# Evaluation of Some Mannich Bases Derived from Substituted Acetophenones Against P-388 Lymphocytic Leukemia and on Respiration in Isolated Rat Liver Mitochondria

**J. R. DIMMOCK *x, K. SHYAM *, N. W. HAMON *, B. M. LOGAN *, S. K. RAGHAVAN *, D. J. HARWOOD *, and P. J. SMITH ‡**

Received March 1, 1982, from the **College of Pharmacy* and ‡*Department of Chemistry and Chemical Engineering, College of Arts and Science, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, Canada.* Accepted for publication July 7, 1982.

**Abstract** □ Series of 3-dimethylamino-1-aryl-1-propanone hydrobromides (IV) and 3-dimethylamino-2-dimethylaminomethyl-1-aryl-1-propanone dihydrobromides (V) were synthesized. Evaluation of these derivatives against P-388 lymphocytic leukemia growth revealed that two compounds show promise as antineoplastic agents. Compounds of the V series were unstable in phosphate buffer (in contrast to series IV), and when the same nuclear substituent was present in both series of compounds, V was ~100 times more active than IV in both the stimulation and inhibition of respiration of mitochondria isolated from rat liver cells. Representatives from both series showed that respiration in mitochondria was affected by changing the pH of the aqueous buffer from 7.4 to 6.9 or 6.4 and by reducing the temperature from 37° to 20°. The compounds showed reactivity toward a biomimetic thiol.

**Keyphrases** □ 3-Dimethylamino-1-aryl-1-propanone hydrobromides—synthesis, activity against P-388 lymphocytic leukemia growth, effect on mitochondrial respiration □ 3-Dimethylamino-2-dimethylaminomethyl-1-aryl-1-propanone dihydrobromides—synthesis, activity against P-388 lymphocytic leukemia growth, effect on mitochondrial respiration □ Antineoplastic agents—potential, 3-dimethylamino-1-aryl-1-propanone hydrobromides, 3-dimethylamino-2-dimethylaminomethyl-1-aryl-1-propanone dihydrobromides, activity against P-388 lymphocytic leukemia growth

When arylalkyl ketones such as propiophenone (I) are attacked by nucleophiles such as thiols, thiohemiketals are formed (1). While the rate of nucleophilic attack may be increased by electron-withdrawing substituents on the aromatic ring, other molecular modifications of these compounds, such as the formation of the corresponding Mannich bases (*e.g.*, II), allow the possibility of increased nucleophilic attack at the carbonyl carbon atom. First, the proton attached to the quadrivalent nitrogen in II is acidic and therefore intramolecular proton transfer to the carbonyl oxygen may occur, expediting nucleophilic attack at the carbonyl carbon atom. Second, such additions as in III, exemplified by the attack of a thiol, lead to a reaction intermediate where there is relief from dipole repulsion between the carbonyl and onium groups.

There are, however, two other ways the Mannich bases could interact with cellular nucleophiles, *per se* or after undergoing molecular modification to give reactive derivatives. First, the strong positive inductive effect of the ammonium group [Taft $\sigma^*$ value for $(H_3C)_3\overset{+}{N}$ is 1.90 (2)] renders the carbon atom of the adjacent methylene group susceptible to nucleophilic attack. Instances may be cited where thiol displacements have occurred (3) although, on occasion, vigorous conditions have been required (4). In the second case, instances have been described whereby Mannich bases have undergone elimination to give the corresponding enones (5, 6); such compounds readily undergo nucleophilic attack (7). Treatment of a variety of 3-dialkylaminopropiophenones with thiophenols gave the corresponding sulfides, possibly formed by an elimination–addition mechanism (8). For these reasons the synthesis of the series of IV compounds for antineoplastic evaluation was contemplated.

When a second dimethylaminomethyl group is introduced into the molecule, as in V, the proton of the methine group adjacent to the carbonyl group is more acidic than the methylene protons adjacent to the carbonyl group in IV. Hence, compounds of the V series have a greater tendency to undergo elimination than IV compounds. Furthermore, after the loss of one onium group in V, the olefin formed may react in a facile manner with nucleophiles, and expedition of the loss of the remaining quaternary ammonium group could occur generating a further center for nucleophilic attack.

Some of a series of Mannich bases (VI) have activity in the P-388 screen. These compounds, which resemble IV, except that an olefinic linkage separates the aromatic ring from the keto function, affected basal respiration in mitochondria isolated from liver cells of male Wistar rats at concentrations as low as 1–5 μmoles (9, 10). One of these compounds [VI, $R^1$ = 3-OH; $R^2$ = H; $R^3$ = $(CH_2)_4CH_3$] elicited a biphasic response: at low dose levels (*e.g.*, 1.25 μmoles), stimulation of respiration occurred, while at higher doses (5 μmoles), inhibition of respiration was noted (11). The stimulation of respiration was shown to be due to the uncoupling of oxidative phosphorylation, while the inhibition was due to the blocking of the electron transport chain between cytochromes b and $c_1$ (11). Thus, it was considered of interest to evaluate the antineoplastic activity and the effect on mitochondrial respiration of series IV and V compounds and to determine whether a corre-

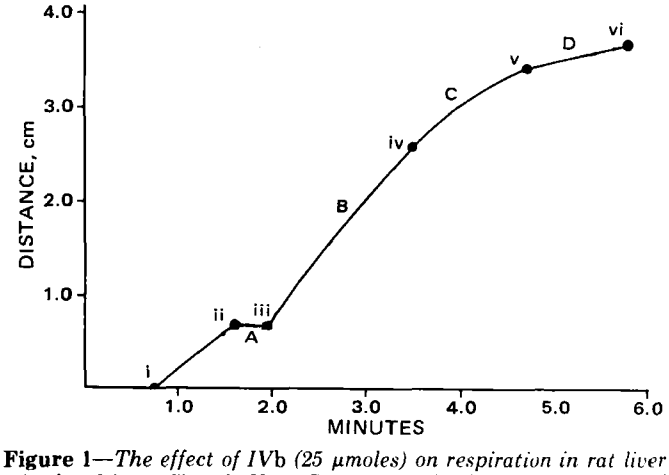



**Figure 1**—*The effect of IVb (25 μmoles) on respiration in rat liver mitochondria at 37° and pH 7.4. Succinate and IVb were added at* i *and* ii, *respectively. The periods A (between* ii *and* iii) *and C (between* iv *and* v) *represent lag periods prior to the constant maximal stimulation (phase B) and constant maximal inhibition (phase D) of respiration.*

