# The Synthesis of Homofolic Acid[1-3]

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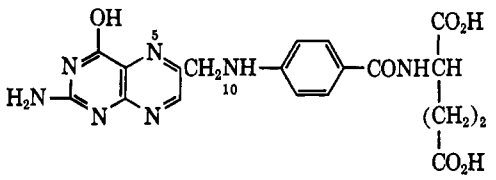
A synthesis of homofolic acid is described. The acid chloride of N-acetyl-N-(*p*-carbethoxyphenyl)-β-alanine was converted to the semicarbazone of 1-amino-4-[N-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone *via* the diazo ketone, chloromethyl ketone, and azidomethyl ketone. Condensation of the aminomethyl ketone semicarbazone with 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine was followed by hydrolysis of the semicarbazone and hydrogenative cyclization of the derived ketone to yield a dihydropteridine. Oxidation of the dihydropteridine, then saponification, afforded homopteroic acid that was blocked and coupled with diethyl L-glutamate. Hydrolysis of the product gave homofolic acid which has been shown to be an efficient and selective inhibitor of the enzyme thymidylate synthetase, derived from *Escherichia coli*.

The vitamin folic acid (I), generally as its tetrahydro derivative ($fH_4$), serves as a one-carbon transfer agent in a variety of biological systems. In the metabolism of the one-carbon fragments, either at the formyl or at the hydroxymethyl oxidation level, five-



I

membered cyclic compounds involving the one-carbon fragment and the N-5 and N-10 atoms of $fH_4$ are important intermediates.[5] The analog of I, homofolic acid (XXIV), in which an additional methylene group is present between N-5 and N-10, would require a six-membered cyclic intermediate for the analogous transfer of one-carbon fragments in its reduced form. The synthesis of XXIV is the subject of this manuscript; the fact that the tetrahydro derivative of XXIV is a selective inhibitor of the enzyme, thymidylate synthetase,[2] may result from the alteration in the geometry of the one-carbon transfer intermediates.

The general approach used by Boon and Leigh[6] for the synthesis of specifically 6-substituted 2-amino-4-hydroxypteridines was used in the preparation of XXIV, thus avoiding the difficult separation of 6- and 7-substituted compounds which plagues many simpler pteridine syntheses. The reaction of ethyl *p*-aminobenzoate and β-propiolactone[7] afforded an excellent yield of the β-alanine derivative II that was converted to the N-acetyl (III) and N-formyl (IV) acids. The acid chlorides V and VIII were prepared conventionally and were treated with excess diazomethane to give the diazo ketones VI and IX. Reaction of VI and IX with hydrogen chloride yielded the chloro ketones VII and X. The chloro ketones VII and X, with excess sodium azide at room temperature, gave good yields of the azido ketones XI and XII. In this series of derivatives from III and IV, the acetyl compounds were oils and the formyl compounds were crystalline solids. Hydrogenation of XI in the presence of hydrochloric acid afforded the amino ketone hydrochloride as a gum that could be converted to a crystalline picrate (XVII). The formyl derivative XII, hydrogenated similarly, yielded a solid hydrochloride (XIX) that could be utilized in the succeeding steps; the crystalline picrate XVIII was also isolated. The N-acetate XVII was converted to the semicarbazone picrate XIV and thence, by ion exchange, to the semicarbazone hydrochloride XV. Compound XIX was converted to the crystalline semicarbazone salt XVI. Although the N-formyl derivatives were largely crystalline compounds, the over-all yields in the formyl series were poorer and the compounds behaved erratically in some of the subsequent steps; consequently, our main efforts were focused on the N-acetyl series.

The condensation of the free base of XV with 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine (XIII), prepared according to Boon and Leigh,[6] was carried out in the presence of *s*-collidine as an acid acceptor in order to utilize XV completely as a reactant. The product XX was converted to the ketone XXI with dilute acid. Hydrogenation of XXI generated a 5-amino substituent that spontaneously reacted with the ketone carbonyl to give the 7,8-dihydropteridine XXII. Treatment of XXII with hydrogen peroxide afforded the pteridine XXV that, by saponification, was converted to homopteroic acid (XXVI). The transformations XXI → XXII → XXV → XXVI were best followed by the striking changes in ultraviolet spectra. In order to provide a molecule capable of coupling with a glutamic ester in order to complete the synthesis of XXIV, it was necessary to block the amino functions of XXVI. Reaction with trifluoroacetic anhydride yielded XXVII which, by further treatment with hot acetic anhydride, afforded the crystalline acid XXVIII.[8] The mixed anhydride obtained from XXVIII and isobutyl chloroformate[9] was treated with diethyl L-glutamate to

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(2) Some of this work has been described in a preliminary communication: L. Goodman, J. DeGraw, R. L. Kisliuk, M. Friedkin, E. J. Pastore, E. J. Crawford, L. T. Plante, A. Al-Nahas, J. F. Morningstar, Jr., G. Kwok L. Wilson, E. F. Donovan, and J. Ratzan, *J. Am. Chem. Soc.*, **86**, 308 (1964).

(3) The systematic name for the title compound is N-{*p*-{[2-(2-amino-4-hydroxy-6-pteridinyl)ethyl]amino}benzoyl}-L-glutamic acid.

(4) To whom reprint requests should be addressed.

(5) For a recent review of folic acid metabolism, see M. Friedkin, *Ann. Rev. Biochem.*, **32**, 185 (1963).

