Mechanism of the Waller Reaction. Investigation of Methods for the Synthesis of Pteridines (1)

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The preparation of aminopterin analogs *via* 6-halomethylpteridine intermediates either were unsuccessful or gave low yields of pteridines by reaction of 2,4,5,6-tetraaminopyrimidine (**1**) with 2,3-dibromopropionaldehyde (**2**), 1,1,3-trichloroacetone (**11**), and 1,1,3-tribromoacetone (**12**), respectively. Similarly, the preparation of a 6-formylpteridine was unsuccessful by the alkylation of **1** with bromomalonaldehyde and by the addition of the 5-acetyl derivative of **1** to 2-bromopropenal (**17**). In contrast, the oxidation of 2,4-diaminopteridine-6-methanol (**23**) with *N,N'*-dicyclohexylcarbodiimide gave the corresponding 6-formylpteridine **20**.

In related work, a mechanism for the Waller reaction was suggested by the identification of some of the products resulting from the reaction of 2-bromopropenal with *p*-aminobenzoyl derivatives.

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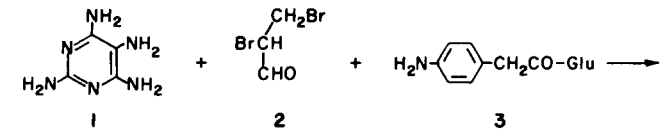
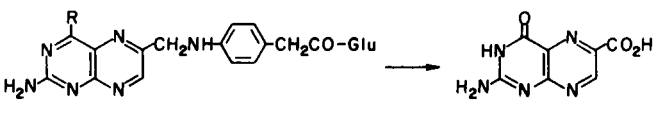
Previous work from this laboratory described the preparation and use of intermediates for the synthesis of folic acid analogs by the procedure of Boon and Leigh (2,3). The latter method is highly successful but requires a long period of time to complete the reaction sequence. In this paper some additional work on the investigation of shorter routes for the preparation of antifolates is reported (4). This study also provided information on the mechanism of the Waller reaction.

The preparation of the phenylacetyl analog **4** of folic acid has been reported (5), and a method was sought for the preparation of the corresponding 4-amino-4-deoxy derivative **5**. The synthesis of this compound was attempted by the procedure described by Waller and coworkers, which was used initially for the preparation of folic acid

