The product was converted to the hydrochloride in absolute ether and recrystallized from alcohol–ether mixture. It then melted at 200–201°.

Bromination of 2,4-Dimethyl-6-hydroxy-5-phenacylpyrimidine.—2,4-Dimethyl-6-hydroxy-5-phenacylpyrimidine (1.00 g., 0.004 mole) was dissolved in 10 ml. of glacial acetic acid and a solution of 0.663 g. (0.004 m.) of bromine in 2 ml. of glacial acetic acid was added dropwise. No reaction occurred until the mixture was heated to 60–70°, when rapid decolorization took place. When a little more than half of the bromine had been added, white crystals began to separate, which redissolved for the most part by the time the remaining bromine had been added. The mixture was allowed to stand and cool for two hours, resulting in the slow formation of a white precipitate again. This was filtered, washed with ether, and dried; weight, 0.4 g. This proved to be a mixture consisting mainly of the hydrobromide of the starting material. On dissolving in water a small amount of insoluble material remained, which was removed by filtration. On neutralization of the filtrate with sodium bicarbonate, a white precipitate formed which, upon drying, melted at 207–209°, contained no halogen, and showed no depression of the melting point with the starting material. The acetic acid liquor yielded a white precipitate upon the addition of ether, but this became sirupy on all attempts at isolation. Upon pouring the acetic acid solution into water, a small amount of white precipitate formed; dry weight, 0.3 g. After purification by recrystallization from alcohol, the substance melted at 167–169°. Analysis indicated it to be a dibrominated base, which was not further identified.

Anal. Calcd. for $C_{14}H_{12}Br_2N_2O$: C, 42.0; H, 3.02; Br, 40.0; N, 7.01. Found C, 42.3; H, 2.97; Br, 40.1; N, 7.31.

Summary

A series of 2- and 5-phenacylpyrimidines has been prepared for pharmacological testing purposes. Condensations of benzoylacetamidine, a new compound, with β-ethoxyacrolein acetal, acetylacetone and ethyl acetoacetate yielded 2-phenacylpyrimidine, 4,6-dimethyl-2-phenacylpyrimidine and 4-hydroxy-6-methyl-2-phenacylpyrimidine, respectively. Condensation of α-phenacylacetoacetic ester with acetamidine yielded 2,4-dimethyl-6-hydroxy-5-phenacylpyrimidine.

The methylene group of 2-phenacylpyrimidine has been shown to be unusually unreactive. Bromination of 2-phenacylpyrimidine produced the 5-bromo derivative only. Mannich and nitrosation reactions failed. The ketone did not yield an oxime.

2,4-Dimethyl-6-hydroxy-5-phenacylpyrimidine was chlorinated to yield the 6-chloro derivative, which reacted with piperidine to form 2,4-dimethyl-5-phenacyl-6-(1-piperidyl)-pyrimidine. Bromination reactions with the 6-hydroxy compound yielded mixtures, from which no monobrominated product was isolated.

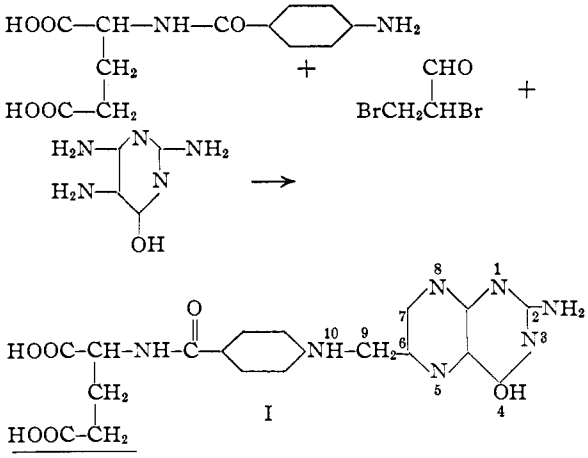
BOUND BROOK, NEW JERSEY RECEIVED AUGUST 24, 1948

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Analogs of Pteroylglutamic Acid. II. 9-Methylpteroylglutamic Acid and Derivatives

BY MARTIN E. HULTQUIST, JAMES M. SMITH, JR., DORIS R. SEEGER, DONNA B. COSULICH AND ERWIN KUH

Pteroylglutamic acid (I) was synthesized by Waller, *et al.*,[1] by the simultaneous reaction of 2,4,5-triamino-6-hydroxypyrimidine,[2] 2,3-dibromopropionaldehyde, and *p*-aminobenzoyl-*l*(+)-glutamic acid[3] in aqueous solution at *p*H 4.



