tion and isomerization of olefins by metal complexes[19,20] involve equilibria (a) between π-olefinic and σ-alkyl complexes and (b) through π-allylic complexes. The isomerization of pent-1-ene by a platinum–tin chloride complex was postulated[21] to involve the addition of the olefin to the hydride complex $[PtCl_x(SnCl_3)_{3-x}H]^{2-}$ (x = 0–2) to form the ion $[PtCl_x(SnCl_3)_{3-x}$ alkyl]. Isomerization would result from reversal of this step, whereas hydrogenation is caused by further reaction with hydrogen with regeneration of the complex hydride ion. A clear insight into the mechanism of complex formation, double-bond isomerization, and hydrogenation with platinum–tin chloride catalysts is not possible until the structure of organometallic intermediates involved in Scheme I can be elucidated and their stoichiometry established.

Experimental Section

Materials.—The preparation and some properties of the hydriodochlorobis(triphenylphosphine)platinum(II), $[(C_6H_5)_3P]_2PtHCl$, and the 1:1 adduct with stannous chloride have been described.[16] Dichlorobis(triphenylarsine)platinum(II), $[(C_6H_5)_3As]_2PtCl_2$, was made by a published procedure.[22] Methyl linolenate was separated from linseed esters by counter double current distribution between *n*-hexane and acetonitrile.[23] Glpc showed 100% triene; infrared showed no isolated *trans*. Conjugated dienes used as standard for glpc were derived from alkali conjugated methyl linoleate and conjugated dienetrienes and conjugated trienes from alkali-conjugated linseed methyl esters.[3e]

Hydrogenation.—Pressure hydrogenations were carried out in a solution of 40% methanol and 60% benzene (v/v) stirred in a Magne-Dash[24] 150-ml, stainless steel autoclave adapted with sampling tube.

Atmospheric hydrogenations were done in methanol solution stirred magnetically in a 50-ml erlenmeyer flask connected to a manometric system under 1 atmosphere hydrogen. Solvent was removed from the hydrogenated products under vacuum on a rotating evaporator. All products were redissolved in petroleum ether treated repeatedly with hydrochloric acid (1:1) to decompose the catalysts, then washed with water once, followed by saturated $NaHCO_3$, and then with water again to neutrality. The solution was dried over sodium sulfate.

Analyses.—Methods for glpc, infrared and ultraviolet spectroscopy, and alkali conjugation were the same as those used previously.[3d,e] Hydrogenation products were separated into monoene, diene, and triene fractions by countercurrent distribution between *n*-hexane and acetonitrile.[25] Monoene fractions were further resolved into *cis* and *trans* isomers by chromatography through a silver-saturated ion-exchange resin column.[26] Dienes were fractionated into nonconjugated isomers by preparative glpc according to a procedure already described.[3c] Position of double bonds was determined in the monoene fractions by potassium permanganate–potassium periodate oxidative cleavage[27] and in the diene fractions by ozonolysis–glpc.[28]

Registry No.—Methyl linolenate, 301-00-8; stannous chloride, 7772-99-8.

Acknowledgments.—The authors express their thanks to V. L. Davison for the oxidative cleavage analyses and to F. L. Little for technical assistance.

(19) G. C. Bond and P. B. Wells, *Advan. Catalysis*, **15**, 211 (1964).
(20) M. Orchin, *ibid.*, **16**, 1 (1966).
(21) G. C. Bond and M. Hellier, *Chem. Ind.*, 35 (1965).
(22) K. A. Jensen, *Z. Anorg. Chem.*, **229**, 225 (1936).
(23) R. O. Butterfield, H. J. Dutton, and C. R. Scholfield, *Anal. Chem.*, **38**, 86 (1966).
(24) Mention of firm names or trade products does not constitute endorsement by the U. S. Department of Agriculture over other firms or similar products not mentioned.
(25) C. R. Scholfield, J. Nowakowska, and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, **37**, 27 (1960).
(26) E. A. Emken, C. R. Scholfield, and H. J. Dutton, *ibid.*, **41**, 388 (1964).
(27) E. P. Jones and V. L. Davison, *ibid.*, **42**, 121 (1965).
(28) V. L. Davison and H. J. Dutton, *Anal. Chem.*, in press.

Bishomofolic Acid. A New Synthesis of Folic Acid Analogs[1]

CAROL W. MOSHER, EDWARD M. ACTON, OSBORNE P. CREWS, AND LEON GOODMAN

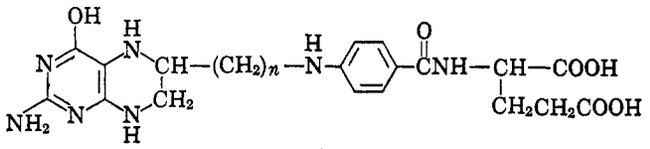
Life Sciences Research, Stanford Research Institute, Menlo Park, California

Received October 26, 1966

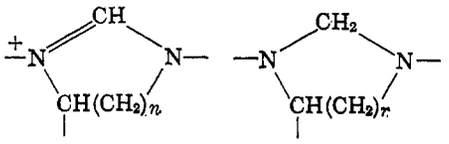
Bishomofolic acid, a folic acid analog where the pteridine and aminobenzoyl groups are joined by three methylenes instead of one, has been synthesized. 5-Bromo-1-pentene and ethyl *p*-tosylamidobenzoate afforded 5-(*p*-carbethoxy-N-tosylanilino)-1-pentene (**3a**). The olefin was converted, through the epoxide, azido alcohol, and azido ketone, to an amino ketone hydrochloride, which as its semicarbazone (**8a**) was condensed with 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine (**10**). The ketone function was regenerated and reductively cyclized with the nitrofunction to form the dihydropteridine. Oxidation afforded the N^{12}-tosylbishomopteroic ester; the 2-N-acetyl-N^{12}-tosyl acid was coupled with diethyl glutamate. The product was treated with base and detosylated with hydrogen bromide–acetic acid to form bishomofolic acid. The sequence was equally applicable to homofolic acid.

Homologs of folic acid with additional methylene groups inserted between the pteridine and aminobenzoyl groups represent an important modification of this important cofactor, but their synthesis constitutes a chemical problem beset with considerable practical difficulties. Homofolic acid (**20c**, which has one additional methylene) was synthesized recently,[2] and its tetrahydro derivative (A, n = 2) showed interesting activity in several biological systems.[3] Conceivably, this activity involved the formation of six-membered cyclic intermediates (B and C, n = 2) with one-carbon fragments, analogous to the five-membered cyclic intermediates formed by tetrahydrofolic acid (A, n = 1) in its function as a one-carbon transfer agent.[4] It was seen that the importance and the geometrical requirements of such intermediates might be tested with the next higher homolog, the so called "bishomofolic" acid (**20a**) having a third methylene, since unfavored seven-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Contract No. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.
(2) J. I. DeGraw, J. P. Marsh, Jr., E. M. Acton, O. P. Crews, C. W. Mosher, A. N. Fujiwara, and L. Goodman, *J. Org. Chem.*, **30**, 3404 (1965).
(3) J. A. R. Mead, A. Goldin, R. L. Kisliuk, M. Friedkin, L. Plante, E. J. Crawford, and G. Kwok, *Cancer Res.*, **26**, 2734 (1966); 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).
(4) For recent review of folic acid metabolism, and leading references, see M. Friedkin, *Ann. Rev. Biochem.*, **32**, 185 (1963).



