Rep. Med. Chem., **9**, 172 (1974).
(3) F. L. Bach, J. C. Barclay, F. Kendl, and E. Cohen, *J. Med. Chem.*, 11, 987 (1968).
(4) R. Hess and W. L. Bencze, *Experientia*, **24**, 418 (1968).
(5) C. Sirtori, A. Hurwitz, K. Sabih, and D. L. Azarnoff, *Lipids*, **7**, 96 (1971).
(6) J. M. Grisar, R. A. Parker, T. Kariya, T. R. Blohm, R. W. Fleming, V. Petrow, D. L. Wenstrup, and R. G. Johnson, *J. Med. Chem.*, **15**, 1273 (1972).
(7) M. R. Boots, S. G. Boots, C. M. Noble, and K. E. Guyer, *J. Pharm. Sci.*, **62**, 952 (1973).
(8) L. J. Chinn and J. S. Mihina, *J. Org. Chem.*, **30**, 257 (1965).
(9) J. Torreilles and A. Crastes de Paulet, *Bull. Soc. Chim. Fr.*, 4112 (1968).
(10) M. Kimura, K. Akiyama, and T. Miura, *Chem. Pharm. Bull.*, **20**, 2511 (1972).
(11) F. Elsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, **43**, 113 (1960).
(12) G. Palazzo, M. Tavella, and G. Strani, *J. Med. Chem.*, **4**, 447 (1961).
(13) R. E. Counsell, P. D. Klimstra, R. E. Ranney, and D. L. Cook, *J. Med. Pharm. Chem.*, **5**, 720 (1962).
(14) (a) J. L. Lowe, D. A. Peak, and T. I. Watkins, *J. Chem. Soc.*, 3286 (1951); (b) G. M. K. Hughes, P. F. Moore, and R. B. Stebbins, *J. Med. Chem.*, **7**, 511 (1964); (c) J. Avigan, D. Steinberg, H. E. Vroman, M. J. Thompson, and E. Mosettig, *J. Biol. Chem.*, **235**, 3123 (1960).
(15) (a) R. A. Edgren, *Proc. Soc. Exp. Biol. Med.*, **92**, 569 (1956); (b) B. L. Rubin, A. S. Dorfman, L. Black, and R. I. Dorfman, *Endocrinology*, **49**, 429 (1951).
(16) (a) R. F. Borne, C. R. Clark, and J. M. Holbrook, *J. Med. Chem.*, **16**, 853 (1973); (b) P. G. Gassman and J. H. Dygos, *Tetrahedron Lett.*, 4745 (1970); (c) J. H. Huffman, T. Kamiya, and C. B. S. Rao, *J. Org. Chem.*, **32**, 700 (1967).
(17) J. Cason, "Organic Syntheses", Collect. Vol. III, Wiley, New York, N.Y., 1955, p 169.

p-Aminobenzoic Acid Derivatives as Inhibitors of the Cell-Free H_2-Pteroate Synthesizing System of *Escherichia coli*

H. H. W. Thijssen*

Institute of Pharmacology, University of Nijmegen, Nijmegen, The Netherlands. *Received March 8, 1976*

A heterogeneous series of compounds, derived from *p*-aminobenzoic acid (PABA), has been investigated for their PABA-antagonistic potency in a cell-free H_2-pteroate synthesizing system of *E. coli*. A prerequisite of compounds, other than sulfones or sulfonamides, to compete with PABA for the enzyme H_2-pteroate synthetase appeared to be the presence of a *p*-aminobenzoyl moiety. Substitution of the carboxyl group of PABA by an ester, an amide, or a ketone function, however, strongly reduces the ability to interact with the PABA binding site on the enzyme. This decrease in affinity probably has to be ascribed to the inability to create a sufficient negative charge in the carbonyl part of these *p*-aminobenzoyl derivatives. The relatively high affinities of L-PABG (**16**), PABP (**22**), and the α-phenyl derivative of **22**, as compared with the other substituted *p*-aminobenzamides and *p*-aminobenzene-1-alkanones, are explained by assuming that these compounds, besides interfering with the PABA receptor site, also interact with an accessory area on the enzyme.

