The alkaloidal material recovered from the next few fractions eluted from the column with chloroform proved to be mixtures when analyzed by countercurrent distribution. The alkaloid fractions eluted with chloroform–1% methanol yielded protoveratrine B on crystallization from ether. The homogeneity of the product was demonstrated by countercurrent distribution,[8] and its identity was confirmed by direct comparison with an authentic specimen of protoveratrine B.[13]

Acknowledgment.—The generous assistance of Eli Lilly and Co., Indianapolis, Ind., in procuring and extracting *Veratrum album* is gratefully acknowledged.

Experimental

Isolation of Neogermitrine and Germitetrine B by Chromatography of *Veratrum album* Amorphous Bases.—The total alkaloids obtained from *Veratrum album* were separated into ether-insoluble and ether-soluble fractions by the procedure described by Craig and Jacobs.[12] The ether-soluble fraction was treated for the removal of inactive alkamines according to the procedure of Fried, White and Wintersteiner.[4a] The residual ether-soluble mixture was designated as the "amorphous bases."

A solution of the amorphous bases (10 g.) in chloroform (100 ml., Merck reagent, containing 0.75% alcohol) was chromatographed on alumina (250 g., in a column of 30 mm. diameter).[11] After a forerun of 150 ml. containing no solid material, the next 100-ml. fraction yielded 150 mg. of yellow oil. The following three 100-ml. fractions eluted with chloroform were combined and evaporated to dryness *in vacuo*. The colorless amorphous residue on crystallization from ether yielded neogermitrine as colorless needles, m.p. 237–239° dec. Recrystallization from acetone–water gave elongated rods, 660 mg., m.p. 236–237° dec., $[\alpha]^{23}D$ −78° (*c* 2.00, pyr.). The mixed melting point with an authentic specimen of neogermitrine[6b] was not depressed, and the infrared spectra of the two samples were identical.

The amorphous material recovered from the following two 100-ml. fractions yielded a crystalline mixture from ether. These were set aside for rechromatography, which afforded additional neogermitrine.

The next three 100-ml. fractions of the chloroform eluate were combined and evaporated to dryness *in vacuo*. Crystallization of the amorphous residue from ether yielded colorless needles, 300 mg., m.p. 230–232° dec. Recrystallization from *n*-butyl chloride yielded germitetrine B in the form of rectangular plates, m.p. 233–234° dec., $[\alpha]^{22}D$ −69° (*c* 2.00, pyr.).

Anal. Calcd. for $C_{41}H_{63}O_{14}N$: C, 62.02; H, 8.00. Found (after drying *in vacuo* at 120°): C, 62.08; H, 7.99.

The mixed melting point with an authentic specimen of germitetrine B[13] was not depressed. The infrared spectra and X-ray diffraction patterns[14] of the respective samples were identical.

Isolation of Protoveratrine A and Protoveratrine B by Chromatography of Protoveratrine.—Crude protoveratrine (the ether-insoluble fraction mentioned above) was purified by repeated crystallization from chloroform–petroleum ether and by reprecipitation with aqueous ammonia from dilute alcoholic acetic acid solution. By this procedure, protoveratrine melting at 265–267° dec. was obtained.

A solution of protoveratrine (5 g.) in chloroform (150 ml.) was chromatographed on alumina (125 g. in a column of 20 mm. diameter). The forerun of 200 ml. of chloroform contained no solid material; the next four 100-ml. fractions eluted with chloroform were combined and evaporated to dryness *in vacuo*. Upon addition of ether to the semicrystalline residue, 1.8 g. of crystalline solid separated. Recrystallization from chloroform–petroleum ether yielded protoveratrine A as colorless plates, m.p. 270–271° dec., $[\alpha]^{25}D$ −40° (*c* 2.00, pyr.). This material did not depress the melting point of an authentic specimen of protoveratrine A,[13] and the infrared spectra of the two samples were identical.