A

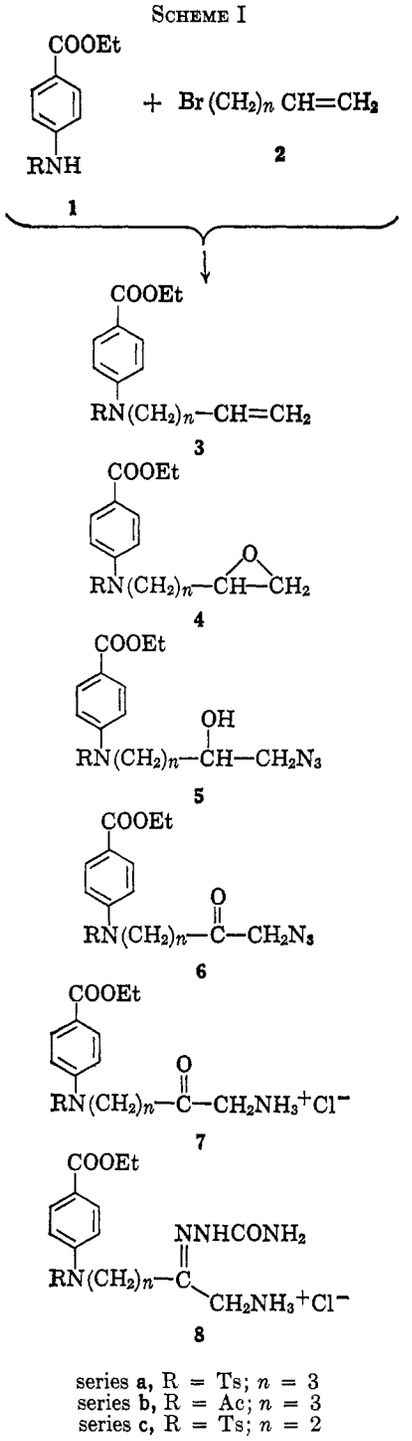


B C

membered rings would then be required from the tetrahydro derivative (A, $n = 3$). If the acitivity were totally dependent on intermediates like B and C, **20a** would be expected to be inactive.

This paper describes the synthesis of bishomofolic acid[5] (**20a**), by a method which seems applicable to folic acid analogs in general, and which has for example permitted a more convenient synthesis of homofolic acid (**20c**). The sequence involved the ready alkylation of ethyl *p*-tosylamidobenzoate[6] (**1a**) with an ω-bromoalkene (**2**). The olefin in the resultant alkenyl compound **3a** was elaborated in three facile steps (Scheme I, series **a**) to an azido ketone (**6a**), which was reduced to the amino ketone hydrochloride (**7a**) needed for the Boon and Leigh synthesis[2,7] of a specifically 6-substituted pteridine. This is a much more direct and convenient approach to such amino ketones than was previously available.[2] The epoxide (**4a**) was formed from **3a**, and was converted to the azido alcohol (**5a**) with sodium azide in the presence of a buffer;[8] there was no evidence (*cf.* series **b**, below) for formation of the isomeric azido primary alcohol. Yields of these oils were quantitative, but oxidation to the azido ketone (**6a**) with chromic oxide in acetic acid occurred in about 60% yield, some material having been lost in basic extracts; though the amount of oxidant was cut to the point where up to 10% of **5a** was unreacted, there was apparently concomitant cleavage to the carboxylic acid with one less carbon. Amine hydrochloride **7a** was converted to the crystalline semicarbazone hydrochloride (**8a**); its free base could be condensed (Scheme II) with either the 5-phenylazo-[2,7] (**9**) or the 5-nitropyrimidine[9] (**10**). Use of **10** was preferred for its shorter reaction time owing to increased reactivity of the 6-chlorine. It was highly advantageous to conduct the following three steps without isolation of intermediates, *i.e.*, cleavage of the semicarbazone **12a** to the ketone **14a** (or **11a** to **13a**), reductive cyclization (of either **14a** or **13a**) to dihydropteridine **15a**, and aromatization to **16a**. This, the N-tosyl ester of bishomopteroic acid, was saponified, the 2-amino group was acetylated, and the resultant **18a** (Scheme III) was coupled with diethyl glutamate by the mixed anhydride procedure. The coupling product (**19a**) was saponified and the tosyl group then removed with

SCHEME I



series **a**, R = Ts; $n = 3$
series **b**, R = Ac; $n = 3$
series **c**, R = Ts; $n = 2$

30% hydrogen bromide in acetic acid containing phenol[10] to form bishomofolic acid (**20a**).

Retaining the N-tosyl-protecting group until the final step provided a number of advantages, compared with the use of an N-acetyl group as in the previous[2] homofolic acid synthesis. For example, the handling of blocking groups in converting **16a** to **18a** was relatively simple. Other advantages (in series **a**) became apparent when the synthesis of bishomofolic acid was studied using an N-acetyl group (series **b**). Alkylation of ethyl *p*-acetamidobenzoate[11] (**1b**) was inconvenient and often resulted in incomplete reaction or deacetyla-

(5) The systematic name for bishomofolic acid is N-(*p*-{[3-(2-amino-4-hydroxy-6-pteridinyl)propyl]amino}benzoyl)-L-glutamic acid. The anilino nitrogen is further designated as N^{12}.

(6) B. R. Baker, D. V. Santi, and H. S. Shapiro, *J. Pharm. Sci.*, **53**, 1317 (1964). Tosyl = *p*-tolylsulfonyl.

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

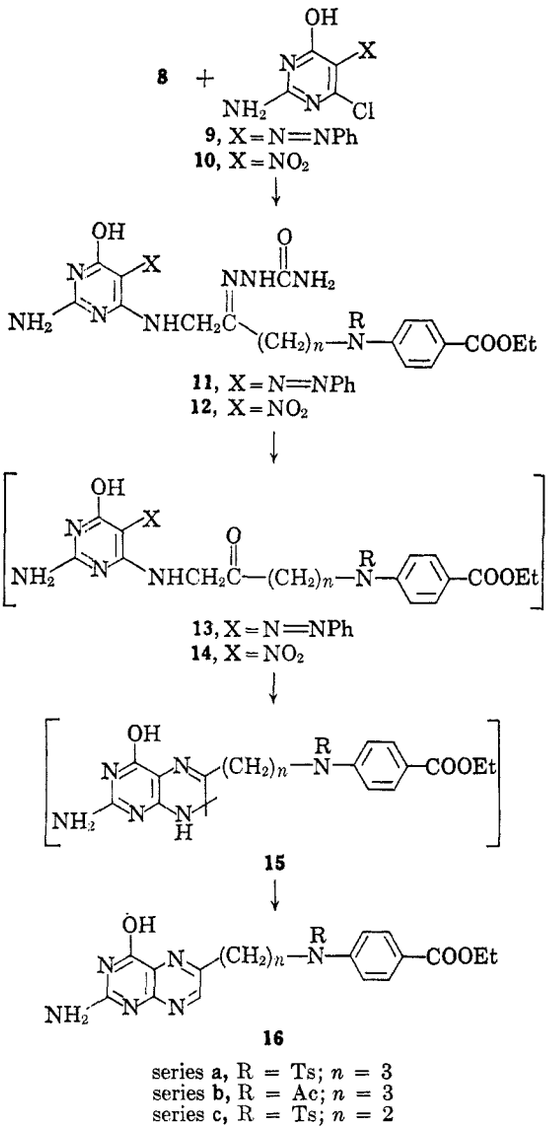
(8) R. D. Guthrie and D. Murphy, *ibid.*, 5288 (1963).

(9) A. Stuart, D. W. West, and H. C. S. Wood, *ibid.*, 4769 (1964). The 6-chlorine was highly labile, and the compound could not be stored without decomposition unless kept cold and protected from moisture.

(10) D. I. Weisblat, B. J. Magerlein, and D. R. Myers, *J. Am. Chem. Soc.*, **75**, 3630 (1953); D. I. Weisblat, B. J. Magerlein, D. R. Myers, A. R. Hanze, E. I. Fairburn, and S. T. Rolfson, *ibid.*, **75**, 5893 (1953).

(11) A. J. Hill and M. V. Cox, *ibid.*, **48**, 3219 (1926).

