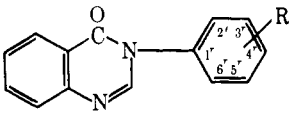
TABLE III

EFFECT OF VARYING CONCENTRATION OF 3-SUBSTITUTED 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′,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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(13) C. T. Beer and J. H. Quastel, *Can. J. Biochem. Physiol.*, **36**, 543 (1958).

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.

(2) H. Otsuka and J. Shoji, *Tetrahedron*, **23**, 1535 (1967), and references cited therein.

(3) H. C. Koppel, I. L. Honigberg, R. H. Springer, and C. C. Cheng, *J. Org. Chem.*, **28**, 1119 (1963).

firming the accuracy of the titration method by determination of the known pK_a values for L-alanine and L-valine. The observed pK_a (27°) values for L-alanine and L-valine were 9.75 and 9.60, respectively (lit.[4,5] (25°) 9.69 and 9.62, respectively). The pK_a (for $>NH_2^+$) values for N-methyl-L-alanine and for N-methyl-L-valine were found to be 9.95 and 9.80, respectively, 0.2 pK_a unit higher than those for the corresponding nonmethylated amino acids.

Therefore, it was concluded that because the N-methylamino acids are more basic than the nonmethylated analogs, the pH of the $NaHCO_3$-buffered reaction mixture was not high enough to liberate the free N-methylamino group from its dipolar form.

Acylations of N-methyl-L-alanine and of N-methyl-L-valine with quinoxaloyl chloride were carried out at 0° and a pH of 11.4, the lowest pH at which acylation was observed to proceed at a reasonable rate, a reflection, possibly, not only of the increased basicities of the N-methylamino groups of the amino acids but also of the increased steric requirements of the reactions. The pH of the systems was carefully controlled with 1 *N* NaOH solution, added *via* a titrimeter whenever the pH of the solution dropped below 11.4. Under these circumstances the desired N-(2-quinoxaloyl)-N-methylamino acids were synthesized in good yields. In contrast to the nonmethylated analogs, the N-(2-quinoxaloyl)-N-methylamino acids showed little tendency to crystallize and exhibited greater solubility in the usual solvents than did their nonmethylated counterparts.

There are two major routes to the preparation of N-(2-quinoxaloyl) dipeptides: the coupling of 2-quinoxalinecarboxylic acid with a dipeptide, and the coupling of an N-(2-quinoxaloyl)amino acid with an amino acid. The former avenue requires the preparation of the dipeptide, followed by its acylation with 2-quinoxaloyl chloride. The latter route requires peptide bond formation between two amino acids, one of which is quinoxaloylated (and therefore N protected). The experience gained with the smooth acylations using an automatic titrimeter for pH control in the syntheses of N-(2-quinoxaloyl)-N-methylamino acids made the technique attractive for the preparation of N-(2-quinoxaloyl) dipeptides *via* the first route described above. Four dipeptides (DL-alanylglycine, glycyl-DL-alanine, L-alanyl-L-valine, and L-valyl-L-alanine) were therefore acylated with 2-quinoxaloyl chloride in the manner described earlier, controlling the pH at 9.6 with NaOH solution.

The reaction of an N-(2-quinoxaloyl)amino acid with a second amino acid unit posed a major problem, because the N-(2-quinoxaloyl)amino acid derivatives have low solubilities in many solvents used for peptide syntheses. Of necessity dimethylformamide was the only solvent available for such condensation reactions.

Because attempted preparation of a series of N-(2-quinoxaloyl)-α-amino acid *p*-nitrophenyl esters led to the formation of dark oils, only one of which, *p*-nitrophenyl N-(2-quinoxaloyl)-L-isoleucinate, could be purified, utilization of such compounds for coupling with free amino acids was abandoned.

(4) E. J. Czarnetsky and C. L. A. Schmidt, *Z. Physiol. Chem.*, **204**, 129 (1932).

(5) S. Miyamoto and C. L. A. Schmidt, *J. Biol. Chem.*, **90**, 165 (1931).

The N-(2-quinoxaloyl) dipeptide esters listed in Table I were prepared from various N-(2-quinoxaloyl)-amino acids and amino acid esters by the use of dicyclohexylcarbodiimide,[6,7] N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide,[8] or N-ethyl-5-phenylisoxazolium 3′-sulfonate.[9]

As a consequence of the experience gained in this study, it was concluded that the most convenient approach to the preparation of quinoxaline antibiotics requires first the preparation of the oligopeptide chain, followed by acylation with 2-quinoxaloyl chloride. Such an approach would alleviate the inability to use the common solvents, except dimethylformamide, for the coupling reactions, and the difficulty of purification of the products obtained when N-(2-quinoxaloyl)-α-amino acids are coupled with C-protected amino acids.