0022-3549/83/0800-0887$01.00/0

**Table I—Physical Data, Activity Against P-388 Lymphocytic Leukemia in Mice, and Murine Toxicity of the Amine and Diamine Hydrobromides IV and V**

| Compound | Yield, % | Melting Point, ° | Formula | Analysis, % Calc. C | Calc. H | Calc. N | Found C | Found H | Found N | T/C%[a] (dose in mg/kg) | Murine Toxicity[b] (dose in mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IV*a* | 60 | 180–181 | $C_{11}H_{16}BrNO$ | 51.17 | 6.24 | 5.43 | 50.76 | 6.17 | 5.41 | 110(100) | 0(200), 6(100) |
| IV*b* | 63 | 183–184 | $C_{11}H_{15}BrClNO$ | 45.14 | 5.17 | 4.79 | 45.11 | 5.26 | 4.79 | 106(50) | 1(200), 5(100), 6(50) |
| IV*c* | 54 | 199–200 | $C_{11}H_{14}BrCl_2NO$ | 40.39 | 4.31 | 4.28 | 40.43 | 4.48 | 4.17 | 107(50) | 1(200), 5(100), 6(50) |
| IV*d* | 41 | 164–166 | $C_{12}H_{18}BrNO$ | 52.94 | 6.67 | 5.15 | 52.63 | 6.68 | 5.12 | 107(100) | 0(200), 6(100) |
| IV*e* | 51 | 178–179 | $C_{12}H_{18}BrNO_2$ | 50.01 | 6.29 | 4.86 | 50.08 | 6.39 | 4.73 | 104(50) | 5(200, 100), 6(50) |
| V*a* | 23 | 187–188 | $C_{14}H_{24}Br_2N_2O$ | 42.44 | 6.11 | 7.07 | 42.32 | 6.29 | 7.03 | 117(25) | 2(50), 6(25) |
| V*b* | 15 | 180–181 | $C_{14}H_{23}Br_2ClN_2O$ | 39.04 | 5.38 | 6.51 | 38.91 | 5.57 | 6.48 | 116(25) | 3(50), 6(25) |
| V*c* | 11 | 188–189 | $C_{14}H_{22}Br_2Cl_2N_2O$ | 36.15 | 4.77 | 6.02 | 36.09 | 4.81 | 5.96 | 108(12.5) | 3(100), 6(50) |
| V*d* | 12 | 184–185 | $C_{15}H_{26}Br_2N_2O$ | 43.92 | 6.39 | 6.83 | 43.68 | 6.64 | 6.82 | 138(25) | 0(50), 6(25) |
| V*e* | 24 | 204–206 | $C_{15}H_{26}Br_2N_2O_2$ | 42.27 | 6.15 | 6.57 | 41.82 | 6.08 | 6.57 | 136(25) | 4(50), 6(25) |

[a] Anticancer activity is expressed as the ratios of the survival time of the treated animals to control animals expressed as a percentage. All of the compounds were initially screened at 200, 100, and 50 mg/kg; if mortalities occurred at these doses, they were reduced to nonlethal levels. A compound should increase the median survival time by ≥20% to be considered active. [b] These figures are survivors out of six mice on the fifth day after commencement of the dosage schedule (nine daily doses given intraperitoneally) except V*b* and *c* in which cases the compounds were administered for five consecutive days only.

lation between these two biological parameters exists. In addition, a comparison of the general bioactivity of IV and V could reveal molecular features associated with efficacy against the growth of P-388 lymphocytic leukemia and effect on mitochondrial function.

The effect of temperature on the pattern of respiration was considered of interest since recent reports indicate alterations in mitochondrial function with changes in temperature (12, 13). The compounds could serve as biochemical tools. For example, if both stimulation and inhibition of mitochondrial respiration occurs at 37° and only inhibition occurs when the temperature is lowered to 20°, the effects of concentration can be determined for inhibition of respiration alone.

A number of investigators have shown that the pH of certain tumors is ~7.0 (14, 15), and in addition, the administration of glucose to tumor-bearing animals has lowered the pH of the neoplasm to 6.4 while the pH of normal tissue was unaffected (14, 15). Earlier work was shown that certain compounds in series VI had increased respiratory-inhibiting properties in mitochondria obtained from both rat liver cells and from the Morris 5123 TCH tumor (16) as the pH was lowered from 7.4 to 6.9 and then from 6.9 to 6.4. The question was posed as to whether a similar variation in effect in mitochondria would occur with series IV and V compounds.

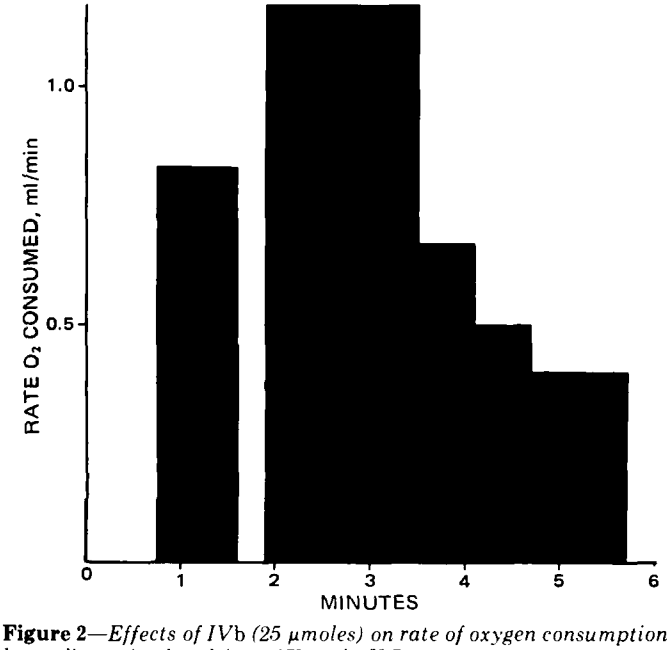
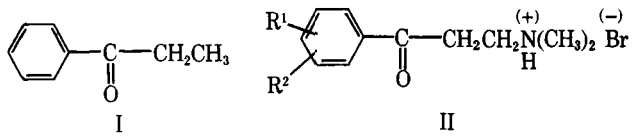



**Figure 2**—*Effects of IV*b *(25 μmoles) on rate of oxygen consumption by rat liver mitochondria at 37° and pH 7.4.*

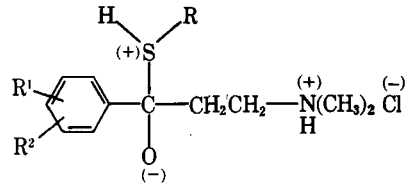
## RESULTS AND DISCUSSION

The two series of compounds (IV and V) were prepared and assessed for activity against P-388 lymphocytic leukemia growth (Table I). The dose schedule was constant for compounds of both series except fewer doses of V*b* and *c* were administered. From the data available, it may be seen that, while the monobasic compounds of series IV were uniformly inactive against murine P-388 lymphocytic leukemia growth, variation in antineoplastic activity in series V compounds was found. Compounds V*d* and *e* showed significant activity in this screen, V*a* and *b* displayed marginal potencies, and V*c* was inactive. The compounds were less active than 5-fluorouracil, which has a T/C% of 182 at 25 mg/kg (20 daily doses) in the P-388 murine screen (17). All of the compounds in Table I showed toxicity; but, whereas no mortalities were found at 50 mg/kg in series IV, only the inactive diamine of the V series (V*c*) did not cause deaths at this dose level. No animals died when V*a–e* were administered at a dose of 25 mg/kg. Thus, with the exception of V*c*, greater antineoplastic activity accompanied by greater murine toxicity was found with series V than with series IV.