I

(1) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948); Angier, *et al.*, *Science*, **103**, 667 (1946).

(2) Traube, *Ber.*, **33**, 1371 (1900).

(3) Van der Scheer and Landsteiner, *J. Immunology*, **29**, 373 (1935).

This reaction has proved to be of wide application and a number of derivatives of I have been prepared by varying one or more of the three components.[4] Previous communications from this Laboratory have described N^{10}-alkyl[5] and 4-amino[6] analogs of pteroylglutamic acid. The present paper deals with a series of analogs in which a methyl group has been introduced on the methylene bridge in the 9-position.

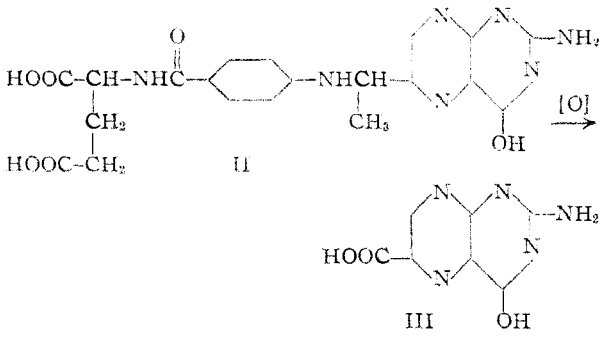
When 2,2,3-trichlorobutyraldehyde ("butyl chloral")[7] was reacted with 2,4,5-triamino-6-hydroxypyrimidine and *p*-aminobenzoylglutamic acid, there was formed N-[4-{[1-(2-amino-4-hydroxy-6-pteridyl)-ethyl]-amino}-benzoyl]-glutamic acid (II), hereafter designated as 9-methylpteroylglutamic acid.

(4) (a) Franklin, *et al.*, *J. Biol. Chem.*, **169**, 427 (1947); (b) Hutchings, *et al.*, *ibid.*, **170**, 323 (1947); (c) Martin, Tolman and Moss, *Archives of Biochemistry*, **12**, 318 (1947); (d) Mowat, *et al.*, THIS JOURNAL, **70**, 1096 (1948); (e) Boothe, *et al.*, *ibid.* **70**, 1099 (1948); (f) Smith, Cosulich, Hultquist and Seeger, *Trans. N. Y. Acad. Science*, [II] **10**, 82 (1948); (g) Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).

(5) Cosulich and Smith, *ibid.*, **70**, 1922 (1948).

(6) Seeger, Smith and Hultquist, *ibid.*, **69**, 2567 (1947).

(7) Pinner, *Ann.*, **179**, 26 (1875). We are grateful to the Westvaco Chemical Corporation for a supply of butyl chloral.



Purification was accomplished by methods similar to those reported for pteroylglutamic acid.[1,4b,d,e,5] Also, it was found that recrystallization of the cyclohexylamine salt of II from aqueous butanol afforded a convenient method of obtaining an analytically pure material. Oxidation of II with hot alkaline permanganate gave solely 2-amino-4-hydroxypteridine-6-carboxylic acid (III).[8] This shows that the point of attachment of the side chain is in the 6-position on the pteridine ring.

9,10-Dimethylpteroic acid (IV) was prepared similarly, using *p*-methylaminobenzoic acid. Other variations in the starting materials resulted in 9,-

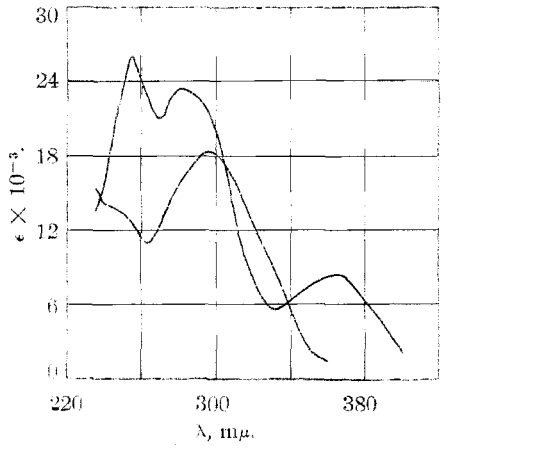


Fig. 1.—Ultraviolet absorption spectra of 9-methylpteroylglutamic acid (II)[a]: ——, 0.1 *N* NaOH; — — —, 0.1 *N* HCl.