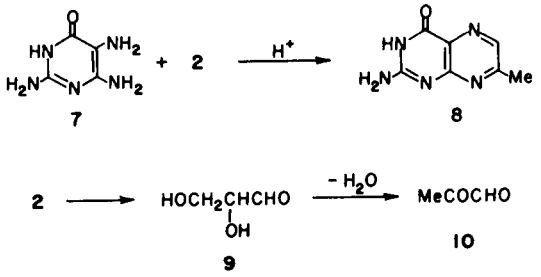


1 **2** **3**

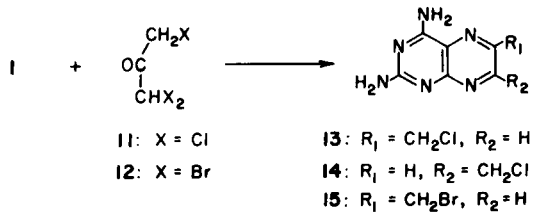


4: R = OH
5: R = NH_2

Glu = $HNCH(CO_2H)CH_2CH_2CO_2H$

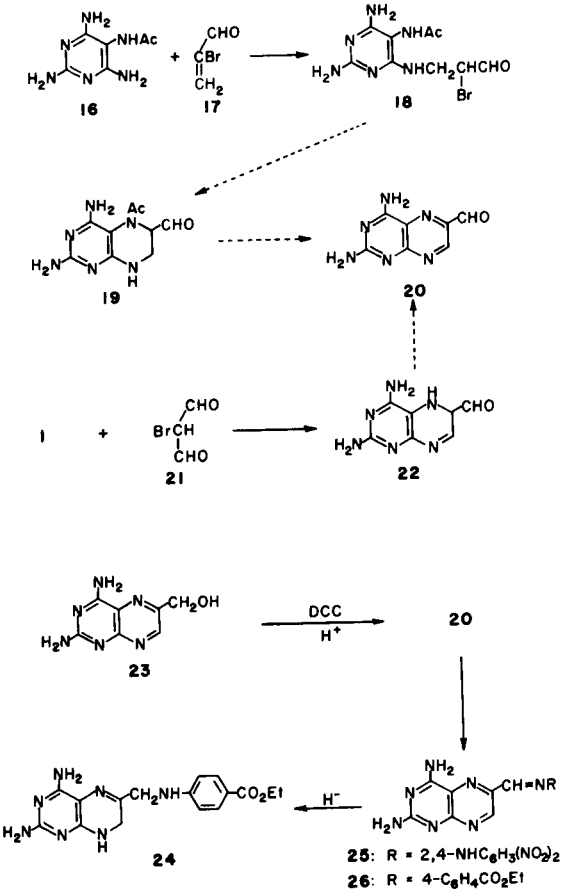


(6) and later for the antifolates aminopterin and methotrexate (7). This method involves the condensation of a tertiary mixture of a 4,5-diaminopyrimidine, a reactive three-carbon compound, and a *p*-aminobenzoyl derivative. Although many types of three-carbon reactants have been used, 2,3-dibromopropionaldehyde (**2**), has been the choice of many investigators. The use of the tetraaminopyrimidine **1** and the phenylacetyl compound **3** (4) in this reaction gave a crude product that was purified by column chromatography to give a sample in 1.5% yield, which was identified as a 2,4-diaminopteridine by its uv and pmr spectra. Oxidation of this sample with potassium permanganate in a basic medium gave the expected pteridine-6-carboxylic acid **6** (7,8), indicating that the side chain was located at the 6- rather than the 7- position of the pteridine ring. However, the condensation product was shown later not to be **5** by comparison of its pmr spectrum and tlc behavior with a sample of **5** prepared in an unambiguous manner (4). The structure of this product was not established, and our attention was directed



toward the preparation of a preformed intermediate, which could be converted to a variety of analogs.

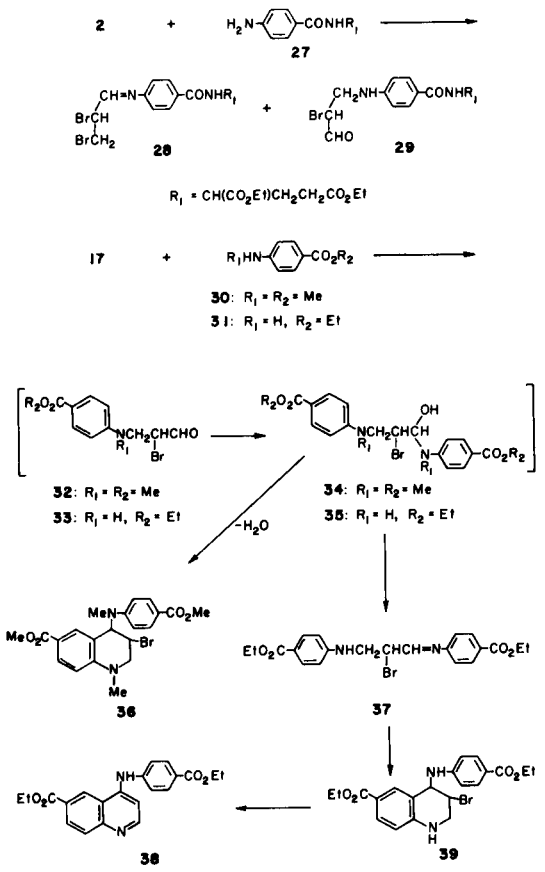
Previously, Waller and coworkers investigated the preparation of an intermediate 6-substituted pteridine by the reaction of **2** with the 6-oxypyrimidine **7** in hydrochloric acid (6). Although the major product of this reaction was shown to be the known 7-methylpteridine **8**, the route by which this compound was formed was not discussed. One possible pathway involved the hydrolysis of **2** to give **9**, which underwent dehydration to give methylglyoxal (**10**)(9). The condensation of **7** with methylglyoxal has been reoprted to give **8** (8). We attempted the preparation of a 6-substituted 2,4-diaminopteridine, but in contrast to the results described above, the reaction of the dihydrochloride of **1** with **2** in glacial acetic acid either at room temperature or at 60° gave no pteridine (uv).



