4-(1-Naphthylethynyl)pyridine.—The procedure is analogous to that reported for the preparation of 4-(1-phenylethynyl)-pyridine.[22] To a refluxing solution of 23.1 g (0.1 mole) of 4-(1-naphthylethenyl)pyridine in 150 ml of $CHCl_3$ was added dropwise with stirring, a solution of 21 g (0.13 mole) of Br_2 in 25 ml of $CHCl_3$. After refluxing for 3 hr, the solvent was evaporated and the dibromo adduct recrystallized (EtOH); mp 145–147° dec. A solution of 19 g (0.05 mole) of the dibromo compound was refluxed for 3 hr in 20% KOH in EtOH, then the solvent evaporated. The residue was extracted with low-boiling ligroin, treated with charcoal, filtered, and evaporated to yield an oil which solidified in the cold. Recrystallization from ligroin yielded 3.5 g of product. *Anal.* ($C_{17}H_{11}N$) C, H. *Anal.* (methiodide XXVI, $C_{18}H_{14}IN$) C, H.

Hydrochlorides.—Hydrochloride salts were prepared by solution of the base in an appropriate ether-miscible solvent to which was then added a solution of HCl in dry Et_2O. Excess Et_2O could be added if necessary to complete precipitation of the salts.

Monomethiodides. Method A.—The substituted pyridine base was dissolved in 3 or more molar equiv of MeI with initial cooling if necessary. The mixture was then refluxed, excess MeI was evaporated, and the crude product was recrystallized from an alcohol (usually *i*-PrOH). Compounds IV and XXVI were prepared in this manner.

Method B.—A solution of a methyl-substituted N-methylpyridinium or -quinolinium iodide (0.02 mole) and 0.02–0.03 mole of the appropriate aldehyde in 30 ± 10 ml of MeOH containing about 5 drops of piperidine was refluxed for 3–6 hr (VIII, IX, XVI, and XX required up to 20 hr). The reaction mixture was cooled and ether was added, if necessary, to complete precipitation of product which was then recrystallized from an alcohol or H_2O. Most of the methoquaternaries were prepared by this procedure.

N-Benzyl-4-(1-phenylethenyl)pyridinium Chloride (VI).—A solution of 3 g (0.017 mole) of 4-styrylpyridine and 3.2 g (0.025 mole) of benzyl chloride in 30 ml of dioxane was refluxed for 15 hr. The crude product which separated on cooling was filtered off and recrystallized several times (*i*-PrOH).

N-(*n*-Hexyl)-4-(1-phenylethenyl)pyridinium Bromide (V).—A mixture of 3 g (0.017 mole) of 4-styrylpyridine and 16.5 g (0.1 mole) of bromohexane was heated to reflux for 17 hr. To the cooled reaction mixture was added 60 ml of Et_2O and the precipitated product was filtered off. The crude preparation was recrystallized from warm *i*-PrOH to which Et_2O had been added to incipient precipitation, followed by cooling.

N-Methyl-2,4-bis(1-phenylethenyl)pyridinium Iodide (XX).—2,4-Lutidine methiodide was prepared by reaction of 2,4-lutidine in excess MeI; mp 128° (from *i*-PrOH). A solution of 12.5 g (0.05 mole) of the methiodide, 20 ml (0.2 mole) of PhCHO, and piperidine as catalyst in 40 ml of MeOH was refluxed overnight. The crude material which separated from the cooled reaction mixture was recrystallized (MeOH).

N-Methyl-3-methyl-4-(1-phenylethenyl)pyridinium Iodide (XVIII).—3,4-Lutidine methiodide was prepared by reaction of the lutidine with excess MeI; mp 124° (from *i*-PrOH). A solution of 12.5 g (0.05 mole) of the methiodide, 20 ml (0.2 mole) of PhCHO, and piperidine as catalyst was refluxed overnight. The crude material obtained was recrystallized from MeOH. The product obtained analyzed well as a monocondensation product. Although unequivocal evidence was not sought, the derivative would be expected to be that resulting from condensation with the 4-methyl group.

Hexamethylene-1,6-bis-N,N′-(1-phenylethenylpyridinium) Dibromide (XXX).—A solution of 5.4 g (0.03 mole) of 4-styrylpyridine, 2.4 g (0.01 mole) of 1,6-dibromohexane, and 40 ml of dioxane was refluxed overnight. The product which separated on cooling was filtered off and recrystallized (EtOH).