[a] ε is the molecular extinction coefficient as defined by $I = I_0 10^{-\varepsilon cl}$ where c is the concentration in moles/liter and l is the cell length in centimeters. Transmittancy (I/I_0) measurements of 10 mg./l. solutions were made in 1-cm. cells at 5 mμ intervals on a Model DU Beckman Spectrophotometer using a solvent filled cell in the reference position. Additional data were obtained at 2 mμ intervals at maxima, minima, and points of inflection.

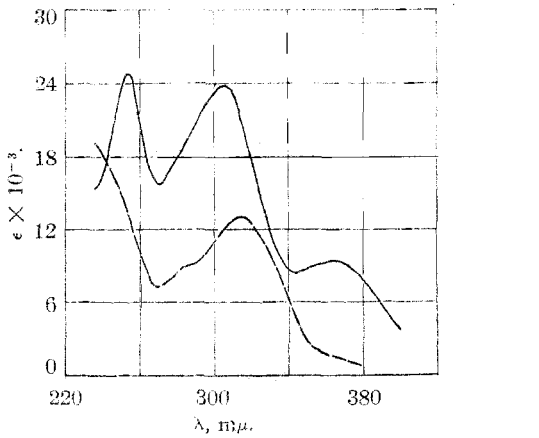


Fig. 2.—Ultraviolet absorption spectra of 9,10-dimethylpteroylglutamic acid (V)[a]: ——, 0.1 *N* NaOH; — — —, 0.1 *N* HCl.

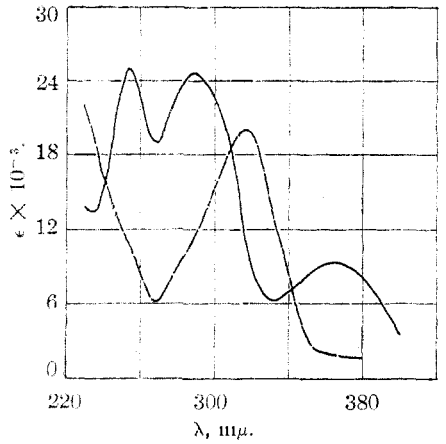


Fig. 3.—Ultraviolet absorption spectra of 9,10-dimethylpteroic acid (IV)[a]: ——, 0.1 *N* NaOH; — — —, 0.1 *N* HCl.

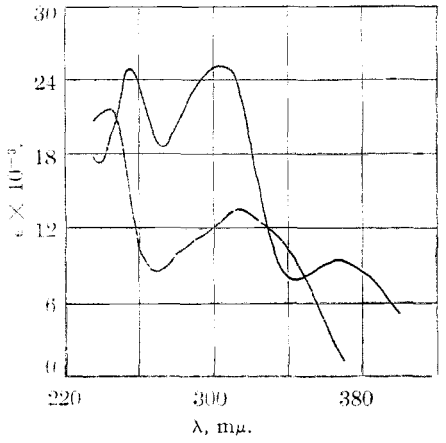
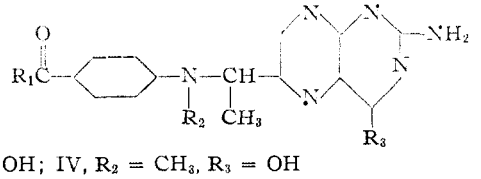


Fig. 4.—Ultraviolet absorption spectra of 4-amino-9,10-dimethylpteroylglutamic acid (VI)[a]: ——, 0.1 *N* NaOH; — — —, 0.1 *N* HCl.



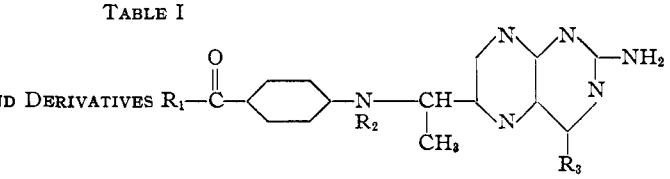
R_1 = OH; IV, R_2 = CH_3, R_3 = OH

R_1 = NHCH(COOH)
CH_2CH_2COOH; { V, R_2 = CH_3, R_3 = OH; VI, R_2 = CH_3, R_3 = NH_2

(8) Mowat, *et al.*, THIS JOURNAL, **70**, 14 (1948).