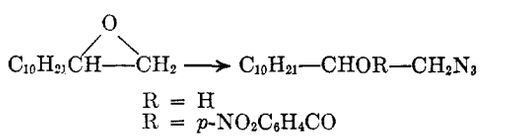
SCHEME II



tion. Epoxidation of olefin **3b** to **4b** did not reach a sharp end point; consequently after the next step, azido alcohol **5b** had to be freed of accumulated impurities by column chromatography. The epoxide opening provided a useful model study for series **a**. The preferential attack of **4b** by azide ion to form only **5b** was expected,[12] and there was no evidence from chromatographic studies or from nmr spectra for presence of the isomeric azido alcohol. It was necessary to buffer the reaction mixture, or the pH gradually rose, the reaction rate became slower and slower, and the base generated (hydroxide or alkoxide) also attacked the ethyl ester.[13]

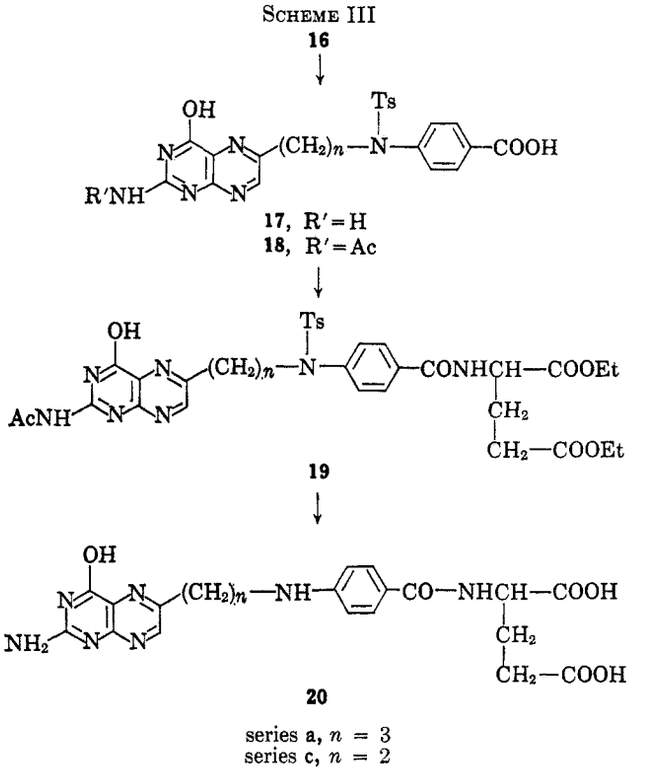
(12) C. A. VanderWerf, R. Y. Heisler, and W. E. McEwen, *J. Am. Chem. Soc.*, **76**, 1231 (1954).

(13) Additional studies were performed, on the conversion of 1,2-epoxydodecane to 1-azido-2-dodecanol. It was instructive to observe chemical shifts of the protons at C-1 and C-2 in the nmr, before and after converting the product to a *p*-nitrobenzoate (an oil), since the protons(s) α to the OH would be more greatly deshielded than the β proton(s). Integration of the

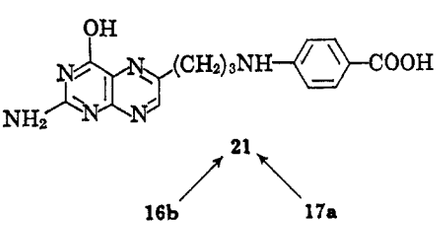


spectra showed only one α proton (a broad multiplet moved from δ 3.6 to 5.2 on *p*-nitrobenzoylation) and two β protons (an unsymmetrical triplet moved from δ 3.25 to 3.50) and excluded the isomeric structure.

SCHEME III



Oxidation of **5b** afforded the crystalline azido ketone (**6b**). Further conversion, as in series **a** and using the phenylazopyrimidine (**9**) in the condensation step, resulted in the bishomopteroic acid derivative (**16b**). Saponification afforded bishomopteroic acid (**21**), identical with a sample obtained by treating the N-tosyl derivative (**17a**) with hydrogen bromide in acetic acid.



Homofolic acid was prepared (series **c**) by the sequence in series **a**, with comparable results.

Experimental Section[14]

Infrared spectra were determined for each compound, the solids in Nujol mull, and the liquids as a film. All the benzoates (**1**, and **3–16** in each series) showed bands at 5.80 (C=O, strong), 6.22 (N-aryl, medium), 7.8 (COC), 9.0–9.1, and 14.1 μ. All the tosyl compounds showed strong bands at 7.4 and 8.6 (both due to NSO_2) and at 12.2 or 12.3 μ (p-C_6H_4). Bands for the other functional groups are listed separately for **3a–8a** and for **3b–8b**; spectra throughout series **c** were nearly identical with those in series **a**. Spectra of the pteridines (**16–20** in each series, and **21**) were of limited utility, in that the bands were often broad and poorly resolved, especially at 5.8–6.0 μ.

(14) Melting points were observed on a Fisher-Johns hot stage. Melting points of the pteridines were above 200° and with decomposition, and were reproducible only to *ca.* ±5°. Organic solutions were dried with magnesium sulfate and were concentrated *in vacuo*. Skellysolve B is a hydrocarbon fraction, bp 62–70°. Paper chromatograms were run by the descending technique on Whatman No. 1 paper and the spots were located by ultraviolet examination. Solvent systems were A, butanol–acetic acid–water (4:1:5); B, the same (5:2:3); C, 2-propanol–2 *M* hydrochloric acid (65:35) with prewashed paper; D, 5% disodium hydrogen phosphate; E, water containing 3% ammonium chloride and 5% ammonium hydroxide. Adenine was the comparison standard (R_{Ad}). Thin layer chromatography (tlc) is described separately; the spots were located by ultraviolet examination.

Nmr spectra were determined with a Varian A-60 spectrometer, using chloroform-*d* solutions (except for **18a** in dimethyl sulfoxide-d_6) containing 4% tetramethylsilane as internal standard. Signals are reported as singlets (s), doublets (d), triplets (t), or quartets (q); chemical shifts are measured from multiplet (m) centers. The *para*-substituted aminobenzoates displayed an A_2B_2 quartet for the four aromatic protons, consisting of doublets at δ 7.95 and 7.12 for **3a**,c–**6a**,c and at 8.13 and 7.26 for **3b–6b** (J = 8.5–9.0 cps). In the N-tosyl compounds, an additional pair of doublets comprising an A_2B_2 quartet was superimposed at δ 7.43 and 7.19 (J = 8.5–9.0 cps); a singlet for the aryl CH_3 was at 2.39. The ethyl esters in **3a**,c–**6a**,c showed a quartet (OCH_2) at δ 4.35 and a triplet (CH_3) at 1.38 (J = 7 cps); in **3b–6b** these signals were at δ 4.40 and 1.40. A singlet for the acetyl methyls in **3b–6b** was at δ 1.86. For any compound, values were within 0.02–0.04 of these figures. Signals for other functional groups are listed by compound. Entire spectra are listed for **18a** and **19a**.

Ultraviolet spectra of the pteridine derivatives at pH 13 permitted certain generalizations. All showed maxima at 252–256 mμ (missing in dihydro compound **15b**). Compounds with a free 2-amino group showed maxima at 360–366, shifted to 350–353 mμ in 2-acetamido derivatives. Compounds with a free anilino NH (*i.e.*, N^{12} in series **a** and **b**, N^{11} in series **c**) showed maxima at 277–281 mμ; these were absent in the N-tosyl derivatives, which instead showed maxima (or shoulders) at 230–232 mμ.

5-(*p*-Carbethoxy-N-tosylanilino)-1-pentene (3a) was prepared from ethyl *p*-tosylamidobenzoate[6] (**1a**) and 2 molar equiv of 5-bromo-1-pentene, by alkylation in dimethyl sulfoxide (DMSO)–potassium carbonate.[6] The reaction was *ca.* 90% complete after 15 hr at 25°, but 5–6 days was required for complete alkylation of **1a**, as determined by tlc in ethyl acetate–benzene on alumina, or by the absence of an NH band in the infrared at 3.1 μ. The product, redissolved in ether, washed, and recovered, was an analytically pure oil, obtained in quantitative yield. Weak olefinic absorption in the infrared was at 3.26, 6.09, 10.03, and (partly) 10.9 μ; nmr showed δ 4.65–5.85 (m, $CH{=}CH_2$), 3.58 (t, NCH_2, J = 7 cps), 1.3–2.3 (m, $CH_2CH_2C{=}$).

Anal. Calcd for $C_{21}H_{25}NO_4S$: C, 65.1; H, 6.50; N, 3.62; S, 8.26. Found: C, 64.9; H, 6.47; N, 3.75; S, 8.28.