Methobromides of Phenanthridine and of Benzo[*f*]quinoline.—Excess MeBr was dissolved in 30 ml of dry dioxane containing 3 g of the aromatic base. After standing at room temperature overnight, the crystalline product was filtered off and recrystallized (hot *i*-PrOH).

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Role of Alkyl Substitution in 2,3-Disubstituted and 3-Substituted 4-Quinazolones on the Inhibition of Pyruvic Acid Oxidation[1]

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Several 2,3-disubstituted and 3-substituted 4-quinazolones have been synthesized to investigate structure–activity relationship of these quinazolones with respect to their ability to inhibit pyruvic acid oxidation by rat brain homogenate. 2-Methyl-3-(*o*-tolyl)-4-quinazolone was used for comparison. In general 2,3-disubstituted quinazolones exhibited greater inhibitory properties as compared to the coresponding 3-substituted quinazolones. Introduction of the alkyl substituent(s) on the phenyl nucleus, attached to the 3 position of the quinazolone molecule, significantly influenced the enzyme inhibitory properties of these quinazolones. In both series, maximum inhibition of the oxidation of pyruvic acid was observed with quinazolones synthesized from 2,4-dimethylaniline. Increase in the concentration of the quinazolones simultaneously increased the enzyme inhibition. Added NAD, responsible for the increase in the respiratory activity of brain homogenate during oxidation of pyruvic acid, reduced the inhibition produced by 2,3-disubstituted and 3-substituted 4-quinazolones.

The structure–activity relationship of various classes of drugs has been attributed to their interaction with receptor surfaces.[2] Such interactions are presumed to be greatly responsible for the inhibitory or stimulatory properties, toxicity, and metabolic fate of drugs where steric differences play an important role in determining their pharmacodynamic effects. Stereoisomeric effects of amphetamine and amphetamine derivatives have recently been reported on the activity of rat liver mito-

(1) This investigation was supported by a research grant from the Council of Scientific and Industrial Research, New Delhi, India.

(2) R. B. Barlow, "Introduction to Chemical Pharmacology," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1964.

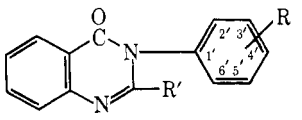
chondrial monoamine oxidase.[3] In a series of 2,3-disubstituted quinazolones possessing hypnotic activity, 2-methyl-3-(*o*-tolyl)-4-quinazolone (I) was found to be a potent hypnotic and anticonvulsant, superior to sodium phenobarbital against pentylenetetrazole seizures.[4,5] Further studies have demonstrated selective *in vitro* inhibition of NAD-dependent oxidations by I with rat brain homogenates and isolated mitochondria.[6,7] In the present study several newer 2,3-disubstituted and 3-substituted quinazolones have been synthesized in an attempt to investigate their structure–activity relationship with respect to their ability to inhibit pyruvic acid oxidation.

Experimental Section

Synthesis of 2,3-Disubstituted and 3-Substituted 4-Quinazolones.—Quinazolones were synthesized by heating equimolar proportions of appropriate acetanthranil or formylanthranilic acid and 2,3-, 2,4-, 2,5-, or 3,4-dimethyl- or 2- or 4-ethylanilines as reported earlier.[8,9] Various 2,3-disubstituted and 3-substituted 4-quinazolones reported in Table I were characterized by their sharp melting points and analyses.

TABLE I

2,3-DISUBSTITUTED AND 3-SUBSTITUTED QUINAZOLONES



R′	R	Mp, °C[a]	Yield, %	Formula[d]
CH_3	2′,3′-Me_2[c]	160–162	60	$C_{17}H_{16}N_2O$
CH_3	2′,4′-Me_2[c]	136–138	65	$C_{17}H_{16}N_2O$
CH_3	2′,5′-Me_2[b]	216–218	70	$C_{17}H_{16}N_2O \cdot HCl$
CH_3	3′,4′-Me_2	196–198	55	$C_{17}H_{16}N_2O$
CH_3	4′-Et[b]	229–232	60	$C_{17}H_{16}N_2O \cdot HCl$
CH_3	2′-Et	240–243	55	$C_{17}H_{16}N_2O$
H	2′,3′-Me_2	136–138	40	$C_{16}H_{14}N_2O$
H	2′,4′-Me_2[b]	205–208	45	$C_{16}H_{14}N_2O \cdot HCl$
H	2′,5′-Me_2[b]	124–126	60	$C_{16}H_{14}N_2O \cdot HCl$
H	3′,4′-Me_2	135	35	$C_{16}H_{14}N_2O$
H	4′-Et	127	40	$C_{16}H_{14}N_2O$

