progesterone was dissolved in 3 ml. of alcohol and 0.3 ml. of hydrochloric acid was added. The solution was refluxed for one-half hour, then concentrated *in vacuo* to give an oily residue which was dissolved in a few drops of acetone. Refrigeration at −10° overnight gave crystals, m.p. 162–168°, which were recrystallized once from acetone–ether, m.p. 164–167°. Infrared spectrum and optical rotation, $[\alpha]^{23}D$ +170°[10] showed this compound to be identical with 11α-hydroxyprogesterone.

Acknowledgment.—We wish to express our thanks to the following people of the Upjohn Research Division for the advice and coöperation in connection with this problem: *viz.*, to Dr. J. L. Johnson, Mrs. G. S. Fonken and Mr. L. Scholten for the spectrographic data, to Mr. W. A. Struck and his associates for all rotations and microanalyses and to Misses Jennie I. Mejeur, Irene N. Pratt and Mr. Glenn Staffen for technical assistance. The authors are indebted to Drs. R. H. Levin and D. I. Weisblat for their helpful and stimulating interest.

(10) D. H. Peterson and H. C. Murray, ref. 1a report $[\alpha]^{23}D$ +179° for pure 11α-hydroxyprogesterone.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

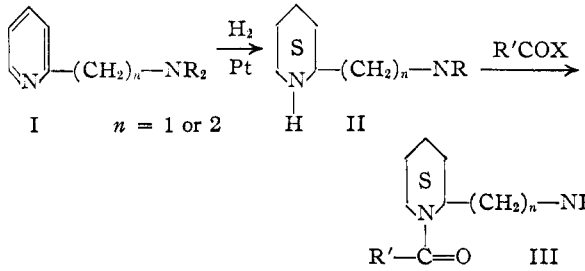
The Preparation of 1-Acyl-2-dialkylaminoalkylpiperidines[1]

BY ARMIGER H. SOMMERS, MORRIS FREIFELDER, HOWARD B. WRIGHT AND ARTHUR W. WESTON

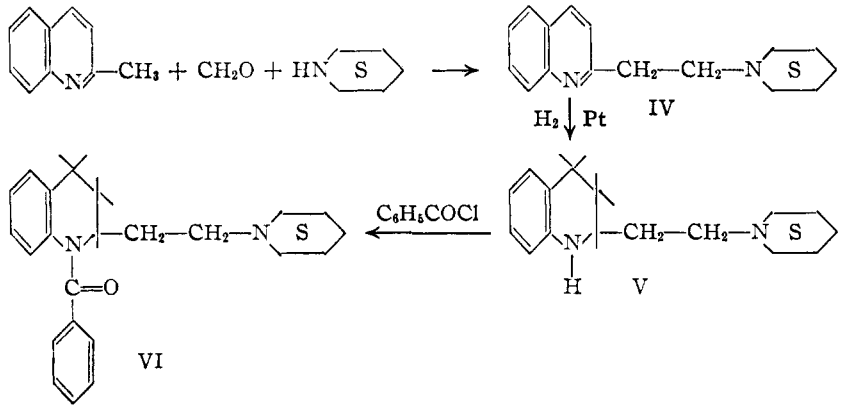
RECEIVED AUGUST 1, 1952

The synthesis of seventeen basic amides of structure III is described. The intermediate diamines were prepared by catalytic hydrogenation of the Mannich type 2-substituted pyridines (I), and by chemical and catalytic reduction of 1-(2′-picolinoyl)-piperidine (VII). The majority of the diamines were acylated by the Schotten–Baumann method.

The local anesthetic activity of 1-benzoyl-2-(β-N-piperidylethyl)-piperidine (III, n = 2, R′ = C_6H_5, NR_2 = N-piperidyl), which was prepared as an intermediate in another program, prompted the synthesis of a series of related basic amides by the reactions shown.

