acid-catalyzed conditions which have proved effective with 17α-hydroxy-20-keto steroids,[31] but only unreacted starting material was recovered.

(31) *Cf.* Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, THIS JOURNAL, **74**, 5394 (1952); R. B. Turner, *ibid.*, **75**, 3489 (1953).

Acknowledgment.—We should like to acknowledge the benefit of a stimulating discussion with Prof. Gilbert Stork of Columbia University.

DETROIT, MICHIGAN

COMMUNICATIONS TO THE EDITOR

THE RESOLUTION OF O-ETHYL ETHYLPHOSPHONOTHIOLIC ACID

Sir:

We wish to record the successful resolution of O-ethyl ethylphosphonothiolic acid ($C_2H_5(C_2H_5O)P(O)SH$).[1] The compound was resolved by fractional recrystallization of its quinine salt (I) from acetone–ether. The more insoluble diastereoisomeric salt (Ia) crystallized as a monohydrate: prisms, m.p. 151–153° (with loss of its water of hydration), $[\alpha]^{26}D$ −96.6 ± 0.8° (α_{obs} −1.990 ± 0.015°, acetone, 2-dcm., c = 1.130), equiv. wt., 492 (calcd. 497 for the monohydrate). When vacuum dried over phosphorus pentoxide for three hours at 100°, Ia gave rise to anhydrous product: m.p. 158–160°, $[\alpha]^{33}D$ −97.6 ± 0.6° (α_{obs} −1.070 ± 0.007°, acetone, 1-dcm., c = 1.096), equiv. wt., 469 (calcd. 479).

The acid was separated from Ia as its sodium salt in an essentially aqueous solution by treating Ia in methanol with an equivalent amount of aqueous base. That the phosphorus atom maintains its tetrahedral configuration in the anion is demonstrated by the optical activity of the product recovered from the sodium salt. The acid was recovered by the addition of an equivalent amount of dilute hydrochloric acid to the sodium salt solution and extraction of the product from the resulting solution with ether. The acid was characterized as its dicyclohexylamine salt: m.p. 159–160.5° $[\alpha]^{25}D$ −7.11 ± 0.23° (α_{obs} −0.153 ± 0.005°, methanol, 1-dcm., c = 2.150), found: C, 57.38; H, 10.00 (calcd. for $C_{16}H_{34}O_3NP$: C, 57.28; H, 10.22).

After the removal of a mixed middle crop of I, the more soluble diastereoisomeric salt (Ib) crystallized as soft anhydrous needles: m.p. 166–168°, $[\alpha]^{25}D$ −81.7 ± 0.6° (α_{obs} −1.613 ± 0.012, acetone, 2-dcm., c = 0.9868); equiv. wt., 475 (calcd. 479). The dicyclohexylamine salt of this enantiomorph of the acid gave m.p. 158–160°, $(\alpha)^{25}D$ +6.85 ± 0.25° (α_{obs} + 0.221 ± 0.008°, methanol, 1-dcm., c = 3.230), found: C, 57.30, H 10.02, mixed melting point with the enantiomorphic dicyclohexylamine salt, above: 163–165°. Racemic O-ethyl ethylphosphonothiolic acid forms a dicyclohexylamine salt, m.p. 166–168°.

This communication represents the first reported resolution of a phosphorus acid, the optical activity of which is due solely to the presence of an asymmetric phosphorus atom. Moreover, the presence of a reactive group directly attached to phosphorus in a resolved compound of this type provides one with a convenient tool applicable to a study of the reactions and stereochemistry of the asymmetric phosphorus atom. Detailed reports on the resolution, reactions and stereochemistry of this and similar compounds will be published at a later date.

(1) The preparation of alkylphosphonothiolic acids will be described in a forthcoming paper by F. W. Hoffmann and co-workers.

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RECEIVED MAY 21, 1956

ELECTROPHORETIC DEMONSTRATION OF THE ISOMERIZATION OF BOVINE PLASMA ALBUMIN AT LOW *p*H

Sir:

Recently much interest has been exhibited in a pronounced conformational change which takes place in bovine plasma albumin at *p*H values acid to the isoelectric point. It was first suggested by Tanford[1] that expansion of the protein molecule results upon titration with acid. Gutfreund and Sturtevant[2] demonstrated a slow thermal effect upon adding acid to this protein. Yang and Foster[3] demonstrated a parallel and reversible enhancement of the optical rotation and intrinsic viscosity acid to *p*H 4, and suggested that there exists an all-or-none equilibrium between two forms of the protein molecule. Tanford[4] has recently shown evidence for an intermediate which he terms the "expandable" form.

We have recently been successful in attaining excellent resolution of two boundaries in the electrophoretic patterns of this protein over the *p*H range 4.6 to 3.5. Heterogeneity of plasma albumins in this *p*H range has been reported previously.[5-8] However, we can now demonstrate that this heterogeneity is due in the main to a *p*H dependent transition of the normal form of the protein into a faster migrating form, presumably of higher positive charge. Results summarized in

(1) C. Tanford, *Proc. Iowa Acad. Sci.*, **59**, 206 (1952).

(2) H. Gutfreund and J. Sturtevant, THIS JOURNAL, **75**, 5447 (1953).

(3) J. T. Yang and J. F. Foster, *ibid.*, **76**, 1588 (1954); **77**, 2374, 3895 (1955).

(4) C. Tanford, J. Buzzell, D. Rands and S. Swanson, *ibid.*, **77**, 6421 (1955).