All compounds listed in Table I, except **9**, were tested by the standard procedures of the CCNSC. None of the tested compounds displayed antitumor activity (tumor weight loss for L1210 lymphoid leukemia in mouse), if a 10% difference in tumor weight is assumed as the confidence limit of this test.

Experimental Section[10]

Materials.—Unless noted otherwise, materials were commercial reagent grade chemicals. The following reagents were purified by procedures described in Fieser and Fieser[11] or Vogel:[12] anhydrous HCl, dicyclohexylamine, DMF, isobutyl chloroformate, MeOH, pyridine, THF, $SOCl_2$, and Et_3N.

2-Quinoxaloyl chloride was prepared from 2-quinoxalinecarboxylic acid[13a] in 89% yield, mp 114–116°, in C_6H_6 (see ref 3); the crude product was purified by sublimation at 80° (0.5 mm).

N-(2-Quinoxaloyl)-N-methyl-L-alanine.—To a cold (0°), stirred solution of 5.2 g of N-methyl-L-alanine[13] and 100 ml of 0.5 *N* NaOH was added 9.6 g of 2-quinoxaloyl chloride; the pH of the solution was maintained at 11.4 with the aid of a Fisher automatic titrimeter Model 36 which dispensed 56 ml of 1 *N* NaOH over a period of 1.5 hr. After treatment with decolorizing carbon and filter aid, the solution (0°) was very slowly brought to pH *ca.* 2 with 3 *N* HCl, whereupon oil separated which crystallized after standing for 24 hr at 5°. The 12.5 g (96.9%) of white crystals, mp 139–140° dec, was recrystallized (H_2O, 23 ml/g); yield 11.0 g (85.2%); mp 140–141° dec; $[\alpha]^{27}D$ −64.6° (*c* 2, 5% $NaHCO_3$); $\lambda_{max}^{95\% \text{ EtOH}}$ 207 mμ (ε 17,520), 240 (24,990), 313 (5858), 324 (6791). *Anal.* ($C_{13}H_{13}N_3O_3$) C, H, N.

N-(2-Quinoxaloyl)-N-methyl-L-valine was prepared in 73% yield, mp 102.5–104.5° dec, from N-methyl-L-valine[13] and 2-quinoxaloyl chloride by the same procedure as that used for N-(2-quinoxaloyl)-N-methyl-L-alanine; after recrystallization (95% EtOH, 10 ml/g; H_2O, 10 ml/g), yield 66.1%, mp 102.2–103° dec, $[\alpha]^{27}D$ −115.9° (*c* 2, 5% $NaHCO_3$), uv absorption bands as expected. *Anal.* ($C_{15}H_{17}N_3O_3$) C, H, N.

L-Alanyl-L-valine and L-Valyl-L-alanine.—Although com-

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(7) J. C. Sheehan, M. Goodman, and G. P. Hess, *ibid.*, **78**, 1367 (1956).

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(10) Uv absorption spectra were obtained from samples at concentrations of 5 mg/l. of solvent with a Bausch and Lomb Spectronic 505 spectrophotometer using 1-cm path silica cells. pK_a values were determined with a Beckman expanded-scale pH meter. All optical activities were observed in a 1-dm polarimeter tube on a Rudolph Model 63 polarimeter. Melting points, determined in a Thomas–Hoover apparatus, were uncorrected. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions are within ±0.4% of the theoretical values.

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(12) A. I. Vogel, "A Textbook of Practical Organic Chemistry," 3rd ed, Longmans, Green and Co., Ltd., London, 1957.