[a] Melting points were taken in open capillary tubes and are corrected. [b] Free bases of these quinazolones could not be crystallized and hence their hydrochlorides were prepared. [c] These quinazolones have been reported earlier but were synthesized using a different method. [d] All compounds were analyzed for C, H, and N and were found within limits.

2-Methyl-3-(2,4-dimethylphenyl)-4-quinazolone.—A mixture of acetanthranil (1.61 g) and 2,4-dimethylaniline (1.21 g) was heated on a free flame for 10 min. On cooling, a jellylike mass separated out, which was washed (10% $NaHCO_3$, H_2O). The solid thus separated was recrystallized from EtOH; mp 136–138°, yield 65%. *Anal.* ($C_{17}H_{16}N_2O$) C, H, N.

3-(4-Ethylphenyl)-4-quinazolone.—A mixture of formylanthranilic acid (1.65 g) and 4-ethylaniline (1.21 g) was refluxed with phenol (20 g) for 4 hr. Excess phenol was distilled off and the resulting mixture was washed (10% NaOH, H_2O). The solid was recrystallized from EtOH; mp 127°, yield 40%. *Anal.* ($C_{16}H_{14}N_2O$) C, H, N. Ir spectra were as expected.

Assay of Pyruvic Acid Oxidation by Rat Brain Homogenate.—Commercial chemicals were used in the present study. Sodium pyruvate, AMP, cytochrome *c*, and NAD were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from BDH, Bombay. Male albino rats kept on an *ad libitum* diet were used in all experiments. Rat brains isolated from decapitated animals were immediately homogenized in ice-cold 0.25 *M* sucrose in a Potter-Elvehjem homogenizer. All incubations were carried out at 37° and the oxygen uptake was measured by conventional Warburg manometric technique with air as the gas phase. Fresh rat brain homogenate equivalent to 125 mg wet weight was added to chilled Warburg vessels containing 6.7 m*M* $MgSO_4$, 20 m*M* Na_2HPO_4 buffer (pH 7.4), 1 m*M* AMP (sodium salt), 33 m*M* KCl, and 500 μg of cytochrome *c* in a final volume of 3 ml unless otherwise stated. The central well contained 0.2 ml of 20% KOH solution. All 2,3-disubstituted and 3-substituted 4-quinazolones were dissolved in propylene glycol (100%). A solution of I in propylene glycol was used for comparison to evaluate structure–activity relationships of the newer quinazolones. An equal volume of propylene glycol was added in the control vessels containing pyruvic acid in the presence and absence of added NAD. These vessels, devoid of 2,3-disubstituted and 3-substituted 4-quinazolones, were used as controls for evaluating inhibitory properties of quinazolones. Pyruvic acid, 10 m*M*, and 0.5 m*M* NAD were used in the final concentrations.

Results

Inhibitory effects of 2,3-disubstituted quinazolones on the oxidation of pyruvic acid by rat brain homogenate are summarized in Table II. All 2,3-disubstituted quinazolones prepared from 2,3-, 2,4-, 2,5-, or 3,4-dimethyl and 2- or 4-ethylanilines inhibited the oxidation of pyruvic acid when used at a final concentration of 1 m*M*. Introduction of an additional methyl group at the 4′ position of the phenyl ring [2-methyl-3-(2,4-dimethylphenyl)-4-quinazolone] significantly increased the ability of I to inhibit pyruvic acid oxidation. However, the enzyme inhibitory property of I decreased when an additional methyl substituent was introduced at either the 3′ or 5′ position on the phenyl nucleus of I, or when the two methyl substituents were present at the 3′ and 4′ positions without a 2′-methyl group [2-methyl-3-(3,4-dimethylphenyl)-4-quinazolones]. A similar decrease in the ability of I to inhibit pyruvic acid oxidation was observed when the 2′-methyl was replaced by a 2′-ethyl substituent. On the other hand substitution of an ethyl group at the 4′ position, without the methyl substituent at 2′ or 4′ [2-methyl-3-(4-ethylphenyl)-4-quinazolone], produced inhibition similar to that observed with I. The degree of inhibition by these 2,3-disubstituted quinazolones remained unaltered with the period of incubation since no significant change in the oxygen uptake was observed during the first 30 min and during the next 30–60-min period. The presence of added NAD decreased the inhibitory effects of quinazolones on the oxidation of pyruvic acid by rat brain homogenates (Table II). These results indicated the protective ability of NAD against the inhibitory effects of 2,3-disubstituted quinazolones. The oxygen uptake in the control experiment, without the quinazolone, decreased during 30–60 min as compared to the first 30 min. Increase in the oxygen uptake during oxidation of pyruvic acid was observed in the presence of added NAD. The inhibition produced by 2-methyl-3-(3,4-dimethylphenyl)-4-quinazolone was significantly reduced by the presence of added NAD. The degree of inhibition on the other hand remained fairly constant throughout the total incubation period of 1 hr.

