EXPERIENTIA

Vol. XIX – Fasc. 5 Pag. 225–272 15. V. 1963

Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

The Zinc Reduction of Benzoin

One of the standard preparations of stilbene involves the reduction of benzoin by means of a modified Clemmensen procedure[1,2]. This reaction, even though it has been extensively employed in this capacity, is still not clearly understood[3,4]. An investigation of the reaction was initiated in an attempt to clarify the reaction mechanism. In an attempt to isolate intermediates, a chromatographic analysis of the benzoin reduction products was undertaken. This analysis disclosed the presence of trans-stilbene (70%), *cis*-stilbene (5%), benzoin 5(%) and deoxybenzoin pinacol (2%). The expected intermediates, benzyl phenyl carbinol, hydrobenzoin or deoxybenzoin, were not isolated. The reduction of these compounds was also attempted. Under reductive conditions[1], the carbinol and hydrobenzoin did not react; the ketone afforded deoxybenzoin pinacol (75%).

Since the α-substituent to the carbonyl group appears to be a prerequisite for the reaction, it was suggested that information on the reaction may be obtained by varying this substituent. Therefore the reduction of benzoin methyl ether, benzoin acetate and benzoate, and desyl chloride were attempted. In each case, the major product was *trans*-stilbene; the yields of olefin were 83, 72, 80, and 71%, respectively. Thus the reaction appears to be a general reduction for benzoin derivatives.

Our explanation for the absence of intermediates in the reduction is that a zinc complex[5] is reduced directly to the olefin.

Zusammenfassung. Es wird die Reduktionsarbeit des Benzoins beschrieben.

G. E. RISINGER, E. E. MACH, and J. H. GARRETT

Department of Chemistry, Louisiana Polytechnic Institute, Ruston (Louisiana, U.S.A.), January 16, 1963.

[1] D. A. BALLARD and W. H. DEHN, J. Amer. chem. Soc. *54*, 3969 (1932).
[2] R. L. SHRINER and A. BERGER, in *Organic Syntheses*, Coll. vol. 3 (John Wiley and Sons, Inc., New York 1955), p. 786.
[3] L. F. FIESER, *Experiments in Organic Chemistry* (D. C. Heath and Co., Boston 1951), p. 179.
[4] J. H. BREWSTER, J. Amer. chem. Soc. *76*, 6364 (1954).
[5] J. H. BREWSTER, J. Amer. chem. Soc. *76*, 6361 (1954).

Free Amino Acids in *Limnaea* II

It has already been found[1] that the amount of free amino acids in the *Limnaea* eggs (fertilized) increases greatly just before hatching. We could not, however, determine the number of amino acids at this stage, for there was absolutely no chromatographic resolution either on Whatman paper no. 1 or on Whatman no. 4. But it would be interesting to ascertain this point, for it might help us to determine exactly when the adult tissues assume their characteristic pattern of free amino acids.

We have therefore tried a slightly different approach, which, though still imperfect, enables us to assert that the number of free amino acids in the mature egg is not more than two. In order to attain this improved result we have carefully separated the individual eggs from the jelly-like substance in which they are embedded. This can easily be accomplished by cutting and scraping with razor blades. These separated eggs, crushed one after another on Whatman paper no. 1, were chromatographed with the solvent system *n*-Butanol:acetic acid:water=4:1:1. The result was better and comparable with the chromatogram of recently hatched snails. There was a spot of slight intensity just above the origin, apparently starting from the origin itself followed by two more recognizable spots with a very marked trailing or streaky effect. This pattern has been noted in young snails[1] up to 45 days after hatching[2].

The densitometric curve corresponding to the egg chromatogram was obtained from a recording densitometer (Figure 1). Because of the excessive trailing effect one should be cautious in evaluating the result; but we may safely say that there are not more than two free amino acids in any remarkable quantity. In case of eggs 1–2 days before hatching, the Rf values corresponding to amino acids were 0.33 and 0.22 (serine) approximately, in a room of constant temperature of about 26°C.

In our earlier work we were surprised to find such a small number of free amino acids, because, according to

[1] R. L. BRAHMACHARY and A. BHATTACHARYA, Exper. *19*, 143 (1963).
[2] R. L. BRAHMACHARY, unpublished data.

the template hypothesis of protein synthesis, which has been recently established by direct experiments with synthetic RNA or polyribonucleotides, a large number of amino acids should 'at one stroke' be incorporated in the protein. One should therefore expect to detect 'pools' of many free amino acids, and these pools should alter markedly at different phases of protein biosynthesis. (On the other hand, HERRMANN et al.[3] have found evidence of embryonic protein biosynthesis from precursors bigger than amino acids.) A sufficient number of amino acids have been detected only in a few cases, e.g. in echinoid embryos.

