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THE EFFECT OF SODIUM CHLORIDE ON THE *alpha*-CHYMOTRYPSIN CATALYZED HYDROLYSIS OF METHYL HIPPURATE

Sir:

The development of the *p*H-Stat[1,2] has made it possible to observe certain enzyme-catalyzed reactions in the absence of conventional buffers and to thereby determine the effect of added salts upon the rates of such reactions at a constant *p*H and at relatively low ionic strengths. In the course of such studies, it has been observed that the initial rates of the α-chymotrypsin catalyzed hydrolysis of methyl hippurate in aqueous solutions at 25.0° and *p*H 7.90 ± 0.01 and at various initial specific substrate concentrations are markedly dependent upon the presence of added sodium chloride. The nature of this dependency is most readily illustrated by a consideration of the separate dependencies of the constants K_S' and k_3' for the above reaction system upon the concentration of added sodium chloride. It will be seen from Fig. 1 that

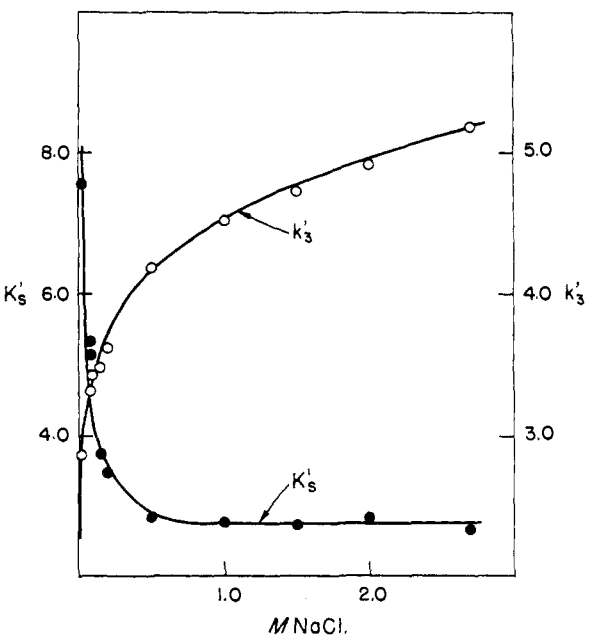

Fig. 1.—Dependence of K's and k'_3 upon concentration of sodium chloride; K's in units of 10^{-3} *M*, k'_3 in units of 10^{-3} *M*/min./mg. protein-nitrogen per ml.

at concentrations of sodium chloride greater than 1 *M* the value of K_S' is essentially constant but as the concentration of sodium chloride is decreased below 1 *M* the value of K_S' begins to increase, slowly and then rapidly, and as the system approaches zero ionic strength the value of K_S' tends to become very large. While the value of k_3' generally decreases with decreasing concentration of sodium chloride, at concentrations below 1 *M* the value of k_3' decreases more rapidly than at concentrations above 1 *M* and as the system approaches zero ionic strength the value of k_3 appears to become very small. Although it is not possible to conduct an experiment in a system of zero ionic strength, it may be inferred from Fig. 1 that in such a system the value of K_S may approach infinity and the value of k_3 may approach zero with the result that no reaction may be observed. The implied inertness of α-chymotrypsin in reaction systems containing no added sodium chloride may be a property of the protein molecule *per se*, or may be due to a transformation of the active enzyme to species that are incapable of combining with the specific substrate. In either case, the addition of sodium chloride leads to the formation of a more active enzyme.

Results similar to but not identical with those summarized in Fig. 1 have been obtained with methyl hippurate and other salts, *e.g.*, lithium chloride, potassium chloride, sodium bromide and magnesium chloride, and with sodium chloride and another ester type of specific substrate, *i.e.*, acetyl-L-valine methyl ester. Therefore, it should be realized that the case involving α-chymotrypsin, methyl hippurate and sodium chloride is not unique but is representative of the general behavior of a number of similar systems.

(1) C. F. Jacobsen and L. Leonis, *Compt. rend. trav. lab. Carlsberg, Ser. Chim.*, **27**, 333 (1951).

(2) J. B. Nielands and M. D. Cannon, *Anal. Chem.*, **27**, 29 (1955).

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THE CONVERSION OF RUSCOGENIN TO 1ξ-HYDROXYPROGESTERONE

Sir:

The isolation of ruscogenin,[1] a steroidal sapogenin from *Ruscus aculeatus L.*, and the recognition that this substance possesses the structure of diosgenin with an additional hydroxyl group,[2] furnished an interesting potential starting material for novel compounds related to physiologically active substances. We wish to report here the synthesis of 1ξ-hydroxyprogesterone.

While it was believed at first that ruscogenin has its second hydroxyl group at C-19,[2] the work of Burn, Ellis and Petrov,[3] as well as subsequent work by Lapin,[4] indicates the hydroxyl to be at C-1. In agreement with these authors, we believe the latter to be the case and wish to offer additional evidence. Authentic (25D)[5] ruscogenin diacetate,

(1) C. Sannié, H. Lapin, F. Eloy and L. Cogolludo Sanchez, *Bull. soc. chim. Biol.*, **39**, 301 (1957), and references listed therein.

(2) C. Sannié and H. Lapin, *Bull. soc. chim. France*, 1552, 1556 (1955).

(3) D. Burn, B. Ellis and V. Petrov, *Proc. Chem. Soc.*, 119 (1957).

(4) H. L. Lapin, *Compt. rend.*, **244**, 3065 (1957).

(5) It is possible to isolate not only the 25D isomer, ruscogenin, but also a 25L compound, neoruscogenin, m.p. of the diacetate 139-141°, from *Ruscus* sapogenins. The two isomers are difficult to obtain completely free from one another. We are indebted to Dr. Lapin for this information.