1,2,3,4,6,7,12,12a-Octahydro-2-phenylpyrazino-[2′,1′:6,1]pyrido[3,4-*b*]indole

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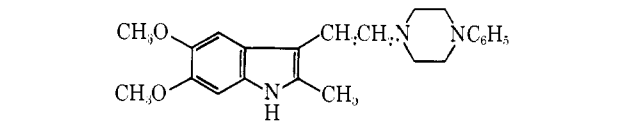
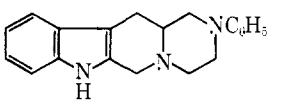
We wish to report the synthesis of the title compound (II) which we believe represents a new ring system. The indole II was of potential interest to us because of its structural relationship to the major tranquilizer oxypertine (I).[1]

The tricyclic ester III was prepared in two steps from tryptophane by a literature method.[2] Treatment of III with $ClCH_2COCl$ furnished the amide IV which was heated with $PhNH_2$ in Cellosolve to afford the cyclized product V. Reduction of V with LAH provided the desired amine II.

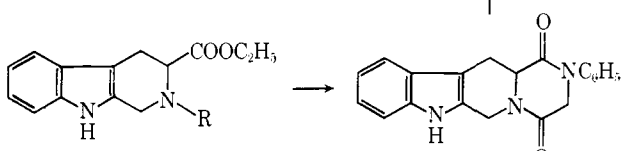
In contrast to oxypertine, compound II is not a CNS depressant. It failed to potentiate hexobarbital at 100 mg/kg po in mice.[1b] At a dose of 1 mg/kg po in mice, II produced a 91% increase in spontaneous activity, whereas oxypertine had caused a marked decrease.[1a,3]



I



II



III, R = H
IV, R = $COCH_2Cl$

V

Experimental Section[4]

Ethyl 2-(Chloroacetyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-*b*]-indole-3-carboxylate (IV).—A solution of 11.3 g (0.10 mole) of $ClCH_2COCl$ in 15 ml of $CHCl_3$ was added over 40 min to a stirred solution of 10.0 g (0.041 mole) of ethyl 1,2,3,4-tetrahydro-9H-pyrido[3,4-*b*]indole-3-carboxylate (III)[2] in 200 ml of $CHCl_3$. The mixture was refluxed for 6 hr, 20 ml of MeOH was added, and the solvent was removed *in vacuo*. The crystalline residue was recrystallized from C_6H_6–*n*-heptane (charcoal) to give 11.0 g (84%) of tan prisms, mp 143–145°. *Anal.* ($C_{16}H_{17}ClN_2O_3$) Cl, N.

2,3,6,7,12,12a-Hexahydro-2-phenylpyrazino[2′,1′:6,1]pyrido-[3,4-*b*]indole-1,4-dione (V).—A mixture of 18.0 g (0.056 mole) of IV (above), 6.7 g (0.072 mole) of $PhNH_2$, and 300 ml of Cellosolve was refluxed 18 hr. The solvent was removed *in vacuo*, the residue was extracted with hot EtOAc, and the extracts were washed with dilute aqueous HCl and aqueous NaCl. Removal of the EtOAc left a residue which could be crystallized directly. However, it was preferable to chromatograph the material on silica. Elution with EtOAc gave a solid which was washed with Et_2O, then recrystallized from EtOAc to furnish 10.3 g (56%) of tan prisms, mp 254–257°. *Anal.* ($C_{20}H_{17}N_3O_2$) C, H, N.

1,2,3,4,6,7,12,12a-Octahydro-2-phenylpyrazino[2′,1′:6,1]pyrido-[3,4-*b*]indole (II).—A mixture of 5.70 g (0.017 mole) of V, 3.3 g (0.088 mole) of LAH, and 500 ml of dry THF was refluxed for 48 hr. After cooling, aqueous THF was added, and the mixture was filtered. The insoluble material was washed with hot THF and the solvent was removed from the combined filtrates to give a dark residue which was chromatographed on silica. Elution with C_6H_6–Et_2O gave crystals which were recrystallized from C_6H_6–heptane to furnish 1.54 g (30%) of tan product, mp 228–232°. *Anal.* ($C_{20}H_{21}N_3$) C, H, N.

(1) (a) S. Archer, D. W. Wylie, L. S. Harris, T. R. Lewis, J. W. Schulenberg, M. R. Bell, R. K. Kullnig, and A. Arnold, *J. Amer. Chem. Soc.*, **84**, 1306 (1962); (b) D. W. Wylie and S. Archer, *J. Med. Pharm. Chem.*, **5**, 932 (1962).

