Summary

1. The dehydration of tertiary alcohols containing a neopentyl system has been studied with relation to rearrangement.

2. In no case was the rearrangement greater than 25%.

3. No rearrangement took place during the dehydration of methylethylneopentylcarbinol.

4. In the case of methyldi-*tert*-butylcarbinol no rearrangement products were found. Over 90% of the normal dehydration product was isolated.

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The Isoelectric Point of Orange Seed Globulin

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A crystalline protein was first isolated from California orange seed in 1931 by Saunders.[1] He obtained the protein in a crystalline form by dialysis of ammonium sulfate solutions of the protein and named it pomelin. The writers have been unable to obtain a crystalline product from Florida or California orange seed by Saunders' method, the product being in all cases in our experience an amorphous mass instead of definite crystals. However, the writers have been successful in preparing a crystalline globulin from orange seed by an adaptation of the method used by Vickery, Wakeman, and Leavenworth[2] for the extraction of the globulin from tobacco seed and its preparation in crystalline form.

Csonka, Murphy and Jones[3] record the isoelectric point of a number of proteins as deduced from data on minimum solubility. A large amount of their data deals with various plant globulins for which the various individual isoelectric points range between *P*H's 5.0 and 5.5.

The point of electrical neutrality of a colloid may be determined in either of two ways, *i. e.*, by the moving boundary method described by Tiselius[4] in which the movement of a column of colloidal particles under a known potential is measured, or the somewhat different method[5] in which the rate of movement of the individual colloidal particle or an aggregate is observed under the microscope. In many cases a substance such as powdered quartz has been added to a sol on which the colloid was supposedly adsorbed and which powder then behaved as the colloid itself.[6]

From the Helmholtz–Lamb equation $V = \zeta HD/4\pi h$, where V is velocity

(1) Saunders, THIS JOURNAL, **53**, 696 (1931).
(2) Vickery, Wakeman and Leavenworth, *Conn. Agr. Expt. Sta. Bull.*, [II] **339**, 625 (1932).
(3) Csonka, Murphy and Jones, THIS JOURNAL, **48**, 763 (1926).
(4) Tiselius, *Nova Acta Reg. Soc. Sci. Upsaliensis*, Series IV, **7**, No. 4 (1930).
(5) Northrup, *J. Gen. Physiol.*, **4**, 629 (1922).
(6) Freundlich and Abramson, *Z. physik. Chem.*, **133**, 51 (1928).

of particle, ζ is electrokinetic potential, H is potential gradient per cm., D is dielectric constant of the dispersion medium, and h is viscosity of the medium. In the present paper the values for D and h have been assumed to be those for water, namely, 81 and 0.009, respectively, at 25°. All quantities in the above equation are expressed in c. g. s. electrostatic units.

Preparation of Material.—Whole seeds of the California orange were coarsely ground in a Wiley mill and extracted repeatedly with cold benzene to remove oil, then air dried and ground to pass a one-millimeter sieve. The protein was extracted from the fat-free meal by warm (55°) molar sodium chloride solution in the proportion of one liter of solvent per 100 g. of meal. The mixture was stirred occasionally and, after two hours standing, the liquid was removed from the undissolved matter in a centrifuge. The somewhat turbid centrifugate was filtered through an asbestos and paper pulp filter, after which treatment it was sparkling clear and amber in color. The filtrate was diluted with five volumes of water at 55° and again filtered through asbestos and paper pulp. Toluene was then added as a preservative and the filtrate allowed to stand twenty-four to forty-eight hours at 0°. The protein crystallized out under this procedure in microscopic octahedral crystals which were separated from the mother liquor by centrifuging.

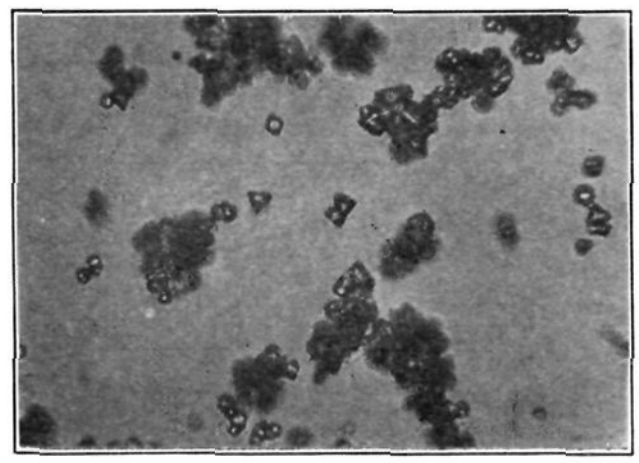

Fig. 1.—Crystalline globulin of orange seed (× 480).