The discovery in 1940 that *p*-aminobenzoic acid (PABA) antagonizes the bacteriostatic action of the sulfonamides[1] initiated the investigation of numerous derivatives of PABA for their antibacterial activity. With the exception of the tuberculostatic *p*-aminosalicylic acid, however, none of these derivatives showed useful antimicrobial activity (for reviews see ref 2 and 3).

The elucidation of the folate-synthesizing system in bacteria,[4-6] plants,[7,8] and protozoa[9-11] definitely showed the sulfonamides to exert their bacteriostatic action by inhibiting competitively the enzyme H_2-pteroate synthetase, the enzyme which catalyzes the condensation of PABA with the pteridine moiety to give 7,8-dihydropteroic acid (H_2-pteroate), the precursor of the biologically important folates.

In today's antiinfectious therapy the sulfonamides had to make way for the antibiotics, not lastly because of some serious side effects that may occur with the former. Yet, inhibitors of the pteroate-synthesizing system, at least in theory, would be ideal chemotherapeutic agents because higher organisms, e.g., mammals, do not possess this biosystem. Therefore, it seemed worthwhile to pay some more attention to derivatives of PABA as potential PABA antagonists.

Earlier investigations already revealed that, because of steric effects, the introduction of substituents into the benzene nucleus of PABA reduces the affinity for the enzyme H_2-pteroate synthetase.[12,13] The present paper deals with the interaction of several PABA analogues, modified in the amino and/or carboxyl group, with the cell-free system of *Escherichia coli*. The results, in general, confirm earlier views about the structural requirements necessary to interfere with the PABA-converting enzyme system.[2,3] In addition, indications are obtained for the existence of an accessory binding area on the enzyme. Such a binding area might be used as a target for further exploration, possibly leading to useful antibacterial drugs of a new structural type.

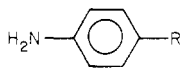
* Address correspondence to the Department of Pharmacology, University of Maastricht, Maastricht, The Netherlands.

Experimental Section

Determination of the Enzyme Activity. Cell-free extracts of *E. coli* B were prepared as described previously.[14] The formation of H_2-pteroate in a reaction mixture was determined by a radioassay method. The mixtures were prepared to contain in a volume of 0.45 ml: 0.5 μmol of ATP, 2 μmol of $MgCl_2$, 0.5 mg of sodium ascorbate, 0.1 mmol of Tris–HCl buffer (pH 8.0), 20 nmol of 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine,[14] and enzyme preparation containing 400–600 μg of protein. After incubation at 37 °C for 10 min, 50 μl of a [7-^{14}C]-PABA solution (0.1–0.3 M) was added and the incubation was continued for another 40 min. The reaction was stopped by adding 1.5 ml of a concentrated citric acid phosphate buffer, pH 3.8, whereafter [^{14}C]-PABA, which was not incorporated into H_2-pteroate, was extracted by ether. The radioactivity remaining in the water layer is a quantitative measure of the H_2-pteroate formed.[12]

Determination of the Inhibition Index. The inhibition of the cell-free H_2-pteroate synthesis was followed at six inhibitor concentrations in duplicate experiments. The inhibition index, I.I. ($=I_{50}/S$), was determined by plotting the percent inhibition vs. the logarithm of the inhibitor concentration. Interpolation for 50% inhibition and dividing the inhibitor concentration (I_{50}) by the substrate concentration (S) will give the relative affinity of the compound, expressed as I.I. These determinations were performed at least at two different PABA concentrations in order