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TABLE I
N-(2-QUINOXALOYL) DIPEPTIDES

No.	N-(2-Quinoxaloyl) deriv of	Formula[d,e]	Yield, %	Recrystn solvent[f]	Mp, °C dec	[α]D (t, °C), deg
1	DL-Alanylglycine	$C_{14}H_{14}N_4O_4$	90.0	E + W	238.0–239.0	...
2	L-Alanyl-L-valine	$C_{17}H_{20}N_4O_4$	94.1	E + W	182.0–183.0	+80.8 (28.0)[g]
3	Glycyl-DL-alanine	$C_{14}H_{14}N_4O_4$	85.0	E + W	239.0–239.5	...
4	L-Valyl-L-alanine	$C_{17}H_{20}N_4O_4$	90.1	E + W	204.0–205.0	+70.9 (28.0)[g]
5	Methyl L-alanyl-L-alaninate[a]	$C_{16}H_{18}N_4O_4$	57.5	B + H	174.5–175.5	+88.5 (27.0)[h]
6	Methyl L-alanyl-L-methioninate[a]	$C_{18}H_{22}N_4O_4S$	62.8	B + H	162.5–163.5	+92.4 (26.0)[h]
7	Methyl L-alanyl-L-valinate[a]	$C_{18}H_{22}N_4O_4$	37.5	B + H	128.0–131.0	+123.2 (26.0)[h]
8	Methyl D-seryl-L-alaninate[a]	$C_{16}H_{18}N_4O_5$	45.6	A + P	184.0	−119.1 (22.0)[i]
9	Methyl D-seryl-L-alaninate[b]	$C_{16}H_{18}N_4O_5$	33.3	A + P	184.4–185.0	−119.3 (22.0)[i]
10	*t*-Butyl D-seryl-L-alaninate[a]	$C_{19}H_{24}N_4O_5$	55.1	B + H	94.0–96.0	−79.2 (27.0)[j]
11	Ethyl DL-serylglycinate[c]	$C_{16}H_{18}N_4O_5$	40.0	M + W	180.0–182.0	...
12	Methyl L-valyl-L-methioninate[a]	$C_{20}H_{26}N_4O_4S$	14.3	A + W	136.0–137.0	+83.5 (26.0)[h]

[a] Synthesized by Woodward's Reagent K. [b] Synthesized by Sheehan's basic carbodiimide reagent. [c] Synthesized by Sheehan's dicyclohexylcarbodiimide reagent. [d] All analyses were for C, H, N; also S when present. [e] Uv absorption bands, $\lambda_{max}^{95\% EtOH}$, mμ (average ε), were as expected: 206–207 (19,765), 244 (36,829), 316–318 (6789), 326–328 (6827). [f] E, 95% EtOH; W, H_2O; B, C_6H_6; A, Me_2CO; M, MeOH; P, 30–60° petroleum ether. [g] (*c* 2, 5% $NaHCO_3$). [h] (*c* 2, DMF). [i] (*c* 1, DMF). [j] (*c* 5, DMF).

mercially available, these dipeptides were prepared in this laboratory by modifications of known general procedures *via* the following sequences: L-alanine, N-carbobenzoxy-L-alanine, *p*-nitrophenyl N-carbobenzoxy-L-alaninate [*via p*-nitrophenyl trifluoroacetate in pyridine, yield 75.6%, mp 78.5–79°, $[\alpha]^{24}D$ −36.6° (*c* 1.4, EtOAc); lit.[14] (*via* tris(*p*-nitrophenoxy)phosphine) mp 79–79.5°, $[\alpha]^{25}D$ −38.1° (*c* 1.4, EtOAc)], N-carbobenzoxy-L-alanyl-L-valine [in DMF maintained at an apparent pH of 9.6 with 0.5 *N* NaOH dispensed from a titrimeter, yield 97.1%, mp 151.5–152, $[\alpha]^{22}D$ −14.4° (*c* 3.6, EtOH); *Anal.* ($C_{16}H_{22}N_2O_5$) C, H, N; lit.[15] (*via* thiophenol active ester) mp 121–124°, $[\alpha]^{22}D$ −12.8° (*c* 3.6, EtOH)], and L-alanyl-L-valine [*via* HBr in AcOH, yield 62.5%, mp 255–255.5° dec, $[\alpha]^{30}D$ −5.9° (*c* 4.4, H_2O); *Anal.* ($C_8H_{16}N_2O_3$) C, H, N; lit.[15] (*via* trifluoroacetic acid) $[\alpha]^{26}D$ −6.5° (*c* 4.4, H_2O)]; L-valine, N-carbobenzoxy-L-valine, *p*-nitrophenyl N-carbobenzoxy-L-valinate [*via p*-nitrophenyl trifluoroacetate in pyridine, yield 40.5%, mp 65–66°, $[\alpha]^{29}D$ −40° (*c* 1, EtOH), $[\alpha]^{29}D$ −25° (*c* 2, DMF); lit.[16] (*via* dicyclohexylcarbodiimide method) mp 66–67°, $[\alpha]^{25}D$ −25° (*c* 2, DMF); lit.[17] (*via* diaryl sulfite method) mp 63°], N-carbobenzoxy-L-valyl-L-alanine [in DMF–H_2O maintained at an apparent pH 9.6 with 0.5 *N* NaOH dispensed from a titrimeter, yield 51.5%, mp 173–174° dec, $[\alpha]^{32}D$ −33.1° (*c* 1, 95% AcOH); lit.[18] (*via* pyrazolone active ester method) mp 179°, $[\alpha]^{20}D$ −19.5° (EtOH); lit.[19] (*via* basic hydrolysis of the ethyl ester) mp 172–173°, $[\alpha]^{25}D$ −32.1° (*c* 1, 95% AcOH)], and L-valyl-L-alanine (*via* ref 19).

