It is well known that denatured proteins react very readily with hæms to form hæmochromogens; indeed, this reaction is a very sensitive test for denaturation occurring in the course of purification of native proteins. Yet the individual constituents of proteins, the amino-acids, with the exception of glycine[7] and histidine[8], are generally believed not to react with hæm. However, it has now been found that, under suitable experimental conditions, most amino-acids do react with hæm to form typical hæmochromogens, showing only slight differences in the positions of their absorption bands. In the course of these reactions, the absorption bands of dihydroxyl-hæm appear first and are then gradually replaced by those of the amino-acid hæmochromogen. Since urohæm is much more soluble than protohæm, it reacts more easily with amino-acids, especially with histidine, lysine, arginine and glycine, which give good hæmochromogens in 1 per cent sodium carbonate without passing through the intermediary stage of dihydroxyl-urohæm. As the reaction of urohæm with other amino-acids requires the presence of sodium hydroxide, the formation of hæmochromogen is, in these cases, preceded by that of dihydroxyl-urohæm. Of the two dicarboxylic amino-acids, only glutamic acid reacted with urohæm; but neither glutamic nor aspartic acid reacted with protohæm, although tested under a great variety of conditions. A spectrophotometric study of the reactions of protohæm with histidine, lysine and glycine showed that two molecules of each amino-acid were required per molecule of protohæm to form the corresponding hæmochromogen. The dissociation constants (K) at 20° C. were found to be $3{\cdot}4 \times 10^{-3}$ for glycine–, $1{\cdot}98 \times 10^{-3}$ for lysine– and $9{\cdot}27 \times 10^{-4}$ for histidine–hæmochromogen.

These results suggest that in the formation of hæmochromogens by denatured proteins such as globin, it is not only the imidazole groups which take part in the reaction, but also probably the free amino-groups as well. In fact, it is known that whereas a molecule of free native globin will combine with only four molecules of hæmatin or hæm to form methæmoglobin or hæmoglobin respectively, denatured globin combines with as many as twenty-four hæms to form globin hæmochromogen[7,9,10]. Since in a hæmochromogen the iron atom (Fe^{++}) of each hæm combines with two nitrogenous groups, the saturation of a globin molecule with twenty-four hæms implies the presence in the globin molecule of a minimum of forty-eight hæmochromogen-forming groups. Amino-acid analysis of horse globin has shown that the molecule contains eighty-eight basic amino-acid residues[11,12] (including thirty-six molecules of histidine) and six terminal NH_2-groups[13], which may be regarded as providing a total of ninety-four potential hæmochromogen-forming groups. Thus the molecular ratio of these groups to hæm was 94 : 24 or about 4 : 1, half of the groups reacting with hæm. On the other hand, in the case of a free amino-acid such as glycine, histidine or lysine, the molecular ratio of amino-acid to hæm must be about 1,000 : 1 for complete hæmochromogen formation. This clearly indicates that the affinity of hæm for free —NH_2-groups in a protein molecule is much greater than its affinity for these groups in individual amino-acids.

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Note added in proof.—A complete account of the reactions of hæms with cyanide, *iso*cyanide and sodium hydroxide has been given elsewhere[14,15].

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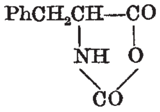
Anhydro-N-Carboxy-DL-β-Phenylalanine

IN view of the increasing importance of anhydro-N-carboxyamino-acids for the synthesis of polypeptides[1,2], it is pertinent to report the following simple preparation of compounds of this type.



I

For example, DL-β-phenylalanine was heated under reflux with a solution of phosgene in toluene (10 per cent w/w) until dissolved, when anhydro-N-carboxy-DL-β-phenylalanine (I) separated in 70 per cent yield on cooling; it melted at 128–129° to a clear liquid which decomposed vigorously at about 155°. This is clearly more convenient than the procedures employed heretofore[3,4].

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Synthesis of 1 : 1 : 1 Trifluoropropyne

THE simple fluorocarbon radicals $CF_3\cdot$ and $CF_3CF_2\cdot$ are obtained by the irradiation of the compounds iodotrifluoromethane and iodopentafluoroethane respectively. These radicals will initiate the polymerization of olefines, and iodotrifluoromethane with ethylene, for example, yields a polymer of general formula $CF_3\cdot[CH_2\cdot CH_2]_n\cdot I$ (where $n = 1, 2, 3$ and 4). With tetrafluoroethylene, longer chain iodofluorocarbons $CF_3\cdot[CF_2\cdot CF_2]_n\cdot I$ ($n = 1$–15) are obtained, and these in turn yield long-chain fluorocarbon radicals, $CF_3(CF_2)_nCF_2\cdot$, when heated or irradiated[1].

The reaction between iodotrifluoromethane and acetylene has now been investigated. A smooth reaction occurred under the influence of ultra-violet light or heat, and the compound 3-iodo 1 : 1 : 1-trifluoropropene, $CF_3CH{=}CHI$, was isolated. This compound formed a solid dichloride which decomposed on standing. Although 3-iodo 1 : 1 : 1-trifluoropropene was the main product from the reaction of iodotrifluoromethane with acetylene, small amounts of material with a higher boiling point were also present, and there is some evidence for the presence of the compound formed by the combination of two molecules of acetylene with one molecule of iodotrifluoromethane (that is, $CF_3CH{=}CHCH{=}CHI$). It therefore appears that the mechanism of the reaction is as follows :

$$CF_3I + h\nu \rightarrow CF_3\cdot + \cdot I$$

$$CF_3\cdot + CH{\equiv}CH \rightarrow CF_3CH{=}CH\cdot \quad \text{(chain initiation)}$$

$$CF_3CH{=}CH\cdot + CH{\equiv}CH \rightarrow CF_3CH{=}CHCH{=}CH\cdot, \text{ etc.} \quad \text{(chain propagation)}$$

$$CF_3CH{=}CH\cdot + CF_3I \rightarrow CF_3CH{=}CHI + CF_3\cdot \quad \text{(chain termination)}$$

The method of chain termination is similar to that proposed for the reaction between iodotrifluoromethane and ethylene and tetrafluoroethylene[1].