Table I. Inhibition Index of 4-Amino-Substituted Benzene Derivatives



Compd	R	I.I.[a]	pK_a'[b]
1	H	nd	4.58
2	CH_3	nd	5.10
3	CH_2COOH	nd	3.60
4	$COOCH_3$	450	2.46
5	$COOC_2H_5$	≈600	2.50
6	$CONH_2$[c]	53	
7	$CONHCH_3$[d]	120	
8	$CON(CH_3)_2$[d]	≈400	
9	$CONH(CH_2)_2C_6H_5$[e]	≈800	
10	$CONHC_6H_5$[c]	≈600	
11	$CONH(CH_2)_2N(C_2H_5)_2$	1000	2.75[f]
12	$COCH_3$	210 ± 11[g]	2.50
13	COC_2H_5	180 ± 7[g]	
14	$COCH_2COOC_2H_5$[h]	105	
15	COC_6H_5	≈800	2.24
16	L-$CONHCH(COOH)CH_2CH_2COOH$	76	
17	D-$CONHCH(COOH)CH_2CH_2COOH$[i]	250	
18	$CONHCH_2COOH$	260	
19	$CONH(CH_2)_3COOH$[j]	700	
20	Diethyl ester of **16**[k]	≈600	
21	Methyl ester of **18**[l]	≈550	

[a] The values are the mean of at least two experiments in duplicate at different PABA concentrations. nd = no detectable interference with the enzymic reaction. [b] Reference 26. [c] Reference 27. [d] Reference 28. [e] Reference 29. [f] Reference 30. [g] ±SD; $n = 4$. [h] Reference 31. [i] Reference 32. [j] Reference 33. [k] Reference 34. [l] Reference 35.

to ensure the constancy of I.I. with respect to the substrate concentration, indicating the inhibition to be competitive.[12] The inhibition index of compounds whose I_{50} were found by extrapolation is marked in Tables I and II by ≈.

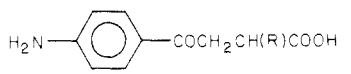
Materials. [7-^{14}C]-PABA (specific activity 10.1 Ci/mol) was purchased from ICN, Calif. Compounds **1–21** were either obtained from commercial sources or were synthesized according to known procedures (for references, see legends to Tables I and II).

β-*p*-Aminobenzoylpropionic Acid (PABP, 22). β-*p*-Acetamidobenzoylpropionic acid was prepared from acetanilide and succinic anhydride.[15] Hydrolysis in 1 N HCl gave PABP, mp 185–187 °C (water). The ethyl ester of PABP (**23**) was obtained by refluxing PABP in 1% sulfuric acid in ethanol: mp 91–92 °C (water–ethanol).

***dl*-α-Phenyl-β-*p*-aminobenzoylpropionic Acid (24).** Attempts to prepare **24** analogously to PABP from acetanilide and phenylsuccinic anhydride at Friedel–Crafts conditions were not successful. The compound has been prepared by hydrocyanic acid addition to benzylidene-*p*-acetamidophenone (4′-acetamidochalcone) and subsequent hydrolysis of the addition product. 4′-Acetamidophenone was prepared by the Claison–Schmidt condensation of *p*-acetamidophenone with benzaldehyde (mp 159–161 °C, water–ethanol). Hydrocyanide addition succeeded following the procedure for HCN addition to benzylidenephenone.[16] The addition product, 1-*p*-acetamidophenyl-3-cyano-3-phenylpropan-1-one (mp 141–143 °C, ethanol), was hydrolyzed to **24** by refluxing the compound in 10% sulfuric acid for 4 h. The acid **24** was isolated by ethyl acetate extraction at pH 3.5 and purified by recrystallization from ethanol–water: mp 166–168 °C.

***d*-α-Phenyl-β-*p*-aminobenzoylpropionic Acid (25).** The *d* isomer of **24** was isolated from the racemate by means of *l*-*N*-methylchinidine hydroxide. (The latter was prepared according to ref 17.) After purification of the acid chinidine salt by repeated crystallization from ether–methanol, compound **25** was isolated from its salt (mp 213–214 °C) by treatment with 2 N HCl and thereupon extraction with ethyl acetate at pH 3.5: mp 166–168 °C; $[\alpha]^{20}D$ +112.8° (*c* 0.96, methanol). Attempts to recover the *l* isomer in an optically pure form from the mother liquids appeared to be troublesome and were not extended. The identity of the compounds and intermediates was proven by elemental analysis, mass and IR spectrophotometry, and TLC.