Another approach involved the condensation of **1** with 1,1,3-trichloroacetone (**11**) (10) under essentially neutral conditions in DMAC. This reaction gave a considerable amount of recovered **1** and a low yield of a mixture of **13** and **14** (pmr) in agreement with a recent report (11). Treatment of the dihydrochloride of **1** with 1,1,3-tribromoacetone (**12**) (12) in hot aqueous acetic acid gave only the 5-acetamidopyrimidine **16**. In DMAC under neutral conditions, **1** and **12** gave a low yield of a pteridine (uv), but this sample was shown to contain none of the desired **15** (tlc) (4). The formation of a trace amount of a 6 (or 7)-bromomethylpteridine was observed in the condensation of the dihydrochloride of **1** with **12** both in aqueous ethanol and in aqueous ethanol strongly acidified with 48% hydrobromic acid (tlc) (13). These results indicated that the formation of either **13** or **15** by the reactants and conditions described above is possible, but that the routes are impractical for large-scale synthesis because of the low yields of the desired products.

Also, the preparation of the pteridine-6-carboxaldehyde **20** was investigated. This compound has been reported to result from side-chain oxidation of both 6-(polyhydroxyalkyl)pteridines (14) and aminopterin (15), but these routes are characterized by the low yields obtained either in the preparation of a intermediate or in the oxidation of the intermediate to give **20**. The preparation of **20** was attempted by the 1,4-addition of **16** to **17** (16) to give **18** followed by treatment of the latter with base to give **19**. However, this approach was terminated when it was found that more than one **17** added to **16**. Also, treatment of **1** with bromomalonaldehyde (**21**) (17) in an aqueous medium gave a product that was initially identified as **22** or a tautomer (mass spectrum). However, this product was apparently either a compound isomeric with **22** or a minor component of the reaction product, since alkaline oxidation with potassium permanganate gave only a trace amount of **6** (7,8). The successful preparation of **20** involved treatment of the pteridine-6-methanol **23** (18) with phosphoric acid and *N,N'*-dicyclohexylcarbodiimide in DMSO (19). This aldehyde was not isolated but was reacted *in situ* with 2,4-dinitrophenylhydrazine to give **25** (15) and with ethyl *p*-aminobenzoate to give **26**. The latter was reduced with borohydride to give the dihydropteridine **24** contaminated with the corresponding heteroaromatic compound (20).

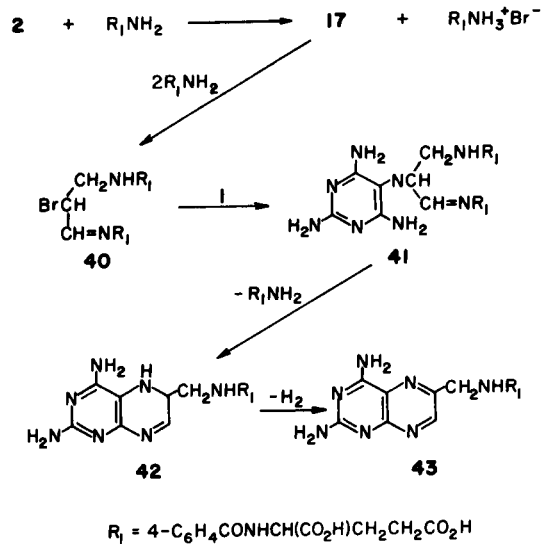
In work carried out simultaneously with that described above, the reactions of **2** with *p*-aminobenzoyl derivatives were investigated. In the Waller reaction the three components of the mixture must react initially by two possible modes to eventually obtain a 6-substituted pteridine. One involves the alkylation of the 5-amino group of the pyrimidine by the *sec*-bromo group of **2** as attempted above. The other route involves the initial reaction of the



side chain with one of the terminal carbons of **2** to give either **28** or **29**. Previously, Waller carried out the reaction of **2** and **27** to give a product that was identified as an anil, presumably either **28** or a derivative of **28** (6). Reaction of this anil with **7** was shown to give folic acid. We attempted the preparation of an intermediate like **29** by treatment of **2** with an equimolar amount of methyl *p*-(methylamino)benzoate (**30**) in ether, but this reaction gave only the hydrobromide salt of the amine. Apparently the other product of this reaction is 2-bromopropenal (**17**) (16). Reaction of **17** and **30** in ether gave one major product that was identified by elemental analyses and its mass and pmr spectra as the tetrahydroquinoline **36**, apparently formed *via* intermediates **32** and **34**. This route is similar to the proposed mechanism of the Doebner-von Miller quinoline synthesis (21). Under aqueous conditions similar to those of the Waller reaction, the interaction of **17** and **30** gave a complex mixture, but one of the major products appeared to be **36** (tlc). These results suggest that compounds like **36** and its precursors are one of the sources of the impurities produced in the Waller reaction.