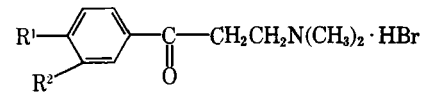
The reasons for the differences in bioactivities between series IV and V were then considered. Lability of a number of Mannich bases to produce the corresponding α,β-unsaturated ketones is well documented (6, 18) and, on occasions, this property has been invoked to explain the bioactivities of a number of Mannich bases (19, 20). Such deamination processes are possible in both IV and V, leading to the formation of substituted acrylophenones which could undergo attack with cellular nucleophiles such as thiols of proteins. Since the rate of deamination is dependent on the nature of the Hammett σ value in the aromatic ring, the stabilities of the compounds with the most divergent σ values namely IV*c* and V*c* ($\sigma = +0.60$) and IV*e* and V*e* ($\sigma = -0.27$) were examined under simulated physiological conditions (buffer of pH 7.4 and 37°). Both IV*c* and *e* were stable for at least 1.5 hr; after 5 min, degradation of V*c* and *e* to VII*a* and *b* occurred as evidenced by extraction of the reaction medium and analysis by NMR. When the temperature was reduced to 5°, V*c* gave rise to a mixture of VII*a* and V*c* as the free base in a ratio of 10:1 after 5 min, while V*e* gave rise to the free base and no olefin. After 1 hr at 5°, V*e* gave a mixture of VII*b* and the free base of V*e* in a ratio of 7:4. Hence, the comparative rates of deamination of V*c* and *e* follow that predicted by a consideration of the electronic effects of the nuclear substituents. The difference between IV and V in ability to undergo elimination, producing the corresponding α,β-unsaturated ketones, is probably due to variation in the acidity of the protons in the β-position to the dimethylamino group, since the rate-determining step in such elimination reactions is the attack of the hydroxyl anion at the methylene carbon atom adjacent to the keto function. This facile elimination could permit reaction with cellular nucleophiles, and reaction of V*c* with a biomimetic thiol (2-mercaptoethanol) was undertaken. Preliminary experiments involving incubation of V*c* and 2-mercaptoethanol in buffer at 37° followed by extraction with chloroform gave a mixture of compounds, as revealed by TLC. However, when V*c* and the thiol were incubated for a short period of time in a mixture of buffer and chloroform, the product



I II

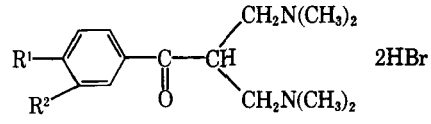


III



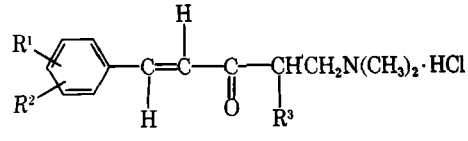
IV

*a*: $R^1 = R^2 = H$ *d*: $R^1 = CH_3$; $R^2 = H$
*b*: $R^1 = Cl$; $R^2 = H$ *e*: $R^1 = OCH_3$; $R^2 = H$
*c*: $R^1 = R^2 = Cl$



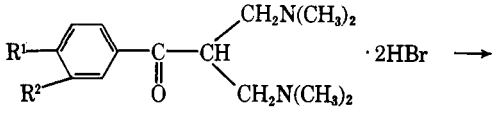
V

*a*: $R^1 = R^2 = H$ *d*: $R^1 = CH_3$; $R^2 = H$
*b*: $R^1 = Cl$; $R^2 = H$ *e*: $R^1 = OCH_3$; $R^2 = H$
*c*: $R^1 = R^2 = Cl$

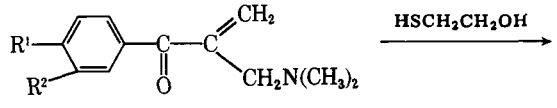


VI

$R^1 = R^2 = H$, Cl, OH
$R^3 = H$, $(CH_2)_4CH_3$

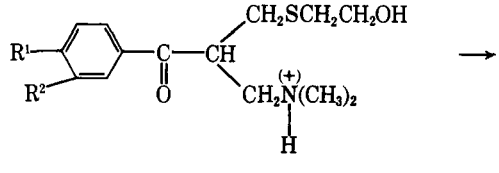


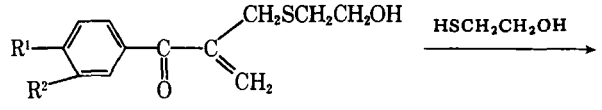
Vc,e

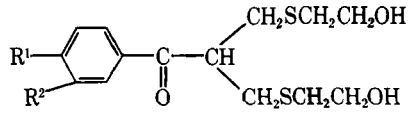


VII

*a*: $R^1 = R^2 = Cl$
*b*: $R^1 = OCH_3$; $R^2 = H$







VIII

*a*: $R^1 = R^2 = Cl$

*Scheme I*

isolated was shown by NMR to be VIII*a*, which could arise as shown in Scheme I. The mass spectral (MS) fragmentation pattern was consistent with the structure of VIII*a*; in particular, a molecular ion corresponding to the dithioether was observed and the absence of a prominent peak at *m/z* 58, which is often the base peak in Mannich bases due to the dimethylmethyleneimmonium ion, was noted. It was considered that the stability of IV compounds in buffer, and hence lack of formation of the corresponding olefins, may have led to inactivity of these compounds towards thiols. The least reactive member of the series, IV*e*, was incubated with 2-mercaptoethanol in the presence of chloroform and buffer, and the mixture was shown by NMR and MS to be principally 3-(2-hydroxyethylthio)-1-(*p*-methoxyphenyl)-1-propanone, *i.e.*, the dimethylamino group had been replaced by a 2-hydroxyethylthio function. Control experiments showed that the reaction between IV*e* and 2-mercaptoethanol occurred in the buffer and not in the organic solvent. Hence, elimination in series IV may occur in the presence of stronger nucleophiles than the hydroxide anion found in the aqueous buffer. It is conceivable, however, that the Mannich bases of IV and V could interact with thiols by a substitution reaction on the carbon atom adjacent to the dimethylamino group, but the synthesis of such derivatives is often accomplished in the presence of virtually nonpolar solvents and elevated temperatures (4). Hence, the elimination–addition mechanism seems more feasible.

The effect of IV and V compounds on respiration in rat liver mitochondria is given in Table II. At low concentrations in both series of compounds, stimulation of respiration occurs. In general, as the concentration is increased, stimulation of respiration increases and then diminishes while inhibition of respiration increases. In addition, the elevation of concentration shortens the time period prior to inhibition by IV and V compounds. A typical result is illustrated in Figs. 1 and 2. While from a qualitative viewpoint, both series of compounds exert similar effects, a quantitative evaluation of IV*a–e* with the compounds bearing a similar nuclear substituent in series V, indicates that IV requires ~100 times the concentration to elicit the same increases in both stimulation and inhibition of respiration as V. For example, doses of 25 μmoles of IV*a–e* cause similar percentage inhibitions of respiration in mitochondria as 0.25-μmole concentrations of V*a–e*. At comparable concentration levels, the olefins VII*a* and *b* have similar respiration-inhibiting properties as their progenitors V*c* and *e*, although the effect on stimulation of respiration appears somewhat different. Two representative compounds in series IV and V were examined for their effect on mitochondria at 20° and, in general, stimulation of respiration was increased and inhibition of respiration diminished at this temperature compared with 37° (Table III). The respiration-inhibitory data generated for V*a* and *c* are comparable to the olefinic analogues VI ($R^1 = R^2 = H$) and VI ($R^1$ = 3-CL; $R^2$ = 4-CL), respectively (9).

A comparison of the effect on respiration in mitochondria between a compound displaying significant activity against P-388 lymphocytic leukemia (V*e*) and an inactive analogue (V*c*) at pH values of 6.9 and 6.4 is summarized in Table IV. With the exception of the 1.0-μmole dose for V*c*, the largest increase in stimulation of respiration is at the pH of normal cells (7.4) in the case of V*c*, while the maximal increase in stimulation for the active compound V*e* is at pH 6.4. If antineoplastic activity is influenced by the effect on stimulation of respiration (*e.g.*, by the uncoupling of oxidative phosphorylation impairing the synthesis of adenosine triphosphate), selective toxicity under the acidic conditions of the tumorous cells is conceivable. When inhibition of respiration was examined, an alternate situation was found; *i.e.*, minimal percentage inhibition of respiration occurs with both V*c* and *e* at pH 6.4. Hence, the bioactivity of V*e* in contrast to V*c* is explicable only in terms of the effect on stimulation and not inhibition of respiration if the effect on mitochondrial function is associated with anticancer properties and the pH of malignant cells is much lower than that of the corresponding normal cells.

