THE SYNTHESIS OF BRADYKININ

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ON March 14, 1960 Dr. D. F. Elliott communicated simultaneously to several research groups, including ours, the following provisional structure for Bradykinin:[1]

H—Arg—Pro—Pro—Gly—Phe—Ser—Phe—Arg—OH

We started at once with the synthesis of this structure and were able to achieve it in a short time. Repetition of this synthesis by two other routes yielded the same final material.[2] All intermediary peptides as well as the final product gave correct elementary and amino acid analyses. They also proved to be pure in all our chromatographic and electrophoretic tests. The schemes of synthesis we had followed excluded any racemization and the action of enzymes proceeded as expected for a peptide of the above mentioned constitution. Nevertheless the synthetic octapeptide proved to be completely devoid of the typical biological activities of bradykinin.[3, 4, 5]

On April 14, 1960, I communicated our negative results to Dr. Elliott. Following his suggestion, we tested the octapeptide immediately after the last step of the synthesis in order to minimize a possible inactivation of the final material for some unknown reasons and tried also to add several inorganic salts, including magnesium salts, in order to allow for the formation of some possible active complexes. But in vain! We also excluded the possibility of the presence of some hypothetical inhibitors by testing a mixture of the synthetic octapeptide with natural bradykinin.

We arrived therefore to the conclusion that the proposed structure was probably partially incorrect. This view was supported by the fact that the behaviour of the synthetic octapeptide toward chymotrypsin differed from the one of bradykinin itself. The synthetic octapeptide was split by chymotrypsin simultaneonsly at the Phe-Ser and at the Phe-Arg bonds, as would be expected for a peptide of this constitution. On the contrary, bradykinin had been found by Elliott *et al.*[1] to be much more rapidly split at the Phe-Arg bond than in any other place of the molecule. Dr. Elliott told us that he was puzzled by the fact that the peptide obtained after a short action of chymotrypsin on bradykinin yielded only phenylalanine on the subsequent action of carboxypeptidase. The presence of Pro between Phe and Ser could have been proposed as an explanation

for these unexpected enzymatic results, but it would then have been in complete contradiction with the combined data obtained by Elliott *et al.*[1] for the Edman's degradation and for the amino acid analyses of bradykinin itself and of the fragments released from it by prolonged chymotryptic digestion.

In presence of this confusing situation, we decided to quiet the torments of our perplexity by devoting ourselves to the synthesis of three alternative structures, which we considered as the most likely, if we admitted that some mistake had occured in the structural work. We were led to the choice of these three structures by the following way of thinking.

We made the assumption that the amino acid composition was correct, for if we should have considered it as incorrect, the number of possible alternative structures would have quite hopelessly increased. We then considered that the sequences of the two first amino acids (H-Arg-Pro-) and of the last two amino acids (Phe-Arg-OH) were correct, for they were proved by several strong independent arguments. We were therefore still facing twenty four possibilities for the sequence of the four remaining residues (Pro, Gly, Phe and Ser) at the middle of the chain. However, we were able to exclude twenty-one of them, as being highly improbable, for each of them was in contradiction with at least two strong independent arguments furnished by the structural work of Elliott *et al.*[1]

This reasoning, which revealed itself later to be partially fallacious, prompted us to the synthesis of the three following octapeptides:

H—Arg—Pro—Pro—Phe—Gly—Ser—Phe—Arg—OH
H—Arg—Pro—Gly—Phe—Ser—Pro—Phe—Arg—OH
H—Arg—Pro—Gly—Pro—Phe—Ser—Phe—Arg—OH

The first and the third of these octapeptides proved completely inactive,[3, 5] but the second one exhibited typical bradykinin-like activities. Those activities however amounted to only about 1/50 of what would have been expected for bradykinin itself.

We communicated at once this first limited but encouraging positive result to Dr. Elliott in a letter dated May 21 and hurried at the same time toward the tempting synthesis of the nonapeptide:

H—Arg—Pro—Pro—Gly—Phe—Ser—Pro—Phe—Arg—OH

This contained one more proline residue than had been found in bradykinin by Elliott *et al.*[1] But on the other side it included in the same molecule the relatively well established left part of the first proposed sequence and the right part of the partially active octapeptide, and was therefore worth trying.

The synthesis of this nonapeptide was accomplished in a few days, for we had already at our disposal a supply of the tetrapeptide containing