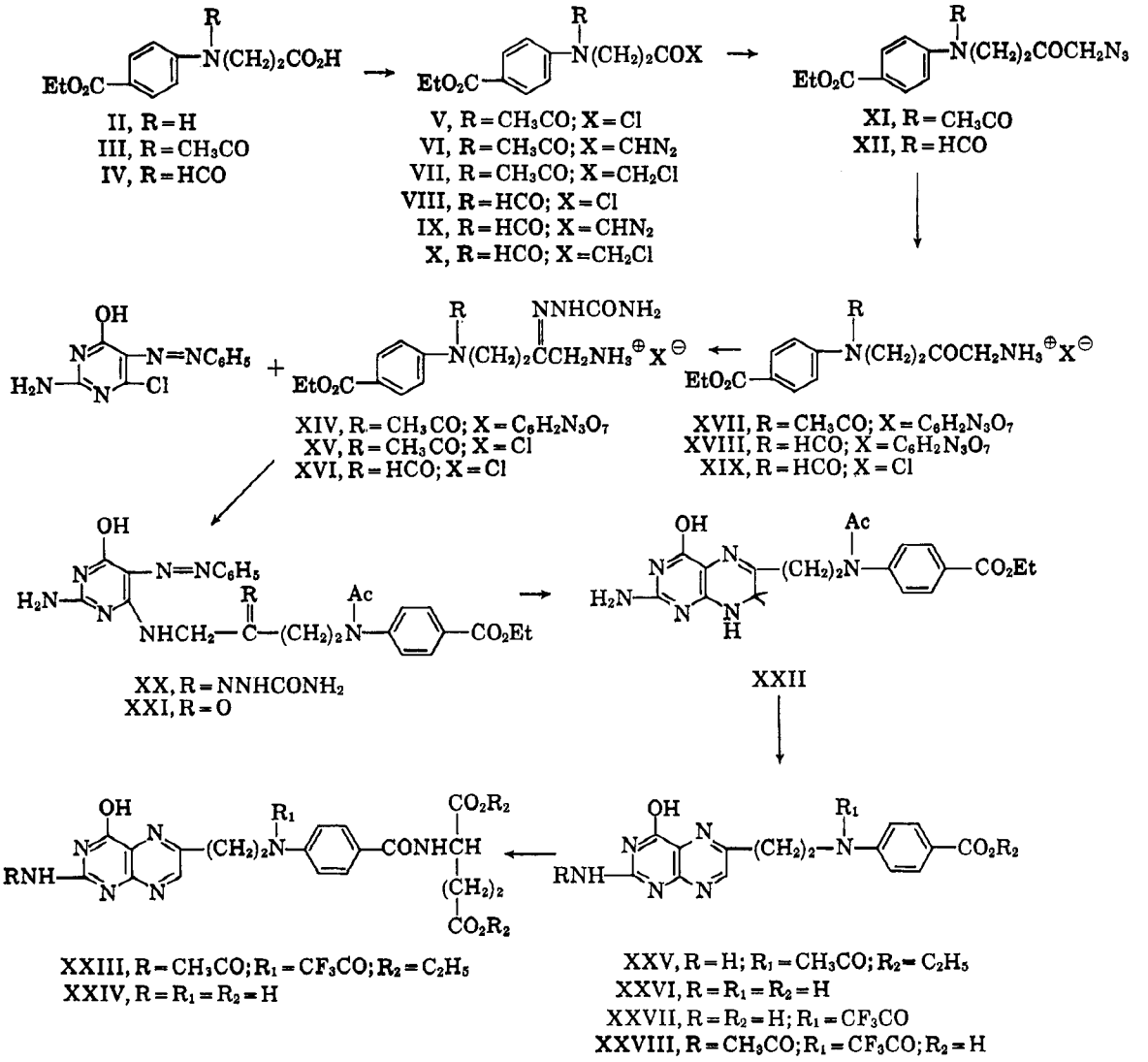
(6) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1497 (1951).

(7) C. Hurd and S. Hayao, *J. Am. Chem. Soc.*, **74**, 5889 (1952).

(8) The singly blocked acid XXVII has been successfully employed in the coupling with diethyl L-glutamate: Dr. L. T. Plante, private communication.

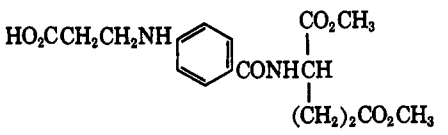
(9) J. R. Vaughan, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

CHART I




form a moderate yield of the completely blocked homofolic acid (XXIII). Saponification of XXIII afforded XXIV as a gel after acidification of the hydrolysis solution. The isolated homofolic acid (XXIV) proved to be a very light-sensitive solid. Reaction of the acid chloride of XXVIII with diethyl L-glutamate followed by saponification of the product gave XXIV in poorer yield. (See Chart I.)

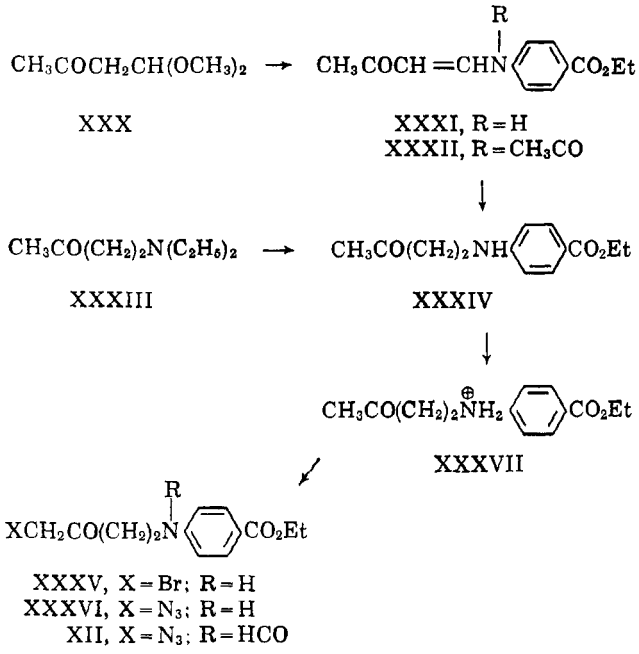
At an early stage in the synthesis of XXIV, the reaction of $\beta$-propiolactone with dimethyl *p*-aminobenzoyl-L-glutamate was investigated as a potential way of shortening the sequence. The crystalline diester XXIX could be obtained, but in a yield so low as to make the sequence impractical.



XXIX

The large quantities of diazomethane that were required to prepare adequate amounts of VI or IX represented a substantial difficulty in the process and impelled us to seek other ways to prepare XI or XII. The methyl ketone XXXIV was prepared by two methods. In the first of these, the acetal XXX was condensed with ethyl *p*-aminobenzoate using the method of Burness[10] to yield the enamine XXXI; this could be acetylated to XXXII and could be hydrogenated to the saturated amine XXXIV. Alternatively, condensation of the Mannich base XXXIII with ethyl *p*-aminobenzoate by Craig's[11] procedure afforded XXXIV directly. Conversion of XXXIV to the hydrobromide salt XXXVII and bromination of the salt gave the bromo ketone XXXV, which, without isolation, was converted to the crystalline azide XXXVI. This technique of selective methyl bromination followed the procedure described by Baker for a similarly substituted methyl ketone.[12] The azide XXXVI decomposed slowly at room temperature but could be converted to the more stable N-formylamine XII, identical with that prepared from IV, with formic–acetic anhydride in pyridine. For reasons we do not understand, the reaction of XXXVI with acetic anhydride did not give a clean product. Unfortunately, the over-all yield of XII from XXXIII was low, at least on the basis of our limited studies, but this route should be

(10) D. M. Burness, *J. Org. Chem.*, **21**, 97 (1956).
(11) J. C. Craig, M. Moyle, and L. F. Johnson, *ibid.*, **29**, 410 (1964).
(12) B. R. Baker and F. J. McEvoy, *ibid.*, **20**, 136 (1955).




considered as an alternative to that based on I in any further large-scale synthesis leading to XXIV.