Table II. Inhibition Index of β-*p*-Aminobenzoylpropionic Acid (PABP) and Derivatives



Compd		R	I.I.
22	PABP	H	55 ± 2 ($n = 5$)
23	Ethyl ester of **22**		≈460
24	*dl*-α-Phenyl-β-*p*-aminobenzoylpropionic acid	C_6H_5	74 ± 3 ($n = 4$)
25	*d*-α-Phenyl-β-*p*-aminobenzoylpropionic acid	C_6H_5	480
26	*l*-α-Phenyl-β-*p*-aminobenzoylpropionic acid	C_6H_5	40[a]

[a] The inhibition index of the *l* isomer was calculated from eq 1.

Results and Discussion

Benzoic acid derivatives not possessing a free *p*-amino group (benzoic acid, *m*-aminobenzoic acid, *p*-acetamidobenzoic acid, *p*-nitrobenzoic acid) showed no inhibition of the enzymic reaction. *p*-Hydroxybenzoic acid, known to inhibit the cell-free H_2-pteroate synthesis in different strains of *Pneumococcus*,[18] showed only weak inhibitory properties (about 10% inhibition occurred at an inhibitor–PABA ratio of 600). The inactivity of the above-mentioned compounds emphasizes once more the importance of the primary *p*-amino group in relation to PABA antagonism and/or agonism.[2,3] The presence of this group, however, does not always seem to be absolutely necessary, for recently Ho et al. showed that diphenylsulfone and derivatives which lack this *p*-amino function inhibited competitively the cell-free H_2-pteroate synthesis of *Neisseria menigitidis* M-166.[19]

Compounds such as aniline (**1**), *p*-toluidine (**2**), and *p*-aminophenylactic acid (**3**) are completely devoid of inhibitory properties toward H_2-pteroate synthesis. Compounds **4–21**, on the other hand, which have a carbonyl function para to the amino group in common, inhibited the enzymic reaction competitively (Table I). However, as can be deduced from the respective inhibition indices, substitution of the carboxyl group by an ester function (**4, 5**), an amide function (**6–11, 16–21**), or a ketone function (**12–15**) strongly reduces the affinity for the enzyme. The β-*N*-diethylaminoethyl ester of PABA (procaine) showed, in comparison with the ethyl and methyl ester **4** and **5** and with procainamide (**11**), a relatively potent antagonistic activity. Further experimentation, however, revealed that this inhibitory activity has to be ascribed to hydrolysis of procaine during the incubation. The resulting PABA manifests itself as a competitive inhibitor of the [^{14}C]-PABA incorporation into H_2-pteroate. For the other PABA esters and amides no hydrolysis at the test conditions could be detected.

Several arguments make it likely that electrostatic interactions should be involved in the complexation of the enzyme H_2-pteroate synthetase and the substrate. (i) PABA (pK_a = 4.68) is almost completely ionized at physiological pH. (ii) The PABA antagonistic potency of ring-substituted PABA derivatives in a cell-free system correlates positively to the acidity of the carboxyl group.[13] (iii) The sulfonamides in their ionized form show enhanced

activity over their molecular form in a cell-free system.[14,20] From these facts it is tempting to conclude that the comparably low affinity of the *p*-aminobenzoyl derivatives **4–21** is mainly because of the inability to create a sufficient negative charge in the carbonyl part of the molecule.

Hotchkiss and Evans,[21] studying the folate synthesis in *Pneumococcus* species, already stressed the importance of an adequate charge distribution in the carbonyl part of such PABA antagonists like *p*-aminoacetophenone. Indeed, compound **14**, in which the ω-carbethoxy group poises the keto–enol equilibrium toward the enolic form thus increasing the negative charge on the carbonyl oxygen, shows enhanced activity over *p*-aminoacetophenone (**12**) and *p*-aminopropiophenone (**13**) (Table I). Consequently, the affinity of the *p*-aminobenzoyl derivatives will probably be determined by the extent of polarization of the carbonyl group. In this view, the poor affinity of the PABA esters **4** and **5** is explicable by the slight polarizability of the ester carbonyl group. The carbonyl group of the amides, on the other hand, is more polarized (tautomerism) explaining the relatively high inhibitory potency of *p*-aminobenzamide (**6**). N′-Substitution will decrease the ability of tautomerism thus decreasing the affinity. Of course, steric factors may play an additional role (compounds **7–11**, **16–21**).