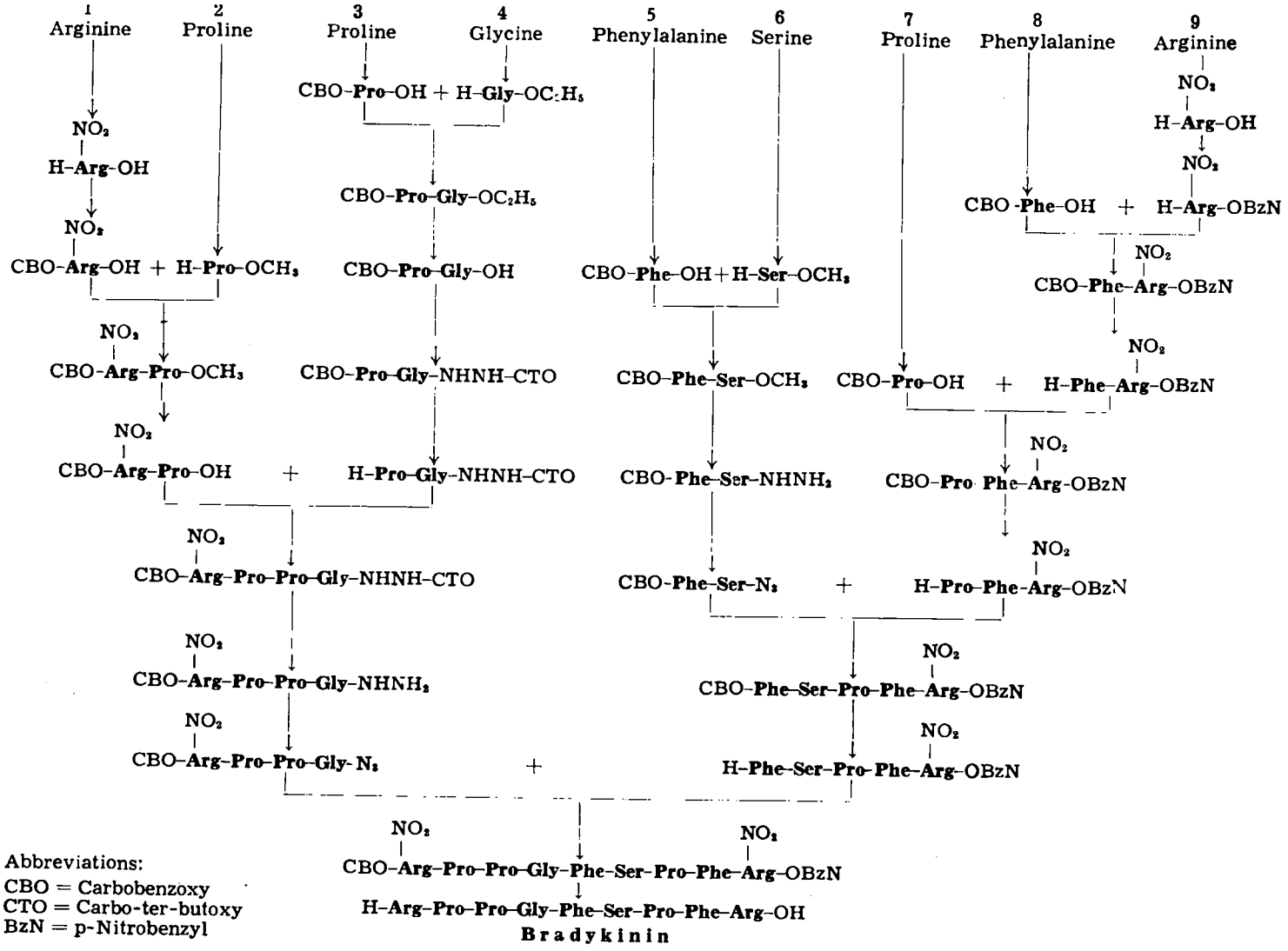

FIG. 1. Scheme of the first synthesis of Bradykinin.

the sequence Arg-Pro-Pro-Gly left from the synthesis of the first postulated octapeptide, and a supply of the pentapeptide with the sequence Phe-Ser-Pro-Phe-Arg left from the synthesis of the partially active octapeptide. The whole scheme of this first synthesis of the nonapeptide[3, 6] is given in Fig. 1. The final product was obtained in an analytically pure state after counter-current distribution. On testing, it showed exactly the same biological properties as bradykinin.[3, 5, 6, 7, 8] Moreover, attack of natural bradykinin and of the synthetic nonapeptide by chymotrypsin yielded exactly the same intermediary and final products.

On June 7, I sent to Dr. Elliott a report with all the evidences for the identity of our synthetic nonapeptide with bradykinin. On July 5, he informed us that he had been able to effectively prove the presence of an additional proline residue between Phe and Ser in natural bradykinin.[9] We acknowledged this information with great satisfaction, for it was the conclusive proof that our synthetic nonapeptide *was* bradykinin.

Thus, starting from a provisional and slightly incorrect octapeptidic structure for bradykinin, we were able, by a method of trial and error quite unusual in organic chemistry, to arrive at the correct nonapeptidic structure. The puzzle would never have been solved so rapidly without the kindness and the openmindness of Dr. Elliott and the close collaboration between our chemical team, which included Dr. Guttmann, Dr. Jaquenoud, and myself, and the pharmacological group composed by Prof. Konzett, and Dr. Stürmer. But luck played also a preponderant part, for it was a happy circumstance that the slightly active octapeptide exhibited just about 1/50 of the activity of bradykinin! Had this activity been higher, we would have mistaken this octapeptide for bradykinin and lost time in fruitless attempts to improve our synthetic methods. Had this activity been lower, we would have neglected it and not been led to the right path.

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