lized from methanol–water and then from petroleum ether. A good yield of very light tan crystals resulted, m.p. 85.0–85.5°.

Anal. Calcd. for $C_8H_9O_3N$: C, 57.5; H, 5.43. Found: Cl, 58.0; H, 5.60.

The methyl ester of 3-aminosalicylic acid prepared in the above manner was found to melt at 85–86° and the melting point of a mixture of the known ester and that from compound II was 85.5–86.5°. Confirmation of the identity of compound II as 3-aminosalicylic acid was obtained by comparison of the infrared spectra of known and isolated samples of the free acid.

Saponification of Antimycic Acid.—Antimycic acid (436 mg.) was dissolved in 4 ml. of aqueous sodium hydroxide (cold saturated) and heated under reflux in a nitrogen atmosphere for two hours. After cooling, the *p*H was adjusted to 2.5 with 5% sulfuric acid, and the mixture was extracted 10 times with ether (20 ml. each). After drying the ether extract over anhydrous sodium sulfate, the solvent was removed *in vacuo* and the resulting residue purified by sublimation at 0.01 mm. and 200° to yield 212 mg. (81%) of 3-aminosalicylic acid (compound II). The aqueous mother liquors contained only a very small amount of ninhydrin-positive material as judged by the feeble color produced after spotting on filter paper.

Acid Hydrolysis of Antimycic Acid.—Antimycic acid (96.5 mg.) was hydrolyzed for 15 hours in 5 ml. of 3 *N* hydrochloric acid at 15 lb. pressure of steam. The resulting solution was concentrated to dryness *in vacuo*, two ml. of water added and the solution again concentrated to dryness. The residue was dissolved in 1 ml. of water and the resulting solution adjusted to *p*H 3 with 5% sodium bicarbonate. A small amount of precipitate (15.2 mg.) was filtered off and the filtrate was extracted with ether (4 × 6 ml.). The ether layer contained 3-aminosalicylic acid. The aqueous phase was adjusted to *p*H 6 and a portion chromatographed by the procedure used for glycine. Its R_f value (0.59) was the same as that of known threonine chromatographed simultaneously. The single spot produced by the unknown was readily distinguishable from that of glycine. Confirmation of the presence of L-threonine was obtained by microbiological assay using *S. faecalis*.[12] By this assay a recovery of more than 85% of the theoretical amount of threonine was obtained on acid hydrolysis. Since this test organism does not respond to D-threonine or to D- or L-allothreonine, the hydrolytic product must have possessed the common L-configuration.

(12) A. M. Violante, R. J. Sirny and C. A. Elvehjem, *J. Nutrition*, **47**, 307 (1952).

MADISON, WISCONSIN

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

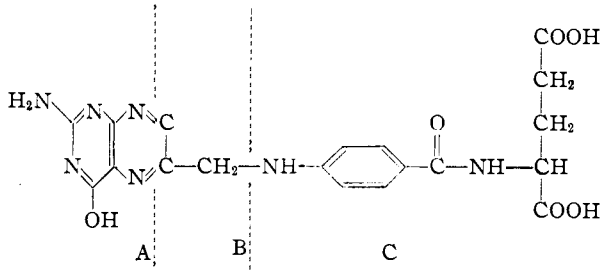
Synthesis of Pteroic and Pteroylglutamic Acids.[1] I.

BY D. I. WEISBLAT, B. J. MAGERLEIN, A. R. HANZE, D. R. MYERS AND S. T. ROLFSON

RECEIVED MARCH 9, 1953

A new general synthesis of pteroic and pteroylglutamic acids is reported wherein an N-tosyl-*p*-aminobenzoate or N-tosyl-*p*-aminobenzoylglutamate is alkylated with a substituted propylene oxide molecule, the product oxidized to a ketone, and condensed with 2,4,5-triamino-6-hydroxypyrimidine. Pteroic and pteroylglutamic acids are then formed from the N^{10}-tosyl compounds by the use of an improved detosylation procedure.

Since the initial publication of the structure and synthesis of pteroylglutamic acid,[2] many papers and patents have appeared describing syntheses of this member of the vitamin B complex.[3] Since the pteroylglutamic acid molecule consists of a 2,4,5-triamino-6-hydroxypyrimidine portion (A), a three carbon system (B), and a *p*-aminobenzoylglutamic acid portion (C), these syntheses of



