This work was supported by the National Institutes of Health and the Howard Hughes Medical Institute.

[10] Fellow of the Kettering Foundation, Dayton, Ohio.

BIOPHYSICS RESEARCH LABORATORY OF
THE DEPARTMENT OF MEDICINE
HARVARD MEDICAL SCHOOL AND
PETER BENT BRIGHAM HOSPITAL
BOSTON 15, MASSACHUSETTS
MARVIN MARGOSHES[10]
BERT L. VALLEE

RECEIVED AUGUST 1, 1957

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 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.

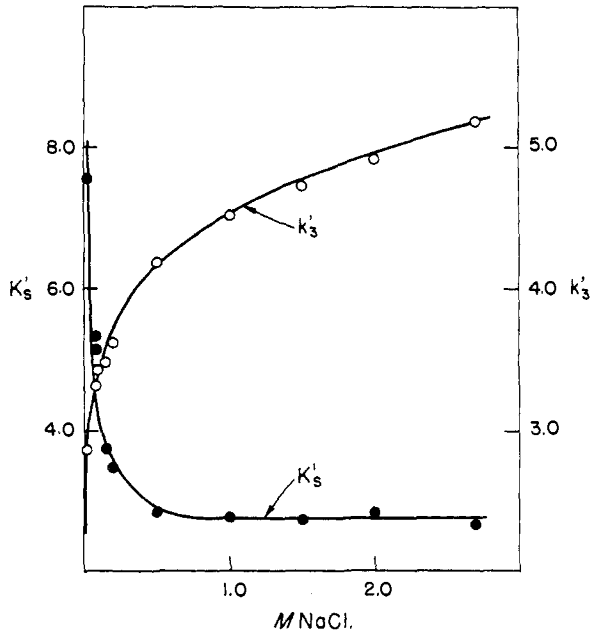

Fig. 1.—Dependence of K'_S and k'_3 upon concentration of sodium chloride; K'_S in units of 10^{-3} M, k'_3 in units of 10^{-3} M/min./mg. protein-nitrogen per ml.

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.

CONTRIBUTION NO. 2227 FROM THE GATES
AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA
R. BRUCE MARTIN
CARL NIEMANN

RECEIVED JULY 18, 1957

[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).

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.

m.p. 199–201°, $[\alpha]^{20}\text{D}$ −82°, kindly furnished by Dr. Sannié, was converted to the $\Delta^{1,4}$-dien-3-one in 20% yield of crystalline material. An infrared spectrum of the mother liquors indicated that they contained approximately another 30% of this material. In contrast to the results obtained by Burn, *et al.*,[3] our substance proved to be identical with the dienone obtained, by selenium dioxide dehydrogenation,[6] from diosgenone.[7] We believe the yield of dienone obtained in our sequence was high enough to preclude its chance origin from an impurity.

Oxidative degradation of the side chain of ruscogenin diacetate,[8] followed by saponification, furnished 1ξ,3β-dihydroxy-$\Delta^{5,16}$-pregnadiene-20-one, m.p. 228–235°, $\epsilon_{240}^{\text{MeOH}}$ 9,100; $\lambda_{\text{max}}^{\text{Nujol}}$ at 2.93, 6.06 and 6.28 μ. Upon hydrogenation in pyridine over palladium-charcoal, one mole of hydrogen was absorbed and there was obtained 1ξ,3β-dihydroxy-Δ^5-pregnene-20-one, m.p. 195–198°, which showed no selective absorption in the ultraviolet between 220 and 300 mμ; $\lambda_{\text{max}}^{\text{Nujol}}$ at 2.96, 3.03 and 5.83 μ, $[\alpha]^{23}\text{D}$ +22.4° (1% in $CHCl_3$). When this substance was subjected to the action of *Flavobacterium dehydrogenans*[9] there was obtained the hydroxylated progesterone, 1ξ-hydroxy-Δ^4-pregnene-3,20-dione, m.p. 155–157°, $\lambda_{\text{max}}^{\text{Nujol}}$ at 2.98, 5.84, 6.01, 6.19 μ; C, 76.58; H, 9.05; $\epsilon_{241}^{\text{MeOH}}$ 15,800. In methanolic alkali at 60°,[10] a shift from 241 to 244.5 m*u* (characteristic of a dienone) was observed. The absence of any selective absorption near 370 mμ[10] under these conditions establishes the formation of a dienone which should result from the dehydration of the proposed structure.

Further 1-hydroxylated hormone analogs are being synthesized, and we are investigating the stereochemistry of this hydroxyl group as well as the relationship of these substances to other 1-hydroxylated steroids.[11,12]

(6) T. Miki and Y. Hara, *Pharm. Bull.* (*Japan*), **4**, 421 (1956).

(7) F. Sondheimer, C. Amendolla and G. Rosenkranz, THIS JOURNAL, **75**, 5932 (1953).

(8) *Inter alia* R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 3592 (1939); D. H. Gould, H. Staeudle and E. B. Hershberg, *ibid.*, **74**, 3685 (1952).

(9) C. Arnaudi, *Zentr. Parasitenk.*, **105**, 352 (1942).

(10) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

(11) W. Schlegel and C. Tamm, *Helv. Chim. Acta*, **40**, 160 (1957).