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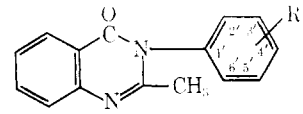
TABLE II

EFFECT OF VARYING CONCENTRATION OF 2,3-DISUBSTITUTED QUINAZOLONES ON THE OXIDATION OF PYRUVIC ACID[a]



	Inhibition, %							
	1 mM				2 mM		3 mM	
	0–30 min		30–60 min					
R	−NAD	+NAD	−NAD	+NAD	−NAD	+NAD	−NAD	+NAD
2′-Me (I)	36.52 ± 0.50	15.20 ± 0.30	35.16 ± 0.17	Nil	49.38 ± 0.42	17.41 ± 0.03	60.48 ± 0.49	28.81 ± 0.37
2′,3′-Me$_2$	25.03 ± 0.92	9.41 ± 0.70	25.15 ± 0.19	10.23 ± 0.42	41.50 ± 0.72	20.57 ± 0.17	58.16 ± 0.43	27.08 ± 0.36
2′,4′-Me$_2$	50.03 ± 0.85	20.46 ± 0.31	49.33 ± 0.65	19.61 ± 0.41	78.57 ± 0.46	33.09 ± 0.74	93.00 ± 0.51	45.88 ± 0.05
2′,5′-Me$_2$	25.07 ± 0.76	14.24 ± 0.47	22.91 ± 0.36	13.43 ± 0.65	44.72 ± 0.29	20.99 ± 0.31	58.06 ± 0.23	29.08 ± 0.11
3′,4′-Me$_2$	7.86 ± 0.95	Nil	9.09 ± 0.70	Nil	16.68 ± 0.17	3.18 ± 0.39	23.86 ± 0.10	13.08 ± 0.72
4′-Et	33.96 ± 0.70	14.86 ± 0.51	30.89 ± 0.24	15.74 ± 0.26	53.04 ± 0.26	25.15 ± 0.32	67.32 ± 0.12	33.62 ± 0.10
2′-Et	20.21 ± 0.46	6.64 ± 0.32	20.50 ± 0.37	8.74 ± 0.45	35.34 ± 0.93	15.31 ± 0.47	49.88 ± 0.90	23.32 ± 0.18

[a] Vessel contents and the assay procedure are as described in the Experimental Section. All experiments were done in duplicate and the values are the mean of three separate experiments. Per cent inhibition and standard errors are calculated from oxygen uptake per 125 mg wet tissue weight. Pyruvic acid and NAD were used at the final concentration of 10 and 0.5 mM, respectively.

Increase in the ability of various 2,3-disubstituted quinazolones to inhibit pyruvic acid oxidation was observed with simultaneous increase in their concentration to 2 mM and 3 mM (Table II). The per cent inhibition of 50.03 ± 0.85 produced by 1 mM 2-methyl-3-(2,4-dimethylphenyl)-4-quinazolone during the first 30 min increased to 78.57 ± 0.46 and 93.0 ± 0.51 with 2 and 3 mM quinazolone, respectively. Effects of substitution, restoration of the inhibition by added NAD, and unaltered degree of inhibition between the first 30 min and the next 30–60-min period at such high concentrations of these quinazolones were found to be similar to those obtained with a low concentration of 1 mM.