In conclusion, the following generalizations regarding chemical structure and bioactivity may be made. First, series V was found to be more active against P-388 lymphocytic leukemia growth and to display greater murine toxicity than series IV, possibly due to the greater fragility

**Table II—Effect on Respiration in Rat Liver Mitochondria Using Succinate as the Substrate at pH 7.4 and 37°**

| Compound | Concentration, μmoles | Stimulation, % | *SE* | Time Prior to Constant Inhibition of Respiration, min | *SE* | Inhibition, % | *SE* |
|---|---|---|---|---|---|---|---|
| IV*a* | 10 | 37.32 | 13.22 | — | — | 0 | — |
| | 25 | 35.57 | 6.09 | 6.72 | 0.90 | 54.18 | 12.21 |
| | 50 | 28.22 | 5.10 | 7.76 | 0.86 | 57.44 | 4.87 |
| | 100 | 28.62 | 2.86 | 4.43 | 0.35 | 72.38 | 2.19 |
| IV*b* | 5 | 35.26 | 7.67 | 3.68 | 0.18 | 48.75 | 8.92 |
| | 10 | 45.03 | 12.18 | 3.36 | 0.16 | 50.36 | 11.79 |
| | 25 | 57.24 | 4.69 | 3.08 | 0.16 | 63.96 | 1.88 |
| | 35 | 27.79 | 5.79 | 3.38 | 0.20 | 86.09 | 2.11 |
| IV*c* | 5 | 68.48 | 15.13 | 3.89 | 0.36 | 84.86 | 2.73 |
| | 10 | 63.27 | 6.26 | 2.22 | 0.19 | 63.38 | 4.73 |
| | 25 | 0 | — | 1.00 | 0.09 | 87.76 | 3.91 |
| IV*d* | 10 | 39.93 | 7.96 | 1.50 | 0.92 | 4.31 | 3.28 |
| | 25 | 92.89 | 6.54 | 5.60 | 0.27 | 62.27 | 3.95 |
| | 50 | 34.23 | 8.47 | 3.58 | 0.29 | 46.83 | 9.68 |
| | 100 | 0 | — | 2.95 | 0.34 | 78.93 | 3.67 |
| IV*e* | 10 | 35.46 | 1.08 | — | — | 0 | — |
| | 25 | 52.18 | 7.82 | — | — | 0 | — |
| | 50 | 64.69 | 8.45 | 6.02 | 0.17 | 29.75 | 15.11 |
| | 100 | 49.11 | 5.61 | 5.22 | 0.21 | 56.58 | 2.26 |
| V*a* | 0.01 | 64.72 | 7.43 | — | — | 0 | — |
| | 0.1 | 38.65 | 5.29 | 2.81 | 1.05 | 9.81 | 4.21 |
| | 0.25 | 33.68 | 4.25 | 3.64 | 0.64 | 53.94 | 5.22 |
| | 1.0 | 20.02 | 1.97 | 5.14 | 1.10 | 70.60 | 4.37 |
| | 5.0 | 34.60 | 4.29 | 1.90 | 0.11 | 85.27 | 1.37 |
| | 10.0 | 12.22 | 3.23 | 1.28 | 0.12 | 87.58 | 2.79 |
| | 25.0 | 0 | — | 1.25 | 0.06 | 96.62 | 1.02 |
| V*b* | 0.01 | 32.14 | 9.19 | — | — | 0 | — |
| | 0.1 | 27.41 | 2.28 | 4.84 | 0.23 | 34.90 | 4.65 |
| | 0.25 | 37.56 | 5.44 | 3.64 | 0.27 | 70.97 | 5.78 |
| | 1.0 | 24.19 | 3.47 | 2.32 | 0.10 | 83.30 | 1.97 |
| | 10 | 16.48 | 1.54 | 0.84 | 0.02 | 89.33 | 2.46 |
| | 25 | 0 | — | 0 | — | 94.47 | 2.31 |
| V*c* | 0.01 | 38.58 | 8.65 | — | — | 0 | — |
| | 0.1 | 63.90 | 8.60 | 4.76 | 0.21 | 79.78 | 1.24 |
| | 0.25 | 79.86 | 9.44 | 1.66 | 0.79 | 79.08 | 4.61 |
| | 1.0 | 0 | — | 1.17 | 0.03 | 89.98 | 1.09 |
| | 10 | 0 | — | 0.20 | 0.03 | 97.35 | 1.16 |
| V*d* | 0.01 | 63.32 | 1.03 | — | — | 0 | — |
| | 0.1 | 28.87 | 6.94 | 3.32 | 0.68 | 23.65 | 5.67 |
| | 0.25 | 57.85 | 5.01 | 6.40 | 0.38 | 51.14 | 1.68 |
| | 1.0 | 56.67 | 4.47 | 2.82 | 0.10 | 64.88 | 4.33 |
| | 10 | 0 | — | 1.22 | 0.07 | 82.82 | 1.37 |
| | 25 | 0 | — | 0.87 | 0.07 | 97.36 | 0.57 |
| V*e* | 0.01 | 54.77 | 14.25 | — | — | 0 | — |
| | 0.1 | 18.41 | 1.99 | — | — | 0 | — |
| | 0.25 | 27.75 | 5.37 | 4.29 | 0.37 | 21.58 | 4.27 |
| | 1.0 | 34.00 | 5.99 | 3.88 | 0.21 | 55.60 | 1.63 |
| | 10 | 33.17 | 3.32 | 2.20 | 0.19 | 75.62 | 4.24 |
| | 12.5 | 60.08 | 7.05 | 1.88 | 0.12 | 78.97 | 6.61 |
| | 15 | 36.92 | 3.44 | 2.32 | 0.30 | 80.52 | 3.12 |
| | 25 | 17.25 | 7.77 | 1.64 | 0.10 | 90.54 | 0.99 |
| | 50 | 0 | — | 1.57 | 0.30 | 97.78 | 0.78 |
| VII*a* | 1.0 | 68.71 | 7.14 | 2.18 | 0.14 | 84.70 | 2.50 |
| | 10 | 0 | — | 0.65 | 0.04 | 90.25 | 1.76 |
| VII*b* | 1.0 | 37.00 | 8.37 | 6.94 | 0.30 | 36.25 | 7.10 |
| | 10 | 69.46 | 4.07 | 2.52 | 0.18 | 74.70 | 2.89 |
| | 25 | 49.34 | 4.84 | 1.42 | 0.02 | 81.85 | 3.06 |