The reaction of **17** with ethyl *p*-aminobenzoate (**31**) gave a mixture that contained one major component (tlc). Column chromatography decomposed most of the sample, but a homogeneous product was obtained in low yield that analyzed correctly for either **37** or the isomeric compound **39**, presumably formed *via* **33** and **35**. The pmr spectrum of this product exhibited only 1,4-disubstituted benzene proton peaks, which provide support for **37**. The mass spectrum showed only one major peak, which was assigned to **38**, presumably formed from **37** *via* **39** during the determination of the mass spectrum.

The results described above suggested the following mechanism for the Waller reaction. Dehydrobromination of **2** by the *p*-aminobenzoyl side chain gave 2-bromopropenal (**17**), which reacted with the side chain to give the intermediate **40**. Alkylation of the 5-amino group of the pyrimidine **1** with **40** gave **41**, which produced **43** by a transamination type of reaction to give **42** followed by either chemical or air oxidation. In the Waller synthesis of methotrexate, presumably an intermediate like **32** or **34** reacted in the same manner as **40**.



EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus. The ^{1}H nmr spectra were determined on DMSO-d_6 solutions with a Varian XL-100 spectrometer at a probe temperature of about 36° with tetramethylsilane as an internal reference; chemical shifts quoted in the case of multiplets were measured from the approximate center. The mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D-3 spectrometer.

Ethyl *p*-[[(2,4-Diamino-7,8-dihydro-6-pteridinyl)methyl]amino]-benzoate (**24**).

To a solution of **23** (1.00 g., 5.21 mmoles) and *N,N'*-dicyclohexylcarbodiimide (3.22 g., 15.6 mmoles) in DMSO (75 ml.) was added under nitrogen crystalline phosphoric acid (2.04 g., 20.8 mmoles), and the whole was allowed to stir at room temperature for 18 hours. After the removal of the precipitate of *N,N'*-dicyclohexylurea (3.50 g.) by filtration, the resulting filtrate was

mixed with ethyl *p*-aminobenzoate (0.860 g., 5.21 mmoles) and stirred under nitrogen first for 18 hours and then for 96 hours in the presence of molecular sieve (Linde 3A). After filtration, the filtrate was evaporated *in vacuo* at 40°. Water (200 ml.) was added to the gummy residue, and the resulting suspension was adjusted with stirring to *p*H 7.2 with 1 *N* sodium hydroxide. The yellow solid that formed was collected by filtration, washed successively with cold water and ether (200 ml.) and dried *in vacuo* over phosphorus pentoxide at 65° to give the crude anil: yield, 0.816 g. (46.6%) (M^+ = 337). A portion of this sample (0.500 g.) in DMAC (15 ml.) was treated under nitrogen with sodium borohydride (57 mg.). After 48 hours, the reaction mixture was treated cautiously (hydrogen) with 1 *N* hydrochloric acid until a drop of the solution in water gave a *p*H 6-7 (paper). This solution was evaporated to dryness *in vacuo* at 40°, and the residue was suspended in methanol and again evaporated to dryness. Next, the residue was suspended in water (40 ml.), and the resulting solid was collected by centrifugation, washed with water, and dried *in vacuo* over phosphorus pentoxide, yield, 0.360 g. This residue was extracted with ethanol (100 ml.) in a Soxhlet apparatus for 1 hour, and the resulting ethanol solution was concentrated to 0.1 volume to deposit the product: yield, 0.135 g., m.p. gradual dec. >150°. Tlc and pmr spectral data indicated that **24** was contaminated with the corresponding dehydro derivative, which was confirmed by the mass spectrum (M^+ = 339) (20).

Anal. Calcd. for $C_{16}H_{19}N_7O_2 \cdot 1/2H_2O$: C, 54.85; H, 5.75; N, 27.98. Found: C, 54.70; H, 5.55; N, 28.04.

2,4-Diaminopteridine-6-carboxaldehyde, 2,4-Dinitrophenylhydrazone (**25**).