The compound 3-iodo 1 : 1 : 1-trifluoropropene has been converted into 1 : 1 : 1 trifluoropropyne, $CF_3C{\equiv}CH$, by dehydroiodination. This fluoroalkyne, which will clearly be of value in synthetic work, is a gas of boiling point *c.* −48°, with an odour reminiscent of that of acetylene. The presence of a triple bond was shown by the addition of two molecules of chlorine to yield $CF_3CCl_2CHCl_2$, a compound which was then further chlorinated to the compound $CF_3CCl_2CCl_3$, identical with a specimen prepared by the vapour-phase chlorination of 1 : 1 : 1 trifluoropropane. Trifluoropropyne affords a copper derivative $CF_3C{\equiv}CCu$, a silver derivative $CF_3C{\equiv}CAg$, and a mercury derivative $(CF_3C{\equiv}C)_2Hg$. The first two acetylides decompose quietly on gentle heating and violently when heated rapidly. The mercury compound is a white crystalline solid of low melting point (42–43° crude), which with iodine yields a compound, probably $CF_3C{\equiv}CI$, with an odour similar to that of diiodoacetylene.

Thus a compound which has a completely fluorinated group adjacent to a reactive triple bond is now available for synthetic work. Its chemistry is being studied and, with full details of its synthesis, will be reported elsewhere.

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A Formate–Carbonate Exchange Reaction Revealed by 'Labelled' Carbon

THE preparation of formates has been examined as part of a programme for the development of reliable syntheses of isotopically labelled organic compounds.

Labelled formates have been obtained in almost quantitative yield by catalytic hydrogenation of bicarbonates[1], in 50–60 per cent yield by the acid hydrolysis of cyanide[2], and by the enzymatic reduction of carbon dioxide by *B. coli* suspensions[3]. We have obtained nearly quantitative yields of formate by the hydrolysis of aqueous solutions of potassium cyanide[4] at 150° for 4 hr., or 175° for 2 hr. The reaction is conveniently carried out in a stainless steel bomb which is shock-heated to the desired temperature in order to minimize the possibility of polymerization. All strengths of solutions up to saturation at room temperature may be used.

Commercial enriched potassium cyanide always contains potassium carbonate, often up to 25 per cent by weight. The presence of carbonate does not affect the yield of formate obtained by hydrolysis of such mixtures ; but the labelled carbon is found to have become distributed between the formate and the carbonate. This is illustrated by the accompanying figures, which refer to the hydrolysis of enriched potassium cyanide in 'Pyrex' glass tubes at 250° for 2 hr.

Nominal atoms % C^{13}	Potassium carbonate content (wt. %)	Atoms % C^{13}			
		CO_3'' before	CO_3'' after	CN' initial	$H.COO'$
16	12·6	1·41	8·1	16·2	15·3
56	22·7	1·76	12·2	53·4	50·8

Preliminary qualitative experiments indicate that the fundamental exchange reaction occurs between formate and carbonate. This is illustrated by the following examples. (1) A solution of 'AnalaR' sodium carbonate and pure labelled sodium formate (15·3 atoms per cent carbon-13), approximately 0·1 *M* with respect to both, was heated in a stainless steel bomb at 250° C. After 2 hr., the carbon-13 content of the carbonate had risen from 1·06 to 2·62, and after 4 hr. to 5·0 atoms per cent. (2) In another experiment a solution of approximately equal weights of carbon-14-labelled sodium carbonate and 'AnalaR' sodium formate (about 50 mgm. per ml. of each) was heated in a 'Pyrex' glass tube at 250° for 4 hr. Barium carbonate precipitated before the experiment had an activity of $4·13 \times 10^{-10}$, and afterwards that of $2·80 \times 10^{-10}$ curies per milligram. (3) It is also perhaps significant that when a solution of carboxyl-labelled sodium acetate (16·3 atoms per cent carbon-13 in the carboxyl group) and 'AnalaR' sodium carbonate (approximately 0·1 *M* with respect to both) was heated at 250° for 5 hr. (stainless steel bomb) there was only a very slight increase in the carbon-13 content of the carbonate (from 1·06 to 1·11 atoms per cent), probably owing to direct decarboxylation.

It was thought that under the minimum conditions for quantitative hydrolysis of potassium cyanide, the exchange reaction might be sufficiently slow as to be unimportant. A mixture of unlabelled potassium cyanide and carbon-14-labelled potassium carbonate containing 20 per cent by weight of the carbonate was therefore hydrolysed at 150° during 4 hr. However, while barium carbonate precipitated from the original solution had an activity of $3·35 \times 10^{-10}$ c./mgm., after the hydrolysis the activity had fallen to $2·49 \times 10^{-10}$ c./mgm. When the carbonate was precipitated by excess baryta before the hydrolysis but left in the solution, the activity at the conclusion of the experiment was $3·02 \times 10^{-10}$ c./mgm. It is therefore necessary to precipitate and remove the carbonate before hydrolysis. After hydrolysis the solution was acidified with excess phosphoric acid and distilled at room temperature in a closed system in high vacuum. Sodium formate was isolated in 98–100 per cent overall yield by neutralization and evaporation of the distillate.

A conceivable mechanism would involve an equilibrium with the symmetrical oxalate ion.

If this is so, there would, in view of the recent work of Lindsay, McElcheran and Thode[5], be expected to