TABLE I

9-METHYLPTEROYLGLUTAMIC ACID AND DERIVATIVES



R_1	R_2	R_3	Ultraviolet absorption spectra[a] Maxima λ mμ 0.1 *N* NaOH	0.1 *N* HCl	Antagonist activity[b]
-l(+)-glutamic acid	-H	-OH	255 283 365	297	0.12
-l(+)-glutamic acid	-H	-OH			0.09[c]
-OH	$-CH_3$	-OH	253 288 365	317	1.12
-l(+)-glutamic acid	$-CH_3$	-OH	253 305 365	315	3.4
-l(+)-glutamic acid	$-CH_3$	$-NH_2$	255 306 369	244 316	1563.0
N^{10}-Methylpteroylglutamic acid[b]			255 302 368	307	100.0

[a] See footnote to Fig. 1. [b] An arbitrary value of 100 is assigned for the antagonist activity of N^{10}-methylpteroylglutamic acid, for half-maximum inhibition of the growth of *Streptococcus faecalis* R. The inhibition ratio for this compound is 2.0 at a concentration of pteroylglutamic acid of 0.1 microgram per 10 ml. (ref. 5). Values for other compounds are reported in terms of the standard. [c] Crude material.

10-dimethylpteroylglutamic acid (V), and 4-amino-9,10-dimethylpteroylglutamic acid (VI).

The biological properties of these compounds have been examined by Dr. E. L. R. Stokstad and Dr. B. L. Hutchings of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. The 9-methyl analogs are antagonists for pteroylglutamic acid in the growth of *Streptococcus faecalis* R., and also in certain animals. Details of this work will be reported elsewhere. The inhibition ratio of 9-methylpteroylglutamic acid for half-maximum growth of *S. faecalis* R is 2000 at a concentration of 0.01 microgram of pteroylglutamic acid per ml.

Table I summarizes the ultraviolet absorption data and relative antagonist activity of these analogs.

Experimental

9-Methylpteroylglutamic Acid (II).—This compound was prepared from 2,4,5-triamino-6-hydroxypyrimidine hydrochloride,[2] 2,2,3-trichlorobutyraldehyde[7] and *p*-aminobenzoylglutamic acid,[3] under the conditions described by Waller, *et al.*, for the synthesis of pteroylglutamic acid[1]. A slurry of 20 g. of crude 9-methylpteroylglutamic acid and 8 g. of lime in 2000 ml. of water was heated at 80° for thirty minutes, clarified and washed with hot water. The filtrate was adjusted to *p*H 10.8 with aqueous zinc chloride, clarified, acidified to *p*H 3 and filtered. This cake was slurried in 2000 ml. of water, made alkaline with sodium hydroxide solution at 80° and then adjusted to *p*H 7 while cooling to 20°. After clarification, the filtrate was acidified to *p*H 3. The product was filtered and dissolved in 1000 ml. of water as the magnesium salt, treated with Darco G-60, clarified and reprecipitated at *p*H 3. The dry product weighed 2.2 g.; purity, 82.5%.

Further purification was accomplished by dissolving 1 g. of this material in 8 ml. of butanol, 2 ml. of water and 1 ml. of cyclohexylamine at 95°, and clarifying to remove some insoluble matter. After boiling to remove part of the water, there was gradually added 7.5 ml. of butanol and 3.5 ml. of cyclohexylamine. On stirring, there crystallized out the cyclohexylamine salt, which was filtered off at 20°. This was recrystallized again, using the above procedure, to give a light yellow, crystalline product.

Anal. Calcd. for $C_{32}H_{47}O_8N_9 \cdot 3H_2O$: C, 54.30; H, 7.55; N, 17.81. Found: C, 54.8; H, 7.55; N, 17.8.

The free acid was recovered from the cyclohexylamine salt by dissolving in water and acidifying to *p*H 3. It was dried at 100° (1 mm.) for seven hours.

Anal. Calcd. for $C_{20}H_{21}O_6N_7$: C, 52.74; H, 4.65; N, 21.53. Found: C, 52.9; H, 4.77; N, 21.5.