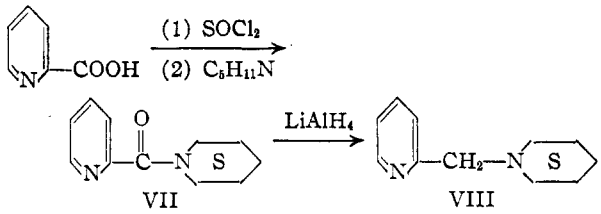


The addition of secondary cyclic amines to 2-vinylpyridine[2] provides, for certain compounds of structure I (n = 2), a synthesis more convenient than the Mannich reaction involving 2-picoline.



Good yields of the desired products were obtained by heating 2-vinylpyridine with piperidine,[2] morpholine and 1-methylpiperazine. Diethylamine, cyclohexylamine, dicyclohexylamine and 2-pipecoline, however, did not react. The Mannich procedure was employed with 2-picoline and quinaldine for the preparation of 2-(β-diethylaminoethyl)-pyridine[3] (I, n = 2, R = C_2H_5) and 2-(β-N-piperidylethyl)-quinoline (IV).[4]

The incorporation of the methylene side chain was accomplished by the scheme



Freshly prepared 2-picolinoyl chloride acted on piperidine to give 1-(2′-picolinoyl)-piperidine (VII) which was reduced to 2-N-piperidylmethylpyridine (VIII) using lithium aluminum hydride.

Catalytic hydrogenation of the pyridine ring in I and IV furnished the corresponding secondary amines (II, V) which on acylation gave the desired amides (III, VI). The basic amides having the β-N-piperidylethyl side chain are described in Table II. The majority were made by the Schotten–Baumann procedure, although compound 1, the formyl derivative, was obtained conveniently by the reaction of the amine with ethyl formate in ethanol,[5]

(1) Presented in part before the Medicinal Division of the American Chemical Society, Milwaukee, Wis., 1952.

(2) W. E. Doering and R. A. N. Weil, THIS JOURNAL, **69**, 2461 (1947).

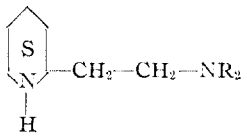
(3) T. Heou-Feo, *Bull. soc. chim. France*, [5] **2**, 105 (1935).

(4) (a) W. O. Kermack and W. Muir, *J. Chem. Soc.*, 3089 (1931); (b) T. Heou-Feo, *Bull. soc. chim. France*, [5] **2**, 100 (1935).

(5) As other examples of this method, see: J. P. E. Human and J. A. Mills, *J. Chem. Soc.*, 1457 (1948), and A. E. Barkdoll, H. W. Gray and W. Kirk, Jr., THIS JOURNAL, **73**, 741 (1951).

TABLE I

SECONDARY DIAMINES



No.	NR_2	B.p., °C.	Mm.	Yield, %	$n^{25}D$	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	$C_5H_{10}N$[a]	107–108	1.2	77	1.4900	$C_{12}H_{24}N_2$	73.42	73.41	12.32	12.32
2	C_4H_8NO[b]	107–108	2.2	54	1.4900	$C_{11}H_{22}N_2O$	66.62	66.41	11.18	11.02
3	$C_5H_{11}N_2$[c]	128–130	1.0	53	1.4918	$C_{12}H_{25}N_3$	[d]			
4	$C_4H_{10}N$[e]	106–109	12	25	1.4753					
5	[f]	143–145	0.5	37	1.5647	$C_{16}H_{24}N_2$	78.63	78.53	9.90	9.06

[a] N-Piperidyl. [b] N-Morpholinyl. [c] 1-Methyl-4-piperazinyl. [d] Calcd.: N, 19.88. Found: N, 20.30. [e] Diethylamino. Prepared by another method by K. Loeffler and M. Kirschner, *Ber.*, **38**, 3335 (1905). [f] 2-(β-N-Piperidylethyl)-1,2,3,4-tetrahydroquinoline. Monohydrochloride salt, m.p. 243°. *Anal.* Calcd. for $C_{16}H_{25}ClN_2$: C, 68.42; H, 8.97; N, 9.98. Found: C, 68.44; H, 8.60; N, 9.62.

and compound 2 by its reaction with acetic anhydride. Compound 9, 1-(*p*-aminobenzoyl)-2-(β-N-piperidylethyl)-piperidine was prepared by hydrogenation of compound 8, the *p*-nitrobenzoyl derivative.