of V in aqueous media in which decomposition to the corresponding enones occurred. The only exception was V*c*, which was inactive against the particular tumor under consideration and appeared to be less toxic than V*a*, *b*, *d*, and *e*. Retrospectively, the five substitution patterns in the aromatic ring in V were the ones recommended for a Topliss analysis (21), and the order of activity found in this series of compounds is claimed by such an approach to indicate the greater importance of the Hammett $\sigma$ value over the Hansch $\pi$ parameter. Second, murine toxicity in both IV and V compounds may be associated, at least partially, with interaction with cellular nucleophiles. Third, both series of compounds affected respiration in mitochondria isolated from rat liver cells, but compounds bearing the same nuclear substituents differed ~100-fold in the doses required to elicit similar effects. A direct correlation between antineoplastic activity and the effect on respiration in mitochondria was absent. Fourth, maximum antineoplastic activity is found with V*d* and *e*, although these do not represent the extremes in regard to either rate of decomposition or effect on respiration in mitochondria. Thus, if decomposition is proportional to the Hammett $\sigma$ value, V*a–c* liberate the corresponding acrylophenones faster than V*d* and *e* while the IV compounds appeared to be stable in aqueous media. In isolated mitochondria, a similar generalization appears valid, *i.e.*, V*a–c* are more active and IV is less active than V*d* and *e*. It is conceivable, therefore, that an optimal rate of breakdown is required for good activity against P-388 lymphocytic leukemia; *e.g.*, although V*c* is rapidly converted to VII*a*, which can affect mitochondria at very low dose levels, it may be inactivated prior to reaching a site of action. On the other hand, the absence of formation of

the acrylophenone or formation at a very slow rate could lead to insufficient compound for anticancer efficacy, which may involve the mitochondria as one of the targets. Relationships between the optimal chemical reactivity required for anticancer activity have been described previously (22), and the evolution of V*d* and *e* from this study permits these compounds to serve as lead compounds (23) in the design of further anticancer molecules.

## EXPERIMENTAL

Melting points are uncorrected. Elemental analyses were undertaken locally[1] and when necessary both solvents and organic extracts were dried with anhydrous magnesium sulfate. No attempt was made to optimize the percentage yields of compounds unless otherwise stated. TLC was carried out using sheets of silica gel with fluorescent indicator[2] and a solvent mixture of chloroform–methanol (10:1). Mass spectra[3] were run at 70 eV and the 60-MHz NMR spectra[4] were determined using tetramethylsilane as the internal standard. High-performance liquid chromatography (HPLC)[5] was undertaken using a column[6] eluted with acetate buffer (0.04 *M*) in acetonitrile.

**Synthesis**—*3-Dimethylamino-1-aryl-1-propanone Hydrobromides* (*IV*a, d, *and* e)—The crude hydrochloride salts of the Mannich bases derived from acetophenone, *p*-methylacetophenone, and *p*-methoxyacetophenone were prepared as previously described (24). The yields obtained for the hydrochloride salts corresponding to IV*b* and *c* were 42 and 16%, respectively. The crude hydrochloride salts of IV*a*, *d*, and *e* were converted to the hydrobromide salts by the procedure described below to give IV*a*, *d*, and *e* as colorless crystals from acetone–methanol. In an attempt to improve the yield of 1-(3,4-dichlorophenyl)-3-dimethylamino-1-propanone hydrochloride, a mixture of 3,4-dichloroacetophenone (9.45 g, 0.05 mole), paraformaldehyde (1.80 g, 0.06 mole) dimethylamine hydrochloride (5.30 g, 0.065 mole), hydrochloric acid (0.1 ml), and 1,2-dimethoxyethane (35 ml) was heated under reflux for 24 hr. The solid that separated on cooling was removed by filtration, dried, and recrystallized from ether–ethanol to give 1-(3,4-dichlorophenyl)-3-dimethylamino-1-propanone hydrochloride (4.2 g, 30%) as colorless crystals; mp 193–195° [lit. (25) mp 193–195°].

*1-(p-Chlorophenyl)-3-dimethylamino-1-propanone Hydrobromide* (*IV*b)—An excess of acetyl chloride was added to *N,N,N′,N′*-tetramethyldiaminomethane[7] (10.2 g, 0.1 mole) in dry methylene chloride (100 ml) at 0° with vigorous stirring. The precipitated Mannich reagent was removed by filtration, washed with methylene chloride, and dried *in vacuo* to give *N,N*-dimethyl(methylene)ammonium chloride (8.5 g). This compound was used without purification.

A solution of *p*-chloroacetophenone (15.50 g, 0.1 mole) in dry acetonitrile (40 ml) was added to the *N,N*-dimethyl(methylene)ammonium chloride (9.35 g, 0.1 mole), and the mixture was stirred at room temperature for 2 hr. The precipitate was removed by filtration, dried, and recrystallized from ether–methanol to give 1-(*p*-chlorophenyl)-3-dimethylamino-1-propanone hydrochloride (21.5 g, 84%) as colorless crystals, mp 173–175° (26).

An aqueous solution of 1-(*p*-chlorophenyl)-3-dimethylamino-1-propanone hydrochloride (6.20 g, 0.025 mole) was basified with aqueous sodium carbonate solution (10% w/v) and extracted with chloroform (3 × 15 ml). The chloroform extracts were combined, washed with water (2 × 5 ml), and dried with anhydrous magnesium sulfate; removal of the chloroform gave a viscous oil which was dissolved in acetone–ether (1:1) and treated with dry hydrogen bromide gas. The crude hydrobromide (5.0 g) which precipitated was removed, and recrystallization from ether–methanol gave 1-(*p*-chlorophenyl)-3-dimethylamino-1-propanone hydrobromide, IV*b* (5.5 g), as colorless crystals. The yield of IV*b* was 63% based on the quantity of *p*-chloroacetophenone utilized.

*1-(3,4-Dichlorophenyl)-3-dimethylamino-1-propanone Hydrobromide* (*IV*c)—This compound was prepared in the same manner as IV*b*. The yield for the formation of 1-(3,4-dichlorophenyl)-3-dimethylamino-1-propanone hydrochloride, mp 193–194° [lit. (25) mp 193–195°] was 72%, and the overall yield for the formation of IV*c* from 3,4-dichloroacetophenone was 54%.

*3-Dimethylamino- 2 -dimethylaminomethyl -1-aryl-1-propanone Dihydrobromides* (*V*)—The general procedure for preparing V is illustrated by the synthesis of V*a*. A mixture of acetophenone (6.0 g, 0.05 mole), an aqueous solution of formaldehyde (37% w/v, 12 ml, 0.15 mole), dimethylamine (25% w/v, 27 ml, 0.15 mole), and ethanol (25 ml) was heated under reflux for 3 hr. After removal of the ethanol *in vacuo*, the mixture was cooled and extracted with ether (3 × 20 ml). The combined ether extracts were washed with water and dried with anhydrous magnesium sulfate; evaporation of the ether gave the crude ketone as a viscous oil. A solution of this oil in dry acetone was treated with hydrogen bromide gas to give a colorless solid which was removed by filtration, washed with acetone, dried, and recrystallized from methanol to give 3-dimethylamino-2-dimethylaminomethyl-1-phenyl-1-propanone hydrobromide, V*a* (4.5 g), as colorless crystals. Compound V*d* was prepared in an identical manner as V*a*, while the times of heating under reflux for V*b*, *c*, and *e* were 16, 30, and 6 hr, respectively. The structures of V*a–e* were confirmed by NMR, and the data generated for a representative compound, V*e*, are as follows: δ 8.1 (d, 2, aromatic protons at $C_2$ and $C_6$), 7.15 (d, 2, aromatic protons at $C_3$ and $C_5$), 3.95 (s, 3, $OCH_3$), 3.2–3.9 [m, 5, $C^2H(CH_2)_2$] and 2.95 [s, 12, $2N(CH_3)_2$]. The base peak in the MS was at 58 AMU.

