NOTE ON THE ERLENMEYER AMINO-ACID SYNTHESIS

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CXV. NOTE ON THE ERLENMEYER AMINO-ACID SYNTHESIS.

By CHARLES ROBERT HARINGTON
and WILLIAM McCARTNEY.

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(*Received June 27th, 1927.*)

The series of reactions, constituting a general synthesis of α-amino-acids, devised by Erlenmeyer [1893] and since employed by many other workers, can be conducted, in most cases, with little trouble and excellent yields except at one stage. The condensation of an aromatic aldehyde with hippuric acid and the conversion of the resulting azlactone into the corresponding α-benzoylaminoacrylic acid proceed almost always in yields of 70 % or over. The ease with which the acrylic acid derivative may be reduced to the corresponding propionic acid varies considerably, however, in different cases. Moreover, even in those cases in which the ultimate yield obtainable is fairly high, the reaction is apt to be somewhat troublesome to carry out. In order to bring about this reduction, Erlenmeyer employed sodium amalgam, in which he has been followed by the majority of subsequent workers, although occasionally [cf. Barger and Ewins, 1917] sodium in alcohol has been used with success.

In the synthesis of thyroxine [Harington and Barger, 1927] where it was desired to utilise Erlenmeyer's method for the preparation of β-3 : 5-diiodo-4-[4′-(hydroxyphenoxy)phenyl]-α-aminopropionic acid, the use of any alkaline reducing agent was precluded by the presence of iodine atoms in the molecule. We attempted at first to surmount the difficulty by using hydrogen and palladium in neutral or acid solution, but our efforts in this direction were entirely without success. It then occurred to us that the desired end might be achieved by the use of hydriodic acid and red phosphorus, since we had previously obtained evidence that the iodine atoms, in the compounds with which we were working, were stable towards these reagents. This experiment was successful, and we were able, by boiling the α-benzoylamino-3 : 5-diiodo-4-(4′-methoxyphenoxy)cinnamic ester with hydriodic acid and red phosphorus, to obtain a 25 % yield of the desired amino-acid.

Later it became necessary, for further work, to prepare larger quantities of this amino-acid, and experiments were therefore undertaken with the object of improving the yield. Without going into the various unsuccessful variations that were tried, it may be said at once that we have succeeded in

improving the above-mentioned yield of 25 % to one of 82 % by the simple modification of substituting for the constant boiling hydriodic acid originally employed a mixture of equal parts of the latter and of acetic anhydride. This mixture is an excellent solvent, and, by its use, the reaction could be made to proceed smoothly and to yield a clean product which could be worked up without difficulty. It may further be remarked that the whole reaction, *i.e.* the reduction and the simultaneous hydrolytic removal of the benzoyl group, was complete after boiling for 1½ hours with the hydriodic acid-acetic anhydride mixture, so that the saving of time effected, as compared with Erlenmeyer's original method, even supposing the latter to have been applicable in this case, was very considerable.

The surprising success of this method in the case under discussion, and its great rapidity, led us to investigate its applicability to the synthesis of some other amino-acids. In particular were we interested to try to prepare in this way 3 : 4-dihydroxyphenylalanine, the synthesis of which by Erlenmeyer's method has been carried out by Funk [1911], but with unsatisfactory yields. Using vanillin as our starting point we were indeed able to prepare this amino-acid in yields which were satisfactory, whilst not being so good as those which we obtained in other cases. We have further applied the method to the synthesis of phenylalanine, tyrosine, and desiodothyroxine, the results in all cases being superior to those obtainable by Erlenmeyer's original method.

It will be noticed in the experimental part that we have employed in some instances the benzoylaminoacrylic acid and in others the ester; we have observed slight but definite differences in the yields of the different amino-acids obtainable from the acid and the ester; it appears to be a matter of experiment to determine, in any given case, which is the more suitable.

Experimental.

β-[3 : 5-Diiodo-4-(4′-hydroxyphenoxy)phenyl]-α-aminopropionic acid.

α-Benzoylamino-3 : 5-diiodo-4-(4′-methoxyphenoxy)cinnamic acid. The azlactone prepared by the condensation of 3 : 5-diiodo-4-(4′-methoxyphenoxy)-benzaldehyde with hippuric acid [cf. Harington and Barger, 1927] was dropped into 100 parts of a boiling solution of 1 % sodium hydroxide in 70 % alcohol; the substance passed rapidly into solution and the reaction was complete after boiling for 5 minutes. The hot solution was acidified with hydrochloric acid, whereupon the acid began immediately to crystallise; after standing a few hours in the ice-chest, the acid was filtered off; the first crop so obtained amounted to 60 % of the theoretical yield; a further 30 % was obtained by heating the filtrate to boiling, diluting with an equal volume of hot water, and again cooling; the total yield was therefore almost quantitative. The acid was moderately soluble in hot alcohol, and sparingly soluble in water; on recrystallisation from 90 % alcohol it formed colourless needles, M.P. 239–241° (decomp.).

Analysis. 18·3 mg. gave 0·38 mg. N (micro-Kjeldahl).
Calculated for $C_{23}H_{17}O_5NI_2$ 2·2 % N; found 2·1 % N.

β-[3 : 5-Diiodo-4-(4′-hydroxyphenoxy)phenyl]-α-aminopropionic acid. 5 g. of the foregoing acid were boiled for 1¼ hours under a reflux condenser with a mixture of 25 cc. hydriodic acid (Sp. Gr. 1·7) and 25 cc. acetic anhydride together with 3 g. red phosphorus. The solution was filtered hot through asbestos into a Claisen flask, the phosphorus being washed with acetic acid. The filtrate was evaporated to dryness *in vacuo*; some water was added and the evaporation repeated. The residue was dissolved in about 40 cc. boiling water; the solution was cooled under the tap, the flask being well shaken, and the precipitate, consisting of the hydriodide of the amino-acid mixed with benzoic acid, was filtered off and washed thoroughly with ether; the aqueous portion of the filtrate was extracted twice with ether, and warmed to remove dissolved ether. The first crop of hydriodide was added to the boiling aqueous solution, a little hydrochloric acid being added, if necessary, to obtain complete solution, and the free amino-acid precipitated by the cautious addition of ammonia; 3·35 g. of the pure amino-acid, or 82 % of the theoretical amount, were obtained.

Phenylalanine.

10 g. of α-benzoylaminocinnamic acid, obtained by the method of Erlenmeyer [1893], were boiled under a reflux condenser with 100 cc. of the hydriodic acid-acetic anhydride mixture and 10 g. of red phosphorus for 1½ hours; the solution was filtered and evaporated to dryness *in vacuo*; water was added and the evaporation repeated; the residue was shaken up with water and ether; the aqueous layer was separated, and, after a second extraction with ether, was heated to boiling and carefully neutralised with ammonia; on cooling there separated, in colourless plates, analytically pure phenylalanine. The yield was 88 % of the theory.

Analysis. 8·8 mg. gave 0·74 mg. N (micro-Kjeldahl).
14·19 mg. gave 2·0 cc. moist N_2 at 19° and 766 mm. (Van Slyke).

	Total N	NH_2-N
Calculated	8·5 %	8·5 %
Found	8·4	8·1

Tyrosine.

In a precisely similar manner there was obtained from ethyl α-benzoylamino-*p*-methoxycinnamate a 60 % yield of tyrosine.

Analysis. 15·5 mg. gave 1·2 mg. N (micro-Kjeldahl).
14·11 mg. gave 1·83 cc. moist N_2 at 19° and 766 mm. (Van Slyke).

	Total N	NH_2-N
Calculated	7·7 %	7·7 %
Found	7·7	7·4

3 : 4-*Dihydroxyphenylalanine.*

Azlactone from vanillin and hippuric acid. An intimate mixture of vanillin (15·2 g.), hippuric acid (17·9 g.), and freshly fused sodium acetate (15 g.) was treated with 30 cc. acetic anhydride and heated on the water-bath for

15 minutes. The reaction product was ground up with water, filtered, and the precipitate well washed. The crude product was crystallised from glacial acetic acid, and formed yellow needles, M.P. 189°. The yield was 75 %.

Analysis. 15·2 mg. gave 0·646 mg. N (micro-Kjeldahl).

Calculated for $C_{19}H_{15}O_5N$ 4·15 % N; found 4·25 % N.

Ethyl α-benzoylamino-3-methoxy-4-hydroxycinnamate. 10 g. of the above azlactone were dissolved in 100 cc. alcohol to which were added 10 cc. concentrated sulphuric acid, and the solution was boiled under a reflux condenser for 15–20 minutes; the greater part of the alcohol was then distilled off under reduced pressure, and the residue was poured into a dish and allowed to stand. After some days crystallisation began and, at the end of a week, was complete; the ester was filtered off and recrystallised from dilute alcohol. It formed colourless prisms, M.P. 128–129°. The yield was 65 % of the theoretical amount.

Analysis. 0·0862 g. gave 0·2109 g. CO_2; 0·0450 g. H_2O.

15·7 mg. gave 0·63 mg. N (micro-Kjeldahl).

	C	H	N
Calculated for $C_{19}H_{19}O_5N$	66·8 %	5·6 %	4·1 %
Found	66·7	5·8	4·0

It will be noted that the analysis indicates that the acetyl group, which was present in the azlactone, was split off in the process of preparation of the ester.

3 : 4-*Dihydroxyphenylalanine.* 5 g. of the ester were boiled for 1½ hours with 25 cc. hydriodic acid, 25 cc. acetic anhydride and 5 g. red phosphorus, in an atmosphere of hydrogen; the solution was filtered and evaporated to dryness under diminished pressure, hydrogen being led into the capillary tube of the Claisen flask; the aqueous solution of the residue, after removal of the benzoic acid by ether extraction, was neutralised with ammonia and evaporated to dryness under the same precautions to avoid access of air; the product was dissolved in a little water and the solution treated with a large excess of alcohol which precipitated the greater part of the pigment; the latter was rapidly filtered off and the filtrate once more taken to dryness. The residue was dissolved in hot water containing a little sulphur dioxide, the solution was boiled with charcoal and filtered, and the filtrate allowed to crystallise in a vacuum desiccator. In this way the amino-acid was obtained almost colourless and in well-formed crystals. The yield was 50 % of the theoretical. The substance melted at 269° (decomp.) and agreed in its properties with the product described by Funk [1911].