The effects of 3-substituted quinazolones, without the 2-CH_3 in the quinazolone ring, on pyruvic acid oxidation by rat brain homogenate are shown in Table III. Introduction of the various substituents on the phenyl nucleus at position 3 of the quinazolone ring exhibited a similar although lesser inhibitory effect, as observed with 2,3-disubstituted quinazolones (Table II). The maximum inhibition among these 3-substituted quinazolones was observed with 3-(2,4-dimethylphenyl)-4-quinazolone. Introduction of methyl groups at the 2′ and 3′, 2′ and 5′, or 3′ and 4′ positions of the phenyl nucleus resulted in lower inhibitory properties of such 3-substituted quinazolones. As was observed with 2,3-disubstituted quinazolones (Table II), introduction of the ethyl group at position 4′ of the phenyl nucleus [3-(4-ethylphenyl)-4-quinazolone] increased inhibitory effects. The presence of added NAD decreased the inhibition which remained unaltered during the total incubation period. The degree of inhibition was found to be concentration dependent both in the presence or absence of the added NAD (Table III).

Introduction of the substituents in the 3-phenyl nucleus exhibited similar inhibitory effects, as observed at low concentrations of these quinazolones. The decrease in the pyruvic acid oxidation was partially restored when NAD was added to the respiratory medium. The per cent inhibition of 70.07 ± 0.29 and 99.33 ± 0.39 by 2 and 3 mM of 3-(2,4-dimethylphenyl)-4-quinazolone observed in the absence of NAD was reduced to 32.28 ± 0.14 and 44.06 ± 0.46 in experiments with added NAD (Table III).

Discussion

Structure-activity relationships of several 2,3-disubstituted quinazolones and 3-substituted quinazolones, with respect to their ability to inhibit pyruvic acid oxidation, were investigated in an attempt to determine site(s) of action of such CNS depressants and to elucidate their biochemical mechanism of action. In the present study I, exhibiting potent anticonvulsant[4] and hypnotic properties,[5] was used for comparison. All 2,3-disubstituted and to a lesser extent 3-substituted quinazolones inhibited oxidation of pyruvic acid by rat brain homogenate where the inhibition increased with higher concentrations. The presence of a methyl group at position 2 of the quinazolone nucleus thus in some way influences the inhibitory properties of these quinazolones and is essential for greater inactivation. These results have indicated a possible competition between the quinazolone and NAD for the active sites on the enzyme during oxidation of pyruvic acid.[6,7] Introduction of the substituents has been shown to play an important role in affecting the ability of these quinazolones to inhibit pyruvic acid oxidation similarly in both 2,3-disubstituted and 3-substituted quinazolones.

The above results can possibly be explained by the work of Sareen, *et al.*,[10] who have indicated that a resonating aromatic nucleus attached to N^3 together with a small alkyl group at position 2 of the quinazolone ring are the essential features for the desired electron availability required for a favorable hypnotic action. However, considerable loss of activity was reported when the methyl group of the 3-*o*-tolyl ring was replaced by other substituents. It appears that the resultant drift of electrons into the ring, due to the presence of a methyl group of the 2′ position of phenyl, leads to the activation of the ring. This presumably results in an increase of electron availability around the 1 position and on over-all molecular morphology for a favorable depressant action. Similar results have been reported by Bianchi and David[11] where 2-methyl-3-*p*-bromophenyl-4-quinazolone was found to be an effective anticonvulsant against electroshock-induced sei-

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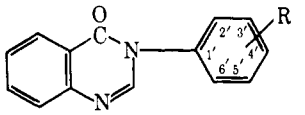
TABLE III

EFFECT OF VARYING CONCENTRATION OF 3-SUBSTITUTED QUINAZOLONES ON THE OXIDATION OF PYRUVIC ACID[a]