(5) J. Luetscher, *ibid.*, **61**, 2888 (1939).

(6) D. Sharp, G. Cooper, J. Erickson and H. Neurath, *J. Biol. Chem.*, **144**, 139 (1942). In this paper it was also shown that heterogeneity disappears below *p*H 3.5, in accord with our own results.

(7) R. Alberty, *J. Phys. Chem.*, **53**, 114 (1949).

(8) L. Longsworth and C. Jacobsen, *ibid.*, **53**, 126 (1949).

the table show clearly that the ratio of fast to slow forms increases regularly with decreasing *p*H. In fact, quantitative analysis of the composition *vs.* *p*H data indicate almost perfect agreement with an equilibrium which is third order in hydrogen ion concentration. This same *p*H dependence was found previously for the optical rotation, though the inflection occurred almost one *p*H unit lower, a result which may be due to the difference in ionic strength or temperature.[3]

TABLE I

SUMMARY OF RESULTS

*p*H	Percentage composition Slow	Fast	Mobilities, cm.²/volt·sec. × 10⁵ Slow	Fast
3.42	0	100		+9.7
3.62	19	81	+7.8	+8.6
3.75	23	77	+6.6	+7.4
3.82	23	77	+5.2	+6.6
3.90	27	73	+4.5	+5.7
4.00	60	40	+3.5	+5.3
4.11	64	36	+2.5	+4.1
4.16	67	33	+2.3	+3.7
4.30	86	14	+1.2	+2.6
4.55	94	6	−1.0 −2.6	+0.8

Electrophoretic resolution indicates the equilibrium reaction to be relatively slow. However, variation of the dialysis time from 19 to 75 hours yielded no trends in composition. The half-life thus cannot exceed a few hours at the temperature of electrophoresis and dialysis (0°). (Very similar, though less complete, results have been obtained at 0° in 0.1 molar chloride, and in 0.02 molar chloride at 25°.)

Electrophoretic analyses were conducted in HCl–NaCl systems of constant ionic strength 0.02. The protein concentration employed was only 0.2% under which conditions the ascending and descending boundaries were reasonably enantiographic, two boundaries of roughly similar area and mobility appearing in both limbs. Some experiments were conducted at 0.05% protein yielding the same qualitative results; however, lack of sensitivity in the Schlieren optical system prohibited quantitative analysis of the composition.[9] At concentrations much above 0.2% enantiography is lost.

The protein preparation employed[10] exhibited relatively little electrophoretic inhomogeneity above *p*H 4.6, being the best in this regard of some half-dozen samples tested. At *p*H 4.6, the main peak showed definite evidence of a split into two components, in agreement with previous findings of three components in the isoelectric region.[7] As noted in the table the "slow" and "fast" forms actually have opposite sign of charge at this *p*H.

The observations reported here should open the way to a much more detailed elucidation of the peculiar low *p*H behavior of this protein. Results of such studies, together with a more detailed exposition of the results outlined here, will appear in due course.

(9) All electrophoretic analyses were performed in a Perkin–Elmer instrument equipped with scanning camera.

(10) Pentex, Lot A 1201. Ultracentrifugal analysis indicated the presence of a few per cent. of a faster sedimenting component, presumably aggregated material.

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THE EFFECT OF ION BINDING ON PROTEIN STRUCTURE. I. INFLUENCE OF ACETATE ON THE ELECTROPHORETIC BEHAVIOR OF SERUM ALBUMIN[1]

Sir:

We wish to describe a new effect of acetate buffer on the structure of proteins. This effect is illustrated by experiments which show that the electrophoretic behavior of bovine serum albumin (BSA) depends upon the concentration of acetate buffer (NaAc–HAc) in the supporting medium. Representative electrophoretic patterns are shown in Figs. 1 and 2. Although these patterns are complex, it is clear that substitution of NaAc–HAc for NaCl in the supporting medium results in an increase in the area under the faster moving bound-

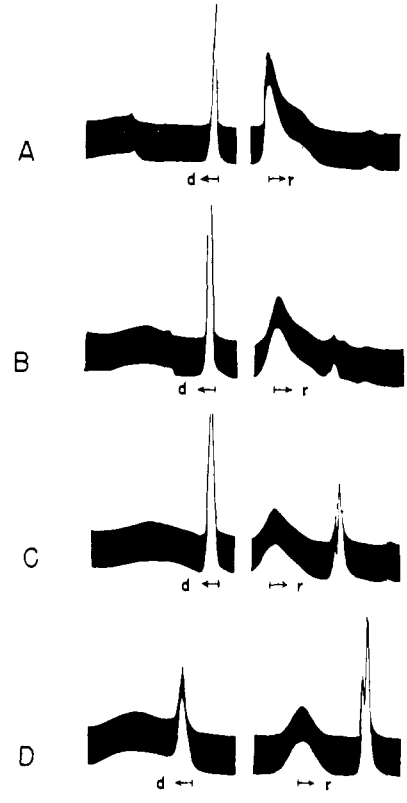

Fig. 1.—Electrophoretic patterns of Armour bovine serum albumin in various buffer solvents at *p*H 4.7: A, 0.002 ionic strength NaAc–HAc + 0.023 ionic strength NaCl; B, 0.005 NaAc–HAc + 0.020 NaCl; C, 0.010 NaAc–HAc + 0.015 NaCl; D, 0.025 NaAc–HAc. Protein migrated toward the cathode. The product of field strength and time is approximately the same in all these experiments.

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