Oxidation of II with Alkaline Permanganate.—A solution of 0.2 g. of II in 60 ml. of *N* sodium hydroxide solution was treated with excess potassium permanganate for one and one-half hours at 95°. The permanganate color was then destroyed with a little sodium sulfite. The mixture was clarified, acidified to *p*H 3, cooled and filtered, yielding 0.075 g. of product which was identified as 2-amino-4-hydroxypteridine-6-carboxylic acid (III) by comparison with an authentic sample.[8]

Oxidation of the cyclohexylamine salt of II gave the same product in approximately the same yield.

9,10-Dimethylpteroylglutamic Acid (V).—This compound was synthesized by the method of Waller, *et al.*[1] using 2,4,5-triamino-6-hydroxypyrimidine, 2,2,3-trichlorobutyraldehyde, and *p*-methylaminobenzoylglutamic acid.[5] A mixture of 30 g. of the crude material, 9 g. of lime, and 2 l. of water was heated at 60° for forty minutes. After filtration with Hyflo-Supercel, the cake was washed with 750 ml. of water at 60°. The filtrate and wash were adjusted to *p*H 10.8–11.0 with 10% zinc chloride and then filtered free of precipitated material. The liquor was adjusted to *p*H 3–3.5 with dilute hydrochloric acid, cooled to 10°, and filtered. The cake was washed with water and then slurried in 1500 ml. of water and enough sodium hydroxide to give *p*H 11–12. After heating at 80° for ten minutes, the solution was adjusted to *p*H 7 while cooling to 20°, and filtered. The filtrate was treated with dilute hydrochloric acid to *p*H 3–3.5. The precipitate was filtered, reslurried in 1 l. of water containing enough magnesium carbonate to obtain about *p*H 9 at 80°, and filtered hot with 2 g. of charcoal. The filtrate at 80° was adjusted to *p*H 3–3.5 with hydrochloric acid and cooled to 10°. Orange microcrystalline material separated which was purified for analysis by repeating (at 1 g./l.) the last step twice more. On cooling to 10° after the second treatment, analytically pure yellow-orange microcrystalline material was isolated (see Table I for biological and ultraviolet absorption data). The analytical sample was dried at 100° and 2 mm. for five hours.

Anal. Calcd. for $C_{21}H_{27}N_7O_6 \cdot H_2O$: C, 51.7; H, 5.13; N, 20.13. Found: C, 51.4; H, 5.22; N, 19.8.

Permanganate Oxidation of 9,10-Dimethylpteroylglutamic Acid (V).—A solution of 0.5 g. of 9,10-dimethylpteroylglutamic acid in 166 ml. of N sodium hydroxide was treated at 80–90° with 5% potassium permanganate solution until the solution maintained a purple color. After adding sodium sulfite to destroy this color, manganese dioxide was filtered off and the filtrate adjusted to *p*H 3–3.5 with dilute hydrochloric acid. The precipitate,

isolated by filtering, washing with water and then acetone, and drying, was shown to be 2-amino-4-hydroxypteridine-6-carboxylic acid (III) by comparison of the ultraviolet absorption curve with that of an authentic sample.[8]

9,10-Dimethylpteroic Acid (IV).—This was prepared similarly to V above, except that *p*-methylaminobenzoic acid[5] was used (see Table I for ultraviolet absorption and biological data).

Anal. Calcd. for $C_{16}H_{16}N_6O_3 \cdot 0.5H_2O$: C, 55.1; H, 4.91; N, 24.1. Found: C, 54.7; H, 4.99; N, 24.0.

N-[{N-[1-(2,4-Diamino-6-pteridyl)-ethyl]-N-methyl-amino}-benzoyl]-glutamic Acid, ("4-Amino-9,10-dimethylpteroylglutamic Acid") (VI).—This compound was prepared by the method of Waller, *et al.*,[1] using 2,4,5,6-tetraminopyrimidine sulfate,[9] 2,2,3-trichlorobutyraldehyde and *p*-methylaminobenzoylglutamic acid.[5] A mixture of 31.6 g. of the crude, 9 g. of lime, and 2 l. of water was heated at 60° for forty minutes. After filtration, the cake was washed with 600 ml. of water at 60°. The combined filtrate and wash liquors were adjusted to *p*H 10.8–11.0 with 10% aqueous zinc chloride. The mixture was filtered, and the filtrate was adjusted to *p*H 3.5–4.0 with hydrochloric acid. After cooling to 5°, the precipitate was filtered, washed with water and slurried in 1500 ml. of water containing sodium hydroxide to give *p*H 11–11.5. After heating it at 60° for ten minutes, the *p*H of the solution was reduced to 7, while cooling the mixture to 20°, and filtered. The filtrate was adjusted to *p*H 3.5–4.0 with hydrochloric acid and cooled to 5°. After filtration, the cake was slurried in 1 l. of water containing enough lime to obtain *p*H 8.5–9 at 60° and filtered hot with 1.5 g. of charcoal. The filtrate was adjusted to *p*H 3.5–4.0 with hydrochloric acid and cooled to 0–5° overnight. The yellow-orange microcrystalline material which separated was isolated by filtration and was analytically pure. The biological and ultraviolet absorption data are shown in Table I.