5-(*p*-Carbethoxy-N-tosylanilino)-1,2-epoxypentane (4a).—A solution of 10.4 g (26.8 mmoles) of olefin **3a** in 60 ml of benzene was mixed with 280 ml of benzene solution containing 35 mmoles, determined iodimetrically,[15] of *m*-chloroperbenzoic acid. After the solution had stood in the dark at 25° for 20 hr, titration of an aliquot showed that 24 mmoles of per acid had been consumed; after 48 hr, 26 mmoles was consumed, *i.e.*, there was no overoxidation on standing. The reaction solution was washed with 3% NaOH solution and with water until the washings were neutral (negative test for peroxides), then was dried and concentrated to form 11.0 g (102%) of yellow syrup. A sample for analysis was redissolved in ether, washed again, dried, and recovered. The infrared spectrum differed from that of IIIa only in the absence of the olefinic bands noted for IIIa; nmr showed δ 3.63 (m, NCH_2), 2.85 (broad, m, epoxy CH), 2.6 m and 2.4 m (epoxy CH_2), 1.6 (broad, CH_2CH_2).

Anal. Calcd for $C_{21}H_{25}NO_5S$: C, 62.5; H, 6.25; N, 3.47; S, 7.94. Found: C, 62.0; H, 6.11; N, 3.40; S, 7.78.

1-Azido-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanol (5a).—A solution of 10.5 g (162 mmoles) of sodium azide and 4.15 g (77.5 mmoles) of ammonium chloride (as buffer) in 30 ml of water was added to a solution of 15.5 g (38.5 mmoles) of epoxide **4a** in 130 ml of 2-methoxyethanol, which was then refluxed for 4 hr, with the gradual precipitation of salts. The mixture was poured into water and extracted with benzene in several portions. The combined benzene extracts were washed with water, dried, and concentrated to form a viscous, brown syrup (17.0 g, 98% yield). The infrared spectrum differed from that of **4a** only in the presence of new bands at 2.83 (broad, OH) and 4.77 μ (N_3); nmr showed δ 3.35–3.90 (broad, NCH_2 plus CH—O–), 3.1–3.3 (unsymmetrical, t, $COCH_2N_3$), 1.53 (broad, s, CH_2CH_2.)

Anal. Calcd for $C_{21}H_{26}N_4O_5S$: C, 56.5; H, 5.87; N, 12.6; S, 7.19. Found: C, 56.0; H, 5.95; N, 12.4; S, 7.03.

1-Azido-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanone (6a).—A solution of 2.92 g (29.2 mmoles) of chromium trioxide in 2 ml of water and 40 ml of acetic acid was added slowly, with stirring, to a solution of 13.0 g (29.2 mmoles) of azido alcohol **5a** in 40 ml of acetic acid. The temperature rose briefly to about 60°, and the solution was stirred and heated at 70–75° for 2 hr. The green solution was poured onto 300 ml of ice and water, and extracted with three 100-ml portions of ether. The combined ether extracts were washed with dilute sodium hydroxide (to remove any acidic cleavage products, as well as acetic acid) until the washings were basic, washed with water until neutral, dried, and concentrated to form 8.20 g (63%) of a yellow syrup which could not be induced to crystallize. It was homogeneous to tlc on silica gel in benzene–ethyl acetate (1:1) with R_f 0.8, *vs.* R_f 0.7 for **5a**. The infrared spectrum, relative to that of **5a**, changed only in loss of the OH band (or of all but a trace) and in but slight intensification of the carbonyl; nmr showed δ 3.98 (s, $COCH_2N_3$, frequently *ca.* 10% low in peak area), 3.60 (broad, t, NCH_2), 2.58 (t, CH_2CO), 1.71 (t or quintet, CH_2 at C-4 of pentyl). The oil darkened on standing for a week and showed a slight decrease in azide absorption in the infrared; a tar was formed on prolonged standing.

Semicarbazone of 1-Amino-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanone Hydrochloride (8a).—A solution of 3.13 g (7.05 mmoles) of syrupy azido ketone **6b** in 15 ml of 1,2-dimethoxyethane and 3 ml of concentrated hydrochloric acid was hydrogenated, as described[2] for reduction of structure **6** where n = 2 and R = Ac, for 8 hr using 0.35 g of 30% palladium–carbon catalyst. The filtered reaction solution was concentrated to form a residual syrup, which was partitioned between water and ethyl acetate. Concentration of the aqueous layer afforded 2.52 g (79%) of **amino ketone hydrohloride 7a** as a viscous syrup, which was free of azide absorption in the infrared. The substance was homogeneous upon paper chromatography in system A with R_f 0.88 (R_{Ad} 1.66); the spot exhibited a white fluorescence under ultraviolet light and a brown color with ninhydrin spray reagent.

A solution of 2.08 g (4.60 mmoles) of amino ketone hydrochloride **7a** in 8 ml of 85% ethanol was treated with a solution of 0.644 g (5.80 mmoles) of semicarbazide hydrochloride in 2 ml of water. The pH was adjusted to 6–7 by addition of saturated, aqueous sodium acetate. Chilling for 1 hr caused the separation of white cyrstals, which were collected on a filter, washed with ethanol, and recrystallized from 20 ml of 95% ethanol. The semicarbazone weighed 1.35 g (57%), mp 171–175°. The substance was very soluble in water. A sample for analysis was obtained by several recrystallizations from 90% ethanol and finally a large volume of absolute ethanol: mp 175–177°; infrared bands were found at 2.88, 2.98, 3.15 strong (NH), 3.7, 3.8, 5.0–5.2 (amine salt), and 5.97–6.02 μ (NCON plus C=N) in addition to benzoate and tosyl bands.

Anal. Calcd for $C_{22}H_{30}ClN_5O_5S$: C, 51.6; H, 5.91; Cl, 6.93; N, 13.7. Found: C, 51.4; H, 5.95; Cl, 6.94; N, 14.1.

In some runs a second form was obtained, mp 148–151°, which was generally similar in the infrared but showed some distinct bands. Mixtures of the two forms, sometimes isolated according to the infrared, ranged in melting point from 160 to 170°; on mixing with the above samples, melting point depression was not observed. All samples were converted into the same semicarbazone amine free base.

Semicarbazone of 1-Amino-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanone.—A solution of hydrochloride **8a** (6.1 g, 11.9 mmoles) in 150 ml of water was made alkaline with sodium hydroxide to form the free base. The sticky gum was repeatedly triturated with water until it solidified. After washing with water, the dried solid weighed 5.0 g (89%, mp 90–93°) and was recrystallized twice (without heating, to avoid consequent yellowing and gumming) from aqueous ethanol: mp 97–99.5°; infrared showed 2.90, 3.00, 3.14–3.3 (NH), 5.93 (NCON plus C=N), 6.27 μ, (aryl), plus benzoate and tosyl bands; absence of all but weak absorption at 11.3 or 11.6 μ confirmed that neither form of hydrochloride **8a** was present.

Anal. Calcd for $C_{22}H_{29}N_5O_5S \cdot H_2O$: C, 53.5; H, 6.33; N, 14.2; S, 6.48. Found: C, 53.7; H, 6.13; N, 14.0; S, 6.64.

Semicarbazone of 1-(2-Amino-4-hydroxy-5-phenylazo-6-pyrimidinyl)amino-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanone (11a).—A solution of 1.02 g (2.00 mmoles) of amino ketone semicarbazone hydrochloride **8a** in 3 ml of dimethylformamide (DMF) was treated with 2.0 ml of 1.0 *M* sodium hydroxide. The resultant clear solution of free base was treated with the red solution of 0.489 g (2.00 mmoles) of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine[2] (**11**) in 5 ml of DMF, and then with 0.53 ml of *sym*-collidine. After being stirred for 5–7 days at room temperature, the reaction mixture containing orange solid was poured into 25 ml of water. The orange product

(15) D. Swern, *Org. Reactions*, **7**, 392 (1953).

was collected on a filter and washed with water, a small amount of alcohol, and finally ether to yield 0.96 g (70%): mp 190–203°; homogeneous in system A with R_f 0.94 (R_{Ad} 1.55), and in system C with R_f 0.98 (R_{Ad} 2.66); infrared showed benzoate and tosylate bands plus 2.88, 2.96, 3.03, 3.2 (NH), 5.92 and 6.06 (partly NCON, C=N), 6.32 μ (aryl). Insolubility prevented study of the ultraviolet spectrum at pH 13.