## Experimental Section[13]

**N-Acetyl-N-(*p*-carbethoxyphenyl)-β-alanine (III).**—To a solution of 9.7 g. (41 mmoles) of the amine II[7] in 30 ml. of pyridine was added 20 ml. of acetic anhydride. The solution was allowed to stand at room temperature for 18 hr., then diluted with 50 ml. of ice water. The mixture was evaporated and the residue was partitioned between 125 ml. of ether and 30 ml. of 1 *N* hydrochloric acid. The ether extract was washed with two 20-ml. portions of water, dried, and evaporated to leave 9.5 g. of a sirup. A portion (8.0 g.) of the sirup was recrystallized from 40 ml. of benzene–Skellysolve B (3:1) affording 4.4 g. (46%) of solid, m.p. 94–98°. An analytical sample, m.p. 99–100°, $\lambda_{max}^{EtOH}$ 241 mμ (ε 13,600), was obtained after two recrystallizations from benzene–Skellysolve B. The compound was homogeneous with $R_{Ad}$ 2.40 in solvent A, easily separable from starting acid (II).

*Anal.* Calcd. for $C_{14}H_{17}NO_5$: C, 60.2; H, 6.14; N, 5.02. Found: C, 60.2; H, 6.17; N, 4.96.

**N-Formyl-N-(*p*-carbethoxyphenyl)-β-alanine (IV).**—To a solution of 0.50 g. of II in 3 ml. of 100% formic acid was added 1.0 ml. of acetic anhydride and the mixture was heated at 60° for 30 min., then cooled in ice, and diluted with 20 ml. of water. The solution at 0°, deposited 0.31 g. (55%) of white crystals, m.p. 97–98°, $\lambda_{max}^{EtOH}$ 264 mμ (ε 15,000), that were homogeneous with $R_{Ad}$ 2.21 in solvent A.

*Anal.* Calcd. for $C_{13}H_{15}NO_5$: C, 58.9; H, 5.70; N, 5.28. Found: C, 59.1; H, 5.84; N, 5.20.

**1-Chloro-4-[N-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (VII).**—To a solution of 10.0 g. (35.8 mmoles) of the acid III in 80 ml. of benzene was added 2.8 ml. (39 mmoles) of thionyl chloride, the mixture was heated at reflux for 25 min. and then evaporated, and the residue was dried *in vacuo* (<1 mm.). The residue, V, was dissolved in 50 ml. of ether and added dropwise during 30 min. to an ice-cold solution of 0.15 mole of diazomethane (prepared from 22.0 g. of N-nitrosomethylurea[14]) in 220 ml. of ether. The yellow solution was allowed to stand for 1 hr. at room temperature, then dry hydrogen chloride was added to the chilled (0–5°) solution for 2.5 hr. during which time 80 ml. of chloroform was added to maintain solubility. The resulting mixture was allowed to warm to room temperature over a 30-min. period and then was filtered, and the filtrate was evaporated to afford 12.0 g. (101%) of a yellow sirup that was suitable for use in the next reaction.

**1-Chloro-4-[N-formyl-(*p*-carbethoxyphenyl)amino]-2-butanone (X).**—The N-formyl acid IV, 3.00 g.(11.3 mmoles), was converted to the acid chloride VIII and thence to the diazo ketone IX as described for the preparation of VII. The diazo ketone IX was a white crystalline solid: $\lambda_{max}^{Nujol}$ 3.22 (CH of $CHN_2$), 4.70 ($N_2$ of $CHN_2$), 5.83 (ester C=O), 5.95 (amide C=O), 6.17 μ (ketone C=O). Without attempt at purification the diazo ketone IX was converted to the chloro ketone X with hydrogen chloride to give 2.99 g. of a white crystalline solid whose infrared spectrum showed the complete loss of the characteristic diazo ketone bands. The solid was extracted with two 50-ml. portions of boiling diisopropyl ether. The chilled extracts afforded 1.80 g. of white solid, m.p. 64–65°. Concentration of the mother liquors gave 0.15 g. more of the solid for a total yield of 58%.

*Anal.* Calcd. for $C_{14}H_{16}ClNO_4$: C, 56.5; H, 5.42; Cl, 11.9; N, 4.71. Found: C, 55.6; H, 5.36; Cl, 11.7; N, 4.58.

**1-Azido-4-[N-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (XI).**—To a solution of 11.9 g. (38 mmoles) of the crude chloro ketone VII in 140 ml. of methanol was added a solution of 12.0 g. (0.17 mole) of sodium azide in 36 ml. of water. The resulting solution, after being held at room temperature for 3.5 hr., was evaporated to remove the methanol. The aqueous residue was diluted with 60 ml. of water and extracted with two 100-ml. portions of ether. The extract was washed with 60 ml. of water, dried, and evaporated, leaving 9.9 g. (81%) of a dark sirup: $\lambda_{max}^{film}$ 4.75 ($N_3$), 5.80 (ester and ketone C=O), 6.00 μ (amide C=O). This material at room temperature slowly changed to a dark resin whose infrared spectrum showed a marked loss of azide absorption at 4.75 μ.

**1-Azido-4-[N-formyl-(*p*-carbethoxyphenyl)amino]-2-butanone (XII).** A.—The chloro ketone X, 1.80 g. (6.03 mmoles), was converted to 1.60 g. (87%) of crystalline XII using the procedure described for the preparation of XI but substituting dichloromethane as the extraction solvent. The residue was recrystallized from a mixture of 5 ml. of benzene and 2 ml. of Skellysolve B, affording 1.17 g. of azido ketone XII: m.p. 88–89°; $\lambda_{max}^{Nujol}$ 4.62 (w) and 4.76 (s) ($N_3$), 5.82 and 5.93 (ester, ketone and amide C=O), 11.60 μ (*p*-$C_6H_4$–); n.m.r. data[15] τ 1.57 (s, —N—CH=O), 1.92 (d) and 2.75 (d, *p*-$C_6H_4$), 5.63 (q) and 8.62 (*t*, $CO_2C_2H_5$), 5.86 (t, —$NCH_2$—), 6.03 (s, O=C—$CH_2N_3$), 7.20 (t, —$CH_2C$=O).

*Anal.* Calcd. for $C_{14}H_{16}N_4O_4$: C, 55.3; H, 5.30; N, 18.4. Found: C, 55.1; H, 5.36; N, 18.1.