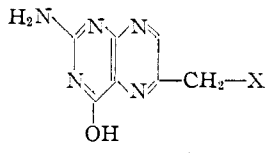
pteroylglutamic acid may be divided into three general classes. The first and most widely investigated type of synthesis employs the simultaneous reaction of the three components, A + B + C, the earliest type of which employed 2,3-dibromopropanal as the B portion.[3a] Other three carbon molecules are 2,2,3-tribromopropanal,[3g] 1,1,3-tribromopropanone-2,[3g,h] glyceraldehyde ditosylate,[3e] dihydroxyacetone[3e] and dichloroacetone.[4] The second type of synthesis, represented as AB + C, involves condensation of the pyrimidine portion (A) with a trifunctional three carbon component (B) to form a product (AB), which in turn is condensed with the *p*-aminobenzoylglutamic acid portion. Representative AB molecules which have been used are N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide (I),[3b] the corresponding bromo derivative II,[3d] the hydroxy derivative III[3e] and the pteridyl aldehyde IV.[3f] In the third

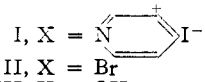
(1) Presented in part before the Division of Biological Chemistry at the XIIth International Congress of Pure and Applied Chemistry, New York, September 10 to 13, 1951.

(2) R. B. Angier, J. H. Boothe, B. L. Hutchings, J. H. Mowat, J. Semb, E. L. R. Stokstad, Y. SubbaRow, C. W. Waller, D. B. Cosulich, M. J. Fahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels and J. M. Smith, Jr., *Science*, **103**, 667 (1946).

(3) (a) C. W. Waller, B. L. Hutchings, J. H. Mowat, E. L. R. Stokstad, J. H. Boothe, R. B. Angier, J. Semb, Y. SubbaRow, D. B. Cosulich, M. J. Fahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels and J. M. Smith, Jr., THIS JOURNAL, **70**, 19 (1948); (b) M. E. Hultquist, E. Kuh, D. B. Cosulich, M. J. Fahrenbach, E. H. Northey, D. R. Seeger, J. P. Sickels, J. M. Smith, Jr., R. B. Angier, J. H. Boothe, B. L. Hutchings, J. H. Mowat, J. Semb, E. L. R. Stokstad, Y. SubbaRow and C. W. Waller, *ibid.*, **70**, 23 (1948); (c) R. B. Angier, E. L. R. Stokstad, J. H. Mowat, B. L. Hutchings, J. H. Boothe, C. W. Waller, J. Semb, Y. SubbaRow, D. B. Cosulich, M. J. Fahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels and J. M. Smith, Jr., *ibid.*, **70**, 25 (1948); (d) J. H. Boothe, C. W. Waller, E. L. R. Stokstad, B. L. Hutchings, J. H. Mowat, R. B. Angier, J. Semb, Y. SubbaRow, D. B. Cosulich, M. J. Fahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels and J. M. Smith, Jr., *ibid.*, **70**, 27 (1948); (e) P. Karrer and R. Schwyzer, *Helv. Chim. Acta*, **31**, 777 (1948); (f) F. Weygand, A. Wacher and V. Schmied-Kowarzik, *Chem. Ber.*, **82**, 25 (1949); (g) F. Weygand and V. Schmied-Kowarzik, *ibid.*, **82**, 333 (1949); (h) F. E. King and P. C. Spensley, *Nature*, **164**, 574 (1949); (i) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 2002 (1949).

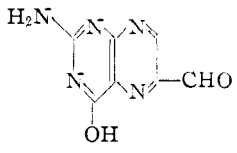
(4) D. I. Weisblat and A. R. Hanze, U. S. Patent 2,560,616 (1951).



I, X = 

II, X = Br

III, X = OH



IV

type of synthesis, represented as A + BC, the three carbon component (B) is allowed to react with a *p*-aminobenzoylglutamate (C) to give a product (BC) which is then condensed with the pyrimidine portion. Examples of the BC molecule are the anils formed by the reaction of the *p*-aminobenzoylglutamate portion with α,β-dibromopropionaldehyde[3a] and with reductone.[3c,i]

In the general synthesis described herein, which is of the third type outlined above, the BC portion of the molecule is formed by alkylating the C-portion with a substituted propylene oxide. In order to alkylate ethyl *p*-aminobenzoate or diethyl *p*-aminobenzoylglutamate with propylene oxide derivatives and to oxidize the resulting secondary alcohols in high yields it was necessary to block the primary amino group. The *p*-toluenesulfonyl (tosyl) group served admirably in this capacity. The outline of the synthesis is given in Fig. 1. The final step, the detosylation of diethyl N^{10}-tosylpteroylglutamate (Xb), was performed by a new detosylation procedure using 30% hydrogen bromide in acetic acid in the presence of phenol.[5]

Diethyl N-[N'-tosyl-*p*-aminobenzoyl]-L-glutamate (Vb), the starting material for the synthesis of pteroylglutamic acid, was prepared from diethyl *p*-aminobenzoyl-L-glutamate by treatment with tosyl chloride or from ethyl *p*-aminobenzoate by tosylation followed by conversion to the acid chloride and reaction with diethyl L-glutamate.