Anal. Calcd for $C_{32}H_{36}N_{10}O_6S$: C, 55.8; H, 5.27; N, 20.3; S, 4.65. Found: C, 56.3; H, 5.05; N, 19.8; S, 4.58.

Semicarbazone of 1-(2-Amino-4-hydroxy-5-nitro-6-pyrimidinyl)-amino-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanone (12a).—The use of 2-amino-4-hydroxy-5-nitro-6-chloropyrimidine[9] (**10**, to be truly equimolar, the stoichiometry was based on its purity as determined by elemental analysis for chlorine) in the same procedure, but heated at 80–90° for 30 min, afforded 71% of product **12a**: mp 143–153°. Infrared showed benzoate and tosylate bands plus 3.02 and 3.10 (NH), 5.9 (broad, C=O), 6.3 (broad, aryl), 6.6 μ (NO_2); $\lambda_{max}^{pH\ 13}$ 229 mμ (ε 37,600), 346 mμ (ε 16,600); R_f 0.87 (R_{Ad} 1.93) in system A, and R_f 0.82 (R_{Ad} 2.50) in system C, when compared with compound **10**, R_f 0.79 (R_{Ad} 1.74) with a weak spot at R_f 0.68 in system A, and R_f 0.76 (R_{Ad} 2.32) in system C with a weak spot at R_f 0.47. Satisfactory analyses could not be obtained for carbon or nitrogen, even after recrystallization from DMF–water which raised the melting point to 165–175°.

Ethyl N^{12}-Tosylbishomopteroate (16a). I. From 11a.—A solution of 13.5 g (19.6 mmoles) of **11a** in 200 ml of glacial acetic acid was treated with 150 ml of 2 *M* hydrochloric acid, and the dark red solution was stirred for 30 min to cleave the semicarbazone to form ketone **13a**. Then 1.35 g of 5% palladium–charcoal catalyst was added and the mixture was stirred for 18 hr under 1 atm of hydrogen (slightly more than the theoretical 2 molar equiv was consumed), while the 5-aminopyrimidine was formed and spontaneously cyclized with the ketone. The catalyst was removed by filtration through Celite, and the light brown filtrate containing **16a** was treated with 21 ml of a solution prepared by mixing 4.4 ml of 30% hydrogen peroxide and 20 ml of water. The solution stood at 25° for 2 hr, darkening in color, and was concentrated to about 25 ml. The residue was diluted to 400 ml with water, and the solution was neutralized (pH 7) with concentrated ammonium hydroxide. The resultant light brown precipitate weighed 8.80 g (86% yield, 90% pure by comparison of ultraviolet extinctions), mp 240–260° dec. Recrystallization from water and washing the solid with acetone yielded 4.7 g (46%), mp 273–277° dec. A second recrystallization afforded the analytical sample: mp 275–278° dec; $\lambda_{max}^{pH\ 13}$ 232 mμ (shoulder, ε 26,400), 252 (33,400), 364 (7540); R_f 0.96 (R_{Ad} 1.97) in system C, R_f 0.80 (R_{Ad} 1.27) in system B.

Anal. Calcd for $C_{25}H_{26}N_6O_5S$: C, 57.5; H, 5.02; N, 16.1; S, 6.12. Found: C, 57.3; H, 4.92; N, 16.2; S, 6.23.

II. From 12a.—Compound **16a** from **12a** was obtained by the same procedure, the initial yield being 71%; $\lambda_{max}^{pH\ 13}$ 230 mμ (shoulder, ε 27,900), 253 (33,200), 363 (7120); R_f values were identical with those in I, but there were traces of fluorescent impurities. A sample recrystallized once (mp 270–273° dec) was analyzed.

Anal. Found: C, 56.9; H, 4.94; N, 16.2; S, 6.32.

N^{12}-Tosylbishomopteroic Acid (17a).—A mixture of 3.98 g (7.63 mmoles) of ester **16a** and 80 ml of 0.25 *M* sodium hydroxide was heated at 100° for 2 hr. The brown solution was clarified by filtration, acidified to pH 3–4 with hydrochloric acid, diluted with water, and cooled. The light brown solid, collected on a filter and washed with water, weighed 3.53 g (94%) and did not melt below 300°. Broad infrared absorptions near 4.2 and 5.2 μ were suggestive of a zwitterion; absence of a distinct band at 7.8 μ qualitatively indicated absence of ester **16a**. Absence of ester was discerned by tlc on silica gel in DMF–water (1:1) where **17a** showed R_f near 0.9 *vs.* **16a** of R_f 0.0. A solution in 0.1 *M* sodium hydroxide was clarified again by filtration and acidified. The gelatinous precipitate was stirred in water for 15 min and collected. The yield was 75% of an analytically pure sample; $\lambda_{max}^{pH\ 13}$ 230 mμ (shoulder, ε 22,000), 253 (28,000), 362 (6500).

Anal. Calcd for $C_{23}H_{22}N_6O_6S$: C, 55.9; H, 4.48; N, 17.0; S, 6.47. Found: C, 55.7; H, 4.22; N, 16.7; S, 6.62, 6.40.

2-N-Acetyl-N^{12}-tosylbishomopteroic Acid (18a).—A suspension of 1.60 g (3.25 mmoles) of acid **17a** in 25 ml of acetic anhydride was stirred and refluxed for 2 hr. The resultant solution was concentrated and the residual syrup was triturated with water. The partly solid residue was dissolved in 20 ml of 2% sodium hydroxide, and the clear solution was adjusted to pH 3 with dilute hydrochloric acid. The precipitate weighed 1.45 g (83%); nmr (in DMSO-d_6 with external tetramethylsilane) showed δ 8.56 (s, pteridine 7-H), 7.80 d and 7.15 d (*p*-C_6H_4, J = 9 cps), 7.28 (s, *p*-C_6H_4 of tosyl), 3.65 (broad, CH_2NTs), 2.85 (broad, pteridine 6-CH_2), 2.28 (s, CH_3 of tosyl), 2.11 (s, CH_3 of acetyl), 1.8 (broad, RCH_2R). On tlc in DMF–water (1:1), the R_f was *ca.* 0.1 on alumina and 0.9 on silica gel. A sample was dried at 60° *in vacuo* for 40 hr: mp 145–155° dec; $\lambda_{max}^{pH\ 13}$ 230 mμ (ε 25,700), 253 (32,200), 352 (6100).

Anal. Calcd for $C_{25}H_{24}N_6O_6S$: C, 56.0; H, 4.51; N, 15.7; S, 5.96. Found: C, 55.9; H, 4.81; N, 15.4; S, 5.87.