Other basic amides which were prepared are described in the Experimental section. These contain diethylaminoethyl, morpholinylethyl, 1-methyl-4-piperazinylethyl and piperidylmethyl side chains. One amide (VI) derived from 2-(β-N-piperidylethyl)-1,2,3,4-tetrahydroquinoline (V) was also synthesized.

The basic amides were usually purified by distillation and converted to hydrochloride salts. These exhibited local anesthetic activity of the order of procaine hydrochloride in animal tests.

Acknowledgments.—We wish to thank Dr. R. K. Richards of the Department of Pharmacology for a preliminary report of his findings. We are grateful to Mr. E. F. Shelberg and staff, of the Microanalytical Department for the analytical values reported in this paper.

Experimental

Secondary Diamines. 2-(β-N-Piperidylethyl)-pyridine was obtained by the method of Doering and Weil,[2] whose general procedure was used also to prepare the two following compounds.

2-(β-N-Morpholinylethyl)-pyridine.—A mixture of 105 g. (1 mole) of 2-vinylpyridine and 174 g. (2 moles) of morpholine was refluxed for 16 hours. The product distilled at 163–171° at 17–18 mm., $n^{27}D$ 1.5233, and weighed 158 g. (82%).

Anal. Calcd. for $C_{11}H_{16}N_2O$: N, 14.57. Found: N, 14.58.

2-(β-1′-Methyl-4′-piperazinyl)-ethylpyridine.—2-Vinylpyridine (10.5 g., 0.1 mole) and 1-methylpiperazine (20 g., 0.2 mole) refluxed 7 hours gave 8.2 g. (40%) of product, b.p. 128–129° at 2.7 mm., $n^{25}D$ 1.5244.

Anal. Calcd. for $C_{12}H_{19}N_3$: N, 20.47. Found: N, 21.06.

2-(β-Diethylaminoethyl)-pyridine and 2-(β-N-piperidylethyl)-quinoline were prepared by procedures found in the literature.[3,4]

The secondary diamines (II, $n = 2$) which are described in Table I were prepared by catalytic hydrogenation in glacial acetic acid over platinum oxide. The reduction was carried out at 75° for compounds 2, 3 and 4, and at room temperature for compounds 1, 5 and 6. The following example illustrates the procedure.

2-(β-N-Piperidylethyl)-piperidine.—A solution of 134 g. (0.7 mole) of freshly distilled 2-(β-N-piperidylethyl)-pyridine in 0.5 l. of glacial acetic acid was shaken with 2.5 g. of platinum oxide at 1800 lb. pressure of hydrogen. After 8 hours the filtered solution was concentrated and made strongly basic with 400 cc. of 30% sodium hydroxide. An organic phase formed. The mixture was shaken with 400-ml. and 200-ml. portions of ether, and the extracts were combined and dried over potassium carbonate. Distillation through a Vigreux head gave 106 g. (77%) of the product described in Table II.

1-(2′-Picolinoyl)-piperidine (VII).—A benzene solution of 2-picolinoyl chloride[6] prepared from 100 g. (0.81 mole) of 2-picolinic acid was added slowly with stirring to a cooled mixture of 86 g. (1 mole) of piperidine and 250 ml. of 10% aqueous sodium hydroxide. The mixture was stirred overnight, the two layers were separated, and the aqueous phase was treated with 25 g. of sodium hydroxide and shaken with 100 ml. of benzene. The combined organic layers were distilled and the fraction boiling at 131–135° at 0.3 mm. was crystallized from 750 ml. of Skellysolve B to yield 48.1 g. (31%) of white prisms, m.p. 76–77°.

Anal. Calcd. for $C_{11}H_{14}N_2O$: C, 69.45; H, 7.42; N, 14.73. Found: C, 69.48; H, 7.12; N, 14.75.

