able[3] which was given the value of 1.0 for the phenyl derivatives and zero for the benzamides. This variable would incorporate factors which differ from one series as a whole to the other series, such as the different geometry of the two sets.) Equation 7 is much more appropriate to test the hypothesis because the values for the variables have a larger variation and there are more observations which thus increases the degrees of freedom. In eq 7 all three electronic parameters contribute significantly to the prediction of antibacterial activity. Thus Cammarata's suggestion that these drugs interact with the receptor in a frontier-controlled reaction is supported by this equation.

The above examples point out the necessity of testing apparent structure–activity relationships with statistical methods. Thus two examples of apparent correlation between electronic factors and inhibitor potency were shown to be not statistically significant. The amount of variation in a factor used in a regression equation must be large enough that experimental error or round off in the computer does not influence the results. Because of the correlation between the various theoretical and empirical parameters one must be cautious in interpreting the meaning of such studies.

β-Amino Ketones as Inhibitors of Pyruvic Acid Oxidation

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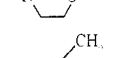
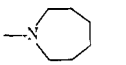
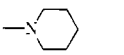
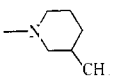
Substituted β-amino ketones (Table I) have elicited a variety of physiological responses; various workers have demonstrated that compounds of this type possess antispasmodic,[2,3] analgetic,[4] local anesthetic,[5–8] and antibacterial[9,10] activity. Luts and Nobles[11] observed anticonvulsant, analgetic, and antiinflammatory activity in a series of cyclic β-amino ketones. Quastel and Wheatley[12] have reported the *in vitro* inhibition of respiration by various anesthetics and CNS depressants; this was related to the selective inhibition of nicotinamide–adenine dinucleotide (NAD) dependent systems by these agents. Such observations led us to prepare a series of β-amino ketones derived from 4-piperidinoacetophenone; such a series would permit us to examine the possible effects on such inhibition as they relate to the specific structure of the β-amino ketones in question. Thus, all amino ketones studied (Table I) had cyclic amine components with one exception (**6**) and all possessed a large cyclic component at the *para* position in the original ketone.

TABLE I

β-AMINO KETONES

No.	NR_1R_2	Mp, °C[a]	Yield, %	Formula[b]
I		185–188	49	$C_{18}H_{28}Cl_2N_2O_2$
II		207–211	37	$C_{20}H_{32}Cl_2N_2O_2$
III		190–192	38	$C_{20}H_{32}Cl_2N_2O$
IV		201–205	40	$C_{19}H_{30}Cl_2N_2O \cdot 0.75H_2O$
V		203–206	44	$C_{20}H_{32}Cl_2N_2O$
VI	$N(CH_3)_2$	194	72	$C_{16}H_{26}Cl_2N_2O \cdot 0.5H_2O$

[a] Melts with decomposition. [b] All compounds were analyzed for C, H, N.

Biochemical Studies.—Male albino rats weighing 100–150 g kept on an *ad libitum* diet were sacrificed by decapitation. Rat brains were immediately homogenized in a Potter-Elvehjem homogenizer. Brain homogenates (10%, w/v) were prepared in 0.25 *M* cold sucrose. O_2 uptake was measured at 37° by the conventional Warburg manometric technique with air as the gas phase.[12] The central well contained 0.2 ml of 20% KOH solution. The reaction mixture in a total volume of 3 ml contained 6.7 m*M* $MgSO_4$, 20 m*M* Na_2HPO_4 in a buffer solution of pH 7.4, 1 m*M* adenylic acid (Na salt), 33 m*M* KCl, 500 μg of cytochrome *c*, and 10 m*M* sodium pyruvate. The compounds, dissolved in double distilled water, were used at a final concentration of 0.5 m*M*.

Results and Discussion

The data in Table II indicate that all the β-amino ketones were found to inhibit the oxidation of pyruvic acid. Such *in vitro* inhibition of respiration has been shown, as noted earlier, to be exhibited by various anesthetics and CNS depressants.[12] These results have seemingly indicated the significance of the cyclic amine moiety in the inhibitory effects thus produced by certain β-amino ketones on pyruvic acid oxidation.