Diethyl 2-N-Acetyl-N^{12}-tosylbishomofolate (19a).—A solution of N-acetyl-N-tosyl acid **18a** (3.07 g, 5.70 mmoles) in 40 ml of DMF (purified by stirring overnight with neutral alumina and molecular sieves) was cooled to 0°, treated with 0.79 ml (5.7 mmoles) of triethylamine (freshly distilled from KOH), then with 0.75 ml (5.7 mmoles) of freshly distilled isobutyl chloroformate, and stirred for 1.5 hr at 0° with exclusion of moisture. A mixture of 1.37 g (5.70 mmoles) of diethyl glutamate hydrochloride and 0.79 ml (5.7 mmoles) of triethylamine in 5 ml of DMF was added, and stirring was continued for 18 hr at room temperature. The reaction mixture was concentrated (50° bath) and the residue was suspended in 30 ml of ethyl acetate. The triethylamine hydrochloride was removed on a filter and the filtrate was washed twice with 15-ml portions of 0.3 *M* hydrochloric acid, once with water, once with saturated sodium bicarbonate, and twice with water. The emulsions encountered in the final water washings were easily broken by adding sodium chloride. Concentration of the dried ethyl acetate solution formed a residual syrup which upon trituration with ether afforded 2.07 g (50%) of a light brown powder. Attempted recrystallization of 1.43 g of 4 ml of dichloromethane and 15 ml of carbon tetrachloride yielded a gel, which was collected on a filter and dried *in vacuo*. After drying for 70 hr at 75°, the resultant powder weighed 1.32 g (46%): $\lambda_{max}^{pH\ 13}$ 230 mμ (shoulder, ε 26,300), 255 (37,500), 350 (7050); nmr ($CDCl_3$) showed δ 12.53 and 11.18 (broad singlets exchangeable with D_2O, amide NH and pteridine N-3–H or C-4–OH), 8.67 (s, pteridine 7-H), 7.75 d and 7.10 d (*p*-C_6H_4), 7.40 d and 7.19 d (*p*-C_6H_4), 4.8 (broad, glutamate NCHCO), 4.17 (symmetrical quintet, from two superimposed quartets, J = 7 cps, OCH_2's from Et's), 3.65 (broad, CH_2NTs), 2.82 (broad, pteridine 6-CH_2), 1.7–2.7 (EtOOC–CH_2CH_2 plus trimethylene RCH_2R), 2.41 s and 2.39 s (CH_3's of tosyl and acetyl), 1.29 t and 1.21 t (CH_3's from Et's, J = 7 cps). If softened to a melt *ca.* 83–95°.

Anal. Calcd for $C_{34}H_{39}N_7O_9S$: C, 56.6; H, 5.45; N, 13.6. Found: C, 56.1; H, 5.40; N, 13.8.

Bishomofolic Acid (20a).—A mixture of 3.53 g (4.90 mmoles) of **19a** and 24.5 ml of 1.0 *M* methanolic potassium hydroxide was refluxed for 30 min and then concentrated. The residual, sticky syrup (salt of N^{12}-tosylbishomofolic acid) was treated with 0.980 g (10.5 mmoles) of phenol and 35 ml of anhydrous 30% hydrogen bromide in glacial acetic acid[10] (*i.e.*, 178 mmoles of HBr). The resultant, clear, yellow solution was stirred at room temperature for 3 hr, turning cloudy as sodium bromide separated. The mixture was poured into 350 ml of ether. The hygroscopic yellow precipitate (HBr salt of **20a**) was collected on a filter, washed rapidly with ether, and dissolved in 50 ml of water (containing a small amount of HCl to increase solubility). The clear, yellow filtrate remaining after removal of small amounts of dark, insoluble tars was adjusted to pH 3 by adding 5 *M* ammonium hydroxide. The gelatinous precipitate was collected by centrifugation (5000 rpm) and repeatedly washed, by stirring vigorously with water and centrifuging, until the washings were free of halide ion. The dried yellow solid weighed 1.43 g (63%). A sample dried *in vacuo* at 100° for 2 hr, then at 75° for 15 hr, was analyzed: $\lambda_{max}^{pH\ 13}$ 253 mμ (ε 25,500), 280 (20,500), 364 (7300). The major spot in system D was ultraviolet absorbing with a blue fluorescent halo, R_f 0.51 (R_{Ad} 1.47), and there was a minor white fluorescent spot at R_f 0.32; in system E the strongly blue fluorescent spot had R_f 0.55 (R_{Ad} 1.72) and there was weak white fluorescence at R_f 0.39.

Anal. Calcd for $C_{21}H_{23}N_7O_6$: C, 53.7; H, 4.94; N, 20.9. Found: C, 53.6; H, 4.81; N, 20.9.

In other runs, yields as high as 87% have been obtained, sometimes as the hemihydrate.

The chromatographically observed fluorescent contaminant could not be detected by other means and had no effect on other properties of the compound. The major spot from a chromato-

gram on Whatman 3 MM paper run in solvent D (as was done[2] for homofolic acid, **20c**) was eluted to give a small sample of **20a**; the ultraviolet spectrum was essentially unchanged [$\lambda_{max}^{pH\ 13}$ 253 mμ (ε 25,600), 280 (19,900), 365 (7200)], but the fluorescent contaminant reappeared when the sample was analyzed again by paper chromatography.

Bishomopteroic Acid (21). I. From 17a.—A mixture of 104 mg (0.21 mmole) of **17a** and 42 mg (0.45 mmole) of phenol in 1.5 ml of glacial acetic acid containing 30% hydrogen bromide was stirred at room temperature for 2 hr and was poured into 40 ml of ether. The solids were collected on a filter and then dissolved in dilute hydrochloric acid. The acid solution (after removal of a little insoluble, brown solid) was adjusted to pH 3 by adding concentrated ammonium hydroxide. The solid, yellow product separated, and was collected, washed with water, and dried (52% yield). Purity was 90% by comparison of ultraviolet extinctions with those of an analytical sample, obtained in another experiment by a second precipitation from sodium hydroxide solution and dried at 140° *in vacuo* for 24 hr: $\lambda_{max}^{pH\ 13}$ 255 mμ (ε 27,800), 277 (23,000), 363 (8020); R_f 0.35 (fluorescent, R_{Ad} 0.95) in system C, R_f 0.25 (fluorescent, R_{Ad} 0.60) in system D, R_f 0.28 (absorbing, R_{Ad} 0.82) in system E.

Anal. Calcd for $C_{16}H_{16}N_6O_3$: C, 56.5; H, 4.74; N, 24.7. Found: C, 56.2; H, 4.82; N, 24.2.

II. From 16b.—As described[2] for homopteroic acid, amide ester **16b** (see below) was saponified with 10% sodium hydroxide and the crystalline sodium salt of **21** was neutralized to form a yellow-brown solid (84%). A sample for analysis was obtained by trituration with aqueous DMF and drying at 100° *in vacuo*: $\lambda_{max}^{pH\ 13}$ 255 (ε 28,900 calcd as hemihydrate), 276 (23,300), 362 (8170); R_f values were the same as in I, above, except that weakly fluorescent spots attributed to trace impurities were seen in systems C (at R_f 0.25) and D (at R_f 0.35, 0.46, and 0.56). The infrared spectrum was nearly indistinguishable from that in I.

Anal. Calcd for $C_{16}H_{16}N_6O_3 \cdot 0.5H_2O$: C, 55.0; H, 4.90; N, 24.1. Found: C, 55.5; H, 4.84; N, 24.4.

5-(*p*-Carbethoxy-N-acetylanilino)-1-pentene (3b).—A boiling solution (dried by azeotropic distillation) of 4.14 g (20.0 mmoles) of ethyl *p*-actamidobenzoate[11] (**1b**) in 100 ml of xylene was treated with 0.72 g (30 mmoles) of sodium hydride (suspended in a few milliliters of xylene, after prewashing to remove mineral oil from the commercial dispersion). Slight foaming occurred. The mixture was stirred and refluxed for 1 hr while a sticky solid separated. 5-Bromo-1-pentene (8.40 g, 56.0 mmoles) was added dropwise, and refluxing with stirring was continued for 6 hr. After standing overnight, the mixture was filtered to remove sodium bromide (washed with a little benzene), and the filtrate was concentrated to form 5.25 g (95%) of a residual orange-brown syrup, which was distilled at 0.04–0.05 mm, bath temperature 180–205° (63% yield). The occurrence of NH bands at 3.0 and 6.5 μ in the infrared were caused either by presence of a little starting amide **1b** (doublets at δ 8.03 and 7.70 in the nmr), or by some deacetylation of the product **3b** (probably owing to presence of moisture in the reaction mixture, and characterized by doublets at δ 7.83 and 6.51 in the nmr for *p*-C_6H_4 of unacylated alkylamino benzoate). Realkylation or reacetylation, respectively, then afforded pure **3b**, free of NH bands. Weak olefinic infrared bands were at 3.27, 10.05, and (partly) 10.95, with strong amide C=O at 5.99 μ; nmr showed δ 4.75–5.9 (m, $CH{=}CH_2$), 3.73 (t, NCH_2), 1.2–2.2 (m, CH_2CH_2).

Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.8; H, 7.69; N, 5.09. Found: C, 69.5; H, 7.89; N, 4.94.

