## Methotrexate Analogs. 4. 7-Methyl Derivatives of Methotrexate and Dichloromethotrexate. A New Synthesis and Some Biological Studies<sup>1,†</sup>

Andre Rosowsky\* and Katherine K. N. Chen

The Children's Cancer Research Foundation and Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115. Received June 3, 1974

The title compounds were synthesized with the aim of obtaining methotrexate and dichloromethotrexate analogs resistant to the catabolic action of hepatic aldehyde.oxidase, an enzyme known to cause significant conversion of dichloromethotrexate into an inactive 7-hydroxy metabolite in man as well as experimental animals. On the basis of antibacterial assays against *Streptococcus faecium* ATCC 8043, mammalian cell culture experiments involving use of P388 murine lymphatic leukemia cells and CCRF-CEM human lymphoblastic leukemia cells, and *in vivo* experimental antitumor evaluation against L1210 lymphatic leukemia in the mouse, it appears that biological activity is severely compromised by 7-methyl substitution. Inhibition studies with purified dihydrofolate reductase derived from *Lactobacillus casei* ATCC 7469 and also the L1210-FR8 tumor suggest that the lack of *in vitro* and *in vivo* activity of these analogs is due to impaired enzyme binding.

3',5'-Dichloromethotrexate (1, DCM),<sup>2,3</sup> a well-known folate antagonist evaluated several years ago as a clinical antileukemic agent in man<sup>4,5</sup> on the basis of promising early trials against rodent tumors,<sup>6,7</sup> has been shown to be an active substrate for the enzyme aldehyde oxidase (aldehyde:oxygen oxidoreductase, E.C. 1.2.3.1) in rodents<sup>8,9</sup> as well as in man.<sup>10</sup> In this respect, DCM differs significantly from methotrexate (2, MTX), which is extensively hydroxylated at the 7 position in the rabbit and guinea pig<sup>11,12</sup> but not appreciably in the mouse<sup>12,13</sup> or in man.<sup>14,15</sup> Present in the supernatant fraction of liver homogenates from diverse mammalian species, aldehyde oxidase is a molybdoflavoprotein enzyme similar to xanthine oxidase in its ability to hydroxylate a variety of N-heterocyclic substrates,<sup>16-18</sup> but its precise physiologic role is not fully elucidated.<sup>19</sup> As with MTX,<sup>20,21</sup> the action of aldehyde oxidase on DCM results in the formation of a metabolite devoid of activity against dihydrofolate reductase.<sup>22</sup> This metabolite has been identified on the basis of spectral evidence and chemical degradation as the 7-hydroxy derivative.<sup>23</sup> Thus, while not a significant metabolic event with MTX in man, hepatic "detoxification" via 7hydroxylation is viewed as an important aspect of the pharmacology of DCM along with other phenomena such as binding to plasma proteins and ease of transport across cell membranes. These considerations prompted us to undertake the synthesis of a DCM analog in which the vulnerable 7 position is blocked. The 7-methyl derivative 3 appeared to be a logical candidate for this purpose, and the synthesis of this compound forms the subject of the present report.



Chemistry. The elegant unequivocal pteridine synthesis described recently by Taylor and coworkers<sup>24-26</sup> was selected in order to test its convenience and generality and because we envisioned that a successful synthesis of compound 3 via this approach might be followed by the subsequent preparation of other 7-alkyl analogs in a similar

manner. Fortuitously, while this work was in progress Loo and coworkers<sup>21</sup> reported an independent synthesis of 7methylmethotrexate (4), the penultimate intermediate in our own reaction scheme (see Scheme I). Their method involved, as a key step, selective reductive debromination of 2,4-diamino-6,7-bis(bromomethyl)pteridine with potassium iodide.