B.—A solution of 5.7 ml. (60 mmoles) of acetic anhydride and 2.4 ml. (59 mmoles) of 97–100% formic acid was warmed to 55–60° for 15 min., cooled, and added to a solution of 1.4 g. (5.1 mmoles) of XXXVI (see below) in 17.5 ml. of dry pyridine. After 16 hr. at 25°, the solution was evaporated and the residue was treated with 30 ml. of toluene, which was removed by evaporation. Similar addition and removal of ether left a crystalline residue, 1.8 g. (116% yield), m.p. 30–68°. Recrystallization from 12 ml. of methanol afforded 1.06 g. (68%), m.p. 87–89°. The infrared spectrum was identical with that from part A. The shift of the *p*-$C_6H_4$– band from its position in the spectrum XXXVI is characteristic for acylation of a *para*-substituted aniline.

**1-Amino-4-[N-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone Picrate (XVII).**—To a solution of 40.0 g. (0.125 mole) of the azido ketone XI in 410 ml. of 1,2-dimethoxyethane was added 15.6 ml. of concentrated hydrochloric acid and, after flushing the vessel with nitrogen, 2.5 g. of palladium black and 3.4 g. of 5% palladium on carbon. Hydrogen was bubbled continuously through the magnetically stirred solution at a slow rate. Progress of the reaction was followed by filtering small aliquots (*ca.* 0.4 ml.) of the reaction mixture and observing the infrared spectrum of the evaporated filtrate as a film. Disappearance of the azide

(13) Melting points are uncorrected and were obtained with a Fisher-Johns apparatus. Magnesium sulfate was used as the drying agent and evaporations were conducted *in vacuo* unless otherwise noted. Skellysolve B is a hydrocarbon fraction, b.p. 62–70°. Paper chromatograms were run by the descending technique on Whatman No. 1 paper and the spots were located by ultraviolet examination. The solvent systems used were A, 5% aqueous disodium hydrogen phosphate; B, 2-propanol–2 *N* hydrochloric acid (65:35); and C, water containing 3% ammonium chloride and 5% ammonium hydroxide. Adenine was used as the chromatography comparison standard.

(14) F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

(15) N.m.r. spectra were determined in chloroform-*d* solutions containing 1% tetramethylsilane as internal standard. Signals are reported as singlets (s), doublets (d), triplets (t), or quartets (q). Chemical shifts are measured from multiplet centers.

absorption at 4.75 $\mu$ generally required about 18 hr. of treatment with hydrogen. The reaction mixture was filtered using a filter aid and the filtrate was evaporated. The residue was partitioned between 170 ml. each of water and ethyl acetate. The ethyl acetate phase was extracted with 50 ml. of water. The combined water solutions were washed with 60 ml. of ethyl acetate, then evaporated partially to remove any ethyl acetate. The aqueous solution then was poured slowly into a warm (50°) solution of 24 g. of picric acid in 2 l. of water. The initially gummy precipitate slowly changed to a crystalline solid when it was scratched intermittently. The collected solid, 41 g. (62%), had m.p. 96–99°.

In the early stages of the research the picrate XVII, obtained by a somewhat different hydrogenation procedure, had m.p. 138.5–140.5° after recrystallization from absolute ethanol.

*Anal.* Calcd. for $C_{21}H_{23}N_5O_{11}$: C, 48.4; H, 4.44; N, 13.4. Found: C, 48.2; H, 4.56; N, 13.5.

In later experiments the crude picrate had m.p. 89–96° and m.p. 91–93° after recrystallization from absolute ethanol and even after seeding with the higher melting form. There was no indication of resolidification and remelting at the temperature of the higher melting form.

*Anal.* Calcd. for $C_{21}H_{23}N_5O_{11}\cdot 0.5H_2O$: C, 47.5; H, 4.61; N, 13.1. Found: C, 47.5; H, 4.54; N, 13.2.

The infrared spectra, taken in Nujol mulls, of the two forms showed substantial differences. The spectrum of the product from the preparative run, described in detail above, was identical with that of the picrate with m.p. 91–93°.

**1-Amino-4-[N-formyl-(*p*-carbethoxyphenyl)amino]-2-butanone Picrate (XVIII).**—To 0.100 g. of platinum oxide were added 0.30 ml. of concentrated hydrochloric acid, 20 ml. of tetrahydrofuran, and 1.06 g. (3.50 mmoles) of azido ketone XII, in that order. The mixture was stirred under 1 atm. of hydrogen for 6.5 hr. at room temperature and then evaporated to dryness, and the residue was partitioned between 12 ml. of water and 10 ml. of ethyl acetate. The ethyl acetate layer was extracted with 5 ml. of water which was combined with the original aqueous phase and extracted with 10 ml. of ethyl acetate. The aqueous solution was filtered and freeze dried, giving 0.737 g. (67%) of white crystalline residue (XIX), whose infrared spectrum showed the absence of azide absorption and which was homogeneous on paper chromatography in 1-butanol–acetic acid–water (4:1:5) with $R_{Ad}$ 1.71, the spot being both ultraviolet absorbing and ninhydrin positive.

The picrate from XIX, after recrystallization from 75% aqueous ethanol, had m.p. 101–103°.

*Anal.* Calcd. for $C_{20}H_{21}N_5O_{11}\cdot H_2O$: C, 45.8; H, 4.42; N, 13.3. Found: C, 45.9; H, 4.46; N, 13.5.

**Semicarbazone of 1-Amino-4-[N-acetyl-(*p*-carbethoxyphenyl)-amino]-2-butanone Picrate (XIV).**—To a solution of 4.3 g. (8.2 mmoles) of the amino ketone picrate XVII in 70 ml. of warm absolute ethanol was added 1.1 g. (9.9 mmoles) of semicarbazide hydrochloride in 50 ml. of water. The resulting solution was allowed to stand 15 hr. during which time a yellow, crystalline precipitate deposited. The mixture was diluted with 50 ml. of water, then filtered. The filter cake was washed with two 20-ml. portions of cold water, then dried to give 3.2 g. (67%) of picrate, m.p. 131–134°. An analytical sample, m.p. 131–133° after recrystallization from 83% ethanol, was obtained from another run.

*Anal.* Calcd. for $C_{22}H_{26}N_8O_{11}\cdot 0.5H_2O$: C, 45.0; H, 4.64; N, 19.1. Found: C, 44.9; H, 4.88; N, 19.2.

**Semicarbazone of 1-Amino-4-[N-acetyl-(*p*-carbethoxyphenyl)-amino]-2-butanone Hydrochloride (XV).**—A mixture of 1.90 g. of the picrate XIV, 9.5 g. of Dowex 2 (Cl), 20 ml. of ethanol, and 95 ml. of water was stirred for 15 hr. The resin was removed by filtration and the filtrate was evaporated to dryness (some of the larger runs were freeze dried), leaving 1.20 g. of a sirup. The sirup was caused to crystallize by trituration with 12 ml. of absolute ethanol. The crystals were collected, washed with 5 ml. of cold ethanol, then dried to afford 0.95 g. (75%) of product, m.p. 205–208°.