2-(N-Piperidylmethyl)-pyridine.—A solution of 47 g. (0.25 mole) of 1-(2′-picolinoyl)-piperidine in 0.7 l. of dry ether was added during 5 hours to a stirred solution of 38 g. (1 mole) of lithium aluminum hydride in 1.5 l. of dry ether, under nitrogen. After 17 hours, 110 ml. of water was cautiously added and the resulting white suspension was filtered. The filtrate and ether washings of the solid were dried over potassium carbonate, concentrated and distilled. The product boiled at 122–124° at 10 mm. and weighed 24.1 g. (55%), $n^{25}D$ 1.5170. A tarry residue remained in the flask.

Anal. Calcd. for $C_{11}H_{16}N_2$: C, 74.95 H, 9.15. Found: C, 74.40; H, 9.19.

The dihydrochloride salt prepared in ether melted over the range 200–215°.

Anal. Calcd. for $C_{11}H_{18}Cl_2N_2$: C, 53.02; H, 7.28. Found: C, 52.89; H, 7.04.

2-(N-Piperidylmethyl)-piperidine.—A solution of 23 g. (0.13 mole) of 2-(N-piperidylmethyl)-pyridine in 175 ml. of acetic acid was shaken with 0.5 g. of platinum oxide at 400 lb. pressure. The product was isolated as described for the higher homolog. It boiled at 104–106° at 5 mm., and weighed 17.5 g. (71%). Analytical results did not agree well with the calculated values for this compound. The hydrochloride salt also gave unsatisfactory results. The base was used as an intermediate without further purification.

Basic Amides

Usually the amides were prepared by shaking together the diamine and an acid chloride in the presence of aqueous sodium hydroxide. After the reaction was completed, the mixture was shaken with ether or benzene to extract the product which was then purified by distillation. The hydrochloride salts were prepared in anhydrous ether and were recrystallized from *n*-propanol or isopropyl alcohol and ether mixtures. Described below are preparations which differed from this general procedure, as well as those of amides not in Table II.

(6) H. Meyer and R. Graf, *Ber.*, **61**, 2201 (1928).

TABLE II: BASIC AMIDES

	R′	B.p., °C.	Mm.	$n^{25}D$	Yield,[a] %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Formula	Hydrochloride M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	H[b]	131–132	0.8	1.5024	95	69.60	69.69	10.78	10.91	$C_{13}H_{24}N_2O\cdot HCl$[c]	169–171	59.86	60.35	9.66	9.96
2	CH_3[b]	124–125	.4	1.5018	86	70.54	70.54	11.00	10.94	$C_{14}H_{26}N_2O$[d]	.....	....	....	....	....
3	n-C_3H_7	144–146	.3	1.4968	52	72.13	71.92	11.35	10.98	$C_{16}H_{30}N_2O\cdot HCl$	169–170	63.44	63.34	10.32	10.06
4	C_6H_5	184	.4	1.5439	68	75.96	75.64	9.39	9.22	$C_{19}H_{28}N_2O\cdot HCl$	214–215	67.73	68.23	8.68	8.53
5	p-$CH_3OC_6H_4$	203–206	.3	....	69	....	....	....	....	$C_{20}H_{30}N_2O_2\cdot HCl$	227–228	65.46	65.53	8.52	8.42
6	p-$C_2H_5OC_6H_4$[e]	231–233	2.0	1.5415	66	....	....	....	....	$C_{21}H_{32}N_2O_2\cdot HCl$	202–203	66.21	66.09	8.73	8.74
7	p-ClC_6H_4	203–204	0.5	....	65	68.14	68.24	8.13	8.01	$C_{19}H_{27}ClN_2O\cdot HCl\cdot 0.5H_2O$	209–210	59.99	60.06	7.69	7.76
8	p-$NO_2C_6H_4$	[f]	....	....	86	....	....	....	....	$C_{19}H_{27}N_3O_3\cdot HCl\cdot H_2O$	200–201	57.06	57.37	7.56	7.27
9	p-$NH_2C_6H_4$	238–242	0.5	....	70	72.34	71.93	9.27	8.88	$C_{19}H_{29}N_3O\cdot HCl\cdot H_2O$[g]	224[h]	61.69	61.76	8.72	8.40
10	$C_6H_5CH_2$	183–185	0.3	1.5393	55	76.39	75.94	9.62	9.41	$C_{20}H_{30}N_2O\cdot HCl$	185–187	68.45	68.69	8.91	8.66
11	$(C_6H_5)_2CH$	[i]	....	....	63	79.95	79.98	8.78	8.58	$C_{26}H_{34}N_2O\cdot HCl$	207–208	73.13	73.41	8.26	8.23