(2) J. LeMen and C. Fan, *Bull. Soc. Chim. Fr.*, 1866 (1959).

(3) Data supplied by the Department of Pharmacology.

(4) Melting points were taken in capillaries and are uncorrected. Analytical results were determined by Mr. K. D. Fleischer and staff. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values. Ir spectra of all compounds are compatible with the assigned structures.

Electronic Factors in Drug–Receptor Interactions

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The recent report by Cammarata[1] presented electronic mechanisms by which a drug and its receptor may interact. Pertinent equations for testing these mechanisms were also included. However, not all of the examples were tested statistically, and in one case a more rigorous analysis of the data was possible, yielding evidence in support of the mechanism. The following calculations have been performed on data in the above paper.

The relationship between frontier orbital charge density at the carbonyl carbon (f) and the potency of some nicotinic acid derivatives as inhibitors of acetylcholine esterase was noted[2] (Table I). The following equation

TABLE I
POTENCY OF NICOTINIC ACID DERIVATIVES AS INHIBITORS OF ACETYLCHOLINE ESTERASE[2]

Compound	Frontier orbital density, f	pI_{50} Obsd	pI_{50} Calcd, eq 1	pI_{50} Calcd, eq 2
Nicotinic acid	0.262	0.3	0.146	
Nicotinamide	0.616	1.2	2.03	1.23
3-Acetylpyridine	0.657	2.3	2.25	2.18
Ethyl nicotinate	0.699	3.1	2.47	3.15

was derived by us from the data by a least-squares technique. The correlation coefficient is not signifi-

$$pI_{50} = -1.247 + 5.322f \quad r = 0.868 \quad F_{1,2} = 6.16 \quad (t = 2.48) \qquad (1)$$

cantly different from zero.[3] If the nicotinic acid is omitted from the series (it is the only compound which would be in an ionic form at the pH of the studies) the

(1) A. Cammarata, *J. Med. Chem.*, **11**, 1111 (1968).

(2) A. Inouye, Y. Shinagawa, and Y. Takaishi, *Arch. Intern. Pharmacodyn.* **144**, 319 (1963).

(3) N. Draper and H. Smith, "Applied Regression Analysis," John Wiley & Sons, Inc., New York, N. Y., 1966.

following equation may be calculated. This equation

$$pI_{50} = 23.1f - 13.0 \quad r = 0.995 \quad F_{1,1} = 104 \quad (t = 10.18) \qquad (2)$$

is also not statistically significant. Thus there is no statistical evidence that there is a relationship between f and pI_{50} in this series. It would be interesting to have a more extensive set of compounds to test in this respect.

A second example of electronic factors in drug interactions is the apparent relationship between the theoretically based values for the total electronic charge on the amide nitrogen (q_N) and the potency of carbamoylpiperidines as cholinesterase inhibitors[1,4] (Table II).

TABLE II
POTENCY OF N-SUBSTITUTED 1-DECYL-3-(CARBAMOYL)PIPERIDINES AS CHOLINESTERASE INHIBITORS[4]

Substituent on carbamoyl nitrogen				pI_{50}	
1	2	q_N	π[a]	Obsd	Calcd, eq 4
H	H	0.137	0	4.206	4.18
CH_3	H	0.212	0.52	4.459	4.47
C_2H_5	H	0.209	1.04	4.864	4.76
CH_3	CH_3	0.276	1.04	4.666	4.76
C_2H_5	C_2H_5	0.273	2.08	5.27	5.35
C_3H_7	C_3H_7	0.270	3.12	5.979	5.93

Purcell, *et al.*,[5] reported that "at least 75% of the observed cholinesterase inhibition can be accounted for from a linear relationship between log I_{50} and log (partition coefficient)," an empirical or experimental value. Electronic factors were also thought to be important but no regression equations were given. In our calculations the following relationships were found. Thus, the empirically derived Hansch π constant[6] for the octanol–H_2O system is the only statistically significant predictor of inhibitor potency. However, there is a significant correlation between the empirical π value and the theoretically based q_N ($r = 0.761$, $t = 3.10$, $p < 0.05$). This relationship illustrates the frequently observed interdependence of the various physical chemical parameters within a series of drug molecules. (For example, Rogers and Cammarata[7] have recently reported on the correlation of superdelocalizability and total absolute charge density with octanol–buffer partition coefficients for a series of aromatic molecules.) Because of such interdependence between what one would label electronic factors and what is thought of as hydrophobic and lipophilic parameters one must be cautious in postulating the mechanism of drug action on the basis of structure–activity studies alone.