N-*p*-Aminobenzoyl-L-glutamic acid (L-PABG, **16**), which can serve as a substrate for the enzyme H_2-pteroate synthetase to generate 7,8-dihydrofolate,[5,6,18,22] apparently does not fit in this general pattern. Its enhanced activity over *N*-*p*-aminobenzoyl-D-glutamic acid (**17**), *p*-aminohippuric acid (**18**), *N*-*p*-aminobenzoyl-γ-aminobutyric acid (**19**), and the other substituted benzamides (Table I) cannot be explained by assuming a more propitious charge distribution nor is it reasonable to assume that L-PABG is less steric restricted by the PABA receptor site than, for instance, *N*′-methyl-*p*-aminobenzamide (**7**). A possible explanation could be that in the case of L-PABG the glutamyl moiety is interacting with areas in the vicinity of the PABA receptor site, in that way contributing positively to the affinity and so counterbalancing the reduction in binding owing to steric hindrance.

If such an accessory receptor site should exist, further exploration could give clues to structures that, because of an effective interference with this site, are potent PABA antagonists. β-*p*-Aminobenzoylpropionic acid (PABP, **22**) and derivatives were designed to meet this purpose.

A Dixon plot of the cell-free H_2-pteroate synthesis in the presence of PABP showed the compound to inhibit the reaction competitively (Figure 1). Its affinity (K_i = 130 μM) is of the same order of magnitude as that of L-PABG (K_i = 170 μM) and about three to four times higher than that of *p*-aminopropiophenone (**13**) (the respective I.I.'s are 55 and 180). Since this augmentation in affinity can hardly be ascribed to a more optimal polarization of charge in the carbonyl part of PABP (**22**), it has to be concluded that, because of its carboxyl group, PABP—in comparison to **13**—is involved in an interaction with an additional site on the enzyme. Esterification of **22**, like esterification of **16**, lowered the affinity (Table II). Modification of the aromatic amino group of **22** (acetylation or substitution by, for instance, hydrogen), or reduction of the keto function, abolished the affinity for the enzyme.

The α-phenyl derivative of **22** [α-phenyl-β-*p*-aminobenzoylpropionic acid (**24**)] was investigated to see whether hydrophobicity may play a role in the interaction with the proposed receptor site. The compound inhibited the H_2-pteroate formation competitively; its affinity (K_i = 150 μM) is comparable to that of PABP (**22**). The dexterorotatory isomer of **24**, however, showed a six times weaker

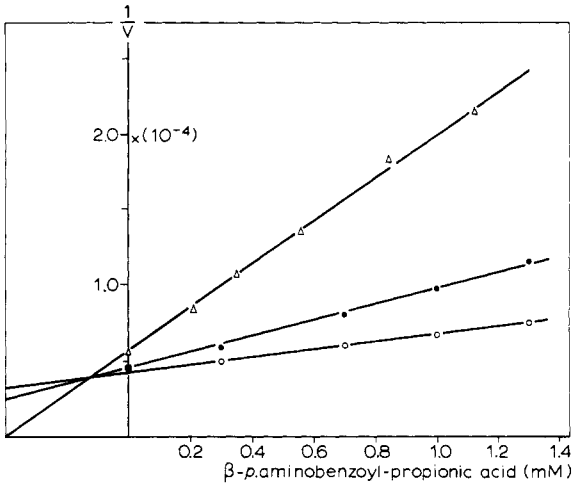


Figure 1. H_2-pteroate synthesis in the presence of β-*p*-aminobenzoylpropionic acid (**22**; Dixon plot). v is the amount of H_2-pteroate formed in disintegrations per minute per hour per 570 μg of protein. PABA: (○—○) 28.8 μM, (●—●) 14.4 μM, (△—△) 7.2 μM.