[a] Calculated on the basis of unrecovered diamine. [b] Prepared by Dr. K. M. Beck of these laboratories. [c] Cl analysis: Calcd.: 13.60. Found: 13.47. [d] The formula is that of the free base. The hydrochloride salt was hygroscopic. [e] Prepared by Dr. M. B. Moore of these laboratories. [f] The base was not distilled but was purified by heating at 190° at 0.3 mm. [g] The dihydrochloride salt melts and resolidifies at 180–190°, not remelting below 300°. *Anal.* Calcd. for $C_{19}H_{29}N_3O\cdot 2HCl$: C, 58.76; H, 8.05. Found: C, 58.45; H, 8.21. [h] Softens 169°. [i] M.p. 100° after recrystallization from Skellysolve B.

1-Formyl-2-(β-N-piperidylethyl)-piperidine.—A solution of 10 g. (0.05 mole) of 2-(β-N-piperidylethyl)-piperidine and 20 g. (0.27 mole) of ethyl formate in 75 ml. of absolute alcohol was heated at reflux for 24 hours and then distilled. The yield was 10.7 g. (95%).

1-Acetyl-2-(β-N-piperidylethyl)-piperidine.—A mixture of 10 g. (0.05 mole) of the above diamine and 30 g. (0.3 mole) of acetic anhydride was boiled for 15 minutes, and then most of the excess anhydride was removed under vacuum. The residue was treated with excess 25% aqueous sodium hyroxide and shaken with ether to give an extract which was dried over potassium hydroxide and distilled. The product weighed 10.5 g. (86%).

1-*p*-Aminobenzoyl-2-(β-N-piperidylethyl)-piperidine.—A solution of 38.2 g. (0.1 mole) of 1-*p*-nitrobenzoyl-2-(β-N-piperidylethyl)-piperidine hydrochloride in 250 ml. of water was shaken with 2 g. of 10% palladium on charcoal under hydrogen at a pressure of 35 lb. until the uptake was 0.3 mole (4 to 5 hours). The filtered solution was evaporated to dryness and the white solid recrystallized from alcohol to give 26.6 g. (72%) of the monohydrochloride monohydrate salt. This was converted to the free base, a light yellow, glassy solid after distillation. The dihydrochloride salt was prepared in isopropyl alcohol and ether.

1-Benzoyl-2-(β-diethylaminoethyl)-piperidine.—A solution of 1.9 g. (0.013 mole) of benzoyl chloride in 10 ml. of dry benzene was added to 2.5 g. (0.013 mole) of 2-(β-diethylaminoethyl)-piperidine in 15 ml. of benzene, and the solution was heated at reflux for three hours. After evaporation of the solvent, treatment of the residue with excess aqueous sodium hydroxide gave an oil which after two distillations yielded 1.3 g. (34%) of oil, b.p. 148–153° at 0.3 mm., $n^{25}D$ 1.5281.

Anal. Calcd. for $C_{18}H_{28}N_2O$: C, 74.95: H, 9.78. Found: C, 74.48; H, 9.70.

The hydrochloride salt was an oil which could not be crystallized.

1-Benzoyl-2-(β-N-morpholinylethyl)-piperidine Hydrochloride.—To a solution of 9.9 g. (0.05 mole) of 2-(β-N-morpholinylethyl)-piperidine in 50 ml. of dry benzene was added 7 g. (0.05 mole) of benzoyl chloride in an equal volume of benzene. The gum which formed became a fine crystalline solid after the mixture had been heated at reflux for 4 hours. The yield of hygroscopic hydrochloride salt was quantitative. A sample melted at 202–203° after recrystallization from ethanol and ether.

Anal. Calcd. for $C_{18}H_{27}ClN_2O_2$: N, 8.27. Found: N, 8.22.