(12) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog and E. B. Hershberg, THIS JOURNAL, **79**, 3922 (1957).

A. L. NUSSBAUM
F. E. CARLON
D. GOULD
E. P. OLIVETO
E. B. HERSHBERG
M. L. GILMORE
W. CHARNEY

CHEMICAL RESEARCH DEPARTMENT
INDUSTRIAL MICROBIOLOGY DEPARTMENT
SCHERING CORPORATION
BLOOMFIELD, NEW JERSEY

RECEIVED JULY 22, 1957

ELECTRIC PROPERTIES OF MACROMOLECULES. II. EVIDENCE FOR A PERMANENT DIPOLE MOMENT IN THE RIB GRASS STRAIN OF TOBACCO MOSAIC VIRUS

Sir:

A previous investigation of the common strain of the tobacco mosaic virus by means of electric birefringence showed that the large Kerr effect in dilute aqueous solutions is due primarily to an induced polarization, and that the permanent dipole moment contribution is negligible.[1] One indication of this was the fact that reversal of a square polarizing pulse after a steady-state orientation was achieved did not produce a transient in the birefringence. In further studies of various strains, similar behavior has been observed with the Masked and Green Aucuba strains, but a large transient was obtained with the Holmes Rib Grass strain (HR). All experiments were carried out in solutions sufficiently dilute to eliminate complications arising from macromolecular interactions. Figure 1 contains oscillograms illustrating the wave form of the polarizing field and the transient electric birefringence signal of HR.

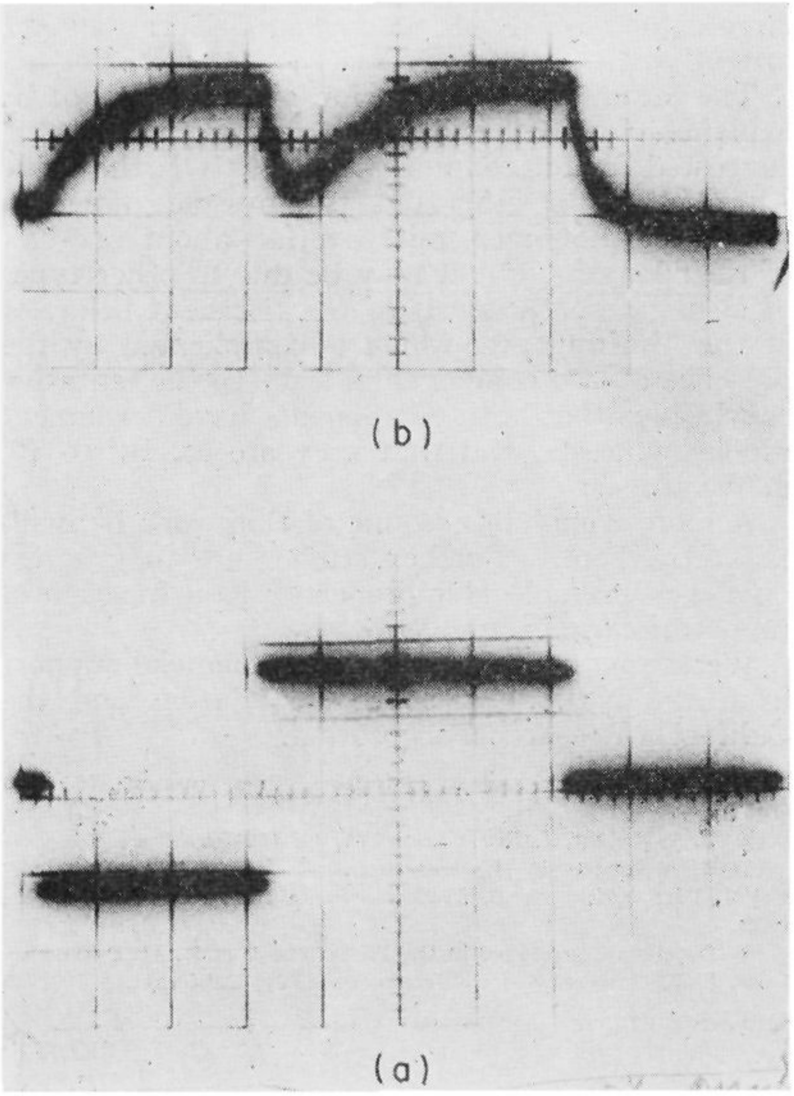

Fig. 1.—Nature of the birefringence transient upon reversal of the field for the Holmes Rib Grass strain of tobacco mosaic virus: (a) oscillogram of the polarizing field; (b) transient response. The central minimum does not occur with the common strain.[1]

The transient upon field reversal was observed with three separate preparations of the Rib Grass strain.[2] Analysis of the field-free decay of the birefringence revealed that each preparation had two relaxation times, corresponding to rotational diffusion constants of 4.8×10^2 and 2×10^2 sec.$^{-1}$. The larger value was assigned to the HR monomer. It is 1.4 times the corresponding value for the common strain, and corresponds to a rod length

(1) C. T. O'Konski and A. J. Haltner (Paper I), THIS JOURNAL, **79**, in press (1957).

(2) Kindly furnished by C. A. Knight of the Virus Laboratory. Prepared by the general method of W. M. Stanley, *ibid.*, **64**, 1804 (1942).