In the first series of reactions investigated ethyl N-tosyl-*p*-aminobenzoate (Va) was caused to react with epichlorohydrin in the presence of a catalytic amount of pyridine to give sirupy ethyl N-tosyl-N-(3-chloro-2-hydroxypropyl)-*p*-aminobenzoate (VIa). This compound was not purified, but was treated with dilute sodium hydroxide in ethanol solution to form the epoxide VIIa in 68% over-all yield. When the epoxide VIIa was refluxed with pyridine hydrochloride in ethanol[6] crystalline halohydrin VIa was obtained in excellent yield.

Oxidation of either the crystalline or sirupy halohydrin VIa with chromic oxide in acetic acid solution gave the crystalline chloroketone VIIIa. Condensation of the chloroketone with 2,4,5-triamino-6-hydroxypyrimidine[7] in glacial acetic acid, followed by detosylation with hydrogen bromide in acetic acid in the presence of phenol,[5] and saponification gave pteroic acid in a weight yield of 18%. The chemical assay for pteroic acid[8] was 34%, making an over-all yield of 6.1%.

(5) D. I. Weisblat, B. J. Magerlein and D. R. Myers, THIS JOURNAL, **75**, 3630 (1953).

(6) P. N. Chakravorty and R. H. Levin, *ibid.*, **64**, 2317 (1942).

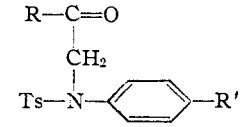
(7) W. Traube, *Ber.*, **33**, 1371 (1900).

(8) B. L. Hutchings, E. L. R. Stokstad, J. H. Boothe, J. H. Mowat, C. W. Waller, R. B. Angier, J. Semb and Y. SubbaRow, *J. Biol. Chem.*, **168**, 705 (1947).

In the course of the above work it was noted that higher yields of pteroic acid were obtained when the crude chloroketone VIIIa rather than the purified compound was used. Therefore, synthesis of other intermediates was undertaken which might give better yields of pteroylglutamic acid. Ethyl N - tosyl - N - (3 - acetoxy - 2 - hydroxypropyl) - *p*-aminobenzoate (VIb) was prepared by alkylation of ethyl N-tosyl-*p*-aminobenzoate (Va) with glycidol acetate or by opening the oxide ring of ethyl N-(2,3-oxidopropyl)-N-tosyl-*p*-aminobenzoate (VIIa) with acetic acid. The corresponding benzoate VId was prepared by the latter method, using benzoic acid in place of acetic acid. The acetoxy VIc and methoxy VIf pteroylglutamic acid intermediates were prepared by alkylation of diethyl N-tosyl-*p*-aminobenzoylglutamate (Vb) with glycidol acetate and glycidol methyl ester, respectively. All of the above intermediates were oxidized with chromic acid and condensed with the 2,4,5-triamino-6-hydroxypyrimidine to give pteroic or pteroylglutamic acids in yields as outlined in Table I. The products were assayed biologically against *Streptococcus faecalis R* or *Lactobacillus casei*, as well as chemically.[8]

TABLE I

YIELDS OF PTEROIC AND PTEROYLGLUTAMIC ACIDS FROM N-ALKYLATED *p*-TOLUENESULFONAMIDES



*Gl = diethyl L-(+) glutamate radical.

R	R'	Yield of folic or pteroic acids, %	Chemical assay, %	Over-all yield, %
CH_2Cl	CO_2Et	18.1	33.6	6.1
CH_2OAc	CO_2Et	23.4	6.4	15.0
CH_2OBz	CO_2Et	20.6	1.2	2.1
CH_2OMe	COGl*	48.0	31.0	14.9
CH_2OH	CO_2Et	48.9	54.2	26.4
CH_2OH	COGl*	55.0	38.9	21.4

The hydrolysis of the chloroketone VIIIa to the hydroxyketone IXa was studied under a variety of conditions. Little product was obtained by the use of potassium formate in methanol, silver nitrate in dioxane, silver oxide in acetone, sodium and potassium acetate in acetic acid or ethanol, silver carbonate in moist hydrocarbon solvents, barium carbonate in dilute acetone, dilute sodium hydroxide, or sodium bicarbonate solutions. In most cases the principal product isolated was ethyl N-tosyl-*p*-aminobenzoate formed by cleavage. The best results were obtained by treating the chloroketone with a dilute solution of sodium bicarbonate, keeping the *p*H under 8.5.[9] The impure product was converted to pteroic acid in 5.4% over-all yield. The best yield of pteroic acid (26% over-all) was obtained using hydroxyketone IXa prepared by ester interchange of the keto acetate in alcohol with *p*-toluenesulfonic acid as the catalyst. Pteroylglutamic acid was prepared from the corresponding hydroxy ketone IXb which was

(9) D. B. Sprinson and A. R. Chargaff, *ibid.*, **164**, 417 (1946).