	Inhibition, %							
	1 m*M*				2 m*M*		3 m*M*	
	0–30 min		30–60 min					
R	−NAD	+NAD	−NAD	+NAD	−NAD	+NAD	−NAD	+NAD
2′,3′-Me$_2$	11.23 ± 0.81	4.88 ± 0.28	11.48 ± 0.49	5.11 ± 0.40	21.76 ± 0.33	7.71 ± 0.22	30.34 ± 0.70	13.96 ± 0.47
2′,4′-Me$_2$	42.14 ± 0.02	19.89 ± 0.45	41.17 ± 0.63	19.35 ± 0.37	70.07 ± 0.03	32.28 ± 0.14	90.33 ± 0.39	44.06 ± 0.46
2′,5′-Me$_2$	14.39 ± 0.70	7.28 ± 0.12	13.83 ± 0.45	6.12 ± 0.58	32.16 ± 0.95	16.14 ± 0.46	41.06 ± 0.35	18.79 ± 0.42
3′,4′-Me$_2$	14.12 ± 0.44	7.61 ± 0.24	15.22 ± 0.35	8.15 ± 0.23	25.16 ± 0.22	12.18 ± 0.31	48.26 ± 0.35	23.68 ± 0.34
4′-Et	19.98 ± 0.85	10.59 ± 0.19	20.87 ± 0.98	10.74 ± 0.33	35.69 ± 0.36	17.11 ± 0.25	60.21 ± 0.52	29.31 ± 0.41

[a] Vessel contents and the assay procedure are as described in the Experimental Section. All experiments were done in duplicate and the values are the mean of three separate experiments. Experimental conditions were essentially the same as shown in Table II.

zures. Such activity is presumably due to the presence of 4-Br in the phenyl nucleus which would also cause a relatively higher electron density at the C-1′. Thus the substitution of an additional 4′-CH_3 in addition to 2′-CH_3 would be expected to have caused an increased electron availability around the nitrogen atom at position 1 of the quinazolone ring and thereby reinforce the inhibitory effects of I. Furthermore, the presence of the 3′-methyl would not be expected to contribute toward a favorable electron density of the 1 position which is reflected by low inhibitory effects of these compounds on pyruvic acid oxidation.

Study of the inhibitory effect of 2,3-disubstituted and 3-substituted quinazolones and their comparison with QZ-2 have indicated possible competition with NAD for the active site(s) on the enzyme[12,13] during the oxidation of pyruvic acid.

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Quinoxaline Studies. XIV.[1a] Potential Anticancer Agents. Some Quinoxaline Amino Acid and Dipeptide Derivatives Related to Quinoxaline Antibiotics[1b]

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2-Quinoxaloyl chloride was utilized to prepare 13 N-(2-quinoxaloyl) derivatives of amino acids and dipeptides related to quinoxaline antibiotics.[2] N-(2-Quinoxaloyl)-L-valyl-L-alanine possessed the most (albeit slight) antitumor activity.

Numerous investigators[2] have reported the presence of the 2-quinoxaloyl (2-quinoxalinecarbonyl) unit in the quinoxaline antibiotics, the quinomycins and triostins. The antibiotics are toxic, but have been reported active against many gram-positive bacteria, protozoans, viruses, and tumor cells.

It was hoped that relatively simple N-quinoxaloyl derivatives of amino acids and peptides would possess the desirable biological qualities of the quinoxaline antibiotics without being toxic. This prompted the syntheses for testing as antitumor agents of N-(2-quinoxaloyl) derivatives of the N-methyl-α-amino acids found in the acidic hydrolysate of desthioechinomycin, as well as some N-(2-quinoxaloyl) dipeptides with either free or blocked C-terminal amino acid groups. Two papers[1a,3] have reported syntheses of 2-quinoxalinecarbonyl (2-quinoxaloyl) derivatives embodying various structural features of the quinomycin and triostin antibiotics.

Attempts to effect acylation of N-methyl-L-alanine and N-methyl-L-valine in aqueous $NaHCO_3$ suspensions of 2-quinoxaloyl chloride (2-quinoxalinecarbonyl chloride), using the earlier published procedure[1a] that led to the preparation of N-(2-quinoxaloyl)-α-amino acids, were unsuccessful. Only 2-quinoxalinecarboxylic acid was isolated.

The pK_a (for $>NH_2^+$) values for both N-methyl-L-alanine and N-methyl-L-valine were ascertained, con-

(1) (a) Paper XIII of this series: S. Gerchakov, P. J. Whitman, and H. P. Schultz, *J. Med. Chem.*, **9**, 266 (1966). (b) We gratefully acknowledge support of this work under National Institutes of Health Grant GM-11968, and thank the Cancer Chemotherapy National Service Center for the biological evaluations of the compounds described. (c) Abstracted in part from the Ph.D. dissertation of S. Gerchakov, University of Miami, Oct 1967.

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