A solution of **23**·HBr (273 mg., 1.00 mmole), crystalline phosphoric acid (392 mg., 4.00 mmoles) and *N,N'*-dicyclohexylcarbodiimide (619 mg., 3.00 mmoles) in anhydrous DMSO (15 ml.) was stirred under nitrogen at room temperature for 18 hours. The precipitate of *N,N'*-dicyclohexylurea (570 mg.) was removed by filtration, and solid 2,4-dinitrophenylhydrazine (198 mg., 1.00 mmoles) was added to the filtrate. This solution was stirred at 35° for 5 hours and evaporated to dryness *in vacuo* (55° waterbath). A suspension of the resulting glassy residue in water (80 ml.) was adjusted to *p*H 7 with 1 *N* sodium hydroxide followed by readjustment to *p*H 6 with 1 *N* hydrochloric acid. The crude product was collected by centrifugation, washed with water (2 x 100 ml.), and dried *in vacuo* over phosphorus pentoxide at 65°, yield, 330 mg. (89%). For analyses, this sample was extracted with EtOH in a Soxhlet apparatus for 5 hours to give a precipitate of the product, yield, 20 mg., m.p. > 260°. Mass spectral analyses indicated the absence of **23** and dicyclohexylurea, and tlc [butanol(5)-acetic acid(2)-water(3)] showed the absence of 2,4-dinitrophenylhydrazine. Pmr (2.5% DMSO w/v): δ 6.86 broad, 7.68 broad (2, 2, NH_2), 8.29 m (2, benzene CHCH), 8.68 (1, 7 H), 8.85 m (1, benzene CH), 9.38 (1, 6-CH), 11.9 broad (1, NH).

Anal. Calcd. for $C_{13}H_{10}N_{10}O_4 \cdot 0.30$ DMSO·0.15 HCl: C, 40.92; H, 3.02; N, 35.09. Found: C, 40.62; H, 2.66; N, 34.80.

From the Soxhlet thimble, 200 mg. of crude **25** was recovered. Elemental analyses indicated that this sample contained sulfur, presumably as DMSO.

Reactions of 2-Bromopropenal with Benzoates.

A. A solution of 2-bromopropenal (410 mg., 3.03 mmoles) and **30** (500 mg., 3.03 mmoles) in anhydrous ether (25 ml.) was stirred at room temperature for 68 hours. The resulting mixture was evaporated to dryness *in vacuo* to give a yellow solid, yield, 570 mg. This sample was stirred vigorously in ether (100 ml.), and the insoluble solid was removed by filtration: yield, 150 mg. Tlc indicated that this sample was a mixture of **36** and the benzoate starting material. The filtrate was cooled in a dry ice-acetone bath to precipitate a homogeneous (tlc) white solid of **36**, yield, 80 mg., m.p. 131-132° dec.; mass spectrum: M^+ 446, 448; M^+ - HBr, 366. Pmr (< 4% DMSO-d_6 w/v): δ 2.72, 3.03 (3, 3, CH_3N, CH_3N); 3.70, 3.80, 3.81 m (8, CH_3O, CH_3O, 2-H_2); 4.81 m (1, 3-H); 5.50 d [1,4-H ($J_{3,4}$ = 8 Hz)]; 6.71 d, 7.38 q, 7.39 d, 7.74 m [7,8-H ($J_{7,8}$ = 9 Hz), C_6H_4, 5-H ($J_{5,7}$ = 2 Hz), 7-H].

Anal. Calcd. for $C_{21}H_{23}BrN_2O_4$: C, 56.39; H, 5.18; N, 6.26. Found: C, 55.96; H, 5.32; N, 6.16.

B. To a solution of **30** (500 mg., 3.03 mmoles) in 1:1 water-acetic acid (36 ml.) containing 1 *N* hydrochloric acid (3 ml.) was added 2-bromopropenal (410 mg., 3.03 mmoles). This residue was refrigerated for 18 hours to give a deposit of a green gum. Tlc indicated that this sample was a complex mixture containing mainly **36** and unreacted **30**.