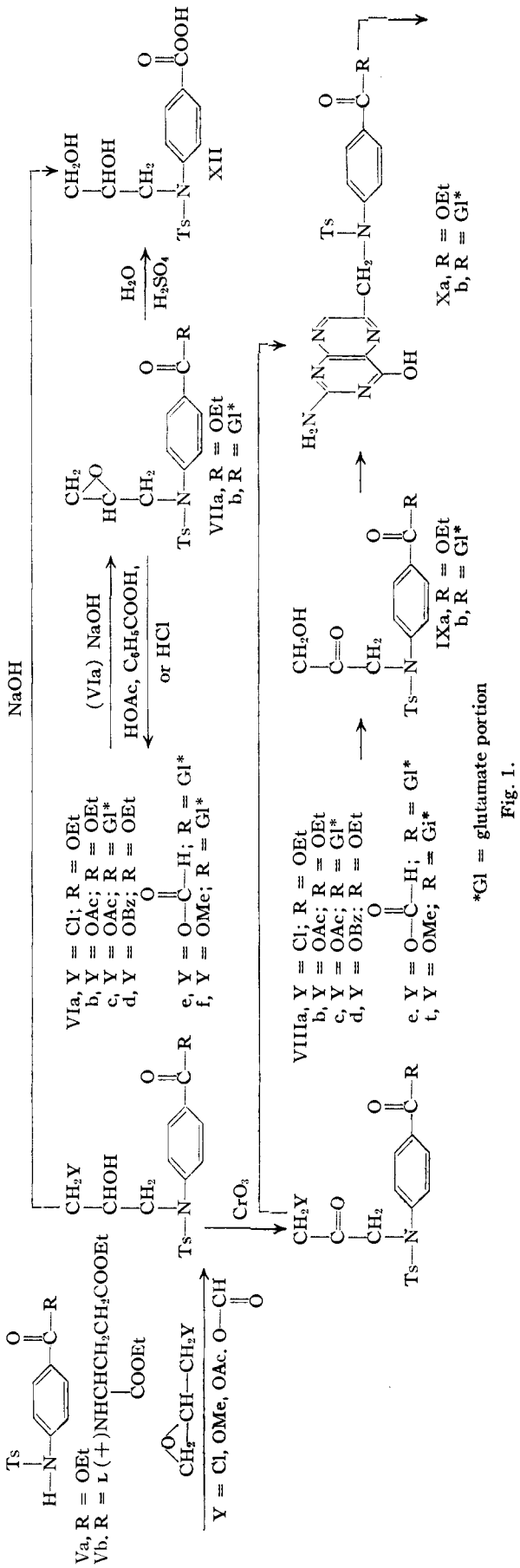
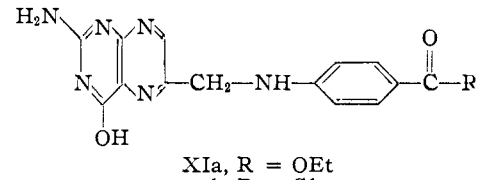


Fig. 1.



XIa, R = OEt
b, R = Gl

obtained in similar manner from the keto acetate VIIIc and keto formate VIIIe.

Purification of the pteroic and pteroylglutamic acids could be effected by the methods described in the literature for the purification of pteroylglutamic acid and other pteridines.[10,3a]

We express our appreciation to H. H. Buskirk and E. M. Stapert for bioassays; to Mrs. E. Saggio and Mrs. M. Katzenberger for chemical assays; to G. Pish and L. Scholten for ultraviolet spectra; to W. A. Struck and associates for microanalyses; and to G. Staffen for technical assistance.

Experimental[11]

A. Preparation of Diethyl N-(N′-Tosyl-*p*-aminobenzoyl)-L-glutamate (Vb). N-Tosyl-*p*-aminobenzoic Acid.—Ethyl *p*-aminobenzoate (165.1 g., 1.0 mole) was dissolved in 450 ml. (4.0 moles) of 2,4-lutidine. Two hundred grams of *p*-toluenesulfonyl chloride (1.05 moles) was added to the well stirred solution at such a rate that the temperature of the solution did not exceed 80°. After all the acid chloride was added, the temperature was maintained at 75–80° for an additional 45 minutes. The hot solution was poured slowly into 1.5 l. of water with vigorous stirring and the mixture cooled to room temperature. The mixture was filtered and the solid washed well with water. The wet solid was added to 100 g. of sodium hydroxide in 1.5 l. of water, the solution brought to boiling and maintained 15 minutes. The hot solution was clarified and added slowly to a vigorously stirred solution of 152 ml. of acetic acid in 400 ml. of water. The mixture was cooled, filtered, washed well with water and dried; yield 286 g. (98%), m.p. 230–232° (dec.).