*2-Dimethylaminomethyl-1-aryl-2-propen-1-ones* (*VII*a *and* b)—Chloroform (20 ml) was added to a solution of V*c* (0.500 g) in 40 ml of Sørensen's phosphate buffer, pH 7.4, (27) and the mixture was incubated at 37° with vigorous shaking. After 1 hr, the chloroform layer was removed and dried with anhydrous magnesium sulfate; evaporation of the organic solvent gave 2-dimethylaminomethyl-1-(3,4-dichlorophenyl)-2-propen-1-one, VII*a* (0.155 g, 56%) as a light yellow semisolid. HPLC showed one major peak (95.3%) plus two minor peaks. NMR: δ 7.85 (s, 1, aromatic proton at $C_5$), 7.7 (s, 2, aromatic protons at $C_2$ and $C_6$), 6.05 (s, 1, $=C^3H_2$), 5.7 (s, 1, $=C^3H_2$), 3.4 (s, 2, $-CH_2N$), and 2.4 [s, 6, $N(CH_3)_2$]; MS: *m/z* 257 ($M^+$, 5%), 173 (9%), and 58 (100%).

*Anal.*—Calc. for $C_{12}H_{13}Cl_2NO$: N, 5.43. Found: N, 5.37.

2-Dimethylaminomethyl-1-(*p*-methoxyphenyl)-2-propen-1-one, VII*b*, was prepared in the same way as a light yellow semisolid in 63% yield. HPLC showed one major peak (94.7%). NMR: δ 7.85 (d, 2, aromatic protons at $C_2$ and $C_6$), 6.9 (d, 2, aromatic protons at $C_3$ and $C_5$), 6.05 (s, 1, $=C^3H_2$), 5.7 (s, 1, $=C^3H_2$), 3.9 (s, 3, $OCH_3$), 3.4 (s, 2, $CH_2N$), and 2.4 [s, 6, $N(CH_3)_2$]; MS: *m/z* 219 ($M^+$, 19%).

*Anal.*—Calc. for $C_{13}H_{17}NO_2$: N, 6.38. Found: N, 5.94.

**Stabilities in Phosphate Buffer**—*Compounds IV*c *and* e—Chloroform (5 ml) was added to a solution of IV*c* (100 mg, 0.306 mmole) in phosphate buffer (pH 7.4, 2 ml), and the mixture was incubated at 37° on a shaking constant-temperature bath[8]. After 1.5 hr, the chloroform layer was separated and dried; removal of the solvent afforded a colorless solid (0.081 g, 95%) identified as the free base of IV*c*. NMR: δ 8.2–7.5 (m, 3, aromatic), 3.3–2.6 (m, 4, $C^2H_2$ and $C^3H_2$), and 2.3 [s, 6, $N(CH_3)_2$].

Similarly IV*e* gave the free base corresponding to IV*e* in 90% yield. NMR: δ 7.9 (d, 2, aromatic protons at $C_2$ and $C_6$), 6.9 (d, 2, aromatic protons at $C_3$ and $C_5$), 3.8 (s, 3, $OCH_3$), 3.4–2.6 (m, 4, $C^2H_2$ and $C^3H_2$), and 2.4 [s, 6, $N(CH_3)_2$].

*Compounds Vc and* e—Compound V*c* (100 mg, 0.215 mmole) was dissolved in a mixture of phosphate buffer (pH 7.4, 2 ml) and chloroform (5 ml) at 37° using a constant-temperature bath[8]. After 5 min, the chloroform layer was separated and dried; removal of the solvent afforded a semisolid which was identified by NMR as predominantly (>95%) VII*a*. Similarly V*e* gave rise to VII*b*.

The experiment was repeated except that V*c* was added to a mixture of buffer and chloroform in an ice bath (5°) and the resultant mixture shaken manually for 5 min. The chloroform layer was separated and dried; removal of the solvent gave a semisolid which was shown from the NMR spectra to be a mixture of VII*a* and V*c* as the free base in a ratio of 10:1. The ratio of these two compounds was determined from the NMR spectra using the following simultaneous equations: $AX_0 + BX_b = Y$ and $CX_0 + DX_b = Z$ where *A* and *B* are the number of aromatic protons in VII*a* and V*c*, respectively, as the free bases; $X_0$ and $X_b$ are the mole fractions of VII*a* and V*c*, respectively, as the free bases; *C* and *D* are the number of protons in the dimethylamino group of VII*a* and V*c*, respectively, as the free bases; and *Y* and *Z* are the respective integrals. When the experiment was repeated with V*e* (100 mg, 0.2346 mmole) at 5° and for 5 min, only the free base of V*e* was obtained. However if the length of time was increased to 1 hr, a mixture of VII*b* and V*e* as the free bases was obtained which was shown by the NMR spectra and the use of the aforementioned simultaneous equations to be in a ratio of 7:4.

[1] Mr. R. E. Teed, Department of Chemistry and Chemical Engineering, University of Saskatchewan.

[2] Eastman-Kodak Co.

[3] AEI MS-12 mass spectrometer, Picker X-Ray Engineering Ltd.; VG Micromass MM16F mass spectrometer with 2025 data system.

[4] Varian T-60 spectrometer, Varian Associates of Canada Ltd.

[5] Waters Associates Model 440 fitted with a UV absorbance detector (254 nm) and a Beckman 110A pump.

[6] Ultrasphere cyanocolumn.

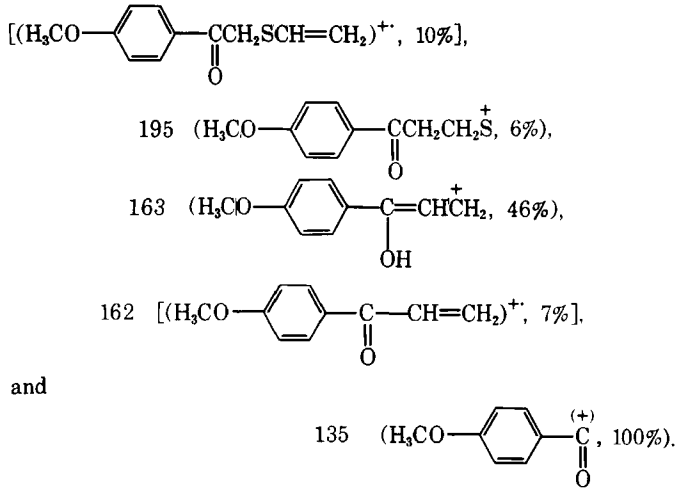
[7] Aldrich Chemical Co.

[8] Dubnoff Metabolic Shaking Incubator.

**Table III—Effect of IV*c, e* and V*c, e* on Respiration in Rat Liver Mitochondria Using Succinate as the Substrate at pH 7.4 and 20°**

| Compound | Concentration, μmoles | Stimulation, % | *SE* | Time prior to Constant Inhibition of Respiration, min | *SE* | Inhibition, % | *SE* |
|---|---|---|---|---|---|---|---|
| IV*c* | 5 | 27.03 | 11.26 | — | — | — | — |
| | 10 | 70.00 | 18.45 | — | — | — | — |
| | 25 | 68.56 | 9.50 | 10.76 | 1.09 | 39.70 | 6.73 |
| IV*e* | 25 | 42.35 | 3.89 | — | — | — | — |
| | 50 | 60.80 | 6.18 | — | — | — | — |
| | 100 | 66.45 | 4.58 | — | — | — | — |
| V*c* | 0.1 | 25.70 | 4.62 | 7.62 | 0.17 | 43.98 | 2.98 |
| | 0.25 | 46.54 | 10.50 | 3.46 | 0.16 | 50.62 | 11.57 |
| | 1.0 | 29.64 | 1.92 | 2.04 | 0.10 | 69.16 | 2.50 |
| | 10 | — | — | 0.62 | 0.13 | 82.75 | 2.46 |
| V*e* | 0.1 | 5.19 | 1.80 | — | — | — | — |
| | 0.25 | 21.17 | 3.54 | — | — | — | — |
| | 10 | 49.86 | 5.87 | 5.78 | 0.45 | 48.30 | 6.69 |
| | 15 | 51.36 | 7.28 | 3.62 | 0.27 | 61.25 | 4.76 |
| | 25 | 49.96 | 8.73 | 2.98 | 0.41 | 68.37 | 4.45 |
| | 50 | — | — | 1.34 | 0.40 | 84.37 | 3.17 |