affinity than the racemate (Table II), indicating the optical isomers to differ in activity. Since the *l* isomer was not isolated as the pure compound, its inhibition index was calculated from the values of the racemate and the *d* isomer by applying the following equation[23]

$$K_i^r = 2\,\frac{K_i^d \cdot K_i^l}{K_i^d + K_i^l} \qquad (1)$$

where K_i^r represents the equilibrium constant of the enzyme–racemate interaction and K_i^d (K_i^l) the equilibrium constants of the isomers. This reveals that the *l* isomer **26** is at least 12 times as potent as the *d* isomer, demonstrating the stereoselectivity of the additional receptor site. However, the phenyl group in **26**, in comparison to **22**, hardly contributes to the affinity (the respective I.I.'s are 40 and 55, Table II); hence, the difference between the isomers, in case of the less active one, has to be ascribed to spatial hindrance of the α-phenyl group.

Since there is little indication in the literature that L-PABG is the natural substrate for the enzyme H_2-pteroate synthetase,[5,6,18,22] the assumed accessory receptor site should not be identified with a specific glutamate binding site. Recently, Tóth-Martinez et al.[25] proposed a multiple enzyme complex for the biosynthesis of folates. This complex, among others, is composed of a glutamate pick-up protein, reversibly attached to a PABA pick-up protein. The latter, in association with a dihydropteridine pick-up protein, functions as the enzyme H_2-pteroate synthetase.[25] Since the enzyme preparation used in this investigation is a more or less crude cell extract, this model of Tóth-Martinez may explain some of the results described.

No clear-cut relation between the basicity of the aromatic amino group and the inhibitory activity of the *p*-aminobenzoyl derivatives can be discerned (Table I). Their pK_a' are of the same order of magnitude as that of PABA (pK_a' = 2.38). The basicity of the amino group of the latter, however, at physiological conditions, will be enhanced about three times because of the ionized carboxyl group.[24]

PABP (**22**) and its α-phenyl derivative **24** showed no antibacterial activity. On the contrary, the compounds promoted the growth of a PABA-requiring strain of *E. coli* (*E. coli* 273) when the latter was cultured in the presence of submaximal PABA concentrations, i.e., below 10^{-7} M (Figure 2). This may indicate that the microorganism is

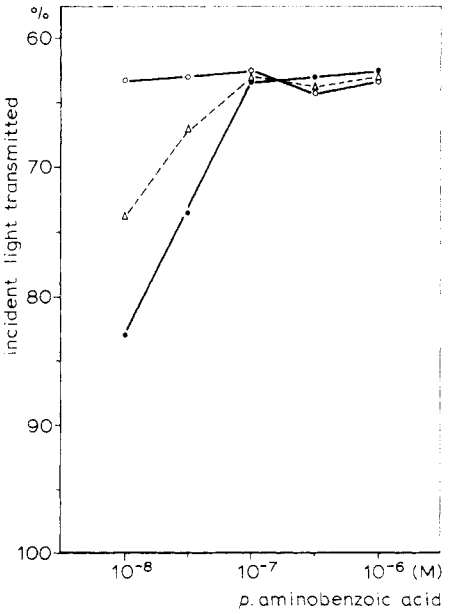


Figure 2. Growth of *E. coli* 273 as a function of the PABA concentration after 22 h of incubation: (●—●) control, (○—○) in the presence of PABP (**22**, 1 mM), (△—△) in the presence of α-Ph-PABP (**24**, 1 mM).

able to convert the compounds to PABA.

Acknowledgment. The author gratefully acknowledges Dr. A. J. Beld, Institute of Pharmacology, University of Nijmegen, for his stimulating discussions and critical review of the manuscript and Professor Dr. E. J. Ariëns, Head of the Institute of Pharmacology, for his valuable advice. Thanks are also due to Mr. P. van Gemert for his excellent help in the synthesis of the various compounds.