The crystalline protein was redissolved in the least quantity of warm molar sodium chloride solution required, filtered as mentioned before, and again precipitated by dilution, this time adding ten volumes of water at 55° instead of five volumes to effect crystallization. The diluted mixture was allowed to stand overnight at 0° for crystallization of the protein. The crystalline precipitate was separated in a centrifuge, suspended in distilled water and electrodialyzed between parchment membranes to remove the last traces of sodium chloride. After the first crystallization, solutions of the protein in salt solution were colorless. Whether the writers' crystalline preparation is a single chemical entity or not and whether it is the same protein obtained from orange seed by Saunders cannot be judged at present. In Fig. 1 is shown a photomicrograph of one of our preparations.

A portion of the crystalline protein was removed before electrodialysis and washed with successive portions of ethyl alcohol of increasing concentrations, finally with ether and then dried in a desiccator. No change in structure could be observed in the crystals under this treatment. For the electrophoretic work to be described, however, electrodialyzed material which had not been alcohol treated or dried was used.

Electrophoresis Experiments.—The solutions of protein were prepared by dissolving about one gram of protein in 100 cc. of each of a series of buffer solutions ($M/30$ after Sörensen) containing the required amount of the proper citrate or phosphate, respectively, to give the desired P_H value over the range P_H 1.5 to 7.8. The actual P_H of each protein solution was determined potentiometrically with the quinhydrone electrode. After preparation the protein solutions were stored a few days at 0° with toluene until the electrophoresis measurements could be completed.

TABLE I

ELECTROPHORETIC VELOCITY AND CALCULATED ζ-POTENTIAL OF ORANGE SEED GLOBULIN

P_H	Velocity μ/sec./v/cm.	Calcd. ζ-potential, millivolts	P_H	Velocity μ/sec./v/cm.	Calcd. ζ-potential, millivolts
1.47	+8.2	+103.0	5.43	−2.2	−27.6
1.67	+7.6	+ 95.5	5.57	−2.2	−27.6
1.69	+8.0	+100.5	5.72	−3.7	−46.5
1.71	+7.8	+ 98.0	5.87	−4.8	−60.3
2.00	+7.8	+ 98.0	5.89	−4.9	−61.6
2.43	+7.3	+ 91.7	5.90	−5.2	−65.3
2.84	+6.3	+ 79.2	6.18	−5.1	−64.1
3.36	+6.1	+ 77.6	6.73	−5.4	−67.9
4.53	+5.2	+ 65.3	7.61	−5.7	−71.6
5.02	+1.8	+ 22.6	7.70	−6.3	−79.2
5.32	−0.7	− 8.8	7.76	−6.7	−84.2

The cell used for the electrophoresis work has been described by Northrup.[5] Velocity observations were always made at the same place in the cell, using a micrometer eyepiece. All measurements recorded for a given P_H are the mean of ten or twelve closely agreeing observations in which the current direction was repeatedly reversed.

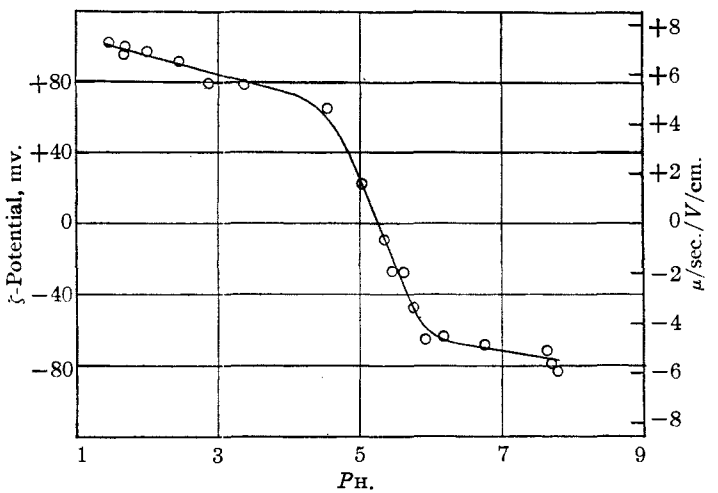

Fig. 2.—ζ-Potential and migration velocity of orange seed globulin.

The data are given in Table I and are presented graphically in Fig. 2. Interpolated from the graph, the isoelectric point of the protein is at P_H 5.23 or C_H 5.9 × 10^{-6}.

Summary

1. The electrophoretic velocity of orange seed globulin has been measured in citrate and phosphate buffer solutions in the P_H range 1.5 to 7.8 and the electrical charge on the particle calculated.

2. The isoelectric point of orange seed globulin was found to be at P_H 5.23 or C_H 5.9 × 10^{-6}.

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