Analysis. 14·3 mg. gave 0·98 mg. N (micro-Kjeldahl).

21·3 mg. gave 2·55 cc. moist N_2 at 17° and 760 mm. (Van Slyke).

	Total N	NH_2-N
Calculated	7·1 %	7·1 %
Found	6·9	6·9

Desiodothyroxine.

The azlactone was prepared in the usual manner from 4-(4′-methoxyphenoxy)benzaldehyde [cf. Harington, 1926] and hippuric acid; there was obtained 70 % of the theoretical amount of a substance which, after crystallisation from glacial acetic acid, formed yellow needles, M.P. 141°.

Analysis. 16·0 mg. gave 0·575 mg. N (micro-Kjeldahl).

Calculated for $C_{23}H_{17}O_4N$ 3·8 % N; found 3·6 % N.

The above azlactone was converted into the acid by boiling for 5 minutes with 100 parts of 1 % sodium hydroxide in 45 % alcohol; the acid, on recrystallisation from dilute alcohol, formed colourless branched needles, M.P. 192·5°. The yield was 95 % of the theory.

Analysis. 15·8 mg. gave 0·554 mg. N (micro-Kjeldahl).

Calculated for $C_{23}H_{19}O_5N$ 3·6 % N; found 3·5 % N.

The preceding compound was converted into the amino-acid in a precisely similar manner to that described for tyrosine and phenylalanine. The yield of amino-acid was 61 % of the theoretical. The product melted at 253–254° (decomp.), and gave a hydrochloride with M.P. 239–240°; it was therefore in all respects identical with the substance previously prepared by Harington [1926].

Analysis. 19·9 mg. gave 1·02 mg. N (micro-Kjeldahl).

26·8 mg. gave 2·22 cc. moist N_2 at 17° and 760 mm. (Van Slyke).

	Total N	NH_2-N
Calculated for $C_{15}H_{15}O_4N$	5·1 %	5·1 %
Found	5·1	4·9

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Funk (1911). *J. Chem. Soc.* **99**, 554.
Harington (1926). *Biochem. J.* **20**, 300.
Harington and Barger (1927). *Biochem. J.* **21**, 169.

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THE SYNTHESIS OF AN ISOMER OF THYROXINE

and of

SOME RELATED COMPOUNDS

by

William McCartney,

A.I.C.

Thesis presented for the Degree of Ph.D.

University of Edinburgh.

September 1928.

CONTENTS

THE SYNTHESIS OF AN ISOMER OF THYROXINE AND OF SOME RELATED COMPOUNDS.

I. INTRODUCTION

As soon as the structure of thyroxine had been elucidated and had been established by synthesis[1, 2] it became of interest to attempt to prepare analogous and isomeric substances. The problems encountered in the synthesis of thyroxine itself had indicated some lines along which progress in the preparation of such compounds might be made, as well as some of the difficulties which would have to be surmounted.

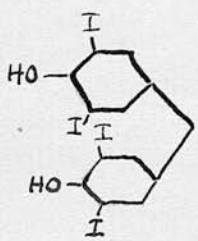
It is to be expected, of course, that some at least of the isomers of thyroxine will have important physiological properties. From the physiological as from the chemical point of view the most interesting of such substances may be supposed to be those in which the distribution of the iodine atoms is different from what it is in the thyroxine molecule, but in which also the constitution of that molecule is otherwise unaltered. For example/

For example, in view of the (unpublished) observation that, as regards physiological activity, the 3:5-di-iodo derivative of desiodothyroxine is comparable rather with thyroxine itself than with 3:5-di-iodotyrosine, the investigation of the physiological properties of:

β- [3:5-di-iodo-4-(2':6'-di-iodo-4'-hydroxyphenoxy)-phenyl] -α-aminopropionic acid

in which the four iodine atoms are grouped symmetrically round the phenyl-ether linkage, might yield interesting results.

The number of possible compounds formed by replacing by iodine four hydrogen atoms of the benzene rings of desiodothyroxine is considerable. But there was evidence that serious obstacles might be met in endeavouring to prepare even one of them. This being so, it was decided to attempt, in the first place, to synthesise an isomer (A) having the structure:

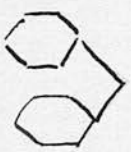
(A.)  $CH.CH(NH_2).COOH.$

"Isothyroxine"

ββ- [Di-(3:5-diiodo-4-hydroxyphenyl)] -α-amino propionic acid, i.e. a compound isomeric with thyroxine/

thyroxine but in which the two benzene rings are linked through carbon instead of, as in thyroxine itself, through oxygen. Such a substance would be expected to result from the direct iodination, in one operation, of the corresponding desiodo compound. Desiodothyroxine, on the other hand, cannot be converted into thyroxine by direct introduction of iodine.

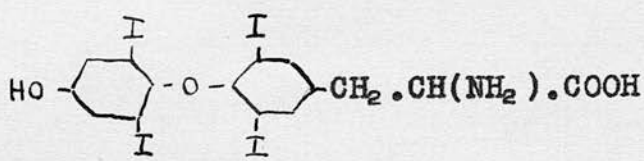
At the same time the preparation of the compound (B), similar to "isothyroxine" but without the hydroxyl groups and iodine atoms was undertaken.

(B)  $CH.CH(NH_2).COOH$

Diphenylalanine

ββ-Diphenyl-α-aminopropionic Acid

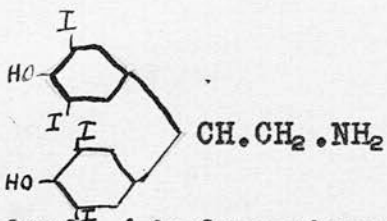
Thereafter it was hoped that at least some advance might be made in the preparation of the thyroxine isomer which was mentioned above, i.e. the one in which the iodine atoms occupy the four positions grouped round the central oxygen atom. This isomer, (C), would have the structure:

(C) 

β- [3:5-diiodo-4-(2':6'-diiodo-4'-hydroxyphenoxy)-phenyl] -α-amino-propionic acid.

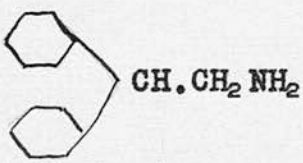
It/

It was intended in addition to prepare, if possible, the amines corresponding to the acids (A) and (B) as well as that corresponding to the non-iodinated acid from which (A) is derived. These amines would be formulated thus:

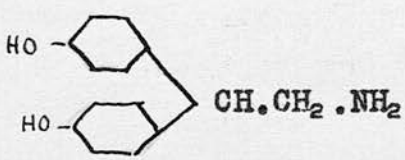


ββ- [Di-(3:5-diiodo-4-hydroxyphenyl)] -ethylamine

"Isothyroxamine"



ββ-Diphenylethylamine

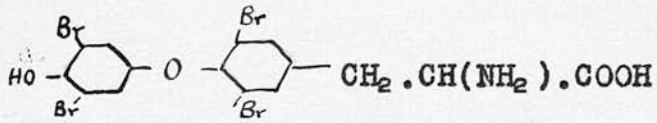


ββ- [Di-(4-hydroxyphenyl)] -ethylamine

"Isodesiodothyroxamine"

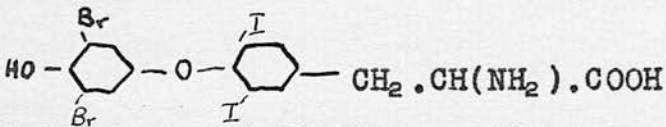
Finally the question presented itself as to whether the substances analogous to thyroxine, but containing bromine instead of iodine, might be synthesised although it was considered probable that such bromine compounds would not have any physiological/

physiological advantages over thyroxine. The synthesis of "tetrabromothyroxine":



β- [3:5-dibromo-4-(3':5'dibromo-4'-hydroxyphenoxy)-phenyl]-α-aminopropionic acid

would proceed, it was thought, along the same lines as that of thyroxine itself, starting with 3:5-dibromo-4-iodonitrobenzene and finishing with a bromination instead of an iodination. But this synthesis was not undertaken, since, from a private communication, it was learned that it had already been carried out in another laboratory. "Dibromothyroxine":



β- [3:5-diodo-4-(3':5'-dibromo-4'-hydroxyphenoxy-phenyl] -α-aminopropionic acid

was very simply obtained, however, by bromination of the precursor of thyroxine —

β- [3:5-diiodo-4-(4'-hydroxyphenoxy)-phenyl] -α-amino propionic acid.

II. Description of the Syntheses and Theoretical Discussion.

General.

At an early stage in the synthesis of thyroxine a phenyl ether condensation had to be carried out and, as will be emphasised later, it was essential that one of the compounds which took part in the condensation should contain iodine atoms in the desired positions. Because of this, certain difficulties, some of which are indicated in the original paper on the subject (2), arose. But, further, the presence of iodine in the phenyl ether, when it was obtained, caused additional difficulties due to the physical properties of the various substances which formed the later steps in the synthesis, to the fact that the iodine atoms would readily be displaced if certain desired reactions were carried out and to other causes.

Isothyroxine is not a phenyl ether derivative, and, as was pointed out in the introduction (see Page 3) it was expected that all its four iodine atoms could be introduced in one operation at the conclusion of the synthesis. Hence none of the difficulties/

difficulties outlined in the preceeding paragraph were likely to arise. In addition, it was fairly certain that any method of synthesising isodesiodothyroxine could equally well be used for the synthesis of diphenylalanine and, in fact, this proved to be so. Those obstacles which were encountered in the synthesis of isothyroxine were of a different kind and are described below. (See pages 8-13). In the case of the isomer C, however, the formidable nature of the difficulties which had to be faced in the thyroxine synthesis, again became significant, and, although the lessons taught by the work involved in that synthesis were very valuable, it was not found possible to proceed very far in the direction of success. The description, given below, (see page 16) of the attempts to prepare isomer C shows to some extent what problems are here involved. Perhaps some procedure very different from those adopted, or it may be some as yet untried modification of one of the methods employed might lead to a solution. But it seems probable that whatever plan may be adopted the route to be followed will be a lengthy and an arduous one.