*Anal.* Calcd. for $C_{16}H_{24}ClN_5O_4$: C, 49.9; H, 6.27; Cl, 9.21; N, 18.2. Found: C, 49.9; H, 6.07; Cl, 9.21; N, 18.0.

**Semicarbazone of 1-Amino-4-[N-formyl-(*p*-carbethoxyphenyl)-amino]-2-butanone Hydrochloride (XVI).**—To a solution of 0.400 g. (1.30 mmoles) of the amino ketone hydrochloride XIX in 6 ml. of 83% aqueous ethanol was added 0.21 g. (1.9 mmoles) of semicarbazide hydrochloride in 1 ml. of water. The resulting solution was allowed to stand at room temperature for 4 hr.; the crystals were collected and washed with ethanol to yield 0.36 g. (76%) of product, m.p. 225–227°. The solid was recrystallized from a mixture of 5 ml. of ethanol and 4 ml. of water yielding 0.30 g., m.p. 224–225°.

*Anal.* Calcd. for $C_{15}H_{22}ClN_5O_4$: C, 48.5; H, 5.97; Cl, 9.54; N, 18.8. Found: C, 48.8; H, 5.85; Cl, 9.36; N, 18.6.

**Semicarbazone of 1-(2′-Amino-6′-hydroxy-5′-phenylazo-4′-pyrimidinyl)amino-4-[N-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (XX).**—To a solution of 0.575 g. (25.0 mg.-atoms) of sodium in 90 ml. of absolute ethanol was added 10.0 g. (26.0 mmoles) of the semicarbazone hydrochloride XV. The mixture was stirred 1 hr. at room temperature, then a solution of 6.25 g. (25.0 mmoles) of 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine (XIII)[6] in 63 ml. of N,N-dimethylformamide (DMF) was added followed by 5.00 ml. (37.5 mmoles) of *s*-collidine. The red mixture was stirred 72 hr. at room temperature, then was diluted with 1500 ml. of water. The yellow, crystalline precipitate was collected, washed with 200 ml. of water, with 100- and 50-ml. portions of ethanol, then dried to leave 10.25 g. (73%) of chromatographically homogeneous solid whose infrared spectrum was essentially identical with that of the analytical sample.

From an earlier run in which the free base of XV served as the acid acceptor in the reaction a 98% yield (based on a requirement of 2 moles of XV/mole of product) of XX was obtained, m.p. 213–215°, $\lambda_{max}^{pH\ 13}$ 388 m$\mu$ ($\epsilon$ 18,700). The compound moved as a single spot in solvent B with $R_{Ad}$ 2.1, easily separable from either of the reactants.

*Anal.* Calcd. for $C_{26}H_{30}N_{10}O_5\cdot 0.5H_2O$: C, 54.7; H, 5.47; N, 24.6. Found: C, 54.8; H, 5.17; N, 24.5.

**1-(2′-Amino-6′-hydroxy-5′-phenylazo-4′-pyrimidinyl)amino-4-[N-acetyl-(*p*-carbethoxyphenyl)amino]-2-butanone (XXI) Hydrochloride.**—To a solution of 0.303 g. of the semicarbazone XX in 3 ml. of glacial acetic acid was added 15 ml. of 2 *N* hydrochloric acid. A reddish gum precipitated which solidified after being stirred for about 15 min. The mixture was stirred at room temperature for 3 hr., then was filtered. The filter cake was washed with 10 ml. of 1 *N* hydrochloric acid and was dried, yielding 0.211 g. (72%) of orange crystals, m.p. 179–181°. Corresponding product from another run was recrystallized from acetic acid affording yellow crystals, m.p. 178–182°, $\lambda_{max}^{pH\ 13}$ 382 m$\mu$ ($\epsilon$ 17,850).

*Anal.* Calcd. for $C_{25}H_{28}ClN_7O_5\cdot H_2O$: C, 53.6; H, 5.40; Cl, 6.34; N, 17.5. Found: C, 53.6; H, 5.47; Cl, 6.43; N, 17.6.

**Ethyl *p*-{N-[2-(2-Amino-4-hydroxy-7,8-dihydro-6-pteridinyl)-ethyl]acetamido}benzoate (Ethyl N$^{11}$-Acetyl-7,8-dihydrohomopteroate, XXII).**—A mixture of 35 ml. of water, 2.03 g. of the phenylazopyrimidinyl ketone XXI, and 0.27 g. of 5% palladium on carbon was stirred under 1 atm. of hydrogen. After 10 hr. the theoretical volume of gas was absorbed. The mixture was acidified with 20 ml. of 1 *N* hydrochloric acid and the catalyst was removed by filtration with the use of filter aid. The yellow filtrate was adjusted to pH 7 with saturated aqueous sodium bicarbonate solution giving a tan, crystalline precipitate which was stirred and chilled (0°) for about 1 hr. The crystals were collected, washed twice with 5-ml. portions of cold water, then dried to give 1.24 g. (83%) of product: $\lambda_{max}^{pH\ 13}$ 234 m$\mu$ ($\epsilon$ 21,200), 277 (7780), 330 (5180).

*Anal.* Calcd. for $C_{19}H_{22}N_6O_4\cdot 0.5H_2O$: C, 56.1; H, 5.71; N, 20.6. Found: C, 56.5; H, 6.20; N, 20.2.

This hydrogenation on occasion was very erratic, requiring as much as a week to complete the uptake of hydrogen and resulting in the absorption of as much as 2.5 times the theoretical hydrogen uptake. However, even in these cases, useful yields of XXII were obtained.

**Ethyl N$^{11}$-Acetylhomopteroate (XXV).**—To 120 ml. of 1.0 *N* hydrochloric acid was added 4.60 g. (11.5 mmoles) of the dihydropteridine XXII and the mixture was stirred a few minutes in order to effect complete solution. A solution of 2.2 ml. of 30% hydrogen peroxide (19.4 mmoles) in 10 ml. of water was added over a period of about 1 min., stirring was continued for 90 min. The mixture was adjusted to pH 6–7 with ammonia and was filtered. The filter cake was washed with 25 ml. of water and with 25 ml. of ethanol and was dried, leaving 4.30 g. (93% of product). A portion was triturated with hot DMF and the insoluble, crystalline solid, which was chromatographically homogeneous in solvent B with $R_{Ad}$ 1.97, was submitted for analysis: $\lambda_{max}^{pH\ 13}$ 253 m$\mu$ ($\epsilon$ 24,750), 366 (7180).

*Anal.* Calcd. for $C_{19}H_{20}N_6O_4$: C, 57.6; H, 5.09; N, 21.2. Found: C, 57.3; H, 5.20; N, 21.2.