The material recovered from the following four 100-ml. fractions proved to be mixtures when analyzed by means of 14-plate countercurrent distribution using chloroform–2% acetic acid solution. Rechromatography afforded additional protoveratrine A.

Chloroform–1% methanol (that is, 99% Merck reagent chloroform +1% methanol) was then added to the column, and four 100-ml. fractions were collected, combined and evaporated to dryness *in vacuo*. After addition of ether to the semi-crystalline residue, 1.3 g. of crystalline solid was obtained. After two recrystallizations from acetone, 1.0 g. of protoveratrine B was obtained as prisms, m.p. 267–269° dec., $[\alpha]^{24}D$ −37° (*c* 2.00, pyr.). The mixed melting point with an authentic specimen of protoveratrine B[13] was unchanged, and the infrared spectra of the two samples were identical.

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

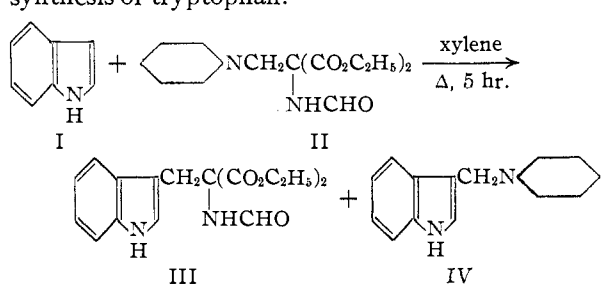
A Study of the Mannich Base–Indole Condensation

BY H. R. SNYDER, CAL Y. MEYERS AND DAVID B. KELLOM

RECEIVED MAY 11, 1953

The condensation of indole with diethyl (1-piperidylmethyl)-formamidomalonate to produce diethyl skatylformamidomalonate apparently proceeds with the intermediate formation of α-(1-piperidyl)-skatole. The scope of the condensation is found to be limited.

Many syntheses of the essential amino acid tryptophan involve the condensation of a Mannich base of indole with a derivative of an active methylene compound.[1–4] Thus, for example, 3-dimethylaminomethylindole can be condensed with formamidomalonic ester[4] to give diethyl skatylformamidomalonate (III), in 96% yield, which can be hydrolyzed and decarboxylated to tryptophan. More recently it has been found that indole (I) can be condensed with the Mannich base diethyl (1-piperidylmethyl)-formamidomalonate (II) to give III,[5] thus providing a new approach to the synthesis of tryptophan.



I II III IV

(1) H. R. Snyder and C. W. Smith, THIS JOURNAL, **66**, 350 (1944).

(2) N. F. Albertson, S. Archer and C. M. Suter, *ibid.*, **66**, 500 (1944); **67**, 36 (1945).

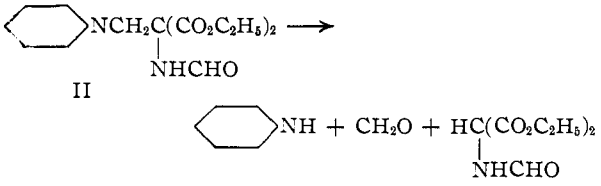
(3) N. F. Albertson and B. F. Tullar, *ibid.*, **67**, 502 (1945).

(4) H. Hellmann, *Z. physiol. Chem.*, **284**, 163 (1949).

(5) A. Butenandt, H. Hellmann and E. Renz, *ibid.*, **284**, 175 (1949).

The Mannich base II was one of a series of "tertiary ester bases" prepared by Butenandt and Hellmann[6] from secondary amines, formaldehyde and an acylaminomalonic ester. Up to the present, these investigators have confined their reports of the reactions of these Mannich bases to those of II.[6,7]

It was found by Butenandt, Hellmann and Renz[5] that the product of the reaction of indole with II depended largely upon the reaction conditions. With anhydrous xylene and a small amount of powdered sodium hydroxide, diethyl skatylformamidomalonate (III) was formed in 76% yield; but in the absence of sodium hydroxide, α-(1-piperidyl)-skatole (IV) was obtained in 70% yield. It was suggested that the formation of IV involved the decomposition of II on heating into piperidine and formaldehyde which can then react with indole. However, this cleavage requires at least a trace of water; since the reaction was observed under anhydrous conditions, it seemed to merit further study.