(A) /

(A) Synthesis of ββ-Di-[(3:5-diiodo-4-hydroxyphenyl)]-α-aminopropionic Acid.

$(HO.C_6H_2I_2)_2CH.CH(NH_2).COOH$

The starting point in the preparation of this compound was the appropriate methoxyphenyl derivative of methyl chloride. This substance was chosen after one or two unsuccessful attempts had been made to prepare a suitable halogen derivative of diphenylmethane. Some of the methods given in the literature for preparing such derivatives were found, in the present instance, to be rather unsatisfactory if not altogether unsuitable.

Di-(4-methoxyphenyl)-methyl chloride was synthesised a few years ago by Straus and Dützmann, (3) and can be obtained in good yield from the product of reduction of the ketone which is formed when anisole acts on 4-methoxybenzoyl chloride in the presence of aluminium chloride. It was expected that the di-(4-methoxyphenyl)-methyl chloride would react with ethyl potassio-malonate in the usual manner giving ethyl di-(4-methoxyphenyl)-methylmalonate; it was then intended to convert the corresponding malonic acid by Fischer's method, i.e. by bromination, decarboxylation and treatment with/

with ammonia, into ββ- [di-(4-methoxyphenyl)] -α-aminopropionic acid. From this latter substance, by demethylation and subsequent iodination the compound desired would result. According to this plan, then, the synthesis would proceed in the manner summarised in the following scheme:

$CH_3O.C_6H_4.COCl \rightarrow (CH_3O.C_6H_4)_2.CO \rightarrow (CH_3O.C_6H_4)_2.CHOH \rightarrow$
$(CH_3O.C_6H_4)_2.CHCl \rightarrow (CH_3O.C_6H_4)_2.CH.CH.(COOC_2H_5)_2 \rightarrow$
$(CH_3O.C_6H_4)_2.CH.CH.(COOH)_2 \rightarrow (CH_3O.C_6H_4)_2.CH.CBr.(COOH)_2 \rightarrow$
$(CH_3O.C_6H_4)_2.CH.CHBr.COOH \rightarrow (CH_3O.C_6H_4)_2.CH.CH.(NH_2).COOH \rightarrow$
$(HO.C_6H_4)_2.CH.CH(NH_2).COOH \rightarrow (HO.C_6H_2I_2)_2.CH.CH(NH_2).COOH$

When an alcoholic solution of di-(4-methoxyphenyl)-methyl chloride was treated with a solution of ethyl potassio-malonate in the same solvent a reaction took place and potassium chloride separated. But the reaction did not proceed in the anticipated direction. The product was poured into water and extracted with ether; the ethereal solution was dried, the ether was removed by distillation and the residue, an oil, was distilled under reduced pressure. The portion of the distillate which did not consist of ethyl malonate, however, was apparently not an ester at all. (It could not be hydrolysed even on prolonged boiling with/

with a 10 per cent. solution of potassium hydroxide in amyl alcohol). It was found later that when the reaction between di-(4-methoxyphenyl)-methyl chloride and ethyl potassio-malonate is brought about in the absence of alcohol (dry xylene being used as a solvent for the methyl chloride and the potassio-malonate being in suspension) the expected product — the ethyl ester of di-(4-methoxyphenyl)-methylmalonic acid — is obtained. This was proved by comparing the melting point of the product with that of the compound formed from 4-methoxyphenyl magnesium iodide and the ethyl ester of 4-methoxybenzilidenemalonic acid (see below, page 13) and also by the determination of a mixed melting point.

Straus and Dützmann (3) had pointed out that the halogen atom of compounds of the di-(4-methoxyphenyl)-methyl chloride type is, as they say, 'ionogenically' bound, that it reacts readily and reversibly with hydroxyl, methoxyl etc. in the following manner:

$$R_2CHCl + HOR' \rightleftharpoons R_2CHOR' + HCl$$

and that altogether, such compounds like the halogen compounds of triphenyl-methyl, may exhibit, to some extent, the properties of electrolytes. They determined/

determined the dissociation constant of di-(4-methoxyphenyl)-methyl chloride dissolved in sulphur dioxide and found it to be $K = 0.84 \times 10^{-4}$. The supposition had been made in the present instance, however, that the reaction between the potassium of the ester and the chlorine of the methyl chloride would have proceeded in preference to that between that chlorine and the hydrogen of the alcoholic hydroxyl group, i.e. that the reaction:

$$R_2CHCl + KCH.(COOC_2H_5)_2 = R_2CH.CH.(COOC_2H_5)_2 + KCl$$

would take place rather than the reactions:

$$R_2CHCl + HOC_2H_5 = R_2CH.O.C_2H_5 + HCl$$

$$HCl + KCH.(COOC_2H_5)_2 = KCl + CH_2.(COOC_2H_5)_2$$

Quite recently Ward (4), investigating the bivalency of carbon, found that diphenylmethyl chloride reacts even at a temperature so low as 25° with ethyl alcohol and with aqueous alcohol either alone or in the presence of sodium hydroxide to give a mixture of diphenylmethyl ethyl ether and benzohydrol. He also found that — at least at the temperatures of 25° and 35° — the sodium hydroxide (or, also, sodium ethoxide) plays no direct part in the displacement of the halogen.

It may, therefore, be assumed that in the case of di-(4-methoxyphenyl)-methyl chloride, one or both of the following reactions had taken place:

(1) /

(1) $(CH_3O.C_6H_4)_2CHCl + HOC_2H_5 = HCl + (CH_3O.C_6H_4)_2CH.O.C_2H_5$

(2) $(CH_3O.C_6H_4)_2CHCl + C_2H_5OH = C_2H_5Cl + (CH_3O.C_6H_4)_2.CHOH$

The examination of the product of the reaction between ethyl potassio-malonate and di-(4-methoxyphenyl)-methyl chloride in the presence of alcohol was not pursued further. No di-(4-methoxyphenyl)-carbinol was detected in it, but a very small amount of a colourless crystalline compound melting at 142° was deposited from the high-boiling portion of the distilled material. It is possible that this substance was di-(4-methoxyphenyl)-methyl ethyl ether.

It was now decided to adopt another method of procedure. Kohler[5] showed many years ago that unsaturated derivatives of ethyl malonate would react with magnesium aryl halides and that the products of reaction could readily be decomposed so as to give saturated substances, esters of substituted malonic acids. From ethyl 4-methoxybenzilidene-malonate and 4-methoxyphenyl magnesium iodide, then, it would be possible to obtain a compound which would yield the ethyl ester of di-(4-methoxyphenyl)-methylmalonic acid on decomposition in the manner indicated below. Thereafter the synthesis would/

would proceed as in the first scheme outlined above (see page 9):

(1) $CH_3O.C_6H_4.CHO + H_2C.(COOC_2H_5)_2 = H_2O +$
$CH_3O.C_6H_4.CH:C(COOC_2H_5)_2$

(2) $CH_3O.C_6H_4.CH:C(COOC_2H_5)_2 + CH_3O.C_6H_4MgI =$

$$(CH_3O.C_6H_4)_2CH.C\begin{matrix}COOC_2H_5\\ \diagup\\ C\\ \diagdown\\ OMgI\end{matrix}\quad\begin{matrix}\\ \diagdown\\ OC_2H_5\\ \\ \\ \end{matrix}$$

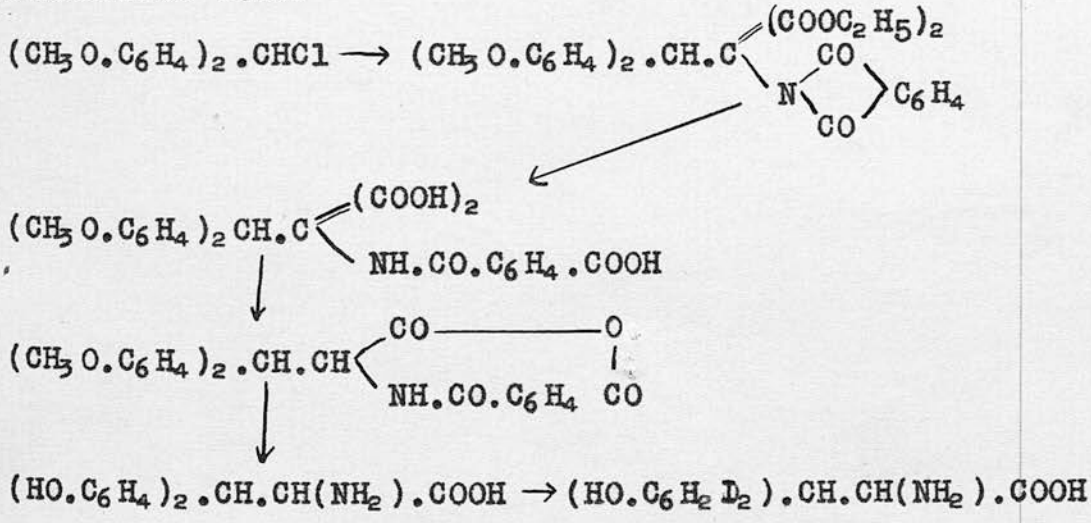
(3) $(CH_3O.C_6H_4)_2\ CH.C\begin{matrix}COOC_2H_5\\ \diagup\\ C\\ \diagdown\\ OMgI\end{matrix}\quad\begin{matrix}\\ \diagdown\\ OC_2H_5\\ \\ \\ \end{matrix}\ + H_2O\ =$

$(CH_3O.C_6H_4)_2CH.CH.(COOC_2H_5)_2 + MgIOH$

Ethyl di-(4-methoxyphenyl)-methylmalonate was prepared in this way without any serious difficulty and the yield was satisfactory. But preliminary experiments with small quantities of material showed that the bromination and subsequent decarboxylation of the malonic acid did not proceed smoothly. Moreover, the replacement of the bromine of compounds of this type by the amino group is sometimes very troublesome. In these circumstances a third method, which eventually proved successful was adopted.