C. To a solution of **31** (1.00 g., 6.06 mmoles) in ether (20 ml.) was added dropwise a solution of 2-bromopropenal (0.41 g., 3.03 mmoles) in ether (10 ml.). After stirring for 6 hours at room temperature, the resulting dark reaction suspension was evaporated to dryness *in vacuo* to give a brown porous glass; yield, 1.41 g. Tlc showed one major product (**37**) contaminated with two other components, one of which was the starting benzoate ester. This sample was eluted from a silica gel column (chloroform) to separate crude **31** (0.27 g.). The appearance of new spots in the tlc indicated that the recovered product (1.03 g.) had undergone some decomposition. The sample was recolumned and thirteen fractions taken, one of which was homogeneous: yield, 0.09 g., m.p., indefinite with softening from about 80°; mass spectrum: M^+-OEt, 401 and 403; M^+-OEt + H, 402 and 404; M^+-HBr-2H, 364 (major). The pmr spectrum of this product in deuteriochloroform was complex, but provided support for **37** by the presence of C_6H_4 peaks and the absence of a CH peak between 6.75 and 7.75 (5-H of **36**, δ = 7.39 ppm).

Anal. Calcd. for $C_{21}H_{23}BrN_2O_4$: C, 56.39; H, 5.18; Br, 17.86; N, 6.26. Found: C, 56.43; H, 4.90; Br, 17.69; N, 6.20.

The major portion of **37** recovered from the other fractions was contaminated with by-products.

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REFERENCES AND NOTES

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(2) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Med. Chem.*, 17, 533 (1974) and references therein.

(3) R. D. Elliott, C. Temple, Jr., J. L. Frye, and J. A. Montgomery, *J. Heterocyclic Chem.*, 10, 1071 (1973).

(4) J. R. Piper and J. A. Montgomery, *ibid.*, 11, 279 (1974).

(5) E. C. Roberts and Y. F. Shealy, *J. Med. Chem.*, 16, 697 (1973).

(6) C. W. Waller, B. L. Hutchings, J. H. Mowat, E. L. R. Stokstad, J. H. Boothe, R. B. Angier, J. Semb, Y. SubbaRow, D. B. Cosulich, M. J. Fahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels, and J. M. Smith, Jr., *J. Am. Chem. Soc.*, 70, 19 (1948).

(7) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *ibid.*, 71, 1753 (1949).

(8) J. H. Mowat, J. H. Boothe, B. L. Hutchings, E. L. R. Stokstad, C. W. Waller, R. B. Angier, J. Semb, D. B. Cosulich, and Y. SubbaRow, *ibid.*, 70, 14 (1948).

(9) J. C. Speck, Jr., *Advan. Carbohyd. Chem.*, 13, 73 (1958).

(10) C. Cloez, *Ann. Chim. Phys.*, [6] 9, 176 (1886).

(11) D. C. Suster, L. V. Feyns, G. Ciustea, G. Botez, V. Dobre, R. Bick, and I. Niculescu-Duvăz, *J. Med. Chem.*, 17, 758 (1974).

(12) F. Weygand and V. Schmied-Kowarzik, *Chem. Ber.*, 82, 333 (1949); H. Watson and E. Yates, *J. Chem. Soc.*, 1207 (1932).

(13) D. Farquhar and T. L. Loo, *J. Med. Chem.*, 15, 567 (1972).

(14) V. A. Wacker and L. Trager, *Z. Naturforsch.*, 17b, 369 (1962); H. G. Petering, U. S. Patent 2,667,484, January 26, 1954; *Chem. Abstr.*, 49, P1825i (1955).

(15) T. L. Loo, R. L. Dion, R. H. Adamson, M. A. Chirigos, and R. L. Kisliuk, *J. Med. Chem.*, 8, 713 (1965).

(16) J. N. Wells and M. S. Strahl, *J. Pharm. Sci.*, 60, 533 (1971).

(17) H. Bredereck, F. Effenberger, and E. H. Schweizer, *Chem. Ber.*, 95, 803 (1962); S. M. McElvain and R. L. Clarke, *J. Am. Chem. Soc.*, 69, 2657 (1947).

(18) C. M. Baugh and E. Shaw, *J. Org. Chem.*, 29, 3610 (1964).

(19) K. E. Pfitzner and J. B. Moffatt, *J. Am. Chem. Soc.*, 87, 5661 (1965).

(20) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, 35, 1676 (1970).

(21) A. Bischler, *Chem. Ber.*, 25, 2864 (1892); M. G. Edwards, R. E. Garrod, and H. O. Jones, *J. Chem. Soc.*, 101, 1376 (1912).