1-Benzoyl-2-(β-1′-methyl-4′-piperazinylethyl)-piperidine Dihydrochloride.—A solution of 6.3 g. (0.03 mole) of 2-(β-1′-methyl-4′-piperazinyl)-ethylpiperidine in 25 ml. of dry benzene was treated with 5.4 g. (0.04 mole) of benzoyl chloride as in the preceding experiment. The product weighed 5.5 g. (47%) and melted at 245–246°.

Anal. Calcd. for $C_{19}H_{31}Cl_2N_3O$: C, 58.76; H, 8.05; N, 10.82. Found: C, 59.12; H, 7.82; N, 10.45.

1-Benzoyl-2-(N-piperidylmethyl)-piperidine.—To a mixture of 9.1 g. (0.05 mole) of 2-(N-piperidylmethyl)-piperidine and 60 cc. of 30% aqueous sodium hydroxide was added, in portions with shaking, 40 g. (0.28 mole) of benzoyl chloride. The mixture was warmed with 100 ml. of benzene until the solid dissolved. Distillation of the organic phase gave 6.6 g. of material boiling at 164–167° at 0.2 mm. Redistillation gave 5.3 g. (37%) of yellow oil distilling at 170–171° at 0.3 mm.

Anal. Calcd. for $C_{18}H_{26}N_2O$: C, 75.48; H, 9.15. Found: C, 75.77; H, 8.70.

The hydrochloride salt, recrystallized from propanol and ether mixture, melted at 255°.

Anal. Calcd. for $C_{18}H_{27}ClN_2O$: C, 66.95; H, 8.43. Found: C, 66.98; H, 8.21.

1-*p*-Anisoyl-2-(N-piperidylmethyl)-piperidine.—By a similar procedure using 7.3 g. (0.04 mole) of 2-(N-piperidylmethyl)-piperidine and 10.3 g. (0.06 mole) of *p*-anisoyl chloride there was obtained 2.7 g. (21%) of viscous oil, boiling at 185–189° at 0.3 mm.

Anal. Calcd. for $C_{19}H_{28}N_2O_2$: C, 72.11; H, 8.92. Found: C, 72.12; H, 8.52.

The hydrochloride salt melted at 244°.

Anal. Calcd. for $C_{19}H_{29}ClN_2O_2$: C, 64.66; H, 8.28. Found: C, 64.90; H, 8.24.

1-Benzoyl-2-(β-N-piperidylethyl)-1,2,3,4-tetrahydroquinoline.—From 6.1 g. (0.025 mole) of 2-(β-N-piperidylethyl)-1,2,3,4-tetrahydroquinoline and 14 g. (0.1 mole) of benzoyl chloride by the same method was obtained an oil which solidified after distillation and was recrystallized from Skellysolve B. The yield was 2.9 g. (33%). It distilled at 188–196° at 0.1 mm., and melted at 102–103°.

Anal. Calcd. for $C_{23}H_{28}N_2O$: C, 79.27; H, 8.10. Found: C, 79.22; H, 7.95.

The hydrochloride salt, recrystallized from alcohol and ether, melted at 236°.

Anal. Calcd. for $C_{23}H_{29}ClN_2O$: C, 71.76; H, 7.59. Found: C, 71.54; H, 7.66.

NORTH CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE UNIVERSITY LABORATORY OF PHYSICAL CHEMISTRY RELATED TO MEDICINE AND PUBLIC HEALTH, HARVARD UNIVERSITY]

Preparation and Properties of Serum and Plasma Proteins. XXIX. Separation from Human Plasma of Polysaccharides, Peptides and Proteins of Low Molecular Weight. Crystallization of an Acid Glycoprotein[1a,b,c]

BY K. SCHMID[1d]

