this protein confirm these reports and indicate a relationship between the solubility of the protein and its composition as defined by the electrophoretic patterns obtained at *p*H 4.7.

β-Lactoglobulin was prepared by the method of Palmer[3] which involves the fractionation of milk whey with ammonium sulfate after the removal of casein with acid. The isolated β-lactoglobulin was recrystallized four times by dialyzing away the salt from sodium chloride solutions at the isoelectric point. Electrophoretic studies on a 1% solution of this material in acetate buffer of ionic strength 0.1, *p*H 4.8, showed the presence of a two component system. The same preparation was electrophoretically homogeneous on the alkaline side of the isoelectric point.

Fractionation experiments on the whey proteins from the filtrate after the partial removal of crystalline β-lactoglobulin gave crystalline fractions with a mobility comparable to that of the usual β-lactoglobulin at *p*H 8.3. Electrophoresis *p*H 4.8, however, indicated a variation in the concentration of the components in each of the fractions. These new crystalline fractions also varied from the standard preparation in that they showed a greater solubility in water and in dilute salt solutions.

Crystallization of the standard preparation by dialysis from an acetate buffer solution, varied with respect to *p*H, gave fractions with a partial separation of the components as indicated electrophoretically. Partial separation of β-lactoglobulin has also been obtained by fractionation with alcohol at low temperatures. The fractions obtained by alcohol have properties in agreement with the data reported in the table below for preparations obtained by the other methods. These results will be reported subsequently.

The solubility experiments were made as described by Grönwall.[1] In every case a suspension of the crystalline material containing 7.6 ± 0.1 mg. protein nitrogen per ml. was equilibrated for a twenty-four hour period. The protein nitrogen was then determined on the supernatant liquid after centrifugation. The table illustrates the variations in the electrophoretic components of a number of preparations at *p*H 4.8 and the solubility of these crystalline fractions at *p*H 5.2 ± 0.1.

Preparation	% Composition, $\mu \times 10^5$		Solubility (mg. N/cc.) at 25°	
	1.4–1.5	2.2–3.2	in H_2O	in 0.02 *M* NaCl
B[a]	28	72	0.08	1.1
A[a]	38	62	.10	1.3
Standard	40	60	.12	1.5
K[b]	46	54	.18	1.7
L[b]	54	46	.13	1.6
M[b]	59	41	.19	1.9

[a] Preparations made from the standard preparation by means of acetate buffer of varying *p*H. [b] From filtrate of standard β-lactoglobulin preparation.

The variation in solubility in salt solutions of preparations with different electrophoretic compositions is an adequate explanation for the divergent solubility data reported by Palmer[3] and Grönwall.[1]

EASTERN REGIONAL RESEARCH LAB. T. L. MCMEEKIN
U. S. DEPARTMENT OF AGRICULTURE B. D. POLIS
AGRICULTURAL RESEARCH ADMINIS. E. S. DELLAMONICA
PHILADELPHIA 18, PA. J. H. CUSTER

RECEIVED DECEMBER 15, 1947

---

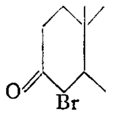
[3] Palmer, *J. Biol. Chem.*, **104**, 359 (1934).

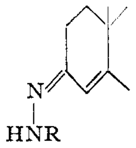
THE PREPARATION OF 3-KETO-Δ^4-STEROIDS

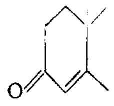
Sir:

For the introduction of a double bond at $C_4 : C_5$ in 3-ketosteroids hydrogen bromide has been eliminated from the 4-bromo derivative by treatment in boiling pyridine.[1] However, the yields have not been good. A new method for dehydrobromination has been found in which the reactivity of the bromine at C_4 is greatly increased through formation of a hydrazone at C_3. For a model compound, methyl 3,11-diketo-12-bromocholanate[2] was brominated to give methyl 3,11-diketo-4,12-dibromocholanate (I), m. p. 157.5–158.5°, $[\alpha]_D$ +13 ± 2° (*c* 0.96 in chloroform). Calcd. for $C_{25}H_{36}O_4Br_2$: C, 53.58; H, 6.48; Br, 28.52. Found: C, 53.56; H, 6.69; Br, 28.5.

When 2,4-dinitrophenylhydrazine (1.2 equivalents) in the absence of molecular oxygen was added to an acetic acid solution of I which contained 5 equivalents of sodium acetate, a hydrazone was formed and hydrogen bromide was eliminated to give methyl 3,11-diketo-12-bromo-Δ^4-cholenate-3-(2,4-dinitrophenylhydrazone) (II). Hydrogen bromide also was eliminated quantitatively without the use of sodium acetate. Red needles, m. p. 238–239°; $\lambda_{max.}$ 387 mμ, log ϵ 4.48 (chloroform); yield, 82%. Calcd. for $C_{31}H_{39}O_7BrN_4$: C, 56.45; H, 5.96; Br, 12.12. Found: C, 56.21; H, 5.89; Br, 11.93.