**Homopteroic Acid (XXVI).**—To 37 ml. of 10% sodium hydroxide was added 2.80 g. of the N-acetyl ester XXV and the mixture, under a nitrogen atmosphere, was heated for 2 hr. on the steam bath. The resulting red-brown solution was chilled 3 hr. and the crystallized sodium salt of XXVI was collected. The salt was dissolved in 30 ml. of hot water and the solution was adjusted to pH 3 with 1 *N* hydrochloric acid. The gelatinous mass was diluted with water to a volume of 130 ml. and the mixture was stirred until it was evenly dispersed and filterable. The resulting yellow solid was collected by filtration, washed thoroughly with water, then acetone, and dried to leave 2.13 g. (93%) of solid. An analytical sample was similarly obtained from another run: $\lambda_{max}^{pH\ 13}$ 256 mμ (ε 26,900), 277 (21,900), 365 (7630); $\lambda_{max}^{pH\ 1}$ 224 mμ (ε 22,300), 310 (14,500). In solvent A the compound showed an ultraviolet-absorbing spot with $R_{Ad}$ 0.80 and in solvent B one at $R_{Ad}$ 1.00.

*Anal.* Calcd. for $C_{15}H_{14}N_6O_3$: C, 55.2; H, 4.32; N, 25.8. Found: C, 54.7; H, 4.62; N, 25.6.

**N$^{11}$-Acetylhomopteroic Acid.**—A solution of 0.54 g. of the N-acetyl ester XXV in 20 ml. of 0.25 *N* sodium hydroxide was heated for 1 hr. on the steam bath under a nitrogen atmosphere. The solution was cooled, adjusted to pH 6–7 with 1 *N* hydrochloric acid, and centrifuged to remove a dark flocculent precipitate. This residue was stirred with 10 ml. of hot water and the mixture was centrifuged. The combined supernatants were adjusted to pH 4 with acid giving a crystalline precipitate that, after being chilled, was collected by filtration. The filter cake was washed with 5 ml. of water and dried to leave 0.355 g. of tan crystals. The compound was dissolved in aqueous sodium bicarbonate and precipitated with 1 *N* hydrochloric acid at pH 3 to furnish the analytical sample: $\lambda_{max}^{pH\ 13}$ 253 mμ (ε 29,300), 365 (7030).

*Anal.* Calcd. for $C_{17}H_{16}N_6O_4 \cdot 0.5H_2O$: C, 54.1; H, 4.54; N, 22.3. Found: C, 54.3; H, 4.52; N, 22.2.

**N$^2$,N$^{11}$-Diacetylhomopteroic Acid.**—A suspension of 0.50 g. of homopteroic acid (XXVI) in 7.5 ml. of acetic anhydride was stirred at reflux for 2 hr., giving an amber solution. The cooled solution was added dropwise to 75 ml. of well-stirred ether affording a crystalline precipitate. The mixture was chilled and the crystals were washed with ether and then suspended in 15 ml. of water. Concentrated ammonia was added giving complete solution at pH 7–8. The solution was adjusted to pH 3 with 1 *N* hydrochloric acid and then centrifuged to remove an amber gum. The supernatant was chilled to afford white crystals that weighed 0.395 g. (63%). Similar acetylation of N$^{11}$-acetylhomopteroic acid gave the same material: m.p. 274–275°; $\lambda_{max}^{pH\ 13}$ 254 mμ (ε 30,900), 353 (6900).

*Anal.* Calcd. for $C_{19}H_{18}N_6O_5$: C, 55.6; H, 4.42; N, 20.5. Found: C, 55.0; H, 4.68; N, 20.6.

**N$^{11}$-Trifluoroacetylhomopteroic Acid (XXVII).**—A stirred mixture of 0.200 g. of homopteroic acid (XXVI) in 4 ml. of trifluoroacetic anhydride was heated at reflux for 1 hr. and then evaporated. The residual sirup was stirred with 10 ml. of water for 30 min.; then the resulting solid was collected, washed with water, and dried, affording 0.244 g. (94%) of off-white crystals: m.p. >300°; $\lambda_{max}^{Nujol}$ 2.99 and 3.12 (NH), 5.82 (amide and acid C=O), 8.20 and 8.60 μ ($CF_3$); $\lambda_{max}^{pH\ 11}$ 256 mμ (ε 25,650), 277 (20,900), 365 (7560). The material moved as a single spot in solvent A with $R_{Ad}$ 1.71.

*Anal.* Calcd. for $C_{17}H_{13}F_3N_6O_4$: C, 48.4; H, 3.10; N, 19.9. Found: C, 48.5; H, 3.70; N, 19.9.

**N$^2$-Acetyl-N$^{11}$-trifluoroacetylhomopteroic Acid (XXVIII).**—A suspension of 1.88 g. of the trifluoroacetyl acid XXVII in 25 ml. of acetic anhydride was stirred at 115° for 5 hr. The mixture was evaporated and the solid residue was dissolved in 18 ml. of hot (115°) DMF. The solution was diluted slowly with 20 ml. of hot water and was allowed to cool slowly to room temperature to destroy any mixed anhydride, then was chilled. The tan crystals were collected, washed with water, then with acetone and dried affording 1.65 g. (80%) of solid: m.p. >300°; $\lambda_{max}^{Nujol}$ 3.10 (NH), 3.75–3.95 (carboxyl H), 5.80 and 5.88 (acid and amide C=O), 8.30 and 8.60 μ ($CF_3$); $\lambda_{max}^{pH\ 11}$ 255 mμ (ε 29,700), 277 (21,500), 353 (7100).

*Anal.* Calcd. for $C_{19}H_{15}F_3N_6O_5$: C, 49.2; H, 3.26; N, 18.1. Found: C, 49.0; H, 3.86; N, 18.1.

The ultraviolet spectra of both XXVIII and XXVII indicate that the N$^{11}$-trifluoroacetyl group has been hydrolyzed in the pH 11 solution. The band at 277 mμ which is present is not found in N$^{11}$-acetylhomopteroic acid but is found in homopteroic acid (XXVI) and represents the *p*-aminobenzoic acid absorption.