II

In the present investigation it was found that the base-catalyzed reaction gave approximately the same yield of the alkylated product III whether carried out under absolutely dry or non-anhydrous conditions. Although the non-catalyzed reaction was more complex because of the formation of a constant-melting mixture of IV and III of varying composition, this reaction also was apparently insensitive to small amounts of water. The Mannich base IV was the major product of the non-anhydrous reaction in the absence of sodium hydroxide. A small amount of the constant-melting mixture and III was also isolated. But in anhydrous xylene the constant-melting mixture was the main product. Since this constant-melting mixture was found in at least one case to be largely IV contaminated with a small amount of III, the yield of IV resulting from the non-catalyzed reaction was about the same under anhydrous and non-anhydrous conditions. Thus it seems unlikely that the reaction proceeds through reversal of the Mannich reaction of II. Also it is obvious that precautions for exclusion of moisture are unnecessary.

In a few cases the xylene, dried over sodium hydride, was pipetted directly into the reaction flask. Even when no sodium hydroxide was used as a catalyst, more of the alkylated product was formed than in the reactions employing the xylene which had been distilled before use. Apparently enough sodium hydride was introduced into the reaction mixture to act as the catalyst.

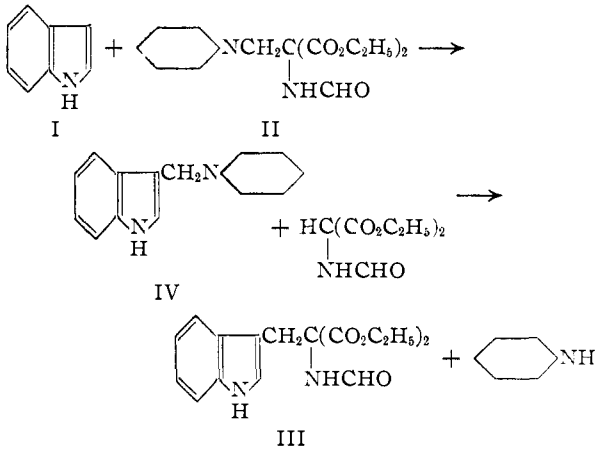
In agreement with the earlier work, the present study indicated that a greater amount of alkylated product III than Mannich base IV was formed in the catalyzed reaction. Under conditions identical to those mentioned above but without a condensation catalyst, the Mannich base IV was the major product. These facts suggested the possibility of the formation of the Mannich base, either with or without catalysis, and then its subsequent condensation with formamidomalonic ester (formed during the reaction) to the alkylated product III, the second step proceeding more rapidly in the presence of base. The data given in Table I indicated that IV can indeed be converted to III in the absence of added base by increasing the reaction time.

TABLE I

REACTION OF INDOLE WITH DIETHYL (1-PIPERIDYLMETHYL)-FORMAMIDOMALONATE (II) IN XYLENE

Reacn. time, hr.	Yield, % Mannich base IV	Alkylated product III
1	22	..
5	29	2
10	5	13
24	..	40

As a further test of the occurrence of IV as an intermediate in the formation of III, a mixture of IV and formamidomalonic ester was heated at reflux for 24 hours under conditions identical with those employed in the reactions given in Table I. From this reaction the alkylated product III was isolated in a yield of 42%. Therefore it is reasonable to assume that IV is an intermediate in the formation of III from indole and II, although the complete details for this reaction sequence are not known.[8]



I II

IV

III

A white crystalline solid which melted at 147° after several recrystallizations from aqeuous ethanol was isolated from a number of the condensations of indole with II. This product, referred to above as the constant-melting mixture, had an infrared spectrum very similar to that of the alkylated product III. Mixtures of this white solid from independent reactions showed no depression of the melting point, although microanalyses indicated that the percentages of carbon and hydrogen varied within the range corresponding to III and IV.