N-Tosyl-*p*-aminobenzoyl Chloride.—Thionyl chloride (59.0 g.) was added slowly to a suspension of 29.1 g. (0.1 mole) of N-tosyl-*p*-aminobenzoic acid in 250 ml. of refluxing benzene containing a few drops of pyridine. When all of the thionyl chloride had been added, the solution was refluxed for 30 minutes. The solution was then cooled with stirring at 10° and allowed to crystallize for 3 hours. The product was filtered and washed with 200 ml. of toluene followed by 300 ml. of Skellysolve F. Recrystallization from toluene containing a trace of thionyl chloride gave material melting at 137–141° (dec.); yield 27.6 g. (89%).

Anal. Calcd. for $C_{14}H_{12}ClNO_3S$: C, 54.3; H, 3.91; Cl, 11.5. Found: C, 54.6, 54.5; H, 4.09, 3.95; Cl, 10.8, 10.9.

Diethyl N-[N′-Tosyl-*p*-aminobenzoyl]-L-glutamate (Vb). (a) From N-Tosyl-*p*-aminobenzoyl Chloride.—Diethyl L-glutamate hydrochloride (56.5 g., 0.24 mole) and N-tosyl-*p*-aminobenzoyl chloride (73.0 g., 0.24 mole) were dissolved in 420 ml. of ethylene dichloride. To this solution was added 52.3 g. of triethylamine in 162 ml. of ethylene dichloride at such a rate that the temperature did not exceed 20° (2–3 hours). When all of the triethylamine solution had been added, the solution was allowed to warm to room temperature and stirred an additional hour. After washing and drying the solution, Skellysolve B was added to opalescence. The mixture was allowed to crystallize, filtered, and the crystals washed with Skellysolve B. The material melted at 124–125° and weighed 94.5 g. (84.3% yield). Recrystallization from dilute ethanol gave material of m.p. 125–126°.

(10) H. Wieland and C. Schopf, *Ber.*, **58B**, 2178 (1925); C. Schopf and H. Wieland, *ibid.*, **59B**, 2067 (1926); H. Wieland and R. Purrmann, *Ann.*, **544**, 173 (1948); B. L. Hutchings, U. S. Patent 2,470,491 (1946); C. W. Waller, U. S. Patent 2,474,022 (1949); J. W. Greiner, A. R. Hanze, R. V. Kline, J. L. Richmond and K. R. Bedell, U. S. Patent 2,630,434 (1953).

(11) All melting points are uncorrected.

Anal. Calcd. for $C_{23}H_{28}N_2O_7S$: C, 58.0; H, 5.9; N, 5.9; S, 6.7. Found: C, 58.0; H, 5.9; N, 6.1; S, 6.9.

(b) From **Diethyl N-*p*-Aminobenzoyl-L-glutamate**.—To an ice-cold solution of 1.61 g. (0.005 mole) of diethyl N-*p*-aminobenzoyl-L-glutamate in 5 ml. of dry pyridine was added 0.95 g. (0.005 mole) of tosyl chloride. The mixture was shaken in an ice-bath for approximately 15 minutes until all of the tosyl chloride dissolved and then allowed to stand at room temperature overnight. The solution was then poured with stirring into 40 ml. of water. Crystallization occurred on stirring and scratching the sirup which separated. The product was recrystallized twice from dilute alcohol; yield 1.73 g. (73%), m.p. 125–126°, $[\alpha]^{25}D$ −12.0° (*c* 2.74, alcohol).

B. Alkylations of Ethyl N-Tosyl-*p*-aminobenzoate (Va) and of Diethyl N-Tosyl-*p*-aminobenzoyl-L-glutamate (Vb) with Substituted Propylene Oxides. Ethyl N-Tosyl-N-(2,3-oxidopropyl)-*p*-aminobenzoate (VIIa).—A mixture of 5.0 g. (0.016 mole) of ethyl N-tosyl-*p*-aminobenzoate and 3.4 ml. (0.045 mole) of epichlorohydrin was heated to 135°. The addition of two drops of pyridine brought about a vigorous reaction which continued for about 5 minutes. The dark brown melt was kept at 135° for 5 minutes, cooled, dissolved in 50 ml. of ethanol, and treated three times with decolorizing charcoal. The ethyl N-tosyl-N-(3-chloro-2-hydroxypropyl)-*p*-aminobenzoate (VIa) failed to crystallize.