1-Chloro-3-oximino-2-butanone (5) was prepared in 30% yield by chlorination of 2,3-butanedione monoxime in the manner prescribed for the lower homolog, isonitrosoacetone.<sup>27</sup> The identity of compound 5 was established rigorously on the basis of its nmr spectrum which revealed that reaction had occurred on the methyl group adjoining the keto rather than the oximino function. Condensation of 5 with aminomalononitrile tosylate<sup>28</sup> proceeded in somewhat lower yield than has been reported by Taylor and Kobayashi<sup>26</sup> for the corresponding reaction of 3-chloro-1oximino-2-propanone, but nonetheless furnished acceptable quantities of the desired 2-amino-5-chloromethyl-3cvano-6-methylpyrazine 1-oxide (6). The latter was characterized by the expected nmr singlets at  $\delta$  2.68 and 4.65 for the methyl and methylene protons, respectively, and a broad signal at  $\delta$  6.0-6.6 for the amino group. The nitrile function was evident in the ir spectrum as a peak of moderate intensity at 2250 cm<sup>-1</sup>.

Reaction of diethyl N-(p-N-methylaminobenzoyl)glutamate  $(7)^{29}$  with compound 6 was brought about readily in aqueous THF containing a small excess of K<sub>2</sub>CO<sub>3</sub>, and the resultant N-oxide 8 was reduced to 9 by treatment with triethyl phosphite in hot DMF.1,30 Purification of the crude gummy product by column chromatography on silica gel afforded a tlc homogeneous yellow solid (48% overall yield from 8) which could be recrystallized for microanalysis but was sufficiently pure to be converted directly into pteridine 10. Optimum yields of 10 (ca. 45%) were obtained by allowing 9 to react with guanidine in boiling EtOH for about 15 min. In the preparation of the guanidine solution, guanidine hydrochloride was added to absolute EtOH containing the precise stoichiometric amount of dissolved sodium for neutralization (excess NaOEt was avoided in order to minimize the possibility of side reactions). Purification of compound 10 was effected via silica gel column chromatography.

Saponification of the ester groups in 10 was achieved in nearly quantitative yield by treatment with 1 N sodium hydroxide in refluxing EtOH (15 min, N<sub>2</sub> atmosphere). The product, 7-methylmethotrexate (4), was isolated from aqueous alkaline solution on acidification to pH 2 with 1 N HCl. The material partially purified in this manner was then chlorinated via the procedure described previously for the conversion of MTX into DCM.<sup>2,3</sup> Following recrystallization from a large volume of MeOH,

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## Scheme I



3',5'-dichloro-7-methylmethotrexate (3) was obtained in approximately 30% yield as a lemon-yellow microcrystalline powder. Tic of the final purified sample of 3 on cellulose, with 3% NH<sub>4</sub>HCO<sub>3</sub> or 5% Na<sub>2</sub>HPO<sub>4</sub> as the developing solvent, verified the absence of unreacted 4 as well as any 2-amino-4-hydroxypteridine which might have been generated during the saponification step.

Ultraviolet spectra of compounds 3 and 4 in 0.1 N NaOH solution (Figures 1 and 2) were entirely consistent



Figure 1. Ultraviolet absorption of MTX and 7-MeMTX in 0.1 N NaOH.

with the spectra reported for DCM and MTX,<sup>3</sup> allowance being made for the expected bathochromic influence of an extra methyl substituent on the pteridine chromophore. On the other hand, a significant difference was noted between the spectrum of 4 and that reported for this compound in the literature<sup>21</sup> which appears more compatible with a 2-amino-4-hydroxypteridine structure (e.g., 7,N<sup>10</sup>dimethylfolate) than a 2,4-diamino derivative.<sup>‡</sup>

The nmr spectrum of compound 3 in DMSO- $d_6$  solution was also examined in order to provide additional structural verification. Peak assignments were made on the basis of comparison with a sample of DCM and with the aid of published nmr spectra of MTX and other folate analogs.<sup>31</sup> Like DCM, compound 3 showed a sharp N-methyl singlet

at  $\delta$  3.16 and a singlet at  $\delta$  8.35 corresponding to the aromatic protons at positions 2' and 6' of the *p*-methylaminobenzoylglutamate moiety. Moreover, in contrast to DCM, which displayed a characteristic strongly deshielded 7-H singlet at  $\delta$  9.31, compound 3 exhibited a 7-Me singlet at  $\delta$  2.70.

