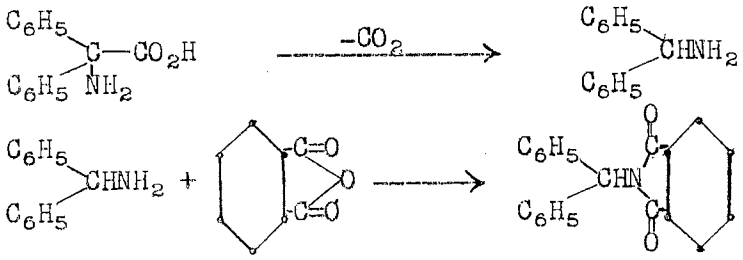
The failure of diphenylhydantoin to hydrolyze was undoubtedly due to its insolubility in barium hydroxide solution. When this hydantoin was added to the hot solution of barium hydroxide a voluminous precipitate of fine needles immediately appeared and the mixture had the consistency of a fluid paste. It was felt that at the temperature attainable at 60 lbs. pressure enough of the barium salt would dissolve to allow hydrolysis to take place, but this evidently was not the case. Hydrolysis of this hydantoin could probably best be carried out with sodium hydroxide, inasmuch as the sulfuric acid method proved to be long and tedious, although it did finally produce the amino acid in small yields.

III. PREPARATION OF THE α-PHTHALIMIDOACETIC ACIDS

The use of phthalic anhydride as a blocking group for amino acids is relatively new, being first reported by Kidd and King (33) in 1948. Most of the investigators who have used this method have reported very good yields and evidently have regarded it as an easy procedure. Billmann and Harting(20) even suggested it as a method of identification of amino acids, although they did admit that if care was not taken many of the phthaloylated amino acids tended to oil out of solution instead of crystallizing. In the case of the present series of branched-chain amino acids, considerable difficulty was encountered in crystallization of the phthaloyl derivatives. The groups which seemed to confer this property were, in order of increasing tendency to cause oiling out of solution, methylethyl, diethyl, methylisobutyl, methylphenyl, methyl-n-pentyl and ethyl-n-

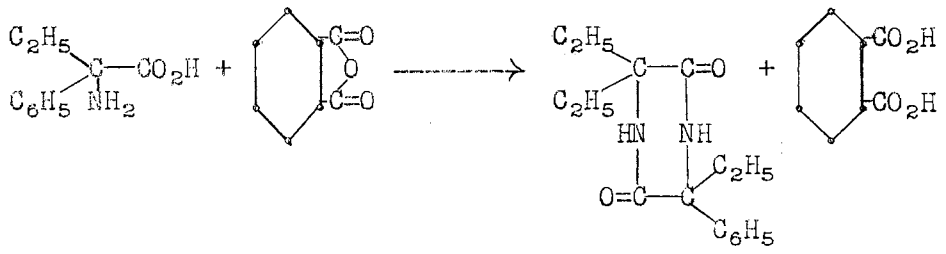
butyl.

Another difficulty encountered was the decarboxylation of the amino acids at the high temperatures used in the fusion with phthalic anhydride. The α,α-diphenyl-α-aminoacetic acid in particular was almost quantitavely decarboxylated to benzhydrylamine which then reacted with the phthalic anhydride.



Of all the amino acids studied, this one would have been predicted as being the easiest to decarboxylate because of the polar effect of the phenyl groups. Since it is unlikely that phthaloylation of the amino acid would enhance the tendency towards decarboxylation, the reaction probably proceeds in two steps, decarboxylation followed by phthaloylation of the amine.

The failure of the α-ethyl-α-phenyl-α-aminoacetic acid to react with phthalic anhydride is not surprising when steric hindrance is considered. It is, however, difficult to explain why this one compound should be so largely converted to the diketopiperazine when other compounds were not appreciably converted. The reaction postulated is:



It is very possible that some diketopiperazines were formed in other cases but if they were present in small amounts they would be unnoticed when the reaction mixture was worked up in the usual manner.

The failure of pentamethylene-α-aminoacetic acid to react may be due to several factors. In the first place, its solubility in melted phthalic anhydride is very low; even on strong heating (200°-210°) of a mixture of this acid and phthalic anhydride there was no evidence of solution. A second point is that the position of the amino group in this acid is different than in the others of the series because the bond angles of the tertiary carbon atom are strained owing to its being part of the cyclohexane ring. This might well result in steric hindrance, which is suggested also by the poor yield obtained when this amino acid was benzoylated. Steric hindrance is particularly plausible since it might stop the reaction of a large entering group such as phthaloyl and still allow the smaller benzoyl group to react.

IV. PREPARATION OF α-BENZAMIDOACETIC ACIDS

Little difficulty was encountered in these reactions. Steiger's method is to be highly recommended for the benzoylation of amino acids. The low yield of pentamethylene-α-benzamidoacetic acid mentioned above might be improved by a slower addition of the sodium hydroxide and benzoyl chloride.