To the boiling alcoholic solution of the halohydrin, containing 3 drops of phenolphthalein, 10% sodium hydroxide solution was added dropwise until the pink color of the indicator persisted. From this solution, after dilution with water, there was obtained 4.1 g. (68% yield) of crude product, m.p. 69–71°. After several recrystallizations from dilute ethanol the compound melted at 71–72°.

Anal. Calcd. for $C_{19}H_{21}NO_5S$: C, 60.8; H, 5.6; N, 3.7. Found: C, 60.6, 61.4; H, 5.8, 5.6; N, 3.9, 3.9.

Ethyl N-Tosyl-N-(3-chloro-2-hydroxypropyl)-*p*-aminobenzoate (VIa).—A solution of 16.0 g. (0.0425 mole) of oxide VIIa and 12.0 g. (0.105 mole) of pyridine hydrochloride in 100 ml. of ethanol and 10 ml. of water was refluxed 30 minutes. After distilling 70 ml. of the ethanol, the solution was diluted with water and extracted with ether. Concentration of the ether solution gave 12.7 g. of crystals, m.p. 77–81° (72.6% yield). Recrystallization from dilute ethanol gave crystals melting at 84–86°.

Anal. Calcd. for $C_{19}H_{22}ClNO_5S$: C, 55.4; H, 5.4; N, 3.4; Cl, 8.6. Found: C, 55.5, 55.7; H, 5.5, 5.3; N, 3.6, 3.6; Cl, 8.6, 8.5.

This crystalline halohydrin could be reconverted to the oxide in excellent yield with dilute sodium hydroxide in ethanol as previously described.

N-Tosyl-N-(2,3-dihydroxypropyl)-*p*-aminobenzoic Acid (XII). (a).—A solution of 1.88 g. (0.005 mole) of ethyl N-tosyl-N-(2,3-oxidopropyl)-*p*-aminobenzoate in 10 ml. of purified dioxane and 3 ml. of water containing 2 drops of concentrated sulfuric acid was heated at 100° for 21 hours. The solvent was distilled *in vacuo* and the sirupy residue dissolved in 5 ml. of 95% ethanol and 10 ml. of 10% potassium hydroxide. The solution was refluxed 30 minutes, cooled and clarified. Acidification gave 1.3 g. (71%) of the diol-acid, m.p. 171–172°. Several recrystallizations from dilute alcohol raised the melting point to 172–174.5°.

Anal. Calcd. for $C_{17}H_{18}NO_6S$: C, 55.9; H, 5.2; N, 3.8. Found: C, 55.9, 56.0; H, 5.3, 5.5; N, 3.8, 3.9.

(b).—The same compound was obtained by saponification of ethyl N-(tosyl)-N-(3-acetoxy-2-hydroxypropyl)-*p*-aminobenzoate with alkali followed by acidification.

Ethyl N-Tosyl-N-(3-acetoxy-2-hydroxypropyl)-*p*-aminobenzoate (VIb). (a) From Ethyl N-tosyl-N-(2,3-oxidopropyl)-*p*-aminobenzoate (VIIa).—A solution of 15.0 g. of ethyl N-tosyl-N-(2,3-oxidopropyl)-*p*-aminobenzoate in 3.0 g. of glacial acetic acid containing 3 drops of pyridine was heated at 120° for 2 hours. The cooled solution was dissolved in ether, washed with dilute hydrochloric acid, sodium bicarbonate, dried and concentrated to give 14.5 g. of viscous, non-crystalline VIb (88% yield). A portion of this material after chromatography over alumina using benzene containing 4% methanol as the eluant gave a colorless glass which resisted crystallization.

Anal. Calcd. for $C_{23}H_{27}NO_8S$: C, 57.9; H, 5.78; N, 3.22. Found: C, 58.1, 57.9; H, 5.63, 5.71; N, 3.45, 3.41.

(b) From **Ethyl N-Tosyl-*p*-aminobenzoate**.—A mixture of 12.3 g. of glycidol acetate and 31.9 g. of ethyl N-(tosyl)-*p*-aminobenzoate was heated to 90°. Five drops of pyridine was added and the temperature raised to 140–145°. The temperature was maintained at this temperature for 20 minutes. The dark colored melt was dissolved in benzene, washed with dilute sulfuric acid, dried and concentrated. There was recovered, by crystallization from ethanol, 2.1 g. of ethyl N-tosyl-*p*-aminobenzoate, m.p. 202–204°, which was identified by mixed melting point. The oily product obtained upon concentration of the filtrate could not be crystallized, but proved to be similar to the product prepared in method A in its chemical reactions.

