Astbury interprets in general that the outer ring represents the backbone distance of a polypeptide chain and the inner ring the average side-chain distance. Both rings are much sharper in the X-ray diagram of plastein than in that of zein. The Bragg spacings corresponding with the rings are about 4.5 Å and 10 Å in zein and about 4.7 Å and 11.1 Å in plastein in which more accurate measurements can be made. From the interpretations of Astbury regarding his X-ray studies we concluded that our X-ray diagrams are in agreement with the idea of Virtanen and his collaborators, based on chemical facts, that plasteins are polypeptides. One of us (V.) asked Prof. Astbury’s opinion of our photograph. Prof. Astbury kindly replied (5. IV. 1950) and by his consent we publish below an excerpt from his letter.

"Plastein from zein. — This is the common type of diagram from a protein after it has been denatured and aggregated. It results from the unfolding or other disorganisation of the specific configuration, the liberated chains now forming more or less regular bundles in the β-configuration. The two rings are now much sharper because the chains (or portions of chains) are trying to lie as parallel as possible, linked more precisely by the sidechain and backbone linkages. The sharpness of the rings is a measure of the extent to which they succeed in doing this, the two main controlling factors being (1) the degree to which the original specific configuration has been unfolded and the chains have been freed, and (2) the average length of the chains (or portions of chains).

In my view, it follows from this line of argument that the sharpness of your plastein diagram indicates that the plastein chains must be relatively long — certainly much longer than the 6-peptides of the pepsin hydrolysate. I am afraid it is not possible to give a more exact estimate, but I can tell you that the plastein diagram is quite as sharp as many of the diagrams I have obtained recently from synthetic polypeptides made by the Leuchs reaction. The molecular weight of these preparations is believed to be of the order of 5,000, say about 50 residues or perhaps rather fewer, which would correspond roughly to the chain-length you estimate for your plastein preparation.

To summarise, I should say that your plastein diagram is pretty good independent evidence of the presence of fairly long peptides, and is consistent both with your own estimate of the probable chain-length and with my own observations on denatured and aggregated proteins in general and on synthetic polypeptides in particular."

The X-ray diagrams thus confirm the picture obtained from plastein in the light of chemical studies as high-molecular polypeptides which are formed from comparatively low-molecular peptides (e.g. 4—6-peptides) by the action of proteolytic enzyme (pepsin). The result supports the reaction scheme introduced earlier.


Received October 20, 1950.

An Improved Method for Carbethoxylations with Ethyl Carbonate

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A very important method for the preparation of β-keto esters, malonic esters, and α-cyano esters is the carbethoxylation of a ketone, an ester, or a nitrile with the aid of ethyl carbonate and sodium ethoxide, as described by Wallingford and his co-workers 1—3.

The disadvantages of this method is that the preparation of dry sodium ethoxide from sodium and alcohol is a rather cumbersome process, and the reaction
in most cases has to be carried out under reduced pressure. This can easily be avoided, however, by the preparation of sodium ethoxide from ethyl carbonate and sodium, a method that has been known for a long time, but which does not appear to have been used for preparative purposes. Wallingford and his co-workers stated: "Any procedure employing sodium or potassium metal suffers from the fact that these metals readily decompose alkyl carbonates." Evidently they thought that the reaction did not stop with the formation of sodium ethoxide, as is shown by the statement: "The reaction of metal alcohohates with alkyl carbonates to form ethers and metal alkyl carbonates increases in rate rapidly at temperatures above about 100° limiting the extent to which one may force the carboxylation." That this reaction does not proceed to a serious extent is shown by the fact that Widequist has obtained ethyl naphtylecyanacetate and ethyl phenylecyanacetate in excellent yields, by adding sodium in pieces to a mixture of ethyl carbonate and a nitrile, with simultaneous removal of the alcohol formed by distillation at atmospheric pressure.

In the present investigation, sodium ethoxide was first prepared from sodium and ethyl carbonate, and a ketone was then added slowly with simultaneous removal of the alcohol formed. In the cases studied, equal or better yields of the β-keto esters than those reported by Wallingford and his co-workers were obtained, indicating that the cleavage of ethyl carbonate by sodium ethoxide is not a very serious side reaction under these condensation conditions.

Experimental: In a 1-l three-necked, round-bottomed flask, fitted with a dropping funnel, a mercury sealed stirrer, and a 30-cm Widmer column connected to a distillation condenser, was placed 400 ml of dry ethyl carbonate. The flask was placed in an oil bath and heated until gently boiling. The oil bath was then removed and 11.5 g (0.5 mole) of sodium was added in pieces to the hot, stirred ethyl carbonate at such a rate that gentle refluxing was obtained. When all the sodium had dissolved, which was a few minutes after the addition of the last piece, the flask was placed in an oil bath at 140°. The ketone (0.5 mole) was added from the dropping funnel over a period of about one hour. The alcohol began to distill after about one third of the ketone had been added and the theoretical quantity 60 ml (1 mole) was collected. The temperature of the oil bath was then raised until the temperature in the top of of the column increased rapidly to over 120°. The oil bath was then removed and the flask allowed to cool to room temperature. The contents were poured over 35 ml of glacial acetic acid and ice. The ethyl carbonate layer was separated, and the water layer extracted with ether. The combined ether and ethyl carbonate layers were washed with water and then dried over anhydrous sodium sulphate. The ether and ethyl carbonate were removed at reduced pressure and the residue fractionated in vacuo.

By this method the following β-keto esters were prepared: Ethyl isovalerylacetate b.p. 90°/10 mm in 75 % yield, ethyl caproylacetate b.p. 114°/10 mm in 65 % yield, and ethyl benzoylacetate b.p. 144 – 147°/8 mm in 76 % yield.


Received October 28, 1950.