N-(2-Quinoxaloyl)-DL-alanylglycine.—To a cold (0°) solution of 1 g of DL-alanylglycine (Mann Research Laboratories), 6.8 ml of 1 *N* NaOH, and 50 ml of H_2O was added 1.2 g of 2-quinoxaloyl chloride. The pH of the vigorously stirred solution was kept at 9.6 with the aid of an automatic titrimeter which dispensed 36 ml of 0.2 *N* NaOH over a period of 1.5 hr. After treatment with decolorizing carbon and filter aid, the cold (0°) solution was acidified slowly to pH ~4 with 6 *N* HCl, yield 2 g (100%), mp 237–238° dec; after recrystallization (95% EtOH, 37 ml/g; H_2O, 75 ml/g), yield 1.8 g (90%), mp 238–239° dec.

This general procedure was used also to prepare the N-(2-quinoxaloyl) derivatives of L-alanyl-L-valine, glycyl-DL-alanine, and L-valyl-L-alanine, data for which are included in Table I.

Methyl N-(2-Quinoxaloyl)-L-alanyl-L-alaninate.—To a cold, stirred suspension of 5.1 g of N-ethyl-5-phenylisoxazolium 3′-sulfonate (Woodward's Reagent K, Pierce Chemical Corp.) in 50 ml of dry DMF at −5° was added in one portion a cold (0°) solution of 4.9 g of N-(2-quinoxaloyl)-L-alanine[13a] in 50 ml of DMF containing 2.8 ml of Et_3N. The resulting orange solution was stirred for 1 hr and then warmed to 25° and stirred for an additional 45 min. The solution was then cooled to 0° and treated with a cold (0°) suspension of 2.8 g of methyl L-alaninate hydrochloride (Mann Research Laboratories) in 50 ml of DMF containing 2.8 ml of Et_3N. Stirring (0°) was continued for 4 hr, then at 25° for 48 hr. The solvent was evaporated under reduced pressure, and the semisolid residue was distributed between 100 ml of H_2O and 200 ml of EtOAc. The aqueous phase was washed with EtOAc; the EtOAc solution was washed successively with 5% citric acid, 18% NaCl, 15% $KHCO_3$, and saturated NaCl. After drying (Na_2SO_4), the solution was evaporated under reduced pressure, yield 4.4 g (68.2%), mp 168–171° dec, which was dissolved in 75 ml of hot C_6H_6, and treated with decolorizing carbon and filter aid. Addition of 125 ml of C_6H_{14} precipitated 4.4 g (68.2%), mp 172–173° dec. Three recrystallizations (C_6H_6, 15 ml/g; C_6H_{14}, 30 ml/g) yielded 3.8 g (57.5%), mp 174.5–175.5° dec.

This general procedure was used to prepare, *via* the use of Woodward's Reagent K, the N-(2-quinoxaloyl) derivatives of methyl L-alanyl-L-methioninate, methyl L-alanyl-L-valinate, methyl D-seryl-L-alaninate, *t*-butyl D-seryl-L-alaninate, and methyl L-valyl-L-methioninate, data for which are included in Table I.