**Biological Evaluation.** Compounds 3 and 4 were evaluated in several biological test systems, as indicated in Table I. Against the folate-requiring microorganism



Figure 2. Ultraviolet absorption of DCM and 7-MeDCM in 0.1 N NaOH.

Streptococcus faecium ATCC 8043,32 the 7-methyl analog 4 of MTX was approximately 100 times less potent than MTX, and similarly the 7-methyl analog 3 of DCM was less than  $4_{00}$  as active as DCM. Against P388 murine lymphatic leukemia in cell culture, the 7-methyl derivatives were likewise less active than MTX and DCM by at least one order of magnitude. Comparable results were obtained, moreover, against CCRF-CEM human lymphoblastic leukemia in continuous spinner culture.<sup>33</sup> The bacterial and mammalian cell assay data were supported by inhibition studies utilizing purified dihydrofolate reductase preparations derived from Lactobacillus casei ATCC 7469 as well as L1210-FR8 mouse leukemia. In contrast to a recently published report<sup>21</sup> that 7-MeMTX and MTX are equipotent against L1210 dihydrofolate reductase, it was observed in both enzyme systems that 7-methyl substitution resulted in a greater than 1000-fold decrease in inhibition as measured by ID<sub>50</sub> values.

<sup>‡</sup>A specimen kindly supplied for comparison by Dr. T. L. Loo and purified by DEAE-cellulose column chromatography differed from our sample of 7-MeMTX (4) in its ir and uv spectral characteristics as well as its migratory aptitude on thin-layer chromatography plates.

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	S faccium			Cell culture, ${ m ID}_{50}$ , $\mu { m g/ml}$			
	S. <i>factum</i> ATCC 8043, $D_{50}, \mu g/ml$ (folate = 0.001	Dihydrofolate ID <sub>50</sub> , m	reductase, <sup>a</sup> nol/l.	P388 murine	CCRF - CEM human		
Compound	$\mu g/ml)$	L1210-FR8	L. casei	leukemia	leukemia		
MTX	0.002	$1.5  imes 10^{-9}$	$3  imes 10^{-9}$	0.01	0.018		
7-MeMTX (4)	0.17	$7 imes 10^{-6}$	$1 imes10^{-5}$	0.1+	0.1+		
DCM	0.01	$1 imes10^{-9}$	$3 imes10^{-9}$	0.007	0.009		
7-MeDCM (3)	1.0*	$4 imes 10^{-6}$	$9 imes10^{-6}$	0.1*	0.1*		

<sup>a</sup>Data kindly supplied by Dr. R. L. Kisliuk, Tufts-New England Medical Center, Boston, Mass.

The lack of activity of 3 and 4 against dihydrofolate reductase can be ascribed to the steric effect of the methyl group at position 7, which might be expected to cause distortion of the  $N^{10}$ -methylaminobenzoylglutamate side chain attached at position 6 and might also interfere with proper positioning of the C-7 and N-8 atoms of the pteridine moiety on the surface of the enzyme.

In accord with the *in vitro* results cited above and the *in vivo* data of Loo and coworkers,<sup>21</sup> compounds 3 and 4 both proved to be inactive against L1210 lymphatic leukemia in the mouse. When given by intraperitoneal injection in aqueous solution on days 1, 4, and 7 after tumor implantation (10<sup>5</sup> cells), compound 3 produced no significant increase in survival ( $\Delta MST = 10\%$ ) at doses of up to 160 mg/kg. On this schedule MTX afforded a significant therapeutic effect ( $\Delta MST > 50\%$ ) at 15 mg/kg, *i.e.*, less than  $\frac{1}{10}$  the dose. Similarly, compound 4 caused only a 10% extension of survival at a dose of 320 mg/kg (the highest dose tested), whereas DCM was active ( $\Delta MST = 50\%$ ) at 120 mg/kg.