Concurrently, it should be noted that β-dimethylamino-4-piperidinopropiophenone (VI), possessing dimethyl substituents in the amine moiety, was found to produce only slight inhibition under similar conditions. On the basis of this observation, it would appear that the cyclic amine group possibly plays an important role in the inhibition of pyruvic acid oxidation. In the com-

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(2) J. J. Denton, R. J. Turner, W. B. Neier, V. A. Lawson, and H. P. Schedl, *J. Am. Chem. Soc.*, **71**, 2048 (1949).

(3) M. Bockmuhe and G. Ehrart, German Patent 865,314 (Feb 2, 1953); *Chem. Abstr.*, **53**, 295 (1959).

(4) P. B. J. Jaussen, U. S. Patent 3,030,372 (April 17, 1962); *Chem. Abstr.*, **59**, 2780 (1963).

(5) C. Mannich and D. Lammering, *Ber.*, **55**, 3510 (1922).

(6) F. F. Blicke and E. S. Blake, *J. Am. Chem. Soc.*, **52**, 235 (1930).

(7) W. Wilson and Z. Y. Kyi, *J. Chem. Soc.*, 1321 (1952).

(8) T. Teshigahara, *Nippon Yakurigaku Zasshi*, **58**, 67 (1962); *Chem. Abstr.*, **59**, 3239 (1963).

(9) H. J. Florestano, M. E. Bahler, and A. D. Chandler, *J. Am. Pharm. Assoc.*, **46**, 539 (1957).

(10) R. S. Varma and W. L. Nobles, *J. Pharm. Sci.*, **57**, 1801 (1968).

(11) H. A. Luts and W. L. Nobles, *ibid.*, **54**, 67 (1965).

(12) J. H. Quastel and A. H. M. Wheatley, *Proc. Roy. Soc.* (London), **B112**, 60 (1932).

TABLE II
INHIBITORY EFFECTS OF β-AMINO KETONES ON PYRUVIC ACID OXIDATION

No.	Inhibition of pyruvic[a] acid, 5×10^{-4} M	
	−NAD	+NAD
I	46.1 ± 0.47	32.3 ± 0.63
II	44.3 ± 0.41	45.3 ± 0.76
III	76.4 ± 0.47	49.1 ± 1.40
IV	34.5 ± 0.70	21.8 ± 1.00
V	61.8 ± 0.94	22.6 ± 0.47
VI	10.3 ± 1.10	13.4 ± 0.65

[a] Values indicate mean per cent inhibition calculated from the decrease in oxygen uptake per 100 mg of fresh weight per hour. All values are mean of four duplicate runs.

pound studies, β-hexamethyleneimono-4-piperidinopropiophenone (III) was found to be the most potent inhibitor of pyruvic acid (Table I).

The inhibition produced by the β-amino ketones was reduced in the presence of added NAD except in the case of II and VI. In these instances, the addition of NAD in no way altered their inhibitory effects. These results are in agreement with those of other investigators in which similar protection was observed by added NAD.[13] It was interesting to note that the inhibition produced by the β-amino ketones having a dimethyl substitutent (VI) could not be protected by added NAD. At present, it is difficult, and, indeed with these limited data, impossible to define the exact role that the substituents play in permitting the added NAD to afford protection against the inhibitory effects of the β-amino ketones. Further studies in progress dealing with the effect of β-amino ketones of this type on other dehydrogenases using purified enzyme preparations may possibly elucidate the site and mechanism of action of these β-amino ketones.

Experimental Section[14]

β-Amino Ketones.—To a mixture of 4-piperidinoacetophenone hydrochloride (0.05 mole), the appropriate secondary amine hydrochloride (0.05 mole), and paraformaldehyde (1.5 g) were added 10 ml of EtOH and two drops of concentrated HCl. The reaction mixture was refluxed on a water bath for 2 hr. At the end of this period 1 g of paraformaldehyde was added and the mixture was further refluxed for 6 hr. After distilling off excess EtOH under vacuum, 50 ml of dry MeAc was added to the viscous mass thus obtained. The product obtained after overnight refrigeration was removed by filtration and recrystallized from EtOH–MeAc. The purity of these compounds was ascertained by their melting points, elemental analysis, and infrared spectra. Data for the various amino ketones are recorded in Table I.