**Homofolic Acid[3] (XXIV).**—A solution of 0.884 g. (1.90 mmoles) of the blocked acid XXVIII in 15 ml. of dry DMF was prepared by warming the mixture on the steam bath. To the chilled (0°) solution was added 0.265 ml. (1.90 mmoles) of triethylamine and 0.249 ml. (1.90 mmoles) of isobutyl chloroformate and the solution was stirred at 0° for 50 min. A solution of 0.465 g. (1.90 mmoles) of diethyl L-glutamate hydrochloride and 0.265 ml. (1.90 mmoles) of triethylamine in 5 ml. of dry DMF was added to the solution containing the mixed anhydride; the resulting solution was stirred at room temperature with exclusion of moisture for 21 hr. and then was evaporated *in vacuo* using a bath temperature of 45°. The residue was suspended in 15 ml. of ethyl acetate and the mixture was filtered through Celite. The flask and filter cake were washed with two 5-ml. portions of ethyl acetate; the combined ethyl acetate solutions were washed with 10 ml. of 0.5 *N* hydrochloric acid, 5 ml. of water, 10 ml. of saturated aqueous sodium bicarbonate solution, and finally 5 ml. of water. Evaporation of the dried ethyl acetate solution afforded 548 mg. of a foam. Extraction of the combined sodium bicarbonate and aqueous washes with 10 ml. of ethyl acetate afforded 136.8 mg. more of a foam. The combined materials from the ethyl acetate extractions were triturated with 20 ml. of diethyl ether. Filtration of the mixture left 564 mg. (45.8%) of a cream-colored foam (XXIII), $[\alpha]^{21}D$ +11.3° (*c* 0.928, in chloroform). On larger runs yields of 61% have been obtained.

*Anal.* Calcd. for $C_{28}H_{32}F_2N_7O_8$: C, 51.6; H, 4.95; F, 8.75; N, 15.1. Found: C, 50.9, 51.1; H, 4.82; F, 8.72; N, 15.1.

Acidification of the sodium bicarbonate extract afforded 51 mg. of the blocked acid XXVIII.

The solid was heated on the steam bath under nitrogen with 130 ml. of 0.1 *N* sodium hydroxide for 25 min. The pale yellow solution was cooled and adjusted to pH 3–4 with 1 *N* hydrochloric acid, giving a gelatinous precipitate. The solid was collected by filtration, washed with 10 ml. of water, and dried, leaving 0.36 g. (40% from XXVIII) of a yellow powder. The material was dissolved in 10 ml. of 1 *N* sodium hydroxide, the mixture was centrifuged, and the supernatant solution was adjusted to pH 3–4 with 3 *N* hydrochloric acid. The resulting gelatinous precipitate was collected by centrifugation and the residue was washed with three 10-ml. portions of water, centrifuging each time at 3900 r.p.m. The pellet was freeze dried to give a yellow powder: $\lambda_{max}^{pH\ 13}$ 255 mμ (ε 22,700), 281 (18,150), 365 (6620). The compound showed one ultraviolet-absorbing spot in solvent A with $R_{Ad}$ 1.38 and in solvent C with $R_{Ad}$ 1.83 accompanied by a number of fluorescent spots. Both these chromatographic systems easily separated XXIV from homopteroic acid (XXVI).

*Anal.* Calcd. for $C_{20}H_{21}N_7O_6 \cdot 0.5H_2O$: C, 51.7; H, 4.79; N, 21.1. Found: C, 51.2; H, 5.09; N, 21.2.

A portion (10 mg.) of product was chromatographed on Whatman 3MM paper in solvent A. The ultraviolet-absorbing spot was eluted with 0.5 *N* ammonia and the homofolic acid (XXIV) precipitated at pH 3–4. The collected material, 2.0 mg., showed $\lambda_{max}^{pH\ 13}$ 255 mμ (ε 24,600), 281 (19,450), 365 (7880).

A sample of XXIV was prepared from the acid chloride of XXVIII by reaction with diethyl glutamate. After a work-up similar to that described in the mixed anhydride method, a low yield of product was obtained whose spectral and chromatographic properties were very similar to those described above.

*Anal.* Calcd. for $C_{20}H_{21}N_7O_6 \cdot H_2O$: C, 50.7; H, 4.90; N, 20.7. Found: C, 50.3; H, 4.67; N, 20.7.

**Dimethyl *p*-(2′-Carboxyethyl)aminobenzoyl-L-glutamate (XXIX).**—A mixture of 1.3 g. (4.4 mmoles) of dimethyl *p*-aminobenzoyl-L-glutamate and 0.65 g. (9.0 mmoles) of β-propiolactone in 7 ml. of acetonitrile was allowed to stand at room temperature for 24 hr. The solution was evaporated and the residual sirup was dissolved in 5 ml. of ethyl acetate. Ether (10 ml.) was added, causing the precipitation of a brown oil. The oil was extracted with 10 ml. of ethyl acetate and the supernatant was added to the original ethyl acetate–ether supernatant solution which was evaporated to give 1.91 g. of sirup. To the sirup was added 5 ml. of water and 5 ml. of chloroform, followed by the dropwise addition of concentrated ammonia to pH 8. The aqueous layer was washed with 5 ml. of chloroform and then adjusted to pH 2–3, giving an oily precipitate which was extracted with 10- and 5-ml. portions of chloroform. The combined chloroform extracts were washed with 5 ml. of water, dried, and evapo-

rated, leaving 0.88 g. of a gum. This was dissolved in 3.5 ml. of warm ethyl acetate; the solution was seeded and chilled affording 0.37 g. (22%) of white crystals, m.p. 90–96°. The analytical sample was obtained after two recrystallizations from ethyl acetate, m.p. 101–102°.

*Anal.* Calcd. for $C_{17}H_{22}N_2O_7 \cdot 0.25H_2O$: C, 55.1; H, 6.13; N, 7.55. Found: C, 55.0; H, 6.18; N, 7.32.

**4-(*p*-Carbethoxyphenyl)amino-3-buten-2-one (XXXI).**—A mixture of 19.8 g. (0.120 mole) of ethyl *p*-aminobenzoate and 15.8 g. (0.120 mole) of 4,4-dimethoxy-2-butanone (XXX) was heated gradually from 100 to 200° over a period of 3 hr., while methanol distilled, and finally heated at 200° for 40 min. The cooled residual product partly crystallized on standing, but was difficult to separate from a small amount of dark polymeric impurity. Column chromatography of a solution in 200 ml. of ether on 600 g. of 90–200 mesh silica gel, by elution with 5 l. of ether, afforded 24 g. (86%) of brown solid. Recrystallization was effected from carbon tetrachloride solution by addition of petroleum ether (b.p. 30–60°) in several portions; any dark oil which initially separated was removed by decantation of the solution. Finally, recrystallization from aqueous methanol afforded 9.3 g. (33%): m.p. 116–118°; $\lambda_{max}^{Nujol}$ 3.07 (NH), 5.81 and 5.93 (C=O), 6.18 and 6.22 (Ar), 7.9 (OBz), 8.49, 8.58, 9.0 (OBz), 10.4 (—C=C—), 11.8 $\mu$ (*p*-$C_6H_4$–).

*Anal.* Calcd. for $C_{13}H_{15}NO_3$: C, 66.9; H, 6.48; N, 6.01. Found: C, 67.2; H, 6.46; N, 5.99.