This/

This was the phthalimide method which was originated by Gabriel[6] and extensively used by Sørensen[7] particularly for the synthesis of diamino acids. Di-(4-methoxyphenyl)-methyl chloride was caused to react with ethyl potassio-phthalimino-malonate and the ester so formed was hydrolysed with potassium hydroxide. The product of hydrolysis, a phthalamino malonic acid, was converted by heating into the anhydride of the corresponding phthalamino-propionic acid, and when this anhydride was boiled with a mixture of hydriodic acid and acetic anhydride[8] the desired amino acid was obtained. It was converted into isothyroxine by direct iodination. The series of reactions involved in this procedure is summarised thus:



In preparing the amine of the non-iodinated acid the method first used was that developed by Johnson and Daschavsky[9] and by Abderhalden and Gebelein[10]

In/

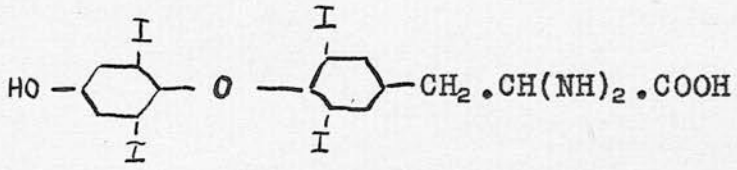
In this method the amino acid is mixed with diphenylamine and heated to about 230°. Later, it was found that better results could be obtained in this case by heating the amino acid alone. From the amine thus produced the corresponding tetraiodoamine was obtained by direct iodination.

(B) Synthesis of ββ-Diphenyl-α-aminopropionic Acid.

$(C_6H_5)_2.CH.CH(NH_2).COOH$

This acid was synthesised in precisely the same manner as that adopted for the preparation of isodesiodothyroxine, starting from diphenylmethyl bromide. The amine corresponding to the acid was prepared by the method of Johnson and Daschavsky[9] as modified by Abderhalden and Gebelein[10]. When diphenylalanine was heated alone a crystalline distillate, sparingly soluble in alcohol, was obtained; but this substance was not diphenylethylamine.

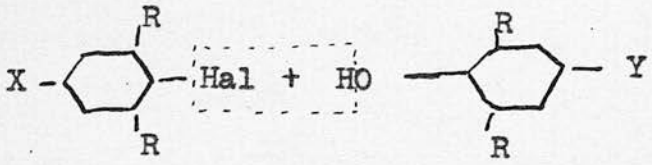
(C) Attempted Synthesis of β- [3:5-Diiodo-4-(2':6'-diiodo-4'-hydroxyphenoxy)-phenyl] -α-amino propionic Acid.



In the synthesis of thyroxine it had been found (2) that p-hydroxy-phenyl ethers (including desiodo-thyroxine itself) could readily be caused to take up two atoms of iodine in the 3':5' positions. But no method could be found for the introduction of more than two such atoms. The iodine atoms (or other replaceable groups) in the 3:5 positions had therefore to be introduced before the phenyl ether synthesis was carried out. It was evident that in the case of the isomeric substance now in view these considerations would apply with even greater force since the introduction of the iodine atoms into the positions 2':6' could not be accomplished directly. This being so, it was obviously desirable to have all four iodine atoms introduced before the phenyl ether synthesis was undertaken. Iodine atoms (or replaceable groups) then, had, if possible, to be present in the ortho positions both to the halogen and to the phenolic group which were

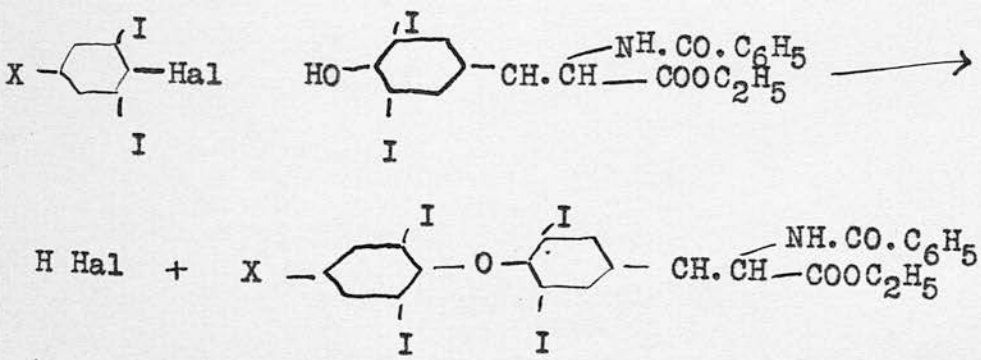
to/

to take part in the phenyl ether condensation, thus



where R represents iodine or atoms or groups replaceable by iodine and X, Y, represent -OH and —CH.CH.(NH_2).COOH or atoms or groups which could be replaced respectively by —OH and —CH.CH.(NH_2).COOH.

If such a synthesis could be carried out at all then the most attractive method would be by combination of the 3:5-diiodo-N-benzoyl derivative of tyrosine with a derivative of benzene containing the two iodine atoms in the desired positions:

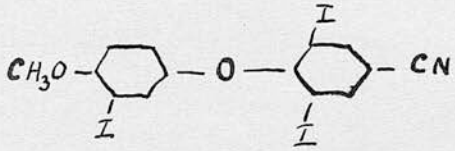


It would only be necessary after that to replace X by —OH and to remove the N-benzoyl and ethyl groups. When a solution of the ethyl ester of 3:5-diiodo-N-benzoyl tyrosine and 3:4:5-triiodonitrobenzene in methyl ethyl ketone was boiled with dry potassium carbonate for over twelve hours, however, it was found/

found that the desired condensation had not taken place and most of the triiodonitrobenzene was recovered unchanged. Nor could the result be achieved by heating at the boiling point for some hours a solution of the triiodonitrobenzene in dry xylene with the potassium salt of the tyrosine ester.

Equally unsuccessful were attempts to condense, in these ways, 3:4:5-triiodonitrobenzene or 3:4:5-triiodotoluene with the ethyl ester of 3:5-diiodo-4-hydroxybenzoic acid (or the potassium salt of this ester).

In a recent patent (11) the claim is made that iodine-substituted diphenyl ether derivatives can be obtained by heating together in sealed vessels at high temperatures iodine-substituted phenol ethers and metallic salts of iodine-substituted hydroxybenzonitriles in the presence of copper as a catalyst, or by treating similarly iodine-substituted mono ethers of dihydric phenols with iodine-substituted benzonitriles or with iodine-substituted nitrobenzenes. Thus 2:4-diiodoanisole is said to condense with the potassium salt of 3:5-diiodo-4-hydroxybenzonitrile in the presence of copper at 220°-230° to give 4-(3'-iodo-4'-methoxyphenoxy)-3:5-diiodobenzonitrile:



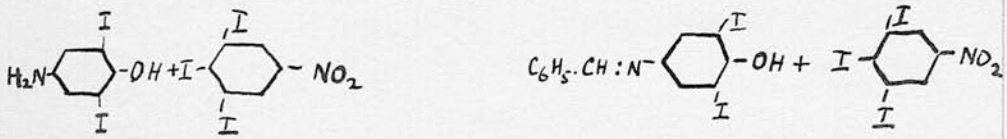
and/

and the same compound results when the potassium salt of the o-iodo monomethyl ether of hydroquinone is similarly treated with 3:4:5-triiodo-benzonitrile. Whether these condensations occur or not it was found that when 3:4:5-triiodonitrobenzene and the potassium salt of ethyl 3:5-diiodo-4-hydroxybenzoate were treated in this manner very extensive decomposition and iodine liberation occurred, the product being a charred mass from which nothing could be isolated.

After a very considerable number of unsuccessful attempts had been made to bring about condensation between pairs of compounds each of which contained two iodine atoms in the appropriate positions it became evident that the ortho-substituted iodine atoms conferred such acidity on the phenolic hydroxyl group that the splitting off of the metal halide would not take place. In these circumstances it seemed that there were three ways in which the difficulty might be overcome: (1) less acidic diiodo phenols might be used; (2) a metal which forms a feebly basic oxide might be substituted for the potassium hitherto used to promote condensation; (3) a non-iodinated phenol might be employed in the hope that the iodine atoms might be introduced at a later stage of the synthesis.

All three possibilities were investigated. For example/

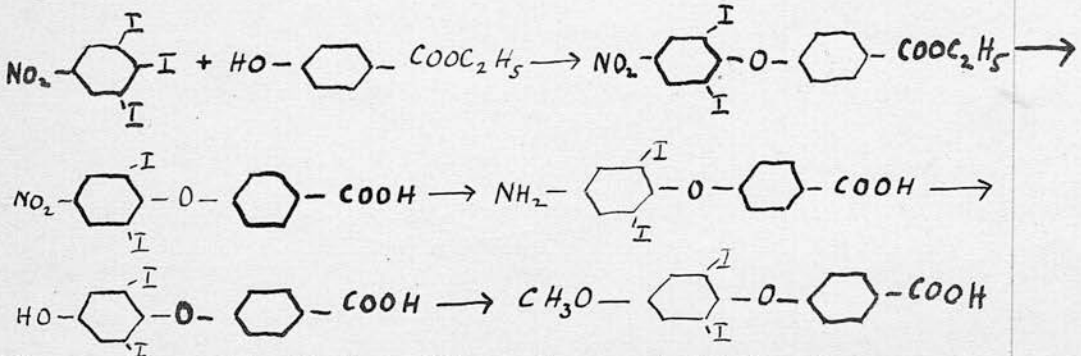
example: (1) 2:6-Diiodo-4-aminophenol[12] was boiled with 3:4:5-triiodonitrobenzene in methyl ethyl ketone solution in the presence of dry potassium carbonate and the same thing was done with the Schiff's base formed from that phenol and benzaldehyde. It was found that the potassium salts of these phenols could not be obtained in the dry state for treatment with the triiodonitrobenzene in boiling xylene because they decomposed suddenly at temperatures near 100°.



(2) Silver carbonate was substituted for potassium carbonate in attempting to condense 3:4:5-triiodonitrobenzene with ethyl 3;5-diiodo-4-hydroxybenzoate, in boiling methyl ethyl ketone.