(6) A. Butenandt and H. Hellmann, *ibid.*, **284**, 168 (1949).

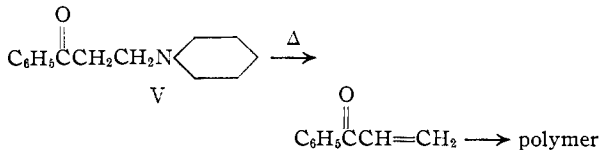
(7) H. Hellmann and E. Brendle, *ibid.*, **287**, 235 (1951).

(8) In a report available to the authors after this manuscript had been submitted, W. Kutscher and O. Klamerth [*Ber.*, **86**, 352 (1953)] proposed an analogous Mannich base exchange and alkylation process for the reaction of tertiary Mannich bases with pyrrole and suggested that the reaction of indole follows a similar course.

From a fractional crystallization of one sample of this solid from high-boiling petroleum ether both III and IV were isolated, 72% of the isolated solid being IV. It was found that physical mixtures of III and IV over a wide range of composition had melting points of between 147–153°. As further evidence for the nature of this material, it was prepared by seeding a solution of III and IV in xylene with a crystal of the solid obtained from a reaction mixture.

To examine the scope of this condensation, xylene solutions of diethyl (1-piperidylmethyl)-formamidomalonate (II) were refluxed with other reactants. β-Naphthol gave mainly a brown polymeric material, although a small amount of α-(1-piperidylmethyl)-β-naphthol was isolated. However, no products could be isolated from the treatment of II with acetophenone, malonic ester or imidazole.

The reaction of indole and phenyl β-piperidinoethyl ketone (V) also gave only a dark viscous polymeric material. Apparently the Mannich base was deaminated and the resulting phenyl vinyl ketone polymerized, this type of reaction being common with Mannich bases having an active hydrogen β to the amino group.[9]



Experimental[10]

Diethyl (1-Piperidylmethyl)-formamidomalonate (II).—Using the method of Butenandt and Hellmann,[8] diethyl (1-piperidylmethyl)-formamidomalonate was prepared from formamidomalonic ester[11] as white needles, m.p. 75.0–76.0 (lit. 77°[6]).

Reaction Conditions.—All solid reagents were dried in a vacuum desiccator and the xylene was distilled from sodium hydride just before use unless otherwise indicated. For the anhydrous reactions the apparatus was oven-dried, dry nitrogen was bubbled into the reaction mixtures and the condenser was protected with a calcium chloride tube. Air-dried apparatus was used for the non-anhydrous reactions, and a drop of water was added to each reaction mixture.

Reaction of Indole and II. A. With Sodium Hydroxide Catalyst.—Under anhydrous conditions a mixture of 1.17 g. (0.01 mole) of indole, 3.00 g. (0.01 mole) of II, 0.13 g. (0.0033 mole) of powdered sodium hydroxide and 8 ml. of xylene was refluxed for 5 hours. After cooling overnight in the refrigerator, 2.1 g. (63%) of crude, orange diethyl skatylformamidomalonate (III) was isolated. Recrystallization of the crude solid from aqueous ethanol using Norit gave white needles, m.p. 177.5–178.5° (lit. 179° uncor.[6]).

This procedure under non-anhydrous conditions gave 1.9 g. (57%) of crude white III, m.p. 178° after recrystallization.

B. **Without Catalyst.**—Under anhydrous conditions a mixture of 0.59 g. of indole, 1.50 g. of II and 8 ml. of xylene was refluxed for 5 hours. This reaction mixture after cooling overnight in the refrigerator gave a white solid when vigorously shaken. The solid, 0.43 g., was collected, washed with petroleum ether and recrystallized from aqueous ethanol using Norit to a constant melting point of 147°. This is a constant-melting mixture of IV and III (see Discussion above and Experimental below).