## **Experimental Section**

Melting points (uncorrected) were determined in Pyrex capillary tubes by means of a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) at a heating rate of approximately 2°/min. Ir spectra were recorded on a Perkin-Elmer Model 137B double beam spectrophotometer and quantitative uv spectra were determined on Cary Model 11 and Model 15 instruments. Nmr spectra were taken by means of a Varian A-60 spectrometer with tetramethylsilane as the reference. Thin-layer chromatography was carried out on silica gel sheets (Eastman 6060, with fluorescent indicator) or cellulose sheets (Eastman 6065, with fluorescent indicator) without prior activation. Unless otherwise specified, solutions were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Where microanalyses are given only by symbols of the elements, the results were within  $\pm 0.4\%$  of theoretical values for those elements. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn

1-Chloro-3-oximino-2-butanone (5). A rapidly stirred solution of 2,3-butanedione monoxime (20 g, 0.2 mol) in CHCl<sub>3</sub> (300 ml) was treated dropwise with an ice-cold solution of Cl<sub>2</sub> (17.5 g, 0.247 mol) in CHCl<sub>3</sub> (400 ml). Addition was conducted at a rate sufficient to maintain the internal temperature of the reaction mixture between 17 and 20°; this required 55 min. Thereupon, the volume was reduced to 250 ml on the rotary evaporator and petroleum ether (bp 30-60°) was added with cooling until a white solid formed. After filtration, further reduction of the volume yielded a second crop. The combined first and second crops weighed 8.2 g (30% yield). The analytical sample, mp 104-106°, was prepared by recrystallization from a CHCl3-petroleum ether mixture: nmr (CDCl<sub>3</sub>)  $\delta$  2.05 (s, 3 H, CH<sub>3</sub>C=NOH) and 4.62 (s, 2 H, ClCH<sub>2</sub>CO). The nmr spectrum of 2,3-butanedione monoxime in CDCl<sub>3</sub> showed two singlets of equal area at  $\delta$  2.00 (CH<sub>3</sub>C=NOH) and 2.40 (CH<sub>3</sub>CO). Anal. (C<sub>4</sub>H<sub>6</sub>ClNO<sub>2</sub>) C, H, Cl, N.

2-Amino-5-chloromethyl-3-cyano-6-methylpyrazine 1-Oxide (6). A mixture of 5 (34.5 g, 0.254 mol) and aminomalononitrile tosylate (66.0 g, 0.261 mol)<sup>28</sup> in *i*-PrOH (840 ml) was stirred at room temperature for 24 hr, then cooled to  $0^{\circ}$ , and suction fil-

tered. Exhaustive trituration of the solid with hot CHCl<sub>3</sub> and concentration of the combined triturates on the rotary evaporator gave several crops totaling 12.6 g (25% yield). The analytical sample was prepared by recrystallization from CHCl<sub>3</sub> (charcoal): mp 191-201° dec;  $R_{\rm f}$  0.59 (silica gel, 95:5 CHCl<sub>3</sub>-EtOH); nmr (CDCl<sub>3</sub>)  $\delta$  2.68 (s, 3 H, CH<sub>3</sub>), 4.65 (s, 2 H, ClCH<sub>2</sub>), 6.0-6.6 (broad, NH<sub>2</sub>). Anal. (C<sub>7</sub>H<sub>7</sub>ClN<sub>4</sub>O) C, H, Cl, N.