5-(*p*-Carbethoxy-N-acetylanilino)-1,2-epoxypentane (4b).—Olefin **3b** was epoxidized as in the preparation of **4a**, except that consumption of peracid did not reach a sharp end point and was allowed to proceed (24 hr) until *ca.* 1.1 moles was consumed per mole of olefin; 5–10% olefin remained according to the nmr. The epoxide was a syrup which differed from **3b** in the infrared only in the loss of olefinic bands; nmr showed δ 3.78 (broad, NCH_2), 2.9 (broad, epoxy CH), 2.4 m and 2.7 m (epoxy CH_2), 1.2–2.2 (m, CH_2CH_2).

1-Azido-5-(*p*-carbethoxy-N-acetylanilino)-2-pentanol (5b) was prepared as was **5a** and purified by column chromatography on silica gel (90–200 mesh). Elution with dichloromethane afforded *ca.* 15% of a fraction containing *para*-substituted benzoate but no azide in the infrared, then *ca.* 4% of olefin **3b**; ethyl acetate eluted **5b** of good purity, obtained as a syrup (64%). The infrared spectrum differed from that of **4b** in the addition of bands at 2.95 (OH) and 4.78 (N_3) and a shoulder at 6.08 μ on the amide C=O; nmr data showed δ 3.5–4.1 (m, NCH_2 plus CH—O–), 3.32 s and 3.23 s (CH_2N_3), 1.2–2.1 (CH_2CH_2).

1-Azido-5-(*p*-carbethoxy-N-acetylanilino)-2-pentanone (6b).—Oxidation as for **6a**, but without heating and for 15 hr, afforded a syrup (67%) which crystallized on standing and was recrystallized from benzene–Skellysolve B, then from chloroform–Skelly B, to form waxy, white crystals (10%), mp 72–74°; infrared showed absence of OH, amide C=O shifted to 6.08 μ (no band at 6.0 μ); nmr showed δ 3.94 (s, $COCH_2N_3$), 3.74 (t, NCH_2, J = 6 cps), 2.52 (t, broad, CH_2CO), 1.83 (s plus m, $NCOCH_3$ plus RCH_2-R).

Anal. Calcd for $C_{16}H_{20}N_4O_4$: C, 57.8; H, 6.07; N, 16.9. Found: C, 57.9; H, 6.12; N, 16.6.

Semicarbazone of 1-Amino-5-(*p*-carbethoxy-N-acetylanilino)-2-pentanone Hydrochloride (8b).—Hydrogenation of **6b** (as the initial syrup) afforded **amino ketone hydrochloride 7b** (90% yield) as a white solid, which was treated with semicarbazide hydrochloride, as described for series **a**. The product (**8b**) crystallized in several crops, after concentration of mother liquors and trituration or recrystallization of the residues with aqueous ethanol. The yield was 48%, mp 209–212°; infrared showed λ_{max} 2.95, 3.05, 3.15, 3.25 (NH), 3.6–3.9 and 5.0 weak (amine salt), 5.90 and 6.00 (NCO, NCON, and C=N), 6.32 μ (aryl C=C), plus benzoate bands. An analytical sample from another run was identical in the infrared, but melted at 203–205°.

Anal. Calcd for $C_{17}H_{26}ClN_5O_4$: C, 51.1; H, 6.56; N, 17.5. Found: C, 50.6; H, 6.69; N, 17.6.

Semicarbazone of 1-(2-Amino-4-hydroxy-5-phenylazo-6-pyrimidinyl)amino-5-(*p*-carbethoxy-N-acetylanilino)-2-pentanone (11b).—According to the method for preparing **11a**, a suspension of **8b** in DMF afforded a yellow product (73%), mp 144–150° dec. Reprecipitation from a hot ethanol solution by addition of water afforded an analytical sample: mp 141–147° dec; λ_{max} 3.0 and 3.17 (NH), 6.0 broad (C=O), 7.8 (COC), 13.1 and 14.4 μ (C_6H_5 of phenylazo); $\lambda_{max}^{pH\ 13}$ 245 mμ (ε 28,600), 382 mμ (ε 23,400). In system C, a strong, yellow spot (deep yellow-brown under ultraviolet light) had R_f 0.95 (R_{Ad} 2.45), with a trace of **9** as a faint yellow spot, R_f 0.56 (R_{Ad} 1.22), and other faint impurities at R_f 0.18 and 0.29.

Anal. Calcd for $C_{27}H_{32}N_{10}O_5$: C, 56.2; H, 5.60; N, 24.3. Found: C, 56.5; H, 5.70; N, 23.6.

Ethyl N[12]-Acetylbishomopteroate (16b).—As described for **16a**, the cleavage of the semicarbazone from **11b**, reductive cyclization of ketone **13b**, and oxidation of dihydropteridine **15b** were performed without isolation of the intermediates to form **16b** in 61% yield (based on **11b**). The purity was 91% relative to that of an analytical sample obtained (25% yield) by recrystallization from 2 ml of DMF and dried at 100°: $\lambda_{max}^{pH\ 13}$ 252 mμ (ε 31,100), 363 mμ (ε 7550); R_f 0.79 (R_{Ad} 2.02) in system C with an extraneous spot of R_f 0.94, both exhibiting blue fluorescence, and weak white fluorescence of R_f 0.21.

Anal. Calcd for $C_{20}H_{22}N_6O_4 \cdot 0.5H_2O$: C, 57.3; H, 5.53; N, 20.0. Found: C, 57.1; H, 5.32; N, 20.3.

4-(*p*-Carbethoxy-N-tosylanilino)-1-butene (3c) was obtained[16] as for **3a** and was recrystallized from ethanol (89% yield): mp 61–62°; nmr showed δ 4.75–6.2 (m, $CH{=}CH_2$), 3.65 (t, NCH_2), and 2.35 (quintet, CH_2C, J = 7 cps).

Anal. Calcd for $C_{20}H_{23}NO_4S$: C, 64.3; H, 6.21; N, 3.75; S, 8.57. Found: C, 64.1; H, 6.16; N, 3.90; S, 8.82.

4-(*p*-Carbethoxy-N-tosylanilino)-1,2-epoxybutane (4c) was obtained as for **4a** in quantitative yield as a syrup; nmr showed δ 3.75 (t, NCH_2, J = 7 cps), 2.9 (broad, m, epoxy CH), 2.7 m and 2.4 m (epoxy CH_2), 1.5–1.95 (m, CCH_2CO).

Anal. Calcd for $C_{20}H_{23}NO_5S$: C, 61.7; H, 5.95; N, 3.60; S, 8.22. Found: C, 61.7; H, 6.08; N, 3.70; S, 8.19.

1-Azido-4-(*p*-carbethoxy-N-tosylanilino)-2-butanol (5c) was obtained in 98% yield; although one sample turned to a waxy solid on standing, it could not be recrystallized and **5c** was generally a syrup; nmr showed δ 3.4–4.2 (m, NCH_2 plus CHO), 3.22 (unsymmetrical, d, CH_2N_3), *ca.* 1.3–1.75 (m, CCH_2CO).

Anal. Calcd for $C_{20}H_{24}N_4O_5S$: C, 55.6; H, 5.59; N, 13.0; S, 7.40. Found: C, 55.8; H, 5.80; N, 12.4; S, 7.40.

1-Azido-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone (6c) was a syrup obtained in 61% yield; nmr showed δ 3.90 (s, $COCH_2N_3$), 3.87 (t, NCH_2), and 2.73 (t, CH_2CO, J = 7 cps). Integration of the singlet at δ 3.90 for slightly less than two protons and

(16) At least 2 moles of 4-bromo-1-butene per mole of sulfonamide was required for optimum yields of **3c**. Yields were only 36% when the ratio was 1.15 to 1, and 54% when the ratio was 1.50 to 1. We are indebted to Mr. Robert B. Bicknell for these observations.

very weak infrared absorption at 2.85 μ indicated that a few per cent of **5c** was still present. The compound darkened upon standing for 1 week with slight decrease in infrared azide intensity, and eventually decomposed to a tar.

1-Amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone hydrochloride (7c) was obtained (as for **7a** under the preparation of **8a**) in 97% yield.