I


II


III

The hydrazone (II) was converted into III by treatment in 20 cc. of chloroform, 30 cc. of pyruvic acid and 2.2 cc. of 2.3 *N* hydrogen bromide in acetic acid at 45°. After two and one-half hours the pyruvic acid 2,4-dinitrophenylhydrazone was removed with aqueous sodium bicarbonate, any C_{24}-carboxyl was esterified and III was separated in about 90% yield. The product was identical with a sample of III prepared by dehydrobromination of I in pyridine, m. p. 190–191°, $[\alpha]_D$ +29 ± 2° (*c* 1.00 in chloroform); $\lambda_{max.}$ 238 mμ, log ϵ 4.22 (methanol). Calcd. for $C_{25}H_{35}O_4Br$: C,

---

[1] Adolf Butenandt und Josef Schmidt, *Ber.*, **67**, 1901 (1934).

[2] R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

62.62; H, 7.36; Br, 16.67. Found: C, 62.44; H, 7.57; Br, 16.77.

A solution of hydrazone II is autoxidizable and should be protected from molecular oxygen with an inert gas. Before autoxidation of the unsaturated hydrazone was recognized dehydrobromination of the 4-bromo intermediates to give the adrenal hormones dehydrocorticosterone acetate (IV) and 17-hydroxydehydrocorticosterone acetate (V) was carried out. The yields of bromide ion, unsaturated hydrazone and IV were 96, 59 and 80% respectively. Slightly lower yields of V were obtained. The preparation of IV and V under more favorable conditions is planned as soon as the essential intermediate compounds again become available.

For the precursors of the adrenal hormones we are indebted to Merck and Co., Inc., Rahway, New Jersey.

DEPARTMENT OF BIOCHEMISTRY
MAYO FOUNDATION
ROCHESTER, MINNESOTA
VERNON R. MATTOX
EDWARD C. KENDALL

RECEIVED JANUARY 19, 1948

VALENCE INDUCTIVITY

Sir:

Experiments conducted in this Laboratory on supported transition group oxides have led to what appears to be a new general principle in inorganic chemistry.

The effect was first observed in supported oxides of manganese. Thermal decomposition of manganous nitrate is well known to yield manganese dioxide. But if the manganous nitrate is first impregnated on high-area *gamma*-alumina, the oxidation state of the manganese becomes dependent on the concentration of the manganese. On an alumina with area of about 200 sq. m. per g., and with a manganese concentration less than 5%, all the manganese is in the +3 oxidation state. At higher concentrations the manganese reverts increasingly to the +4 state. The oxidation state is established both by direct titration, and by measurement of the magnetic moment.

This observation suggests that the manganese oxide tends to assume the crystal structure of the alumina, even to the extent of changing oxidation state in order to do so. It is well known that the *gamma* form of manganese(III) oxide is isomorphous with *gamma*-alumina.

Confirmation of this view is obtained by supporting the manganese on a high area (94 sq. m. per g.) rutile, which is isomorphous with pyrolusite. In this case the oxidation state of the manganese remains at +4, even at very low concentrations.

The experiments leading to the above results were performed by Marylinn Ellis and Kathryn Wethington. The effect described is strikingly shown by supported nickel in experiments performed by Fred N. Hill.

Magnesia is isomorphous with nickel(II) oxide. Supported nickel prepared by impregnation and ignition of high area magnesia is all in the +2 oxidation state. But dilute nickel oxide supported on high area alumina has the nickel in the +3 oxidation state. This state is established by direct quantitative uptake of hydrogen during reduction, and by magnetic measurements. It has proved possible to obtain up to 10% of nickel, all in the +3 state, by using multiple impregnation. This supported oxide is pale blue in color.

On the other hand, copper supported on alumina shows no tendency to assume the +3 state, nor does supported silver. It appears, therefore, that the effect is shown only when the supported positive ion may fairly readily assume a charge and radius similar to that of the positive ion in the support.

The effect illustrated for manganese and nickel may be described as a leading of the supported ion to take a different oxidation state than it would normally assume. Following a suggestion by Prof. Robert L. Burwell, Jr., we offer "valence inductivity" as a name for this effect. The general principle illustrated may be described as an induced change of valence brought about when a transition group ion is supported on a high area surface with which it may become isomorphous, and in which the ions may become isometric. The effect is somewhat related to the phenomenon of oriented overgrowth. It may be expected to aid in the understanding of several problems in catalysis and promoter action, and to be useful in the fields of mineralogy, crystallography, and coprecipitation.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS
P. W. SELWOOD

RECEIVED JANUARY 29, 1948

EXCHANGE REACTION BETWEEN FERRIC AND FERROUS IONS IN PERCHLORIC ACID USING A DIFFUSION SEPARATION METHOD

Sir:

Equilibrium exchange was found to occur by Nahinsky in Professor Ruben's laboratory[1] when ferrous and tagged ferric ions mixed in perchloric acid solution were immediately separated by a chemical method. In a chemical separation of the ions, other ionic or molecular species formed through chemical change during the separation may exchange. For the perchlorate system, for example, ether–hydrochloric acid extraction of iron (III) leads to erroneous conclusions, because in 6 *M* hydrochloric acid ferrous and ferric iron exchange rapidly.[2] If separation is achieved by a physical method, without changing the environment of the ions, exchange incidental to chemical changes during separation may be avoided. Since catalytic effects of trace impurities and surfaces cannot be excluded, the results must still be interpreted cautiously.

---

(1) Nahinsky, Ph.D. Thesis, University of California, 1942.

(2) Seaborg, *Chem. Rev.*, **27**, 256 (1940).