The filtrate was concentrated *in vacuo* and diluted with petroleum ether precipitating an orange oily solid. After several crystallizations from aqueous ethanol, a small amount of III was isolated, m.p. 178°.

The above reaction was repeated under non-anhydrous conditions. From the cold reaction mixture 0.31 g. of yellow pellets and 0.04 g. of a white solid were isolated by filtration and then separated physically. On recrystallization from aqueous ethanol using Norit, the yellow pellets gave white plates, m.p. 156–157°. No depression was observed in a mixed melting point of this material with an authentic sample of α-(1-piperidyl)-skatole (IV). The yield of crude IV was 29%. The white solid after recrystallization from aqueous ethanol had a melting point of 147° and did not depress the melting point of the previously obtained solid melting at 147°.

Further cooling of the filtrate yielded 0.03 g. of III, m.p. 178° after recrystallization from aqueous ethanol. Concentration of the mother liquor to a few milliliters and cooling gave 0.09 g. of a white solid, m.p. 148–154°. Three recrystallizations of this solid from aqueous ethanol gave crystals of m.p. 151–154°, probably a mixture of IV and III.

C. **Reaction in Undistilled Xylene.**—From a similar experiment in which 8 ml. of xylene dried over sodium hydride was transferred to the reaction flask with a pipet, 0.66 g. (40%) of recrystallized III, m.p. 178°, was obtained after 5 hours of reflux under anhydrous conditions.

From a similar reaction but under non-anhydrous conditions 0.45 g. of white solid was isolated from the cold reaction mixture. Recrystallization of this solid from aqueous methanol and ethanol gave white crystals, m.p. 147°. A very small amount of III, m.p. 178° after recrystallization, was isolated after additional cooling of the filtrate from the reaction mixture.

D. **Effect of Reflux Time on Reaction B.**—Under anhydrous conditions a series of reactions using 0.59 g. of indole, 1.50 g. of III and 8 ml. of xylene were run with varying times of reflux. The following results were obtained.

One-hour Reflux.—From the cold reaction mixture, 0.24 g. (22%) of crude IV, m.p. 156–157° after recrystallization from aqueous ethanol, was isolated.

Ten-hour Reflux.—A solid separated from the cold mixture only after scratching of the walls of the flask. The solid was collected, washed with petroleum ether and recrystallized from aqueous ethanol giving a mixture of white needles and plates, m.p. 159–163°. This mixture was heated with 30 ml. of high-boiling petroleum ether and the white needles that separated were collected. The needles, m.p. 177–178°, gave no depression in a mixed melting point with authentic III. The yield was 0.21 g. (13%). Concentration of the petroleum ether filtrate *in vacuo* gave small yellow plates which were recrystallized from aqueous ethanol using Norit giving 0.05 g. (5%) of IV, m.p. 157°. There was no depression of the melting point of authentic IV.

Twenty-four-hour Reflux.—The white solid that separated on cooling the reaction mixture was collected and recrystallized from aqueous ethanol to give 0.67 g. (40%) of III, m.p. 178°.

Preparation of α-(1-Piperidyl)-skatole (IV).—An authentic sample of α-(1-piperidyl)-skatole was prepared by a modification of the method of Kuhn and Stein,[12] the modification being similar to the method of Snyder, Smith and Stewart[13] for the synthesis of gramine. To 8.8 g. (0.105 mole) of piperidine cooled to 5° was added 14.0 g. (0.233 mole) of cold glacial acetic acid. The mixture was held at 5° and 7.6 g. of formalin (equivalent to 0.1 mole of formaldehyde) was added. After thorough agitation this mixture was added to 11.7 g. (0.1 mole) of indole. Shaking was continued until the solution became clear and the temperature stopped rising. Then it was allowed to stand at room temperature for 10 hours before the addition of a solution of 14 g. (0.35 mole) of sodium hydroxide in 100 ml. of water. From the cooled reaction mixture, 17.1 g. (79%) of crude IV was isolated. Two recrystallizations from aqueous methanol produced white crystals, m.p. 157.5–158.5° (lit. 161°,[12] 158–159°[14]).