Diethyl N-[N'-Tosyl-N'-(3-acetoxy-2-hydroxypropyl)-*p*-aminobenzoyl]-L-glutamate (VIc).—In a manner similar to the preparation of ethyl N-tosyl-N-(3-acetoxy-2-hydroxypropyl)-*p*-aminobenzoate (VIb), diethyl N-[N'-tosyl-N'-(3-acetoxy-2-hydroxypropyl)-*p*-aminobenzoyl]-L-glutamate was prepared by alkylation of Vb with glycidol acetate. The oil which was obtained resisted all attempts at crystallization. Chromatography over alumina gave a clear sirup which was analyzed.

Anal. Calcd. for $C_{28}H_{36}N_2O_{10}S$: C, 56.8; H, 6.12; N, 4.73. Found: C, 57.57; H, 6.13; N, 4.68.

Diethyl N-[N'-tosyl-N'-(3-formyloxy-2-hydroxypropyl)-*p*-aminobenzoyl]-L-glutamate (VIe) and diethyl N-[N'-tosyl-N'-(3-methoxy-2-hydroxypropyl)-*p*-aminobenzoyl]-L-glutamate (VIf) were prepared in a similar manner, using glycidol formate and glycidol methyl ether, respectively, as alkylating agents on diethyl N-[N'-tosyl-*p*-aminobenzoyl]-L-glutamate. We were unable to crystallize either of these compounds and the sirups were used for the oxidation.

Ethyl N-Tosyl-N-(3-benzoyloxy-2-hydroxypropyl)-*p*-aminobenzoate (VId).—In a manner similar to the preparation of VIb above ethyl N-tosyl-N-(3-benzoyloxy-2-hydroxypropyl)-*p*-aminobenzoate, m.p. 133–135° was prepared in 70% yield by the action of benzoic acid on ethyl N-tosyl-N-(2,3-oxidopropyl)-*p*-aminobenzoate.

Anal. Calcd. for $C_{26}H_{27}NSO_7$: C, 62.7; H, 5.5; N, 2.8. Found: C, 63.0, 63.1; H, 5.3, 5.4; N, 2.7, 2.9.

C. CrO_3 Oxidations. Ethyl N-Tosyl-N-(3-chloro-2-ketopropyl)-*p*-aminobenzoate (VIIIa).—Crude ethyl N-tosyl-N-(3-chloro-2-hydroxypropyl)-*p*-aminobenzoate (VIa) from 34.0 g. (0.107 mole) of ethyl N-tosyl-*p*-aminobenzoate, was dissolved in 200 ml. of acetic acid. Over a period of 2 hours a solution of 22.4 g. (0.224 mole) of CrO_3 in 10 ml. of water and 300 ml. of acetic acid was added. The temperature was maintained at 15–20°. After stirring at room temperature for 16 hours the acetic acid was distilled under reduced pressure keeping the bath temperature less than 40°. To the green residue was added 500 ml. of water and 100 ml. of ethyl acetate. After stirring for 15 minutes the crystals were filtered. The crystals were washed three times with 150 ml. of water, and once with 30 ml. of ethanol. The yield was 24.0 g. (50.4%), m.p. 101–106°. Recrystallization from *n*-propyl alcohol raised the melting point to 116–122°. Several recrystallizations from benzene gave an analytical sample, m.p. 122–124°.

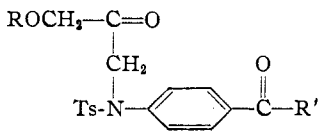
Anal. Calcd. for $C_{19}H_{20}ClNSO_5$: C, 55.7; H, 4.9; N, 3.4; Cl, 8.7. Found: C, 55.6, 55.5; H, 5.1, 4.9; N, 3.9, 4.0; Cl, 8.6, 8.5.

Oxidation of 1.20 g. of crystalline ethyl N-tosyl-N-(3-chloro-2-hydroxypropyl)-*p*-aminobenzoate under similar conditions gave 1.10 g. (92.0% yield) of chloroketone, m.p. 112–118°. Recrystallization gave a pure sample whose mixed melting point with the above analytical sample was not depressed.

In similar manner the other alkylation products were oxidized to the corresponding ketones, which have the constants recorded in Table II.

D. Pteroic Acid. (a) From Ethyl N-Tosyl-N-(3-acetoxy-2-ketopropyl)-*p*-aminobenzoate (VIIIb).—A solution of 100 mg. of 2,4,5-triamino-6-hydroxypyrimidine sulfate and 340 mg. of crude ethyl N-tosyl-N-(3-acetoxy-2-ketopropyl)-*p*-aminobenzoate in 20 ml. of acetic acid was refluxed for two hours. The solution was concentrated and the residue dissolved in 2 ml. of 30% hydrogen bromide in glacial acetic acid containing 100 mg. of phenol. After two hours at room temperature the mixture was poured into 30 ml. of anhydrous ether. The precipitate was dried under vacuum. It was dissolved in 2 ml. of methanol and 15 ml. of 0.25 *N*

TABLE II



Gl* = diethyl glutamate.