The faliure of α,α-diphenyl-α-aminoacetic acid to react was probably, as stated, due to the insolubility of the sodium salt. It is entirely possible that this compound also requires a longer reaction time because of a sluggish amino group. The use of a larger volume of a more concentrated solution of sodium hydroxide might help this reaction to proceed.

It is interesting to note that the amino acids which gave uncrystallizable oils on phthaloylation showed the same tendency in benzoylation. The α-methyl-α-n-pentyl, α-methyl-α-phenyl, and α-methyl-α-isobutyl benzoylated amino acids all were first isolated as gums which later crystallized. The α-ethyl-α-n-butyl benzoylated amino acid was an oil which did not become solid even when subjected to the hot-water treatment previously mentioned. The oil had to be dissolved in methanol, treated with just a small amount of water, and allowed to stand for twenty-four hours before crystallization took place.

V. PREPARATION OF ACID CHLORIDES AND AMIDES

These compounds were prepared in the classical manner as stated and gave little difficulty in most cases. Some reactions which failed in ether succeeded in benzene, presumably because of the higher boiling point of the latter. It was noticed that if the substituted amino acid tended to be gummy or oily when the phthaloylated derivative was isolated, this tendency was enhanced in the amide. The amides of α-methyl-α-n-pentyl-α-benzamido- and α-methyl-α-isobutyl-α-benzamidoacetic acids had to be very carefully treated before they would crystallize. The α-ethyl-α-n-butyl-α-benzamidoacetic acid remained an oil in spite of all attempts to crystallize it. This tendency toward difficulty of crystallization was more apparent in the phthalimido derivatives than it was in the benzamido derivatives.

It must be admitted that the calculated and found values for carbon content do not agree well for four of the benzamidoacetamides: the methyl ethyl, methyl isobutyl, methyl phenyl, and ethyl phenyl derivatives. However, the purity of the benzamido acids used as reagents, as shown by neutral equivalents, and the satisfactory

agreement of calculated and found values for hydrogen and nitrogen in the compounds in question remove any real doubt of their identity or purity. The nitrogen has evidently made difficulty in the carbon determination.

VI. PREPARATION OF ACID UREIDES

The preparation of acid ureides of substituted acetic acids has been reported to be a rather sluggish reaction. For example, a benzene solution of the acid chloride and urea requires three to four hours refluxing for completion. This evidently is due to the fact that the acid chlorides are not particularly active and that urea is a weak base.

The acylaminoacetyl chlorides were evidently less reactive since long refluxing with urea gave no indication of reaction. Other methods of synthesis are available for trial if the corresponding amides which have been made prove to be active drugs.

The amides which have been prepared are being tested by the pharmacology division of Lederle Laboratories Inc., a division of American Cyanamide. A report of their findings will be published elsewhere. Until such testing shows some of the hoped-for hypnotic activity in these compounds, any further discussion of their structure in relation to physiological effect would be premature.

SUMMARY

A number of α-phthalimido and α-benzamido derivatives of α,α-disubstituted acetic acids and amides have been synthesized for use as possible hypnotics. Such compounds have a structural similarity found in many known and used hypnotics.

The phthaloylation of branched-chain α-amino acids was studied and attempts made without much success to modify the reaction as reported in the literature so as to fit this class of compounds. The phthaloylation reaction was found to proceed with difficulty when applied to the branched-chain α-amino acids. When this reaction was applied to some of the acids in this series oily products were formed which could not be purified by crystallization. α,α-Pentamethylene-α-aminoacetic would not react presumably because of steric hindrance. Benzoylation of the branched-chain α-aminoacetic acids was found to proceed normally and gave better yields than did the phthaloylation. Some of the substituents in the alpha position were found to impart a tendency to oiliness to the phthaloylated and benzoylated acids and amides. This was particularly true of the ethyl-n-butyl and methyl-n-pentyl groups.

All the amino acids prepared are known compounds with the exception of α-methyl-α-n-pentyl-α-aminoacetic acid, but only a few of the α-phthalimido- and α-benzamidoacetic acids are known. The new α,α-disubstituted-α-phthalimido acids are: diethyl, methylisobutyl, and methylphenyl. The new α,α-disubstituted-α-benzamidoacetic acids are: methylethyl, diethyl, methylisobutyl, methyl-n-pentyl, methylphenyl, ethyl-n-butyl, and ethylphenyl. The α-phthalimido and α-benzamidoacetic acid amides prepared are all new compounds

not reported previously in the literature.

Attempts were made to prepare the ureide analogs of this series. Methods tried which were reported as successful when applied to α,α-disubstituted acetic acids would not work when α,α-disubstituted amino acids were used.

The compounds prepared will be tested for hypnotic activity by the pharmacology division of Lederle Laboratories, Inc., a division of American Cyanamide.

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