In another experiment, a different crystal form was isolated, after further recrystallization from carbon tetrachloride–petroleum ether, as a light yellow solid: m.p. 80–82°; $\lambda_{max}^{Nujol}$ 5.82 (ester C=O), 6.06 (ketone C=O), 6.27 and 6.38 (Ar), 7.9 (OBz), 8.32, 8.47, 8.56, 9.1 (OBz), 11.8 $\mu$ (*p*-$C_6H_4$–); no NH absorption was seen near 2.8–3.1 $\mu$. The crude samples also existed in this form.

*Anal.* Found: C, 67.1; H, 6.35; N, 5.71.

Both forms were identical in ultraviolet and n.m.r.[15] spectra: $\lambda_{max}^{EtOH}$ 234 m$\mu$ ($\epsilon$ 8580), 261 (3680), 348 (44,600); $\tau$ −1.65 (d, NH), 2.03 (d) and 3.02 (d, *p*-$C_6H_4$–), 2.80 (q, N–CH=C), 4.67 (d, C=CH–C=O), 5.70 (q) and 8.64 (t, –$COOC_2H_5$), 7.87 (s, $CH_3C$=O) ($J_{NH-CH-C}$ = 13 c.p.s., $J_{CH-CH}$ = 8 c.p.s.).

**4-[N-Acetyl(*p*-carbethoxyphenyl)amino]-3-buten-2-one (XXXII).**—A solution of 12 g. of XXXI in 125 ml. of acetic anhydride was refluxed for 4 hr. and then evaporated. The residual product crystallized and was triturated under 125 ml. of ether to remove soluble colored impurities. Recrystallization of the undissolved solid from 35 ml. of methanol–water (5:2) afforded 9.4 g. (66%), m.p. 113–116°. The infrared spectrum of a sample for analysis melted at 114–115° and was nearly identical with that of the initial residue in the infrared. Carbonyl bands (Nujol) were at 5.80 (s), 5.84 (s), and 5.96 $\mu$ (m). N.m.r.[15] data were $\tau$ 1.55 (d, N–CH=C), 1.88 (d) and 2.77 (d, *p*-$C_6H_4$–), 5.08 (d, C=CH–C=O), 5.64 (q) and 8.60 c.p.s. (t, –$COOC_2H_5$), 7.85 (s, =C–$COCH_3$), 8.03 (s, N–$COCH_3$) ($J_{CH-CH}$ = 14 c.p.s.).

*Anal.* Calcd. for $C_{15}H_{17}NO_4$: C, 65.4; H, 6.22; N, 5.09. Found: C, 65.5; H, 6.31; N, 4.97.

**4-(*p*-Carbethoxyphenyl)amino-2-butanone (XXXIV). A.**—XXXIV was prepared from ethyl *p*-aminobenzoate, according to the procedure[11] for 1-(*p*-anisidino)-3-butanone. Upon cooling the reaction solution, the product crystallized and was collected on a filter and washed with water, 74% yield, m.p. 70–91°. Recrystallization from 95% ethanol (4 ml./g.) afforded 50%: m.p. 97–99°; $\lambda_{max}^{Nujol}$ 2.93 (NH), 5.79 (ketone C=O), 5.89 (ester C=O), 11.91 $\mu$ (*p*-$C_6H_4$); n.m.r.[15] data $\tau$ 2.15 (d) and 3.48 (d, *p*-$C_6H_4$), 5.29 (indistinct t, –NH), 5.72 (q) and 8.68 (t, –$COOC_2H_5$), 6.62 (q, N–$CH_2$–), 7.33 (t, C–$CH_2$–C=O), 7.91 (s, $CH_3C$=O).

*Anal.* Calcd. for $C_{13}H_{17}NO_3$: C, 66.4; H, 7.28; N, 5.95. Found: C, 66.3; H, 7.33; N, 6.06.

**B.**—A solution of 1.16 g. of XXXI in 18 ml. of 95% ethanol was hydrogenated at 1 atm. with 110 mg. of 5% palladium-on-charcoal catalyst for 11 hr. at 25°. After removal of the catalyst by filtration and evaporation of the ethanol, the white crystalline residue (1.15 g., 98%) melted at 93–95°. Infrared and n.m.r. spectra suggested a trace of ethyl *p*-aminobenzoate may have been present, but were otherwise identical with the spectra from part A.

**1-Azido-4-(*p*-carbethoxyphenyl)amino-2-butanone (XXXVI).**—A suspension of 16.8 g. (0.0715 mole) of XXXIV in 45 ml. of 30% hydrogen bromide–glacial acetic acid solution was treated with 4.8 ml. (0.093 mole) of bromine in 10 ml. of glacial acetic acid and stirred for 2.5 hr. at 25° while gradual solution occurred. Ether (500–550 ml.) was added with swirling until separation of the sirupy hydrobromide was complete. The ether was decanted, and any ether remaining was evaporated from the sirup along with excess hydrogen bromide. After further drying by evaporation (bath 35°) of a solution in an equal volume of dichloromethane, the sirup weighed 24 g. This crude hydrobromide of the bromo ketone XXXV was dissolved in 200 ml. of methanol and treated with 24 g. (0.37 mole) of sodium azide dissolved in 66 ml. of water. After 2 hr. at 25°, the solution was concentrated to remove methanol, with the resultant gradual separation of a pale yellow solid, which weighed 11 g. after being collected on a filter, washed with water, and dried. The n.m.r. spectrum indicated the presence of 66% of XXXVI, 4% of XXXIV, and 30% of ethyl *p*-aminobenzoate. Recrystallization from 80 ml. of methanol–water (3:1) afforded 6.0 g. (30% yield) of XXXVI: m.p. 83–85°; $\lambda_{max}^{Nujol}$ 2.99 (NH), 4.58 (w), 4.68 (s), and 4.77 (m, $N_3$, in a liquid phase, only the strong band at 4.68 $\mu$ appeared), 5.80 and 5.95 (C=O), 11.96 $\mu$ (*p*-$C_6H_4$); n.m.r. data $\tau$ 2.10 (d) and 3.42 (*p*-$C_6H_4$–), 5.43 (broad, NH), 5.68 (q) and 8.65 (t, $COOC_2H_5$), 6.06 (s, O=C–$CH_2N_3$), 6.48 (t, N–$CH_2$–), 7.26 (t, –$CH_2C$=O).

*Anal.* Calcd. for $C_{13}H_{16}N_4O_3$: C, 56.5; H, 5.84; N, 20.3. Found: C, 57.2; H, 5.85; N, 19.2.

The compound decomposed on standing on an open shelf for 1–2 weeks. A slight pressure developed in a stoppered bottle, there was a strong odor of ammonia, and $-N_3$ and –NH absorption was nearly absent from the infrared.

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