(3) 3:4:5-triiodonitrobenzene was condensed with ethyl p-hydroxybenzoate in boiling methyl ethyl ketone, dry potassium carbonate being present. The condensation product, which was obtained in good yield, was hydrolysed with concentrated sulphuric acid, and the nitro group was reduced by pouring an excess of a solution of stannous chloride in concentrated/

concentrated hydrochloric acid into a suspension of the nitro compound in boiling acetone[12]. Then the hydrochloride of the amino compound thus obtained was suspended in glacial acetic acid and diazotised with amyl nitrite. The diazonium salt was precipitated with ether and made into a cream with water. To convert the diazo group into a phenolic group this cream was stirred vigorously in small portions, into a large volume of a solution of sodium sulphate in fairly concentrated sulphuric acid at 150°. After purification the hydroxy compound which had been formed was methylated with methyl sulphate. The series of reactions may be represented thus:



It was thought that iodine atoms might be introduced (say by nitration followed by reduction and subsequent diazotisation) into the desired positions in the methoxy acid which had been prepared in this way (or into a derivative of it) and if so the tetraiodo compound formed might conceivably be converted into/

into the thyroxine isomer. But, at the best, the process seemed likely to be very tedious and this route was not further explored.

Much time was spent on these and other similar attempts and eventually some preliminary steps were also taken towards preparing phenyl ethers by quite different methods. But finally it had to be concluded that the problem of the synthesis of this isomer could not, for the present, be solved.

(D) Synthesis of "Dibromothyroxine".

β-[3:5-Diiodo-4-(3':5'-dibromo-4'-hydroxy-phenoxy)-phenyl]-α-aminopropionic acid.

$HO.C_6H_2Br_2.O.C_6H_2I_2CH_2.CH(NH_2).COOH$

The steps in the synthesis of this compound were exactly the same as those in the preparation of thyroxine except that bromination was substituted for the final iodination. The bromination was carried out in much the same way as in the case of the preparation of dibromotyrosine.[13]

III. EXPERIMENTAL

(A) Synthesis of ββ-[di-(3:5-diiodo-4-hydroxyphenyl)]-α-aminopropionic acid $(HO.C_6H_2I_2)_2CH.CH(NH_2).COOH$ and of the corresponding amine, ββ-[di-(3:5-diiodo-4-hydroxyphenyl)]-ethylamine, $(HO.C_6H_2I_2)_2.CH.CH_2NH_2$.

(1) 4-Methoxybenzoyl Chloride.

$CH_3O.C_6H_4.COCl$.

Anisic acid was boiled under reflux on the water-bath with five times its weight of thionyl chloride until hydrogen chloride ceased to be evolved. The excess of thionyl chloride was then distilled off on the water-bath, the last traces being removed under reduced pressure. Next the acid chloride was distilled under reduced pressure (about 15 mm.) over a smoky flame. At about 150° a colourless liquid, which afterwards solidified to an almost colourless crystalline mass, passed over. The yield was 75% of the theoretically possible quantity.

A. Schoonjans[14] describes this compound as a colourless, highly refractive liquid, which crystallises in needles melting at 22° and boiling at 145° (14 mm.).

(2) /

(2) Di-(4-methoxyphenyl) Ketone.

$(CH_3O.C_6H_4)_2.CO.$

Anisole (11 grams) was mixed with one part of dry carbon disulphide and the mixture was run into a large flask, into which also 15 grams of powdered anhydrous aluminium chloride were then rapidly weighed out. The flask was fitted with a reflux condenser and placed in ice. 4-Methoxybenzoyl chloride (21.7 grams), dissolved in one part of carbon disulphide was then run in, drop by drop, from a tap-funnel. Meanwhile the flask was shaken and occasionally removed from the ice so as to prevent over-cooling. Evolution of hydrogen chloride took place, at first slowly, eventually vigorously and the contents of the flask set to a solid mass. The flask was now warmed at 50°-70° for half an hour and then ice and cold water were added with shaking. Next the mixture was steam-distilled till the distillate was passing over as a clear liquid. The solid residue in the flask was filtered off, washed successively with sodium hydroxide solution, dilute hydrochloric acid, and water, and dried in the steam oven. The yield was almost theoretical and the material obtained was sufficiently pure to be used for the next stage in the synthesis. A small sample, /

sample, further purified by crystallisation from absolute alcohol, melted at 143°-144°. Schnackenberg and Scholl, who prepared this ketone[15] in a somewhat similar way, give 144° as its melting point.

(3) Di-(4-methoxyphenyl) carbinol.

$(CH_3O.C_6H_4)_2.CHOH$.

For the preparation of this compound the method of Schnackenberg and Scholl[15] was adopted. The corresponding ketone (15 grams) was boiled on the water-bath for two hours with zinc dust (10 grams) and potassium hydroxide (5 grams) in 95% alcohol (25 grams). During this operation the mixture was frequently shaken vigorously. Dilute alcohol (12 c.c. of 50%) was then added and the mixture was brought to the boil, filtered rapidly and the filtrate allowed to stand. The hydrol crystallised as the liquid cooled and from the mother-liquor a further quantity of it was obtained by addition of water followed by cooling in the ice-chest for a time. Yield, almost theoretical. Melting point 72°. The product was thoroughly dried in a vacuum desiccator.

(4) /

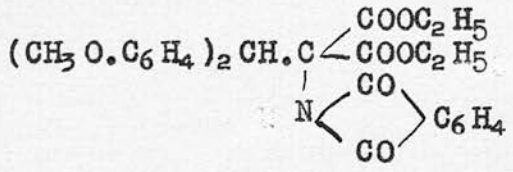
(4) Di-(4-methoxyphenyl)-methyl Chloride.

$(CH_3O.C_6H_4)_2CHCl$.

The carbinol (10 grams), prepared in the manner just described, was dissolved in dry benzene (100 c.c.), a quantity of granulated anhydrous calcium chloride was added, and the mixture was cooled in ice while dry hydrogen chloride was passed in to saturation. After this the mixture was allowed to stand for four hours in a cool place. As much as possible of the excess hydrogen chloride was removed by drawing dry air through the liquid and the clear benzene solution was rapidly poured off from the calcium chloride into a distillation flask whence the benzene was driven off by distillation under reduced pressure. The solid residue was crystallised from petrol ether (boiling point 60°-80°). The product, which was brown in colour, but almost pure, was obtained in 83% yield. A small sample of the carefully recrystallised material, formed perfectly colourless needles melting at 83°. (Straus and Dützmann (3) give 82-82.5°).

(5) /

(5) Ethyl-di-(4-methoxyphenyl)-methylphthaliminomalonate.



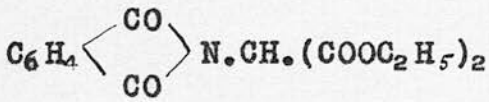
(a) Ethyl Bromomalonate.

$BrCH.(COOC_2H_5)_2$

Ethyl malonate (1 mol. dissolved in one part of carbon tetrachloride) was mixed with bromine (1 mol. dissolved in one part of carbon tetrachloride) and the mixture was exposed to sunlight. After some time bubbles of hydrogen bromide were evolved and a vigorous reaction took place. When this had subsided the mixture was allowed to stand for half an hour, washed with water containing sodium carbonate and a little sodium bisulphite, then with pure water and dried over calcium chloride. From the dried solution carbon tetrachloride was removed by distillation on the water-bath, first at atmospheric, later under reduced pressure. Finally the bromo ester was distilled under reduced pressure over a smoky flame. The boiling point at about 15 mm. was in the neighbourhood of 125°.

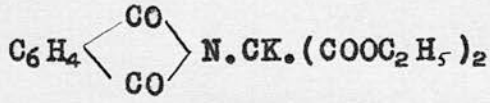
(b) /

(b) Ethyl Phthaliminomalonate.



Potassium phthalimide and ethyl bromomalonate in the proportions 1 mol.: 1.1 mol. were heated together in a metal bath at 150° for forty-five minutes with occasional stirring. The cooled product was dissolved in ether-water mixture and the ethereal portion was separated and dried over calcium chloride. Then the ether was distilled off. On pouring the thick syrupy residue into a beaker and rubbing it with a little alcohol rapid crystallisation took place. The cooled crystalline mass which formed was rubbed well on a porous plate and dried in a vacuum desiccator. Yield - almost theoretical. The melting point of a recrystallised sample was 75°.

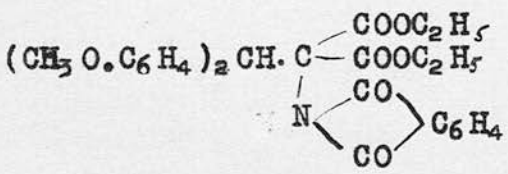
(c) Ethyl Potassiophthaliminomalonate.



This compound was prepared according to the method described by Stephen and Weizmann[16]. The ethyl phthaliminomalonate (1 mol.) was dissolved in hot alcohol (1 part) and added slowly to a solution of potassium (1 mol.) in alcohol (10-20 parts). The bright yellow paste of potassium compound/

compound which was formed almost at once was completely freed from alcohol as follows. Most of the alcohol was distilled off on the water-bath, first at atmospheric, later under reduced pressure. Next the flask containing the material was heated under reduced pressure at 110° for a time. The last traces of alcohol were removed by shaking the yellow cake of potassio-ester with sodium-dried xylene, distilling the hydrocarbon off on the water-bath under reduced pressure and removing the last traces of it by heating the flask at 110° for a time under reduced pressure in a metal-bath. Finally the distillation with dry xylene was repeated and the dry potassio-ester was used without further treatment for the next stage of the synthesis.

(d) Ethyl Di-(4-methoxyphenyl)-methylphthaliminomalonate



Still following the procedure of Stephen and Weizmann[16] di-(4-methoxyphenyl)-methyl chloride (calculated quantity) was dissolved in one part of sodium-dried xylene and poured on to the potassium compound/

compound prepared in the manner just described. The mixture was then heated in a metal bath at 145° for four hours with occasional shaking, care being taken that good contact was maintained between the xylene solution and the insoluble potassio-ester. The mixture was now poured into water and well stirred when a large part of the product separated in the solid state and was filtered off, washed successively with dilute potassium hydroxide solution and water, dried in a vacuum desiccator and crystallised from alcohol. The rest of the product remained dissolved in the xylene which was likewise successively washed with dilute potassium hydroxide and water and dried over calcium chloride. After the xylene had been distilled off under reduced pressure there remained a thick syrup which was rubbed with alcohol till a semi-solid paste formed and then crystallised from alcohol. The crystals were dried in a vacuum desiccator. The yield was about 75% of the theoretical quantity. The melting point of the pure substance is 106°. It separates from alcohol in colourless prisms.