Methyl N-(2-Quinoxaloyl)-D-seryl-L-alaninate.—To a cold, stirred (0°) solution of 1.12 g of methyl L-alaninate hydrochloride (Mann Research Laboratories) and 2.09 g of N-(2-quinoxaloyl)-D-serine[13a] in 25 ml of dry DMF was added 1.1 ml of Et_3N and 1.55 g of N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride.[8] After stirring at 0° for 2 hr the ice bath was removed and stirring was continued for 70 hr at 25°. The solvent was evaporated under reduced pressure, and the residual oil was distributed between 30 ml of EtOAc and 20 ml of 1 *N* HCl. The aqueous phase was washed and worked up as described above to give 1.5 g (54.3%), mp 145–147° altered, 181–182.5° dec; after recrystallization (Me_2CO, 35 ml/g; ligroin, bp 30–60°, 100 ml/g), 0.9 g (33.3%) of light yellow product, crystalline structure altered at 152–153° and decomposed at 184.5–185°. The mixture melting point of this product with the one obtained *via* Woodward's Reagent K was not depressed. Physical data are in Table I.

Ethyl N-(2-Quinoxaloyl)-DL-serylglycinate.—To a solution of 0.7 g of ethyl glycinate hydrochloride (Eastman Organic Chemicals), 1.3 g of N-(2-quinoxaloyl)-DL-serine[3] (mp 215–216°), and 0.7 ml of Et_3N in 15 ml of DMF was added 1.1 g of dicyclohexylcarbodiimide. The solution was stirred at 25° for 66 hr; the white solid which had formed was filtered and washed with DMF. The DMF solution was cooled to 0° and 100 ml of H_2O was added in small portions. After cooling at 5° for 12 hr, the precipitated solid weighed 1.6 g (92%), mp 155–157° dec; after recrystallization (MeOH, 15 ml/g), yield 0.7 g (40%), mp 177–178° dec. For analysis the product was recrystallized (MeOH, 30 ml/g; H_2O, 30 ml/g) to give 66% recovery of analytically pure product, mp 180–182° dec. Physical data are in Table I.

N-Carbobenzoxy-N-methyl-L-alanine.—To a stirred, cold (0°) solution of 5.2 g of N-methyl-L-alanine[13] and 20 g of $KHCO_3$ in 200 ml of H_2O was added 8.5 g of carbobenzoxy chloride (Mann Research Laboratories) in one portion; stirring was continued for 2 hr at 0° and 30 min at 25°. Cooling to 0° and addition of another portion of 8.5 g of carbobenzoxy chloride was repeated.

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Stirring at 0° was continued for 30 min, then at 25° for 20 hr. The opaque solution was washed with EtOAc, cooled to 0°, and brought to pH ~2 with 6 *N* HCl. The oil that separated was extracted with EtOAc which was washed with saturated NaCl, dried (Na_2SO_4), and evaporated under reduced pressure, yield 7.9 g (70.5%), mp 62–64.5°; after recrystallization (C_6H_6, 6 ml/g; C_6H_{12}, 20 ml/g), yield 6.9 g (61.6%), mp 63.2–65.5°. Repeated recrystallization yielded 5.3 g (47.9%), mp 64.5–66°; $[\alpha]^{25}D$ −33.1° (*c* 2, AcOH). *Anal.* ($C_{12}H_{15}NO_4$) C, H, N.

N-Carbobenzoxy-N-methyl-L-alanine Dicyclohexylamine Salt.—A solution of 9.5 g of N-carbobenzoxy-N-methyl-L-alanine and 80 ml of EtOAc was cooled to 0° in an ice bath and 9.8 ml of redistilled dicyclohexylamine was added; after 12 hr at 25° and 5 hr at 5°, the white solid was filtered and washed with EtOAc, yield 16.7 g (100%), mp 141–142°; after recrystallization (Me_2CO, 25 ml/g), 16 g (95.6%), mp 141°, $[\alpha]^{25}D$ −13.8° (*c* 2, 95% EtOH). *Anal.* ($C_{31}H_{38}N_2O_4$) C, H, N.

***p*-Nitrophenyl N-(2-Quinoxaloyl)-L-isoleucinate.**—A solution of 2.9 g of N-(2-quinoxaloyl)-L-isoleucine[1c] and 10 ml of pyridine was treated with 2.4 g of *p*-nitrophenyl trifluoroacetate (Aldrich Chemical Co.) in a flask protected with a drying tube. The solution was stirred for 1 hr and poured into 100 ml of H_2O, extracting the dark oil with $CHCl_3$. The $CHCl_3$ solution was washed successively with saturated NaCl, 5% citric acid, and saturated NaCl, then dried (Na_2SO_4) and evaporated under reduced pressure. The residual brown oil was dissolved in 50 ml of absolute EtOH, treated with decolorizing carbon and filter aid, and filtered. After the addition of 200 ml of ligroin (bp 30–60°), the solution was kept at 0° for 10 hr, yield 1 g (24.5%), mp 114–115° dec; after recrystallization (EtOH, 20 ml/g; ligroin, bp 30–60°, 70 ml/g); yield 0.8 g (19.5%); mp 115–116° dec; $[\alpha]^{26}D$ +7.6° (*c* 2, DMF); $\lambda_{max}^{95\%\ EtOH}$ 207 mμ (ε 23,940), 244 (35,290), 278 (9232), 318 (7516). *Anal.* ($C_{21}H_{20}N_4O_5$) C, H, N.