Anal. Calcd. for $C_{21}H_{24}N_8O_5 \cdot 2H_2O$: C, 50.0; H, 5.55; N, 22.2. Found: C, 50.4; H, 5.42; N, 21.5.

Acknowledgment.—We are indebted to Mr. Richard L. Shepard for technical assistance in the preparation of certain of these substances, to Miss Ruth Abbott for the ultraviolet absorption data, and to Mr. O. Sundberg and coworkers for the microanalyses.

Summary

N-[4-{[1-(2-Amino-4-hydroxy-6-pteridyl)-ethyl]-amino}-benzoyl]-glutamic acid (II), designated herein as 9-methylpteroylglutamic acid, has been synthesized and isolated in pure form. Corresponding analogs which have also been prepared are 9,10-dimethylpteroylglutamic acid (V), 4-amino-9,10-dimethylpteroylglutamic acid (VI), and 9,10-dimethylpteroic acid (IV).

These compounds exhibit antagonism for pteroylglutamic acid in the growth of *S. faecalis* R.

BOUND BROOK, NEW JERSEY
RECEIVED SEPTEMBER 27, 1948

(9) Traube, *Ber.*, **37**, 4545 (1904).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

Mechanical Properties of Substances of High Molecular Weight. V. Rigidities of Polyisobutylene Solutions in Various Solvents[1]

BY J. N. ASHWORTH[2] AND JOHN D. FERRY

Many concentrated solutions of linear polymers behave as highly viscous liquids in steady flow, but possess solid-like rigidity in small oscillating deformations; transverse waves can be propagated in them at audio frequencies. From the wave length of these waves the rigidity can be calculated, and from the damping the mechanical loss, or the dynamic viscosity, can be derived. Measurements of this sort on solutions of polystyrene in xylene have been reported previously.[3]

In seeking to understand the mechanism of rigidity in polymer solutions, it is desirable to study a variety of molecular types. In this paper, measurements are reported for a non-polar polymer for which complications due to intermolecular forces should be at a minimum: polyisobutylene. As in the earlier work on polystyrene, the dependence of rigidity on requency, concentration, and temperature has been studied. Also, four different solvents have been employed.

(1) Supported in part by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation, and in part by a grant from Research Corporation.

(2) Present address: Rohm and Haas Co., Philadelphia, Pa.

(3) J. D. Ferry, THIS JOURNAL, **64**, 1323 (1942).

Materials

The polyisobutylene was kindly furnished by Dr. John Rehner, Jr., of the Esso Laboratories. It was designated 303-92-1. From measurements of viscosities of dilute solutions in di-isobutylene, the intrinsic viscosity in this solvent at 20° was found to be 2.85 (g./100 cc.)$^{-1}$, corresponding to a viscosity-average molecular weight of 1,200,000 on the basis of the interpolation equation of Flory.[4] Intrinsic viscosities in the solvents used for the rigidity studies were also determined at 20° and 30°; these are given in Table IV below. The details of the intrinsic viscosity measurements are presented elsewhere.[5]

In most cases, the polymer was used without purification. However, it contained a small amount of tiny fibrous foreign particles which, though negligible in quantity, were optically anisotropic and interfered somewhat with optical measurements of waves. Accordingly, a portion of the polymer was dissolved in toluene at a concentration of about five per cent., clarified by filtration through Whatman No. 4 filter paper on a

(4) P. J. Flory, *ibid.*, **65**, 372 (1943).

(5) J. N. Ashworth, Ph.D. Thesis, University of Wisconsin, 1948.