RECEIVED JULY 7, 1952

A method is described for the separation into five fractions of the components of human plasma which remained in solution following precipitation of the major plasma proteins by procedures formerly published. All the proteins not previously precipitated were concentrated with the aid of zinc hydroxide (Fraction VI); among them were hitherto little known glycoproteins of low molecular weight. Unknown polysaccharides with specific blood group activity were selectively rendered insoluble with calcium hydroxide (Fraction VII), and amino acids and peptides were adsorbed on an ion exchange resin (Fraction VIII). After concentration of the final solution, the blood constituents were separated into a lipophilic (Fraction IX) and a hydrophilic fraction (Fraction X). In addition a method is described for subfractionation of the proteins precipitated as Fraction VI. An acid glycoprotein has been separated in a homogeneous state, as judged by electrophoretic and ultracentrifugal analyses, over the *p*H range 1.9 to 9.6. It was isoelectric, in a phosphate buffer solution of ionic strength 0.1, at *p*H 2.7. Its sedimentation constant, $S_{20,w}$, in 0.15 *M* NaCl solution at *p*H 6.5, extrapolated to zero concentration, was 3.5 *S*. The chemical composition and physico-chemical properties of the acid glycoprotein differed widely from those of other plasma proteins. Its concentration in normal plasma was 0.5 g. per liter. The acid glycoprotein has been crystallized as a lead salt.

I. Introduction

A method for the fractionation of human plasma in ethanol–water mixtures at low temperatures[2,3] was introduced in 1941–1944 by which the plasma proteins were separated into six principal fractions: I, II + III, IV-1, IV-4, V and VI. Recently this method has been greatly improved by transferring immediately all proteins to the solid state in order to prevent further chemical, especially enzymatic, changes. In Method 10[4] the albumins, α_1-lipoproteins, α_2-glycoproteins, and the β_1-metal-combining protein were precipitated in an ethanol–water mixture of mole fraction 0.066, and *p*H 5.8 at −5°, by the addition of zinc acetate, following precipitation of the less soluble plasma globulins in Fraction I + II + III.[4,5]

The components which remained in solution included small amounts of certain very soluble blood constituents totalling 1.5 to 2% of the plasma proteins. A method for the concentration and fractionation of these blood components into five fractions denoted as VI, VII, VIII, IX and X is reported in this paper and represents an extension of Method 10.[1c] One of the major components of Fraction VI has been further purified and crystallized as a lead salt.

The separation of these very soluble plasma components was based on selective precipitation by ions and hydroxides of alkaline earths and heavy metals such as calcium, barium, zinc, cadmium and lead. Our experiments indicated that complex formation with low concentration of ions of heavy metals did not denature the investigated plasma proteins.

II. Materials and Methods

The solution remaining after precipitation of over 98% of the protein (Fractions I–V) from pooled normal human plasma[4] was the *starting material* for these investigations. In Method 10 the volume of this supernatant solution was five times that of the original plasma volume. It contained 0.066 mole fraction ethanol (19% ethanol at 25°) and slightly

(1) (a) This work has been supported by funds of Harvard University and the Eugene Higgins Trust, by grants from the Rockefeller Foundation and the National Institutes of Health, and by contributions from industry. (b) This paper is No. 98 in the series "Studies on the Plasma Proteins" from blood collected by the American Red Cross, on products developed by the University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University. (c) A preliminary report of some of this work was presented at the 119th Meeting of the American Chemical Society in Boston, Massachusetts, April 1–5, 1951. See Abstracts of this meeting, p. 28 C. (d) Present address: Research Laboratories of the Medical Clinic, Massachusetts General Hospital, Boston, Massachusetts.

(2) E. J. Cohn, J. L. Oncley, L. E. Strong, W. L. Hughes, Jr., and S. H. Armstrong, Jr., *J. Clin. Invest.*, **23**, 417 (1944).

(3) E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, THIS JOURNAL, **68**, 459 (1946).

(4) E. J. Cohn, F. R. N. Gurd, D. M. Surgenor, B. A. Barnes, R. K. Brown, G. Derouaux, J. M. Gillespie, F. W. Kahnt, W. F. Lever, C. H. Liu, D. Mittelman, R. F. Mouton, K. Schmid and E. Uroma, *ibid.*, **72**, 465 (1950).

(5) In a more recent method the human plasma proteins have been fractionated in an aqueous system. A fraction closely resembling Fraction I+II+III was rendered insoluble by complexing with zinc ions. From the remaining "stable plasma protein solution" (S.P.P.S.) a fraction similar to Fraction IV + V was precipitated by complexing with mercuric ions.[4]