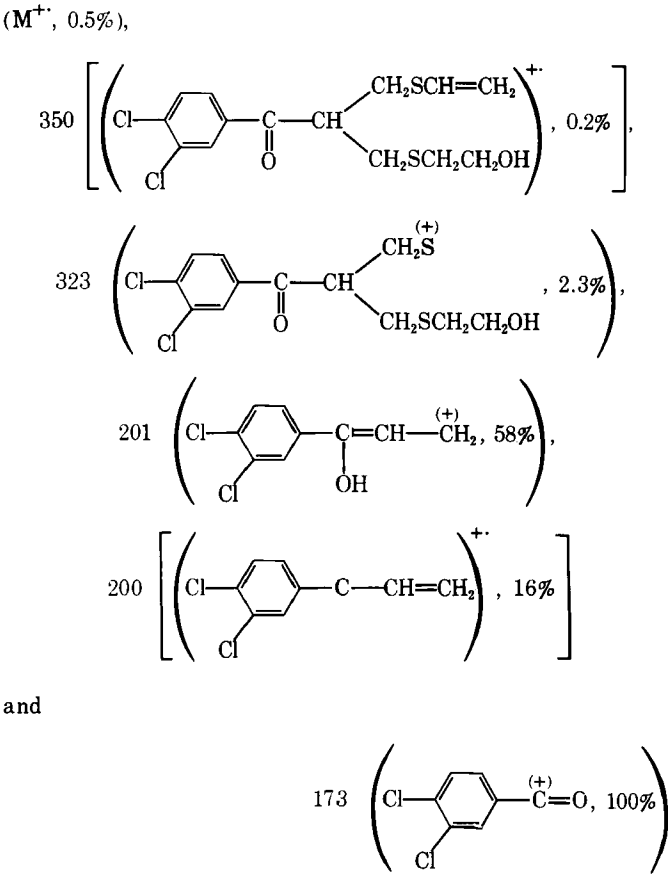
**Reaction of IV*e* with 2-Mercaptoethanol**—Chloroform (10 ml) was added to a solution of IV*e* (200 mg, 0.694 mmole) and 2-mercaptoethanol (167 mg, 2.1 mmoles) in phosphate buffer (pH 7.4, 4 ml), and the mixture was incubated at 37° on a shaking constant-temperature bath[8]. After 1 hr, the chloroform layer was separated and dried; removal of the organic solvent and excess of 2-mercaptoethanol under reduced pressure gave a bright-yellow viscous oil, the NMR spectrum of which showed the retention of the aromatic and methoxy protons but the absence of the dimethylamino protons. Other unidentified peaks were present and the oil appeared to be a mixture of compounds, although predominantly 3-(2-hydroxyethylthio)-1-(*p*-methoxyphenyl)-1-propanone. MS gave a parent peak corresponding to this ketone (*m/z* 240, 0.4%) with major ions at *m/z* 222:




The intensity of the peak at *m/z* 58 was 1%.

The experiment was repeated twice: in the absence of phosphate buffer and when chloroform was omitted. In the absence of buffer, a 98% recovery of IV*e*, identified by NMR and melting point, was found. In the absence of chloroform, IV*e* and 2-mercaptoethanol were incubated at 37° in buffer (5 ml) for 1 hr, and the mixture was extracted with chloroform (3 × 10 ml). The organic extract was saturated with dry hydrogen chloride gas and dried. Removal of the chloroform gave a residue which, when triturated with dry ether, gave IV*e* as the hydrochloride salt (0.042 g), identified by NMR. Evaporation of the ether gave a residue which was shown by NMR to contain 3-(2-hydroxyethylmercapto)-1-(*p*-methoxyphenyl)-1-propanone along with minor amounts of contaminants.

**Preparation of 3-(2-Hydroxyethyl)mercapto-2-[(2-hydroxyethyl)mercapto]methyl-1-(3,4-dichlorophenyl) - 1 - propanone (VIII*a*)**—A solution of 2-mercaptoethanol (125 mg, 1.6 mmoles) in phosphate buffer, pH 7.4 (13 ml), was added to a solution of V*c* (250 mg, 0.538 mmole) in phosphate buffer, pH 7.4 (10 ml). After the addition of chloroform (50 ml), the mixture was incubated for 15 min at 37° with vigorous stirring. The chloroform layer was removed, washed with water, and dried with anhydrous magnesium sulfate, and both chloroform and excess 2-mercaptoethanol were removed *in vacuo* to give a yellow viscous oil (0.120 g) which was predominantly 3-(2-hydroxyethyl)mercapto-2-[(2-hydroxyethyl)mercapto]methyl-1-(3,4-dichlorophenyl)-1-propanone. MS: *m/z* 368:




58 AMU (4%) was noted.

*Anal.*—Calc. for $C_{14}H_{18}Cl_2O_3S_2$: C, 45.53; H, 4.91; N, 0.00. Found: C, 44.43; H, 5.18; N, 0.27.

**Effect of IV and V on Respiration in Rat Liver Mitochondria**—Compounds IV and V were dissolved in Sørensen's buffer and kept ice-cold prior to the addition to the previously prepared mitochondrial suspension. The mitochondria were obtained from male Wistar rats (200–250 g) and the effect of IV and V on respiration was measured using a previous procedure (9). The buffer used in isolating the mitochondria and the

**Table IV—Effect of Vc and e on Respiration in Rat Liver Mitochondria Using Succinate as the Substrate at pH 6.9 and 6.4 and 37°**

| Compound | Concentration, μmoles | Stimulation: pH 6.9 % | pH 6.9 SE | pH 6.4 % | pH 6.4 SE | Levels of Significance (p) Between pH Values of: 7.4 & 6.9 | 6.9 & 6.4 | 7.4 & 6.4 |
|---|---|---|---|---|---|---|---|---|
| Vc | 0.01 | 12.23 | 2.71 | 30.41 | 7.15 | 0.05 | 0.10 | 0.50 |
| | 0.1 | 0 | — | 34.47 | 7.06 | <0.001 | 0.005 | 0.01 |
| | 0.25 | 0 | — | 40.76 | 6.73 | <0.001 | <0.001 | <0.001 |
| | 1.0 | 0 | — | 49.53 | 7.96 | >0.50 | <0.001 | <0.001 |
| Ve | 0.25 | 19.57 | 4.11 | 54.22 | 1.85 | 0.20 | <0.001 | 0.005 |
| | 1.0 | 17.48 | 2.98 | 58.65 | 6.86 | 0.10 | <0.001 | 0.01 |
| | 10 | 9.27 | 4.97 | 43.33 | 6.89 | 0.25 | 0.005 | 0.50 |