Analysis:

0.1533 grams gave 0.3802 grams CO_2 and 0.0801 grams H_2O

20.5 mgm. gave 0.594 mgm. N (micro-Kjeldahl)

Found /

	C	H	N
Found:	67.7%	5.5%	2.4%
Calculated for $C_{30}H_{29}O_8N$	67.8%	5.8%	2.6%

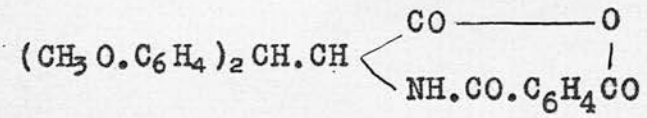
(6) Di-(4-methoxyphenyl)-methylphthalaminomalonic Acid.

$$(CH_3O.C_6H_4)_2\ CH.C\begin{cases}=(COOH)_2\\ NH.CO.C_6H_4COOH\end{cases}$$

The ester (29.5 grams) was moistened with alcohol and treated with a hot solution of potassium hydroxide (37 grams) in water (75 c.c.). The mixture was stirred well and was heated on the water-bath with further frequent stirring for one hour. After about fifteen minutes the potassium salt of the acid began to separate. At the end of the hour this salt was filtered off, washed twice with absolute alcohol and then dissolved in hot water. The aqueous solution was cooled as rapidly and as much as possible without precipitation of the salt and then, with continued cooling, was cautiously acidified, first with dilute acetic acid and finally with hydrochloric acid. In this way the acid was precipitated and after standing for some time in the ice-chest it was filtered off and dried in the steam/

steam oven. Neither the acid itself nor its potassium salt could be obtained in a condition sufficiently pure for analysis.

(7) Anhydride of ββ- [Di-(4-methoxyphenyl)] -α-phthalaminopropionic Acid.



The crude phthalaminomalonic acid (22 grams) was heated in a metal-bath under reduced pressure at 180°-200° for about one hour. (The pressure was about 15 mm.). As decarboxylation proceeded the material first became soft and afterwards hardened again. A minute amount of phthalic anhydride sublimed into the neck of the flask. The solid cake which remained was dissolved in the minimum amount of boiling glacial acetic acid and the boiling solution was diluted with boiling water until a very faint turbidity appeared. On allowing the solution to cool slowly the anhydride separated in colourless prisms. Yield - 75% (calculated on the malonic ester). A sample of the product, twice crystallised from acetic acid with/

with dilution in the manner described, formed colourless prisms melting at 209°-210°.

Analysis:

0.1172 grams gave 0.2984 grams CO_2 and 0.0505 grams H_2O

25.6 mgm. gave 0.743 mgm. N (micro-Kjeldahl)

	C	H	N
Found	69.4%	4.8%	2.9%
Calculated for $C_{25}H_{21}O_6N$	69.4%	4.9%	3.1%

(8) ββ-[Di-(4-hydroxyphenyl)]-α-aminopropionic Acid (Isodesiodothyroxine).

$(HO.C_6H_4)_2CH.CH(NH_2).COOH$

The anhydride of the phthalamino acid was boiled for two hours with a mixture of acetic anhydride (5 parts) and hydriodic acid (sp. gr. 1.7; 5 parts). Then the whole was evaporated to dryness on the water-bath under reduced pressure, some water was added to the residue and distillation to dryness was repeated. The partially crystalline residue was dissolved in hot water and the cooled aqueous solution was extracted twice with ether. Ether was then boiled off from the extracted aqueous solution and concentrated ammonia solution was added to/

to it, while still boiling, till neutralisation was just exceeded. Once more the solution was taken to dryness by distillation on the water-bath under reduced pressure. The residue was dissolved in just sufficient boiling alcohol. When the alcoholic solution had cooled and had stood for some time in the ice-chest the product separated in the form of colourless crystals. These were filtered off, dried in a vacuum desiccator and purified by dissolving in just sufficient boiling water, adding a little charcoal, boiling for a few minutes and filtering rapidly through a hot funnel. The pure amino-acid crystallised slowly from the filtrate after it had been cooled in the ice-chest for a time. Further small quantities were obtained both from the aqueous and alcoholic mother-liquors by concentration. The acid forms very fine colourless needles which soften at 190°-200° and melt with decomposition at 241°. On exposure to the atmosphere it rapidly absorbs moisture. The amount of water thus absorbed is equivalent to about six molecules, but experiment showed that, apparently, no definite hydrate was formed. Yield - about 60%.

Analysis/

Analysis.

0.1445 grams gave 0.3481 grams CO_2 and 0.0738 grams H_2O.

15.2 mgm. gave 0.762 mgm. N (micro-Kjeldahl)

16.3 mgm. gave 1.5 c.c. N at 20° and 750 mm. (van Slyke)

	C	H	N	Amino N
Found	65.7%	5.7%	5.02%	5.2%
Calculated for $C_{15}H_{15}O_4N$	65.9%	5.5%	5.13%	5.13%

(9) ββ-[Di-(3:5-diiodo-4-hydroxyphenyl)]-α-amino-propionic Acid. (Isothyroxine)

$(HO.C_6H_2I_2)_2CH.CH(NH_2).COOH$

The amino acid was dissolved in just sufficient concentrated ammonia (sp. gr. 0.880); the solution was cooled in ice-water while the calculated amount of the strongest iodine in potassium iodide solution (2.54 N) was slowly added drop by drop. At first the iodine was taken up fairly rapidly but towards the end of the addition the uptake was rather slow, so the addition of iodine was interrupted and the mixture was allowed to stand in the ice-water until the brown colour of the halogen had disappeared.

Throughout/

Throughout the process the mixture was frequently well shaken. When all the iodine had been added the contents of the vessel in which the iodination had been carried out were transferred to a Claisen flask, the iodination flask being washed with a little alcohol and the washings added to the main solution. Next the liquid in the Claisen flask was entirely removed by distillation on the water-bath under reduced pressure. The residue was washed out of the flask with water and acetic acid on to a Buchner funnel, filtered with suction and washed with the filtered liquid. It was then dissolved in a considerable quantity of hot water containing a little dilute hydrochloric acid, just sufficient alcohol being also added to bring about solution of the salt formed. (A small quantity of flocculent material remained undissolved). The liquid was boiled with animal charcoal for a few minutes and filtered while hot. To the filtrate, heated to boiling, on the water-bath, saturated sodium acetate solution was added until the liquid was no longer acid to congo-red paper, when the tetra-iodo acid separated almost immediately. The hot suspension was cooled to room temperature and then placed in the/

the ice-chest for a time. Finally the almost pure product was filtered off, washed with water, and dried in a vacuum desiccator. It was later further purified by redissolving in hot water with dilute hydrochloric acid and alcohol followed by re-precipitation with saturated sodium acetate solution. In this way an almost colourless sphaero-crystalline product was obtained. Its melting point was 218° (decomposition). The yield was about 60%.

Isothyroxine is insoluble or almost insoluble in water, cold or hot, and it is only very slightly soluble in alcohol. It is likewise insoluble in dilute hydrochloric acid (2 N) and in dilute sulphuric acid (2 N) though it is readily soluble in these acids when sufficient alcohol is added to the warm suspension of the substance in the hydrochloric or sulphuric acid. In dilute sodium carbonate solution (2 N) and in dilute sodium hydroxide solution (2 N) it is easily soluble in the cold.

Analysis:

62.5 mg. gave 1.104 mg. N (micro-Kjeldahl)

1.66 mg. required 10.2 c.c. N/200 sodium thiosulphate (Kendall, 1914)

	N	I
Found	1.77%	65.1%
Calculated for $C_{15}H_{11}O_4NI_4$	1.81%	65.3%

(10) ββ- Di-(4-hydroxyphenyl) -ethylamine (Isodesiodothyroxamine)

$(HO.C_6H_4)_2.CH.CH_2.NH_2$

Isodesiodothyroxine (0.5 grams) was heated with twenty times its weight of diphenylamine in a metal-bath for about one and a half hours, the temperature being gradually raised to 275°. During the heating a current of hydrogen was passed through the flask containing the mixture. The acid, which did not dissolve in the molten diphenylamine, began to decompose at about 210° and bubbles of carbon dioxide appeared. Eventually the evolution of this gas became fairly brisk and when the maximum temperature was reached almost all the acid had disappeared. The mixture was then allowed to cool and was poured off from the small quantity of solid residue. Benzene (15-20 c.c.) was added and the diphenylamine mixture was stirred into it after which petrol (15-20 c.c.; boiling point 100°-120°) was added. After the well stirred mixture had been allowed to stand for one hour the amine which had separated was filtered off and allowed to dry in air. The yield of the material thus obtained was about 60%. It was further purified in the following manner. It was heated/

heated in portions weighing about 0.5 grams in a sublimation apparatus in a metal-bath, the pressure being reduced to about 1 mm. and the heating was continued till no more material distilled. The maximum temperature attained was about 315°. The crystalline material which distilled was boiled with ethyl acetate, filtered from a little undissolved material, and the boiling filtrate was treated with petrol (boiling point 100°-120°) till a faint turbidity appeared. On cooling the petrol-ethyl acetate solution, first to room temperature, then in the ice-chest a crystalline precipitate appeared. This was filtered off and dried in the steam oven. The yield of purified material, however, was only 40% of the theoretical amount and it was afterwards found that a yield of about 60% could be obtained by heating the dried recrystallised amino-acid directly in the sublimation apparatus under reduced pressure (about 1 mm.) in the manner just described for the product from the diphenylamine treatment. The product was washed with a little boiling ethyl acetate and was then dissolved in the minimum of boiling water. (A rather large volume of water was required and a small amount of material remained undissolved). The boiling aqueous solution was decolorised as much as possible with charcoal and filtered/

filtered rapidly through a hot funnel. As the filtrate cooled the amine separated in the form of rosettes of fine, short, colourless needles and when cooling was continued in the ice-chest for a time a further quantity of it separated. It was filtered off, washed with water and dried in vacuo. The yield of recrystallised substance was about 55%.