Epimeric 2-Hydroxy-2-phenylquinolizidines[1]

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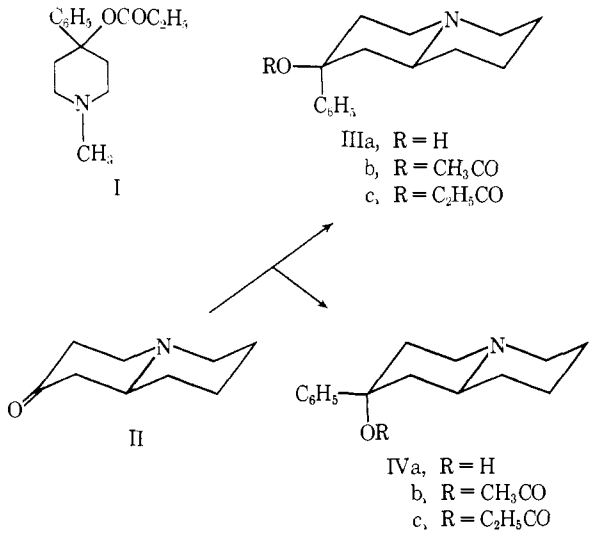
The preparation of epimeric 2-hydroxy-2-phenylquinolizidines and the corresponding acetates and propionates is described. Ir and nmr data were utilized for the elucidation of the stereochemical structures. Preliminary analgetic screening of the esters demonstrated marked activity in both axial and equatorial esters.

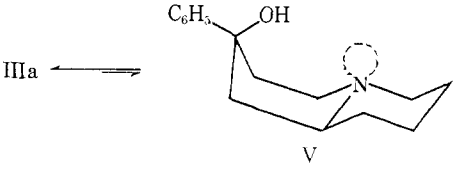
The potential biological properties inherent in substituted phenylhydroxyalkylamines[3] and the importance of stereochemical characteristics[4] on biological action led to the investigation of epimeric 2-hydroxy-2-phenylquinolizidines (IIIa, IVa) and their esters (IIIb, c, IVb, c). The preparation and structural relationship of epimeric 1-hydroxy-1-phenylquinolizidines to biologically active phenethylamines were reported earlier.[5]



The isomeric 2-substituted derivatives not only are related closely to the phenylalkylamines but also to the potent analgetic piperidines (I).[6]

The reaction of C_6H_5MgBr with 2-ketoquinolizidine (II) provided a 1:3 mixture of epimeric hydroxyphenylquinolizidines (IIIa, IVa). Elution chromatography provided first the 2(a)-hydroxy-2(e)-phenylquinolizidine (IVa) followed by 2(e)-hydroxy-2(a)-phenylquinolizidine (IIIa). The epimers were identified by means of ir and nmr spectroscopy (Tables I and II). The ir spectra of both IIIa and IVa are very similar; however, with high dilutions IIIa exhibits some intramolecular hydrogen bonding (broad weak band at 3350 cm^{-1} attributed to V). A distinguishing characteristic in the ir spectra is the absorption at higher wave numbers (770–762 cm^{-1}) in the monosubstituted aromatic region of the axial phenylquinolizidines (III, VI) in contrast to the absorption at lower wave numbers (762–750 cm^{-1}) of the corresponding equatorial phenylquinolizidines (IV, VII).

The similarity of the nmr spectra of both epimers did not provide features which could be used for the identification of either IIIa or IVa. Additional evidence for the configuration of the epimers, however, was provided by the nmr spectra (Table II) of the corresponding



acetates (IIIb, IVb). The methyl protons of the axial acetate (IVb) absorb downfield from the corresponding equatorial acetate (IIIb). This is consistent with ob-

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(2) National Institutes of Health Predoctoral Fellow, 1966–.

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