Diethyl N-[p-[N-(2-Amino-3-cyano-6-methyl-5-pyrazinylmethyl)-N-methylamino]benzoyl]glutamate (9). A solution of 6 (10.8 g, 0.0543 mol) and diethyl p-(N-methylamino)benzoylglutamate (18.2 g, 0.0543 mol)<sup>29</sup> in THF (165 ml) was treated with a solution of  $K_2CO_3$  (11.3 g, 0.0815 mol) in water (165 ml). The reaction mixture was stirred vigorously during the addition and kept cool by means of a cold water bath. After 2.5 hr, water (550 ml) was added and the product was extracted with CHCl<sub>3</sub> (6  $\times$ 200 ml). The combined CHCl<sub>3</sub> extracts were washed with water, dried, and evaporated under reduced pressure to a thick syrup which was heated directly in a mixture of DMF (54 ml) and triethyl phosphite (81 ml) at 125° (internal temperature) for 45 min. Volatile materials were removed by means of a vacuum pump, and the dark amber-colored syrup was purified by passage through a column of silica gel (180 g). Elution was performed with EtOAc-benzene mixtures ranging from 1:4 to 1:1 in composition, and the course of purification was monitored carefully by tlc. After removal of some fast-moving components, the main product was obtained as an amorphous yellow solid,  $R_{\rm f}$  0.20 (silica gel, 1:1  $EtOAc-C_6H_6$ ). A portion of the crude syrup could also be made to crystallize spontaneously from 1:4  $EtOAc-C_6H_6$  without prior chromatography. The total yield was 12.5 g (48%). The analytical sample was prepared by repeated crystallization from EtOAc- $C_6H_6$ : mp 73-75°; uv  $\lambda_{max}$  (EtOH) 225 nm ( $\epsilon$  20,500), 250 (22,370), 300 (26,870), 365 (6910). Anal.  $(C_{24}H_{30}N_6O_5 \cdot H_2O) C$ , H. N.

Diethyl N-[p-[N-(2,4-Diamino-7-methyl-6-pteridinylmethyl)-N-methylamino]benzoyl]glutamate (10). Na metal (0.575 g, 0.025 g-atom) was dissolved in absolute EtOH (242 ml) and guanidine hydrochloride (2.39 g, 0.025 mol) was added. After 15 min the NaCl was filtered off and the filtrate was added to aminonitrile 9 (10.5 g, 0.0218 mol). The mixture was refluxed for 15 min under a nitrogen atmosphere and then refrigerated until crystallization occurred. The crude product was filtered and chromatographed on a column of silica gel (200 g). Elution was performed with mixtures of CHCl<sub>3</sub> and EtOH ranging in composition from 9:1 to 4:1. A small quantity of unchanged starting material was eluted as a fast-moving band. The major fraction yielded 10 as a tlc-homogeneous bright yellow solid (5.12 g, 45%):  $R_f$  0.28 (silica gel, 7:3 CHCl<sub>3</sub>-EtOH). The analytical sample, mp 208-210°, was obtained after recrystallization from 1:1 EtOH-benzene: uv  $\lambda_{max}$ (EtOH) 266 nm ( $\epsilon$  38,000), 303 (33,950), 384 (7950);  $\lambda_{max}$  (pH 1, EtOH) 253 nm (¢ 33,760), 300 (32,830), 348 (7720), 363 (7400). Anal.  $(C_{25}H_{32}N_8O_5 \cdot 1.2H_2O) C, H, N.$ 

N-[p-[N-(2, 4-Diamino-7-methyl-6-pteridinylmethyl)-N-methylamino]benzoyl]glutamic Acid (7-MeMTX, 4). The diester 10 (2.56 g, 0.0048 mol) was suspended in a mixture of EtOH (116 ml) and 1 N NaOH (48 ml) and stirred under reflux (N<sub>2</sub> atmosphere) for 15 min. The EtOH was evaporated under reduced pressure and a minimum of water was added in order to dissolve the solid residue. Treatment with a small quantity of charcoal and acidification to pH 2 with 1 N HCl at 0° gave a yellow solid which was collected, washed with ice-cold water, and dried (4.43 g, 92% yield). For microanalysis, a portion of this material was dissolved in a minimal volume of 3% NH<sub>4</sub>HCO<sub>3</sub> and passed through a col-