References and Notes

(1) D. D. Woods, *Br. J. Exp. Pathol.*, **21**, 74 (1940).
(2) E. H. Northey, "The Sulphonamides and Allied Compounds", Reinhold, New York, N.Y., 1948.
(3) J. K. Seydel, *J. Pharm. Sci.*, **57**, 1455 (1968).
(4) G. M. Brown, *J. Biol. Chem.*, **237**, 536 (1962).
(5) T. Shiota, M. N. Disraely, and M. P. McCann, *J. Biol. Chem.*, **239**, 2259 (1964).
(6) P. J. Ortiz and R. D. Hotchkiss, *Biochemistry*, **5**, 67 (1966).
(7) H. Mitsuda, and Y. Suzuki, *J. Vitaminol.*, **14**, 106 (1968).
(8) O. Okinaka and K. Iwai, *Anal. Biochem.*, **31**, 174 (1969).
(9) R. Ferone, *J. Protozool.*, **20**, 459 (1973).
(10) R. D. Walter and E. Konigk, *Hoppe-Seyler's Z. Physiol. Chem.*, **355**, 431 (1974).
(11) J. L. McCullough and T. H. Maren, *Mol. Pharmacol.*, **10**, 140 (1974).
(12) H. H. W. Thijssen, *Anal. Biochem.*, **53**, 579 (1973).
(13) H. H. W. Thijssen, Thesis, University of Nijmegen, The Netherlands, 1973.
(14) H. H. W. Thijssen, *J. Pharm. Pharmacol.*, **26**, 228 (1974).
(15) J. P. Englisch, R. C. Crapp, Q. P. Cole, and J. Krapcho, *J. Am. Chem. Soc.*, **67**, 2263 (1945).
(16) W. Davey and D. J. Tivey, *J. Chem. Soc.*, 1230 (1958).
(17) J. Knabe and R. Kräuter, *Arch. Pharm. (Weinheim, Ger.)*, **298**, 1 (1965).
(18) P. J. Ortiz, *Biochemistry*, **9**, 355 (1970).
(19) R. I. Ho, L. Corman, S. A. Morse, and H. Schneider, *Antimicrob. Agents Chemother.*, **7**, 758 (1975).
(20) G. H. Miller, P. H. Doukas, and J. K. Seydel, *J. Med. Chem.*, **15**, 700 (1972).
(21) R. D. Hotchkiss and A. H. Evans, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **19**, 912 (1960).
(22) D. P. Richey and G. M. Brown, *Biochim. Biophys. Acta*, **222**, 237 (1970).
(23) W. D. M. Paton and H. P. Rang, *Proc. R. Soc. London, Ser. B*, **163**, 1 (1965).
(24) P. H. Bell and R. O. Roblin, *J. Am. Chem. Soc.*, **64**, 2905 (1942).
(25) B. L. Tóth-Martinez, S. Papp, Z. Dinya, and F. J. Hernádi, *BioSystems*, **7**, 172 (1975).
(26) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution", Butterworths, London, 1973.
(27) *Beilstein*, **14**, 425 (1931).
(28) H. Wenker, *J. Am. Chem. Soc.*, **60**, 1081 (1938).
(29) J. v. Braun, F. Dengel, and A. Jacob, *Ber.*, **70B**, 994 (1937).
(30) J. Büchi and X. Perlia in "Drug Design", Vol. III, E. J. Ariëns, Ed., Academic Press, New York, N.Y., 1973, Chapter 6.
(31) C. Coll, A. Mila, and J. Pascual, *Chem. Abstr.*, **57**, 12860c (1957).
(32) E. Auhagen, *Z. Physiol. Chem.*, **277**, 197 (1943).
(33) W. Reppe, *Justus Liebigs Ann. Chem.*, **596**, 222 (1955).
(34) D. I. Weisblat and A. R. Hanze, *Chem. Abstr.*, **46**, 1595*h* (1952).
(35) M. Miyataka and S. Kaga, *J. Pharm. Soc. Jpn.*, **72**, 1160 (1952).