Isodesiodothyroxamine is insoluble in cold water but dissolves in a rather large volume of boiling water from which it separates in colourless needles. These appear to contain water of crystallisation and they darken somewhat on keeping. The amine is soluble in alcohol and in methyl alcohol, sparingly soluble in benzene even at the boil and insoluble, or almost so, in ether and in chloroform. In dilute sulphuric acid and in dilute hydrochloric acid it is readily soluble and separates from the solutions, when they are made alkaline with ammonia, in the form of clusters of short needles.

For purposes of analysis the amine was dried to constant weight over phosphorus pentoxide, in vacuo, at 80°. Dried thus it had the melting point 207-208°.

Analysis/

Analysis:

0.1218 grams gave 0.3273 grams CO_2 and 0.0727 grams H_2O

15.4 mg. gave 0.934 mg. N (micro-Kjeldahl)

	C	H	N
Found	73.3%	6.6%	6.1%
Calculated for $C_{14}H_{15}O_2N$	73.4%	6.6%	6.1%

The salts of isodesiodothyroxamine are very soluble and hence difficult to prepare in a pure condition from small quantities of material. When larger amounts of the base are available it will be possible to prepare pure salts and other derivatives.

The Hydrochloride. Dry hydrogen chloride was passed to saturation into an alcoholic solution of the amine and the solution was then diluted with a large volume of dry ether which produced a fairly dense turbidity. When the turbid liquid had been allowed to stand in the ice-chest over night the hydrochloride of the amine was found to have separated in the form of fairly large thin leaflets. These were filtered off, washed with dry ether, and dissolved in a little alcohol. The alcoholic solution was boiled with charcoal, filtered, and allowed to evaporate almost to dryness in a vacuum. When /

When the solution thus obtained was cooled for some time in the ice-chest the product separated as a mass of colourless crystals which were dried, first on a porous plate and then in the steam oven.

Isodesiodothyroxamine hydrochloride is very easily soluble in water and in alcohol. It melts at 275°.

The Hydrobromide. To an alcoholic solution of the amine a few drops of a concentrated solution of hydrogen bromide in glacial acetic acid were added followed by sufficient dry ether to produce a rather deep turbidity. The turbid liquid was allowed to cool in the ice-chest for a week . At the end of that time an almost colourless crystalline deposit had appeared but the amount of the impure salt thus formed was too small for purification; the melting point and other properties could not be determined.

(11) ββ-Di-(3:5-diiodo-4-hydroxyphenyl)-ethylamine.

(Isothyroxamine)

$(HO.C_6H_2I_2)_2.CH.CH_2.NH_2$

Isodesiodothyroxamine (1 gram) was dissolved with gentle warming in concentrated ammonia (sp.gr. 0.880; about 150 parts) and was treated with the/

the theoretical amount of the strongest solution of iodine in potassium iodide in the same manner as was used for the iodination of isodesiodothyroxine except that cooling was not employed. The product began to separate after rather less than half the iodine had been added. At the end of the addition the mixture was allowed to stand for a few hours, diluted with water and the product filtered off. This was then suspended in a rather large volume of dilute sulphuric acid, free iodine was removed by the addition of a very little sodium bisulphite, and an equal volume of alcohol was added. When the aqueous-alcoholic solution was boiled most of the base went into solution and the residue was separated by filtration. To the filtrate about its own volume of dilute sulphuric acid (2 N) was added, most of the alcohol was boiled off and the liquid was filtered rapidly through a hot funnel in order to remove some tarry matter which had formed. As the filtrate cooled the sulphate of the base crystallised and was filtered off when it had ceased to separate. It was then dissolved in just sufficient hot dilute alcohol (50%)(a rather large volume was required) and enough ammonia solution was added to dissolve the precipitate first formed. Finally the solution was boiled till the base began

to/

to separate and allowed to cool first to room temperature, then in the ice-chest. The amine was then filtered off and washed with a little dilute alcohol (50%). For further purification it was dissolved in a considerable volume of dilute ammonia, filtered from a little undissolved material and the solution boiled till ammonia ceased to be given off. The mixture thus obtained was cooled to room temperature and then in the ice-chest and the product which had separated was filtered off, washed well with water and dried over phosphorus pentoxide in vacuo at 100°. Thus obtained isothyroxamine forms clusters of short, almost colourless needles which melt with decomposition and iodine liberation at 232°-233°. It is insoluble in water, alcohol, dilute hydrochloric acid or dilute sulphuric acid. In dilute sodium hydroxide and in mixtures of dilute hydrochloric acid or dilute sulphuric acid with alcohol it is easily soluble. The yield was about 30% but could almost certainly have been improved by working up the various mother-liquors. Salts or other derivatives of this base have not yet been prepared in the pure state.

Analysis/

Analysis:

50.2 mg. gave 0.944 mg. N (micro-Kjeldahl)

1.44 mg. required 9.3 c.c. N/200 sodium thiosulphate (Kendall, 1914)

	N	I
Found	1.88%	68.5%
Calculated for $C_{14}H_{11}O_2NI_4$	1.91%	69.3%

(B) Synthesis of ββ-Diphenyl-α-aminopropionic Acid $(C_6H_5)_2CH.CH(NH_2).COOH$, and of the corresponding amine, ββ-Diphenyl-ethylamine $(C_6H_5)_2CH.CH_2NH_2$.

(1) Benzohydrol.

$(C_6H_5)_2CHOH$. This compound was prepared by two methods.

(a) Method of Marschalk (17)

Benzophenone (10 grams), calcium turnings (30 grams) and absolute alcohol (about 400 c.c.) were placed in a flask fitted with a reflux condenser and heated to boiling on the water-bath. At first the reaction was very slow but after about half an hour, became suddenly very vigorous. When this occurred heating was at once stopped and the reaction gradually subsided. Next the contents of the flask were largely diluted with water and acidified with hydrochloric acid. The hydrol separated from the cold mixture first as an oil, then as a crystalline solid. It was filtered off, dried in a vacuum desiccator and twice crystallised from petrol ether (boiling point 60°-80°). Yield - about 32%. The recrystallised substance melted at 66°-68°.

(b) /

(b) Method of Elbs.[18]

Benzophenone (45 grams) was dissolved in 95% alcohol (625 c.c.) and zinc dust (340 grams) was added followed by a solution of potassium hydroxide (10 c.c. of a solution of 20 grams potassium hydroxide in 20 grams of water). The mixture was well shaken and allowed to stand in the incubator (temperature about 40°) for three days with occasional shaking and with the addition of potassium hydroxide (10 c.c. of the solution already used). Then the solid matter was filtered off and carbon dioxide was passed through the filtrate till all the zinc present had been precipitated. When this precipitate had been separated the solution was diluted with a large quantity of water which precipitated the hydrol. It was separated by filtration and dried in vacuo. Yield - 73% of material sufficiently pure for use in the next stage of the synthesis. The melting point of the product was 61°-65° whereas pure benzohydrol melts at 68°.

(2) Diphenylmethyl Bromide. $(C_6H_5)_2.CHBr$.[19]

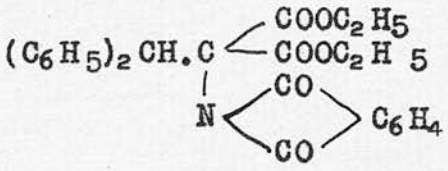
The hydrol (15.75 grams) was dissolved in three times its weight of carbon tetrachloride and phosphorus/

phosphorus tribromide (10.1 grams = 3.6 c.c.) was added. The mixture was allowed to stand for one day in a flask having a calcium chloride tube in the stopper. Then the flask was attached to a reflux condenser and was warmed on the water-bath at 60°-70° for six hours. After cooling, the solution was poured off from a small quantity of syrupy material, washed with ice-water and with sodium acetate solution and dried over calcium chloride. The carbon tetrachloride was then distolled off on the water-bath. When the residue was distilled under reduced pressure (about 15 mm.) over a smoky flame, the bromo compound passed over at about 180° as an almost colourless liquid, which, on cooling, solidified to a crystalline mass. The melting point of the crystals was about 39°. Yield 76%.

Except for slight modifications which are mentioned at the appropriate points, the subsequent stages in the synthesis of ββ-diphenyl-α-amino-propionic acid were exactly similar to the corresponding ones in the synthesis of isodesiodo-thyroxine.

(3) /

(3) Ethyl Diphenylmethylphthaliminomalonate.



This ester is more soluble in xylene than the corresponding dimethoxy ester and does not separate in the solid state when the xylene solution is poured into water. The residue after the removal of the xylene was a thick syrup which was dissolved in a small amount of boiling alcohol. The crystalline solid which separated on cooling the alcoholic solution was filtered with suction and washed well with alcohol which had been cooled in a freezing mixture. Yield - 57%. Melting point of a sample twice crystallised from 90% alcohol: 117° (with softening from 95° upwards). The pure material separates from alcohol in colourless prisms.

Analysis:

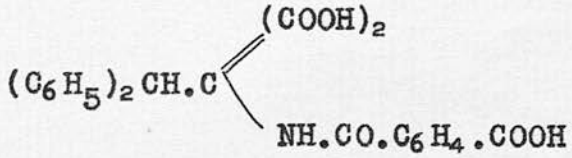
0.1503 grams gave 0.3909 grams CO_2 and 0.0710 grams H_2O.

50.8 mgm. gave 1.51 mgm. N (micro-Kjeldahl)

Found /

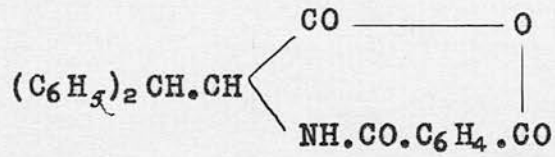
	C	H	N
Found	70.9%	5.3%	2.97%
Calculated for $C_{28}H_{25}O_6N$	71.3%	5.3%	2.97%

(4) Diphenylmethylphthalaminomalonic Acid.



Like the corresponding di-(4-methoxyphenyl) acid, this compound could not be obtained in the pure condition.

(5) Anhydride of ββ-Diphenyl-α-phthalaminopropionic Acid.



Crystallised in colourless prisms melting at 214°-215°. Yield - almost theoretical (calculated on the crude malonic acid).