The **picrate** was recrystallized from ethanol, mp 158–164°, and analyzed.

Anal. Calcd for $C_{26}H_{27}N_5O_{12}S$: C, 49.3; H, 4.30; N, 11.1; S, 5.06.

Found: C, 49.5; H, 4.23; N, 11.2; S, 4.99.

Semicarbazone of 1-amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone hydrochloride (8c) was obtained from crude **7c** in three crops (diluting the initial filtrate with absolute ethanol), which were combined and recrystallized from 85% ethanol (10 ml/g), 25% yield, mp 197–201° dec. An analytical sample melted at 199–201° dec; λ_{max} 2.93, 3.11 (NH), 3.7, 3.8, 5.0 (amine salt), 5.99 (NCON), 6.33 (aryl), 10.9 μ, (tosyl), plus other tosyl and benzoate bands.

Anal. Calcd for $C_{21}H_{28}ClN_5O_5S$: C, 50.7; H, 5.67; Cl, 7.12; N, 14.1; S, 6.44. Found: C, 50.6; H, 5.75; Cl, 7.48; N, 14.2; S, 6.43.

The **picrate** was recrystallized from ethanol, mp 171–173° dec.

Anal. Calcd for $C_{27}H_{30}N_8O_{12}S$: C, 47.0; H, 4.38; N, 16.2; S, 4.64. Found: C, 47.1; H, 4.41; N, 16.1; S, 4.70.

Semicarbazone of 1-(2-Amino-4-hydroxy-5-nitro-6-pyrimidinyl)-amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone (12c).—The procedure for **11a**, as modified for **12a**, afforded **12c** in 81% yield: mp 155–165° dec; $\lambda_{max}^{pH\ 13}$ 228 mμ (ϵ 33,500), 345 mμ (ϵ 14,900); R_f 0.81 (R_{Ad} 1.67) in system A, compared to R_f 0.80 (fluorescent, R_{Ad} 1.64) for **7c** and R_f 0.72 (R_{Ad} 1.47) for **10**; R_f 0.84 (R_{Ad} 2.56) in system C. Satisfactory analyses could not be obtained for carbon or nitrogen, after two recrystallizations from DMF–water, which did not change the melting point.

Ethyl N^{11}-Tosylhomopteroate (16c).—A mixture of 6.34 g (10.3 mmoles) of **12c** and 150 ml of glacial acetic acid was treated with 35 ml of 6 *M* hydrochloric acid and was stirred for 30 min. The clear, light brown solution of **14c** was treated with 650 mg of 5% palladium–carbon catalyst and hydrogenated with the gradual precipitation of **15c**; 42 hr was required. The solids were collected on a filter; **15c** was separated from the catalyst by extracting with five 50-ml portions of hot 50% acetic acid. The combined orange solutions were added to the original filtrate and treated with 11.2 ml of a solution prepared from 2.2 ml of 30% hydrogen peroxide and 10 ml of water. After 3 hr, product **16c** was isolated (as described for **16a**) in 70% yield, mp 180–200° dec. After one recrystallization from DMF–water, the yield was 52%: mp 220–230° dec; $\lambda_{max}^{pH\ 13}$ 238 mμ (shoulder, ϵ 29,200), 253 (31,400), 366 (7750).

Anal. Calcd for $C_{24}H_{24}N_6O_5S$: C, 56.7; H, 4.76; N, 16.5; S, 6.30. Found: C, 56.3; H, 4.94; N, 16.5; S, 6.27.

N^{11}-Tosylhomopteroic acid (17c) was obtained as described for **17a** in 88% yield. Purity was 95% by comparison of ultraviolet extinctions with those of an analytical sample obtained from a solution in 0.1 *N* sodium hydroxide; upon acidification, a small, brown, flocculent precipitate formed at pH 7 and was removed, and at pH 6 the purified yellow product precipitated: λ_{max}^{pH13} 236 mμ (shoulder, ϵ calcd as monohydrate 27,000), 253 (29,500), 366 (7500).

Anal. Calcd for $C_{22}H_{20}N_6O_5S\cdot H_2O$: C, 53.0; H, 4.45; N, 16.9; S, 6.42. Found: C, 53.0; H, 4.30; N, 17.3; S, 6.56.

2-N-Acetyl-N^{11}-tosylhomopteroic acid (18c) was obtained in 79% yield, as for **18a**. It was purified by dissolving in 2 *M* ammonium hydroxide and acidifying; at pH 7 a dark solid precipitated and was removed, and at pH 3 the light brown product precipitated: mp 172–180° dec; $\lambda_{max}^{pH\ 13}$ 232 mμ (shoulder, ϵ 27,600), calcd as monohydrate 255 (32,600), 355 (7760). Deacetylation of **18c** can occur upon storing basic solutions at room temperature, as evidenced by shift of the maximum from 355 to 365 mμ.

Anal. Calcd for $C_{24}H_{22}N_6O_6S\cdot H_2O$: C, 53.3; H, 4.48; N, 15.6; S, 5.92. Found: C, 53.7; H, 4.46; N, 16.0; S, 5.64.

Diethyl 2-N-Acetyl-N^{11}-tosylhomofolate (19c).—Using the procedure for preparing **19c**, but with dichloromethane instead of ethyl acetate to dissolve the product, the yield was 58%. A sample for analysis was dried at 100° for 24 hr *in vacuo*: $\lambda_{max}^{pH\ 13}$ 230 mμ (shoulder, ϵ 24,900), 256 (31,300), 356 (6340).

Anal. Calcd for $C_{33}H_{37}N_7O_9S$: C, 56.0; H, 5.27; N, 13.9; S, 4.53. Found: C, 55.8; H, 5.12; N, 13.6; S, 4.28.

Homofolic Acid (20c).—Alkaline and then acidic hydrolysis of **19c**, as in **a**, afforded 55% of **20c** (without H_2O of hydration): $\lambda_{max}^{pH\ 13}$ 255 mμ (ϵ 26,400), 279 (20,000), 364 (8250). The infrared spectrum and paper chromatographic behavior were identical with those of a sample obtained by the previous procedure,[2] and very similar to those of **10a**.

Optical rotations of 20a and 20c could not be studied with a Perkin-Elmer Model 141 automatic polarimeter, and were not an analytical tool. Erratic results were apparently caused by by color of the solutions (yellow or orange) at the concentration (0.25–0.5%) required for a measurable angle. With a visual polarimeter and 0.5% solution in 0.1 *M* sodium hydroxide, bishomofolic acid (**20a**) showed $[\alpha]^{25}D$ 12 ± 4° and homofolic acid (**20c**) showed $[\alpha]^{25}D$ 12 ± 2°.

Registry No.—**20a**, 10098-06-3; **3a**, 10083-94-0; **4a**, 10098-07-4; **5a**, 10076-34-3; **6a**, 10083-95-1; **8a**, 10083-96-2; semicarbazone of 1-amino-5-(*p*-carbethoxy-N-tosylanilino)-2-pentanone, 10076-35-4; **11a**, 10083-97-3; **12a**, 10127-42-1; **16a**, 10076-36-5; **17a**, 10076-37-6; **18a**, 10076-38-7; **19a**, 10076-39-8; **21**, 10083-98-4; **3b**, 10083-99-5; **4b**, 10127-43-2; **5b**, 10084-00-1; **6b**, 10084-01-2; **8b**, 10084-02-3; **11b**, 10084-03-4; **16b**, 10118-31-7; **3c**, 10084-04-5; **4c**, 10084-05-6; **5c**, 10084-06-7; **6c**, 10084-07-8; 1-amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone, 10084-08-9; picrate of 1-amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone, 10084-09-0; semicarbazone of 1-amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone, 10098-08-5; **8c**, 10084-10-3; picrate of the semicarbazone of 1-amino-4-(*p*-carbethoxy-N-tosylanilino)-2-butanone, 10127-44-3; **12c**, 10084-11-4; **16c**, 10084-12-5; **17c**, 10084-13-6; **18c**, 10098-09-6; **19c**, 10076-40-1; **20c**, 3566-25-4.

Acknowledgment.—The authors thank Dr. Peter Lim and staff for the spectroscopy and the paper chromatographic data.