Characterization of the Constant-melting Mixture.—From several of the reactions of indole and II, a white solid, m.p.

(9) C. Mannich and G. Heilner, *Ber.*, **55**, 356 (1922).

(10) All melting points are corrected. Microanalyses were carried out by Miss Emily Davis, Mrs. Jean Fortney and Mrs. Katherine Pih. The infrared spectra were obtained by Miss Elizabeth Petersen.

(11) A. Galat, THIS JOURNAL, **69**, 965 (1947).

(12) H. Kuhn and O. Stein, *Ber.*, **70**, 567 (1937).

(13) H. R. Snyder, C. W. Smith and J. M. Stewart, THIS JOURNAL, **66**, 200 (1944).

(14) E. E. Howe, A. J. Zambito, H. R. Snyder and M. Tishler, *ibid.*, **67**, 38 (1945).

147°, was isolated. The infrared spectrum of this solid suggested the presence of III, and microanalyses for three samples from different reactions indicated a varying mixture of III and IV.

Anal. Calcd. for $C_{17}H_{20}N_2O_5$: C, 61.45; H, 6.07. Calcd. for $C_{14}H_{18}N_2$: C, 78.46; H, 8.46. Found: C, 68.24, 71.00, 75.98; H, 7.24, 7.74, 8.21.

There was no depression of the melting point when the various samples were mixed. Also physical mixtures of III and IV over a wide range of composition had melting points of between 147–153°.

A 0.1-g. sample of the constant-melting mixture was heated with 25 ml. of high-boiling petroleum ether. The insoluble crystals, 0.021 g., were isolated and recrystallized from aqueous ethanol, m.p. 178°. There was no depression in a mixed melting point with authentic III. Concentration of the petroleum ether filtrate gave 0.056 g. of light tan crystals. Three recrystallizations from aqueous ethanol gave white plates, m.p. 157°, which did not depress the melting point of authentic IV.

To synthesize this constant-melting mixture, 0.200 g. of III and 0.300 g. of IV were dissolved in 4 ml. of hot xylene. Then this mixture was placed in an ice-bath and seeded with a crystal of the constant-melting mixture. From the cold solution 0.465 g. of white solid was recovered, m.p. 146.5–155.0°. A portion was recrystallized from aqueous ethanol, m.p. 146.0–148.0°.

Reaction of α-(1-Piperidyl)-skatole (IV) with Formamidomalonic Ester.—A solution of 0.214 g. (0.001 mole) of IV and 0.203 g. (0.001 mole) of formamidomalonic ester in 5 ml. of xylene was refluxed for 24 hours and then cooled in the refrigerator. The white solid was collected, washed with petroleum ether and recrystallized twice from aqueous ethanol giving 0.14 g. (42%) of III, m.p. 177–178°.

Reaction of β-Naphthol with Diethyl (1-Piperidylmethyl)-formamidomalonate (II).—A solution of 0.36 g. (0.0025 mole) of β-naphthol, 0.75 g. (0.0025 mole) of II and 0.06 g. (0.0015 mole) of powdered sodium hydroxide in 6 ml. of anhydrous benzene was heated to reflux for 5 hours and then cooled in the refrigerator. The frozen mixture was allowed to melt and the residual slightly yellow solid was collected. After two crystallizations from aqueous ethanol and one from benzene, 0.07 g. of small white crystals was obtained, m.p. 189–191°. The structure of this material has not yet been established. Its infrared spectrum in chloroform indicated the presence of naphthyl, hydroxyl and carbonyl groups.