umn of DEAE-cellulose (Whatman DEAEC-32) which had been previously converted into the bicarbonate form and equilibrated with 3% NH<sub>4</sub>HCO<sub>3</sub>. Elution with 3% NH<sub>4</sub>HCO<sub>3</sub> gave rise to two well-separated yellow bands, the larger of which was removed from the column first. The fractions comprising this band were pooled and lyophilized, and the residue was dissolved in a minimal volume of water. Addition of glacial AcOH to approximately pH 4 produced a gelatinous yellow solid which was isolated by centrifugation, addition of a small amount of water, recentrifugation, and transfer to a Büchner funnel with the aid of a mixture of EtOH and Et<sub>2</sub>O. After thorough pulverization in a mortar and drying overnight at 70° (0.05 mm), the analytical sample was obtained as a bright yellow microcrystalline powder: mp 202-210° dec; Rf 0.7 (cellulose, 5:2:3 BuOH-AcOH-H2O), 0.9 (cellulose, 5% Na<sub>2</sub>HPO<sub>4</sub>); uv  $\lambda_{max}$  (0.1 N NaOH) 261 nm ( $\epsilon$  38,410), 297  $(29,290),\,379\,(8820).\,Anal.\,(C_{21}H_{24}N_8O_5{\boldsymbol{\cdot}}2H_2O)\,C,\,N.$ 

N-[p-[N-(2,4-Diamino-7-methyl-6-pteridinylmethyl)-N-methylamino]-3',5'-dichlorobenzoyl]glutamic Acid (7-MeDCM, 3). The product obtained in the preceding experiment (1 g, 0.002 mol) was dissolved in formamide by gentle warming on a steam bath. The solution was then cooled to  $5^\circ$  and gaseous  $\text{Cl}_2$  was bubbled through with occasional swirling until 0.94 g (0.013 mol) had been absorbed (30 min). After an additional 1 hr in the ice bath, water (80 ml) was added and NaOAc (1 g) was added to bring the pH to about 3.5. The mixture was stored overnight in the refrigerator and then filtered. Washing of the crude solid with a little water followed by rinsing with acetone and finally ether gave a brownish solid. Recrystallization from MeOH, with the aid of a small amount of decolorizing carbon, afforded 0.31 g (29% yield) of lemon-yellow microcrystalline solid: mp 218-221°;  $R_f$  0.8 (cellulose, 5% Na<sub>2</sub>HPO<sub>4</sub>); uv  $\lambda_{max}$  (0.1 N NaOH) 262 nm ( $\epsilon$ 42,800), 290 infl (18,180), 379 (10,070). Anal. (C<sub>21</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>8</sub>O<sub>5</sub>) C, H. Cl. N.

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## References

- M. Chaykovsky, K. K. N. Chen, N. Papathanasopoulos, A. Rosowsky, E. J. Modest, R. L. Kisliuk and Y. Gaumont, J. Med. Chem., 17, 1212 (1974) (paper 3).
- (2) D. B. Cosulich, D. R. Seeger, M. J. Fahrenbach, B. Roth, J. H. Mowat, J. M. Smith, Jr., and M. E. Hultquist, J. Amer. Chem. Soc., 73, 2554 (1951).
- (3) R. Angier and W. V. Curran, J. Amer. Chem. Soc., 81, 2814 (1959).