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(13) P. K. Seth and S. S. Parmar, *Can. J. Physiol. Pharmacol.*, **43**, 1019 (1965).

(14) Melting points were taken in capillary tubes on a Thomas–Hoover apparatus and are uncorrected. Elemental analyses were obtained from Galbraith Laboratories, Inc., Knoxville, Tenn. Infrared spectra were recorded in Nujol mull on a Perkin-Elmer Model 137 Infracord spectrophotometer and were as expected.

Synthesis of 6-Trifluoromethyl-1,4-dihydrazinophthalazine

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In a previous paper[1] we described the synthesis of various 1-hydrazinophthalazines carrying a fluorine atom on different positions of the benzene ring, in order to investigate the effect of this substitution on the pharmacological activity. We have now prepared 6-trifluoromethyl-1,4-dihydrazinophthalazine dihydrochloride (V).

The preparation of V was accomplished according to the general procedure for the synthesis of hydrazinophthalazines[2,3] with minor modifications. 4-Trifluoromethylphthalic anhydride (I), described as a mobile liquid by Loev[4] and as a dark solid by Lombardino,[5] was obtained in a pure state by distillation *in vacuo*; a solid product resulted, mp 65°. By condensing I with hydrazine hydrate 6-trifluoromethyl-2,3-dihydro-1,4-phthalazinedione (II) was obtained. This was converted into 5-trifluoromethyl-1,4-dichlorophthalazine (III), which with an equivalent amount of sodium methoxide furnished 6-trifluoromethyl-1(4)-chloro-4(1)-methoxyphthalazine (IV). By action of hydrazine hydrate on IV the desired 6-trifluoromethyl-1,4-dihydrazinophthalazine was obtained and then transformed into the more stable dihydrochloride (V).

Pharmacology.—Compound V was studied for hypotensive activity in conscious renal hypertensive rats[6] and dogs[7] and in anesthetized normotensive dogs, for adrenergic blocking activity in dogs, and for cardiac action on isolated guinea pig hearts (Langendorff). Acute oral toxicity was investigated in mice and dogs.

Blood pressure was measured in renal hypertensive rats and dogs by an indirect method, before treatment by the oral route and 2 and 4 hr later, for 4 consecutive days; the minimal effective dose is 3 mg/kg in both species. A drop of 13 mm was produced even by 0.5 mg/kg iv. The hypotensive effect increases proportionally with dosage. In the anesthetized dogs a reduction of the pressor response to epinephrine was induced by doses as low as 0.1 mg/kg iv. In isolated guinea pig hearts doses of 5 and 20 μg increased the coronary flow by 21 and 50%, respectively, and did not modify the heart rate and amplitude of contractions.

The approximate LD_{50} by the oral route in mice is 280 mg/kg; in dogs it produced convulsions and death in three out of four animals treated with 100 mg/kg orally, while only sedation and anorexia were observed in two animals treated with 75 and 50 mg/kg.

Dihydrazinophthalazine was tested in comparison with V in hypertensive rats and dogs. In rats the minimal effective oral dose was 3 mg/kg. In two dogs oral dosing with 2 mg/kg induced a drop in blood

(1) B. Cavalleri and E. Bellasio, *Farmaco, Ed. Sci.*, **24**, 833 (1969).
(2) S. Biniecki and J. Z. Izdebski, *Acta Polon. Pharm.*, **15**, 421 (1957).
(3) A. Hirsch and D. Orphanos, *Can. J. Chem.*, **43**, 2708 (1965).
(4) B. Loev, *ibid.*, **42**, 1488 (1964).
(5) J. G. Lombardino, *J. Org. Chem.*, **32**, 1989 (1967).
(6) A. Grollmann, *Proc. Soc. Exp. Biol. Med.*, **57**, 102 (1944).
(7) H. Goldblatt, J. Lynch, R. F. Hanzel, and W. W. Summerville, *J. Exp. Med.*, **59**, 227 (1937).