$$pI_{50} = 3.04 + 8.116q_N \quad r = 0.696 \quad F_{1,4} = 3.77 \quad (t = 1.94) \qquad (3)$$

$$pI_{50} = 4.17 + 0.561\pi \quad r = 0.992 \quad F_{1,4} = 279 \quad (t = 16.71) \qquad (4)$$

$$pI_{50} = 4.47 + 1.63q_N + 0.623\pi \quad (t = 2.06)\ (t = 16.16) \quad r = 0.997 \quad F_{2,3} = 255 \qquad (5)$$

Cammarata[1] also considered the frontier-controlled interaction of a series of sulfanilamides against *Escherichia coli* (Table III). The equation which was reported for the phenyl derivatives contained no statistically significant terms. Thus a combination of frontier electron density, total charge density, and the square of the total charge density does not "explain" the variation in activity. However, using six compounds to fit an equation with three terms results in only two degrees of freedom, and therefore a correlation of these terms might be possible if more data were obtained.

TABLE III
MINIMUM INHIBITORY CONCENTRATION OF SULFONAMIDES AS A FUNCTION OF ELECTRONIC CHARACTER OF THE CORRESPONDING ANILINES AND BENZAMIDES

Substituent	σ_N	q_N	q_N^2	pC_m Obsd	pC_m Calcd, eq 7
Phenyl Derivatives					
4-NH_2	0.383	0.077	0.006	4.35	4.25
4-OCH_3	0.453	0.081	0.007	4.47	4.40
4-CH_3	0.453	0.081	0.007	4.57	4.40
H	0.477	0.083	0.007	4.80	5.25
4-Cl	0.459	0.082	0.007	4.80	4.78
4-NO_2	0.484	0.106	0.011	5.85	5.73
Benzoyl Derivatives					
3-CH_3–4-OCH_3	0.041	0.148	0.022	5.25	5.27
4-OCH_3	0.035	0.148	0.022	5.40	5.22
3,4-CH_3	0.042	0.148	0.022	5.40	5.28
4-CH_3	0.036	0.148	0.022	5.40	5.23
3-CH_3	0.018	0.149	0.022	5.40	5.39
H	0.000	0.149	0.022	5.25	5.23
4-Cl	0.027	0.148	0.022	5.10	5.15
4-CN	0.000	0.151	0.023	4.05	4.14
4-NO_2	0.000	0.153	0.023	4.50	4.78

[a] The negative log of the minimum inhibitory concentration *vs. E. coli*.

The equation reported by these workers for the benzoyl derivatives could not be confirmed. Our equation is the following. All terms in this equation are statistically significant. However, q_N varies by only 6% in this series, and q_N^2 values in the original report and in these calculations were rounded off to two significant figures. If one calculates such an equation using q_N^2 calculated from q_N rather than taken from the table (which were rounded off) q_N and q_N^2 are so highly correlated ($r = 0.999$) that it is unreasonable to include both in the same equation in the absence of any theoretical reason for doing so. Thus, although the equation (6) *appears* to be statistically valid, it is a quirk of rounding off plus extremely small variation in one variable under examination which produced such results.

$$pC_m = 6.54 + 8.86\sigma_N + 237.92q_N - 1671.07q_N^2 \quad (t = 2.75)\ (t = 3.71)\ (t = 7.76) \quad r = 0.987 \quad F_{3,5} = 63.59 \qquad (6)$$

A better test of the influence of electronic factors on the mimimum inhibitory concentration is to include all 15 compounds in one equation. (D is a dummy variable.)

$$pC_m = -4.3089 + 8.759\sigma_N + 319.31q_N - 1733.10q_N^2 - 9.08D \quad (t = 3.66)\ (t = 5.77)\ (t = -5.44)\ (t = 5.16) \quad r = 0.939,\ F_{4,10} = 18.65 \qquad (7)$$

(4) W. P. Purcell, *J. Med. Chem.*, **9**, 294 (1966).
(5) W. P. Purcell, J. G. Beasley, R. P. Quintana, and J. A. Singer, *ibid.*, **9**, 297 (1966).
(6) J. Iwasa, T. Fujita, and C. Hansch, *ibid.*, **8**, 150 (1965).
(7) K. S. Rogers and A. Cammarata, *ibid.*, **12**, 692 (1969).

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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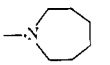
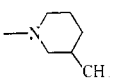
Substituted β-amino ketones (Table I) have elicited a variety of physiological responses; various workers have demonstrated that compounds of this type possess antispasmodic,[2,3] analgetic,[4] local anesthetic,[5–8] and antibacterial[9,10] activity. Luts and Nobles[11] observed anticonvulsant, analgetic, 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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