We therefore performed acid hydrolysis of adult *Limnaea* (proteins) and detected only six to eight amino acids (Figure 2). However, prolonged (16 h) acid hydrolysis can destroy two particular amino acids[4]. Therefore, at most, the adult *Limnaea* have only ten amino acids. Direct crushing of bigger snails had of course revealed a streaky band of weak colour extending up to higher regions[1] of Rf value. In the present investigation, we once found such an effect even in a small snail (0.50 cm).

The small number of free amino acids is thus partly explained but it is surprising that there are only one or two free amino acids even in the mature egg where the embryo has been largely formed[5].

Résumé. De nouvelles recherches ont amené les auteurs à constater l'existence de seulement 2 ou 3 acides aminés dans les œufs de la Limnée (Gastéropode), un jour avant l'éclosion. Ces acides sont très semblables à ceux que l'on trouve chez les jeunes escargots. Dans les produits hydrolytiques des protéines de l'escargot adulte, on constate la présence de 9 acides aminés.

R. L. BRAHMACHARY and A. BHATTACHARYA

Research and Training School and Gerontology Unit, Indian Statistical Institute, Calcutta (India), October 16, 1962.

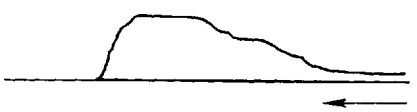

Fig. 1

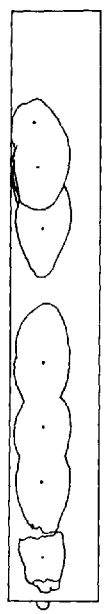

Fig. 2

[3] H. HERRMANN, in *Fundamental Aspects of Normal and Malignant Growth* (Elsevier, 1960), p. 497.
[4] *Biosynthesis of E. Coli* (Carnegie Institution of Washington Publications 1955), p. 27.
[5] We take this opportunity of thanking Dr. P. R. PAL, Dr. J. GHOSH, and Mr. K. K. BOSE for their kind help.

The Effect of Radiation on the Mitosis-Stimulating Activity of Hepatectomized Rat Serum

It has been demonstrated that in the serum of mammals there exists a factor both stimulating and inhibiting mitosis. FRIEDRICH-FREKSA and ZAKI[1] have produced cell division in the liver tissue by administering serum from rats whose livers were just regenerating after partial hepatectomy. ADIBI et al.[2] have demonstrated a considerable increase in the liver mitotic index after having injected serum from the hepatic vein of a hepatectomized rat into another partially hepatectomized rat.

In our experiments we have been studying the effect of X-radiation on the mitosis-stimulating factor in the serum of partially hepatectomized rats. There is still much discussion about the significance of the humoral factor responsible for the lowering of the mitotic activity following irradiation, as well as about the so-called distant effect of radiation, but no conclusive explanation has been established as yet (BACQ and ALEXANDER[3]).

In our experiments we used white Wistar rats, each weighing about 200 g. Partial hepatectomy was performed according to the technique of HIGGINS and ANDERSON[4]. Each group of experimental animals was irradiated 20 h after hepatectomy with a dose of 700 r (180 kV, 15 mA, dose rate 60 r per min, 0.5 mm Cu filter). 24 h after hepatectomy, blood was drawn with a syringe from the hepatic vein below the diaphragm of the animals. The serum was then separated from this blood by centrifugation. The mitosis-stimulating factor was tested on another group of partially hepatectomized animals in such a manner that 1.5 ml of serum was injected intraperitoneally 24 h after operation. Colchicine (0.1 mg per 100 g, Merck) was administered subcutaneously to the rats 18 h after the injec-

The mitotic index and standard error in regenerating liver

Rats were injected with:	Number of animals	Mitotic-index
1.5 ml of physiologic saline	3	1.0 ± 0.34
1.5 ml of serum from irradiated rats	5	3.8 ± 0.21
1.5 ml of serum from non-irradiated rats	5	9.5 ± 0.66

[1] H. FRIEDRICH-FRESKA and F. ZAKI, Z. Naturforsch. *9* b, 394 (1954).
[2] S. ADIBI, K. E. PASCHKIS, and A. CANTAROW, Exper. Cell Res. *18*, 396 (1959).
[3] Z. M. BACQ and P. ALEXANDER, *Fundamentals of Radiobiology*, Chapter 18 (Pergamon Press, Oxford 1961).
[4] G. M. HIGGINS and R. M. ANDERSON, Arch. Pathol. *12*, 186 (1931).