Analysis/

Analysis:

0.1527 grams gave 0.4151 grams CO_2 and 0.0652 grams H_2O.

30.2 mgm. gave 1.142 mgm. N (micro-Kjeldahl)

	C	H	N
Found	74.2%	4.7%	3.8%
Calculated for $C_{23}H_{17}O_4N$	74.4%	4.6%	3.8%

(6) ββ-Diphenyl-α-aminopropionic Acid (Diphenylalanine.)

$(C_6H_5)_2CH.CH(NH_2).COOH$

This acid is considerably less soluble in water than isodesiodo-thyroxine and is precipitated almost immediately when ammonia is added to the solution of the hydriodide, so that subsequent evaporation to dryness and solution in alcohol is not necessary. The compound was purified by being dissolved in a little more than sufficient dilute ammonia and precipitated from the boiling solution by the addition of enough acetic acid to make the liquid faintly acid. It separated rapidly and almost completely from the hot solution in the form of fairly large, flat, colourless prisms which were filtered off and washed well with cold water. When precipitated

thus/

thus from dilute solutions the substance contained water of crystallisation (equivalent to rather less than one molecule) but it separated from more concentrated solutions apparently without such water.

Diphenylalanine is insoluble, or almost insoluble, in water cold or hot. It is fairly readily soluble in hot alcohol and somewhat less soluble in boiling methyl alcohol. From these two solvents it crystallises on cooling in the form of fine needles. It is also soluble in cold dilute sulphuric acid, in hot dilute hydrochloric acid, in glacial acetic acid, in dilute sodium carbonate solution and in dilute sodium hydroxide solution. From the hot solution in dilute hydrochloric acid, small prism-shaped crystals, presumably of hydrochloride, separated on cooling but these were rather badly formed and may have been mixed with crystals of the free acid.

Diphenylalanine melts with decomposition (evolution of gas and frothing) at 236°. The yield was 77%.

Analysis:

0.1195 grams gave 0.3720 grams CO_2 and 0.0682 grams H_2O

21.8 mg. gave 1.222 mg. N (micro-Kjeldahl)

	C	H	N
Found	74.6%	6.3%	5.6%
Calculated for $C_{15}H_{15}O_2N$	74.7%	6.2%	5.8%

(7) ββ-Diphenylethylamine.

$(C_6H_5)_2.CH.CH_2.NH_2$

Just after the preparation of this compound had been begun it was found that some previous syntheses of it had been overlooked. As long ago as 1890 Freund and Immerwahr[23] reduced diphenylacetonitrile with sodium and alcohol and obtained a very small amount of the amine. Konowalow and Jatzewitsch[24] also seem to have prepared it, in 1905, in an impure condition. More recently it has been prepared by Sieglitz[25], by Rupe and Gisiger[26], and by Lipp[27]. All of these workers used methods quite different from that described here, however, and the yields obtained, except by Rupe and Gisiger, were very poor.

Diphenylalanine (2 grams) was mixed with twenty times its weight of diphenylamine and the mixture was slowly heated for an hour in a metal-bath in a current of hydrogen till the temperature reached 250°. Bubbles of carbon dioxide first began to appear at about 200° and at 230° the gas was being freely evolved. As the amine has a low melting point (about 40°) the hydrogen which passed through the reaction-flask was led into dilute sulphuric acid (2 N) before being allowed to escape and this acid was afterwards used to extract the/

the benzene solution of the amine. At the end of the time of heating all the amino acid had disappeared and no more carbon dioxide was coming off. The reaction mixture was cooled and was dissolved in benzene, the benzene solution was extracted with dilute sulphuric acid and the acid extract, after being filtered from a little solid matter, was made alkaline with dilute sodium hydroxide solution. The oily material which was thus precipitated was extracted from the aqueous liquid with ether, the ethereal solution was dried over anhydrous sodium sulphate and the amine was obtained from solution as a slightly brown coloured oil by distilling off the ether. On allowing the liquid amine to stand in the ice-chest for some time partial crystallisation took place but the material was naturally not quite pure. In view of the probably large loss which would have occurred in dealing with such a small amount of substance it was not distilled. The yield of the not quite pure, oily amine was 75%. Four salts of the amine were prepared.

The Hydrochloride. The amine was dissolved in dilute hydrochloric acid and the solution was taken to dryness by distilling off the liquid on the water-bath under reduced pressure. The solid residue/

residue was dissolved in hot alcohol, and the alcoholic solution was boiled with charcoal, filtered and concentrated. From the cooled concentrated solution the hydrochloride of the amine separated in colourless needles. When further purified by recrystallisation from boiling alcohol these melted at 259°. (Freund and Immerwahr[23] give 255°, Sieglitz[25] gives 253° and Lipp[27] 263°-265°.

A sample of the hydrochloride was also prepared in needle-shaped prisms by precipitation from alcohol with a large volume of ether.

The Picrate.

Alcoholic solutions of picric acid and the amine hydrochloride were mixed and the mixture was allowed to stand for several days. Long yellow needle-shaped prisms of the picrate of the amine separated slowly. These melted, after darkening and shrinking at a somewhat lower temperature, at 216°-217° (Sieglitz[25] gives 212°-213°). From more concentrated solutions of picric acid and amine hydrochloride the picrate separated fairly quickly in smaller crystals.

The Gold Salt.

When aqueous solutions of gold chloride and the amine hydrochloride were mixed the gold salt

was/

was precipitated at once in the form of bright yellow, irregular plates.

The Platinum Salt.

This salt was easily and immediately precipitated from the concentrated aqueous solution of the hydrochloride by the addition of platinum chloride solution. Crystallised from a rather large volume of hot water it formed rectangular yellow tables.

(C) Synthesis of "Dibromothyroxine"

$HO.C_6H_2Br_2.O.C_6H_2I_2.CH.CH(NH_2).COOH$

β- [3:5-Diiodo-4-(3':5'-dibromo-4'-hydroxy-phenoxy)-phenyl] -α-aminopropionic Acid.

The diiodo acid, β-[3:5-diiodo-4-(4'hydroxyphenoxy)-phenyl] -α-aminopropionic acid (8) (0.53 grams) was mixed with glacial acetic acid (1.7 c.c.) and to the mixture there was added, slowly, a solution of bromine (0.1 c.c.) in glacial acetic acid (0.3 c.c.). Heat was developed and a clear solution produced. This solution, after being cooled in ice, was allowed to stand over-night at room temperature and the crystalline material (presumably the hydrobromide) which had separated was filtered off, washed with a little glacial acetic acid and dried in vacuo. (Yield of presumed hydrobromide - 0.5 grams). The dry material was then dissolved in a little ammonia solution (about 2 N) with the addition of an equal volume of 95% alcohol and the solution was filtered. To the boiling filtrate sufficient glacial acetic acid to make the solution acid was added. Almost at once the crystalline product separated. After the mixture had been cooled/

cooled in the ice-chest for some time the dibromo acid was separated by filtration, washed with water, and dried in vacuo. The yield was 45% of the theoretical quantity. It was not found possible to obtain the acid in well-formed crystals. The melting point was 244.5° (decomposition).

Analysis:

52.8 mgm. gave 1.104mgm. N (micro-Kjeldahl)

3.03 mgm. required 10.7 c.c. N/200 sodium thiosulphate (Kendall, 1914)

	N	I
Found	2.1%	37.4%
Calculated for $C_{15}H_{11}O_4N\ Br_2\ I_2$	2.1%	37.2%

IV. PHYSIOLOGICAL

As yet there is very little to record concerning the physiological properties of the more important substances described in the preceding sections. Some observations may be made, however, about the kind of experiments which might be made, taking into account the effects known to be produced by analogous compounds and also the fates of these compounds in the animal organism.

Isothyroxine. Since thyroxine has such a powerful effect on the metabolic rate, it would naturally be of interest to compare isothyroxine physiologically with thyroxine itself, although the fact that the phenyl ether linkage of the latter is absent in the former would lead to the supposition that there might be considerable difference between the two compounds. Gaddum (20) has carried out some preliminary quantitative observations on the effect of thyroxine and allied substances on tadpoles and has found that, as far as his experiments went, isothyroxine shows none of the activity exhibited by thyroxine. It is not impossible, however/

however, that other methods of examination might show that the isomer has some effect on metabolic rate.

"Dibromothyroxine". This substance might also be compared with thyroxine especially in view of the fact, already mentioned in the introduction (see page 2) that the 3:5-diiodo derivative of desiodo-thyroxine physiologically resembles thyroxine to some extent. No information about the properties of "tetrabromothyroxine" has so far been received but should it have any noteworthy physiological effects the intermediate position which "dibromo-thyroxine" occupies, chemically, between thyroxine and the tetrabromo compound would lend additional interest to the investigation of the bromoiodo compound.

Diphenylalanine. Diphenylalanine would, of course, be compared with phenylalanine; it would be of considerable interest to know whether the two benzene rings of the diphenyl compound would be oxidised in the same way as is the single ring of phenylalanine. The diphenyl compound might also be compared with phenylacetic acid and with diphenyl-acetic acid. Miriam, Wolf and Sherwin (21) have recently/

recently investigated the fate in the animal body of the latter substance and found that it is resistant to biological oxidation, being recovered, for the most part, unchanged. It had been found earlier (22) that phenylacetic acid and its derivatives were remarkable in this respect.

Isodesiodothyroxine. Just as isothyroxine would be compared with thyroxine so this substance would be compared with desiodothyroxine, which, in Gaddum's experiments (20) appeared to be quite devoid of specific thyroid action.

Isodesiodothyroxamine. Isothyroxamine.

When the effects of the corresponding substances in the thyroxine series have been investigated, it might be worth while to determine the physiological properties of these two amines. In addition, experiments might be made to discover if the pressor effect produced by tyramine is also produced by isodesiodothyroxamine and if the action of isothyroxamine is similar to that of diiodotyramine.

Diphenylethylamine. The sympathomimetic action of phenylethylamine might also be looked for, to some extent/

extent at least, in this diphenyl compound. Although both phenylpropylamine and benzylamine are considerably less active than phenylethylamine itself the effect of the presence of another phenyl group would probably be different from that produced by alteration in the length of the chain.

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