R	R'	M.p., °C.	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Yield, %
$CH_3C{=}O$	OC_2H_5	Sirup							82.3
$CH_3{-}C{=}O$	Gl*	Sirup	56.9	56.65	5.80	5.80	4.74	4.63	78.4
$C_6H_5{-}C{=}O$	OC_2H_5	70–72°	63.0	63.2	5.1	5.2			68
$H{-}C{=}O$	Gl*	Sirup							75.2
CH_3	Gl*	87–92°	57.64	57.46	6.09	6.14	4.98	5.02	61

NaOH added. After one hour at room temperature the *p*H was adjusted to 3.0, the precipitate collected, washed with water and then three times with acetone. There was obtained 51 mg. (23.4%) of dark colored solid which showed 6.4% of pteroic acid when assayed against *Streptococcus faecalis R.*

(b) From **Ethyl N-Tosyl-N-(3-hydroxy-2-ketopropyl)-*p*-aminobenzoate (IXa).**—Three grams of crude ethyl N-tosyl-N-(3-acetoxy-2-ketopropyl)-*p*-aminobenzoate was dissolved in 40 ml. of absolute ethanol and 100 mg. of *p*-toluenesulfonic acid added. The solution was refluxed six hours during which time a portion of the solvent was distilled and fresh solvent added. Saponification of the distillate showed the presence of 77.1% of the theoretical amount of ethyl acetate. Further heating failed to liberate more ethyl acetate. The solution was poured into water, extracted with ether, washed, dried and concentrated to give 2.5 g. of oil (96.5% yield). This oil could not be crystallized, but is presumed to be chiefly the hydroxy ketone IXa. Reaction of 380 mg. of this oil with 150 mg. of 2,4,5-triamino-6-hydroxypyrimidine and 115 mg. of sodium acetate in 15 ml. of acetic acid followed by detosylation and saponification as described above, gave 136 mg. (62.0%) of brown solid which contained 36.5% of pteroic acid by assay against *Streptococcus faecalis R.*

If the crude IXa was dissolved in dilute methanol and treated at 50° with a slight excess of copper acetate the resulting product could be converted to pteroic acid in slightly higher yields.

In similar manner the other oxidation products were condensed with 2,4,5-triamino-6-hydroxypyrimidine to give the yields indicated in Table I.

E. Pteroylglutamic Acid. (a) From Diethyl N-[N'-tosyl-N'-(3-hydroxy-2-ketopropyl)-*p*-aminobenzoyl]-L-glutamate (IXb).—A solution of 2.34 g. of diethyl N-[N'-tosyl-N'-(3-acetoxy-2-ketopropyl)-*p*-aminobenzoyl]-L-glutamate and 0.1 g. of *p*-toluenesulfonic acid in 40 ml. of absolute alcohol was refluxed for three hours. Then approximately 30 ml. of the ethanol–ethyl acetate mixture was distilled and 40 ml. of absolute ethyl alcohol added. This solution was heated under reflux an additional three hours and then concentrated under reduced pressure to an oil. The oil was dissolved in ethyl acetate and washed with water three times and with saturated sodium chloride solution once. The solution was dried over anhydrous sodium sulfate, filtered and concentrated to an oil; yield 2.3 g. (IXb).

A portion of the above oil (425 mg., 0.77 millimole), 150 mg. (0.7 millimole) of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride and 115 mg. (1.4 millimoles) of sodium acetate reacted in 7 ml. of glacial acetic acid at 25° in a nitrogen atmosphere for one hour. The mixture was concentrated under reduced pressure to a paste which was detosylated by treatment with 3.5 ml. of 30% hydrogen bromide in acetic acid and 130 mg. of phenol for 100 minutes at room temperature. The mixture was then poured into 30 ml. of dry ether and the solid which separated was washed with fresh ether by centrifugation. The solid was dried over phosphorus pentoxide and saponified for 70 minutes in a mixture of 5 ml. of methyl alcohol and 20 ml. of 0.25 *N* NaOH. The pteroylglutamic acid was precipitated by adjusting the *p*H to 3.0. The solid was centrifuged and washed by centrifugation with water and acetone; yield 170 mg. (55% by weight). The material assayed 38.9% *vs. Streptococcus faecalis R* and 35.5% *vs. Lactobacillus casei*, making the over-all yield 19.5 to 21.4%.

The material may be purified by the methods previously cited.[10,3a]

Condensations of the other chromic acid oxidation products with 2,4,5-triamino-6-hydroxypyrimidine were carried out in similar manner in yields reported in Table I.

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