- (4) L. R. Shroeder, Proc. Amer. Ass. Cancer Res., 3, 160 (1965).
- (5) E. Frei, III, C. L. Spurr, C. O. Brindley, O. Selawry, J. F. Holland, D. P. Rall, L. R. Wasserman, B. Hoogstraten, B. I. Shnider, O. R. McIntyre, L. B. Matthews, Jr., and S. P. Miller, Clin. Pharmacol. Exp. Ther., 6, 160 (1965).
- (6) A. Goldin, J. M. Venditti, S. R. Humphreys, and N. Mantel, J. Nat. Cancer Inst., 19, 1133 (1957).
- (7) A. Goldin, S. R. Humphreys, J. M. Venditti, and N. Mantel, J. Nat. Cancer Inst., 22, 811 (1959).
- (8) V. T. Oliverio and J. D. Davidson, J. Pharmacol. Exp. Ther., 137, 76 (1962).
- (9) T. L. Loo and R. H. Adamson, Biochem. Pharmacol., 11, 170 (1962).
- (10) J. D. Davidson and V. T. Oliverio, Clin. Pharmacol. Exp. Ther., 6, 321 (1965).
- (11) H. M. Redetzki, J. E. Redetzki, and A. L. Elias, *Biochem. Pharmacol.*, 15, 425 (1966).
- (12) D. G. Johns, A. T. Iannotti, A. C. Sartorelli, and J. R. Bertino, Biochem. Pharmacol., 15, 555 (1966).
- (13) E. S. Henderson, R. H. Adamson, C. Denham, and V. T. Oliverio, *Cancer Res.*, 25, 1008 (1965).
- (14) D. G. Johns, J. W. Hollingsworth, A. R. Cashmore, I. H. Plenderleith, and J. R. Bertino, J. Clin. Invest., 43, 621 (1964).
- (15) E. S. Henderson, R. H. Adamson, and V. T. Oliverio, Cancer Res., 25, 1018 (1965).
- (16) D. G. Johns, A. I. Iannotti, A. C. Sartorelli, B. A. Booth, and J. R. Bertino, *Biochim. Biophys. Acta*, 105, 380 (1965).
- (17) T. L. Loo, C. Lim, and D. G. Johns, Biochim. Biophys. Acta, 134, 467 (1967).
- (18) D. G. Johns, J. Clin. Invest., 46, 1492 (1967).
- (19) D. G. Johns, D. Farquhar, M. K. Wolpert, B. A. Chabner, and T. L. Loo, Drug Metab. Disposition, 1, 580 (1973).
- (20) D. G. Johns and T. L. Loo, J. Pharm. Sci., 56, 356 (1967).
- (21) D. Farquhar, T. L. Loo, and S. Vadlamudi, J. Med. Chem., 15, 567 (1972).
- (22) D. K. Misra, R. H. Adamson, T. L. Loo, and V. T. Oliverio, *Life Sci.*, 407 (1963).
- (23) T. L. Loo and R. H. Adamson, J. Med. Chem., 8, 513 (1965).
- (24) E. C. Taylor, K. L. Perlman, I. P. Sword, M. Séquin-Frey, and P. A. Jacobi, *J. Amer. Chem. Soc.*, **95**, 6407 (1973).
- (25) E. C. Taylor, K. L. Perlman, Y.-H. Kim, I. P. Sword, and P. A. Jacobi, J. Amer. Chem. Soc., 95, 6413 (1973).
- (26) E. C. Taylor and T. Kobayashi, J. Org. Chem., 38, 2817 (1973).
- (27) J. Armand, J.-P. Guette, and F. Valentini, C. R. Acad. Sci., Ser. C, 263, 1388 (1966).
- (28) J. P. Ferris, R. A. Sanchez, and R. W. Mancuso in "Organic Syntheses," Collect. Vol. V, Wiley, New York, N.Y., 1973, p 32.
- (29) D. V. Santi, J. Heterocycl. Chem., 4, 475 (1967).
- (30) M. Chaykovsky, A. Rosowsky, and E. J. Modest, J. Heterocycl. Chem., 10, 425 (1973).
- (31) E. J. Pastore, Ann. N.Y. Acad. Sci., 186, 43 (1971).
- (32) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N.Y. Acad. Sci.*, 76, 413 (1958).
- (33) G. E. Foley and H. Lazarus, Biochem. Pharmacol., 16, 659 (1967).