Concentration of the benzene filtrate *in vacuo* caused the separation of tan crystals. These were recrystallized from aqueous ethanol to give 0.11 g. (18%) of white plates, m.p. 94–95°. There was no depression in a mixed melting point with an authentic sample of α-(1-piperidylmethyl)-β-naphthol, m.p. 94.5°.[15]

This reaction carried out in xylene gave in most cases only an amorphous brown polymeric material. However, in one instance an orange solid was recovered after removing the polymeric material and concentrating the xylene solution. Recrystallization from benzene gave 0.11 g. of white crystals, m.p. 194°, which were soluble in dilute sodium hydroxide, but insoluble in acid. The infrared spectrum in chloroform was identical with that of the unidentified material from the benzene reaction although the spectra in nujol mull were different.

Anal. Found: C, 69.69; H, 6.82; N, 8.45.

(15) J. Decombe, *Compt. rend.*, **197**, 258 (1933).

URBANA, ILLINOIS

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

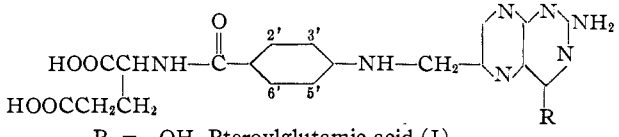
Analogs of Pteroylglutamic Acid. IX. Derivatives with Substituents on the Benzene Ring[1]

BY DONNA B. COSULICH, DORIS R. SEEGER, MARVIN J. FAHRENBACH, KENNETH H. COLLINS, BARBARA ROTH, MARTIN E. HULTQUIST AND JAMES M. SMITH, JR.

RECEIVED APRIL 27, 1953

A series of pteroylglutamic acid derivatives has been synthesized with halogen and methyl substituents in the benzene ring moiety. The nitration of pteroylglutamic acid and 4-aminopteroylglutamic acid has been investigated. A number of new nitrobenzoic and nitrobenzoylglutamic acid derivatives have been synthesized as intermediates and reference compounds.

A series of 3′,5′-dihalo analogs of pteroylglutamic acid (PGA, I) was described in a previous publication from this Laboratory.[2] Interesting activity in the inhibition of neoplasms in experimental animals was shown by some of these compounds; therefore, the synthesis of other pteroyl derivatives with substituents on the benzene moiety was undertaken in an effort to enhance this activity. The 3′,5′-dihalo derivatives were readily prepared by the direct halogenation of the appropriate pteroyl derivative in aqueous acid.[2] The nitration of PGA[3a] and 4-amino PGA (II)[3b] was examined briefly, and appeared to yield 3′,5′-dinitro derivatives. For other benzene ring substituents it was necessary to investigate different approaches which involved ultimately the preparation of the 4-aminobenzoylglutamic acids with suitable substituents, and subsequent condensation with a 4,5-diaminopyrimidine and a 3-carbon intermediate to yield the desired pteroyl compounds. The latter were purified in some cases, and in others the crude or partially purified materials were submitted for screening against tumors in experimental animals. Analogs which were obtained in pure form were 3′-chloropteroylglutamic acid, 3′-methylpteroylglutamic acid and the 2′-chloro-, 3′-chloro- and 3′-methyl analogs of 4-aminopteroylglutamic acid. No enhancement of activity in the inhibition of neoplastic disease in experimental animals was observed with any of the compounds described in this paper over that of 4-amino-3′,5′-dichloropteroylglutamic acid.[2]



R = –OH, Pteroylglutamic acid (I)
R = –NH_2, 4-Aminopteroylglutamic acid (II)

(1) For the preceding paper in this series see THIS JOURNAL, **78**, 2869 (1951).

(2) D. B. Cosulich, *et al.*, *ibid.*, **73**, 2554 (1951).

(3) (a) R. B. Angier, *et al.*, *Science*, **103**, 667 (1946); (b) D. R. Seeger, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **69**, 2567 (1947).