| Compound | Concentration, μmoles | Time Prior to Constant Inhibition of Respiration: pH 6.9 min | pH 6.9 SE | pH 6.4 min | pH 6.4 SE | Levels of Significance (p) Between pH Values of: 7.4 & 6.9 | 6.9 & 6.4 | 7.4 & 6.4 |
|---|---|---|---|---|---|---|---|---|
| Vc | 0.01 | 4.74 | 0.26 | 0 | 0 | <0.001 | <0.001 | >0.50 |
| | 0.1 | 3.42 | 0.11 | 10.22 | 0.41 | 0.005 | <0.001 | <0.001 |
| | 0.25 | 3.50 | 0.21 | 6.20 | 0.25 | <0.001 | <0.001 | <0.001 |
| | 1.0 | 1.90 | 0.09 | 3.65 | 0.13 | <0.001 | <0.001 | <0.001 |
| Ve | 0.25 | 1.78 | 1.11 | 0 | 0 | 0.10 | 0.10 | 0.01 |
| | 1.0 | 3.26 | 0.97 | 0 | 0 | 0.50 | 0.005 | <0.001 |
| | 10 | 3.93 | 0.31 | 7.97 | 0.19 | <0.001 | <0.001 | <0.001 |

| Compound | Concentration, μmoles | Inhibition: pH 6.9 % | pH 6.9 SE | pH 6.4 % | pH 6.4 SE | Levels of Significance (p) Between pH Values of: 7.4 & 6.9 | 6.9 & 6.4 | 7.4 & 6.4 |
|---|---|---|---|---|---|---|---|---|
| Vc | 0.01 | 11.35 | 2.49 | 0 | — | <0.001 | <0.001 | >0.50 |
| | 0.1 | 67.43 | 3.79 | 24.13 | 9.30 | 0.20 | <0.001 | <0.001 |
| | 0.25 | 87.56 | 2.00 | 46.09 | 5.36 | 0.20 | <0.001 | <0.001 |
| | 1.0 | 94.45 | 1.45 | 82.29 | 3.04 | 0.20 | 0.005 | 0.025 |
| Ve | 0.25 | 7.33 | 4.51 | 0 | — | 0.05 | 0.50 | 0.025 |
| | 1.0 | 12.14 | 4.57 | 0 | — | <0.001 | 0.005 | <0.001 |
| | 10 | 73.01 | 1.87 | 49.30 | 6.72 | <0.50 | 0.005 | 0.005 |

respiration media employed were those described previously (9), except that Sørensen's buffer and not tromethamine hydrochloride was used. The data generated at 37° (Table II) and the percentage stimulation and inhibition of respiration are both in relation to the original respiration rate. A minimum of five determinations were made at each concentration; in the case of IVc (5.0 μmoles), Va (0.1 μmole), Vd (0.1 μmole) and Ve (0.25 and 25 μmoles) a total of 12 determinations were performed.

In both Figs. 1 and 2, the data shown indicate differences in oxygen uptake compared with control mitochondria (no succinate or compound added). The lag period (A) was also found with IVa (10 μmoles), IVd (10 μmoles), IVe (10, 25, and 50 μmoles), Va (0.01, 0.1, and 0.25 μmole), Vb (0.01 μmole), Vc (0.001, 0.01, 0.1, and 0.25 μmole), Vd (0.01, 0.1, 0.25, and 1.0 μmole), Ve (0.01, 0.1, 0.25, 1.0, and 10 μmoles), and VIIb (1.0 μmole). In some cases, during interval A the slope of the line was identical to that after the addition of succinate; *i.e.*, no difference in oxygen utilization was noted after addition of the compound. This phenomenon occurred with IVb (5 and 10 μmoles), Va (1.0 μmole), Ve (12.5 and 15 μmoles), and VIIa (1.0 μmole). Inhibition of respiration occurred prior to stimulation of respiration during interval A in the case of IVa (25 μmoles), IVb (25 μmoles), IVc (5 and 10 μmoles), IVd (25 and 50 μmoles), IVe (100 μmoles), Va (10 μmoles), Vb (0.01 and 0.1 μmole), and VIIb (10 and 25 μmoles). In the remaining cases, the lag period (A) was absent and addition of the compound led to effects noted in one or more of the time periods B, C, and D.

The effect on respiration at pH values of 6.9 and 6.4 is given in Table III. At pH 6.9, respiration continued at the same rate as after addition of succinate during interval A in the case of Vc (0.01 μmole) and Ve (0.25 and 1.0 μmoles). Inhibition of respiration during interval A occurred with Ve (10 μmoles). The lag period was absent at concentrations of 0.1, 0.25, and 1.0 μmole of Vc. At pH 6.4, submaximal stimulation of respiration was noted with Vc (0.01 and 0.1 μmole) and Ve (0.25 and 1.0 μmole). Inhibition of respiration during interval A was found with Vc (0.25 and 1.0 μmole) and Ve (10 μmoles).

Table IV indicates the effect of various concentrations of IVc and e, Vc and e on respiration in rat liver mitochondria at 20°. The same procedure (9) was employed, using a constant-temperature bath[9]. Submaximal stimulation occurred during the lag period for IVc (5 μmoles) and Vc (0.1, 0.25, 10, 15, and 25 μmoles). Respiration was identical to that after the addition of succinate for IVe (25 μmoles) and Vc (0.25 μmoles). Inhibition of respiration during interval A was noted for IVc (10 and 25 μmoles), IVe (50 and 100 μmoles), and Vc (1.0 μmole). The lag period was absent in the cases of Vc (10 μmoles) and Ve (50 μmoles).

**Screening of Compounds**—The anticancer screening was carried out by the Drug Research and Development Division of the National Cancer Institute, Bethesda, Md., using their protocols (28). Male or female $CD_2F_1$ mice were used. The compounds were administered by the intraperitoneal route in saline except for IVc, for which hydroxypropylcellulose was employed.

## REFERENCES


(1) D. S. Kemp and F. Vellaccio, "Organic Chemistry," Worth, New York, N.Y., 1980, p. 295.

(2) K. B. Wiberg, "Physical-Organic Chemistry," Wiley, New York, N.Y., 1964, p. 410.

(3) M. Tramontini, *Synthesis*, **1973**, 703 and references cited therein.

(4) J. R. Dimmock, L. M. Smith, and P. J. Smith, *Can. J. Chem.*, **58**, 984 (1980).

(5) J. R. Dimmock and M. L. C. Wong, *Can. J. Pharm. Sci.*, **11**, 35 (1976).

(6) J. A. Mollica, J. B. Smith, I. M. Nunes, and H. K. Govan, *J. Pharm. Sci.*, **59**, 1770 (1970) and references cited therein.

(7) R. C. Fahey, P. A. Myers, and D. L. Distefano, *Bioorg. Chem.*, **9**, 293 (1980).

(8) R. Andrisano, A. S. Angeloni, P. De Maria, and M. Tramontini, *J. Chem. Soc. (C)*, **1967**, 2307.


[9] Lauda K2R instrument, Messgeräte-Werk Lauda, West Germany.

(9) N. W. Hamon, D. L. Bassendowski, D. E. Wright, J. R. Dimmock, and L. M. Noble, *J. Pharm. Sci.*, **67**, 1539 (1978).
(10) J. R. Dimmock, N. W. Hamon, L. M. Noble, and D. E. Wright, *J. Pharm. Sci.*, **68**, 1033 (1979).
(11) N. W. Hamon, D. L. Kirkpatrick, E. W. K. Chow, and J. R. Dimmock, *J. Pharm. Sci.*, **71**, 25 (1982).
(12) T. Psenakova and J. Kolek, *Biologia*, **36**, 1109 (1981).
(13) I. A. Goroshinskay, A. A. Krichevskaya, and Z. G. Bronovitskaya, *Bull. Exp. Biol. Med.*, **91**, 464 (1981).
(14) M. Eden, B. Haines, and H. Kahler, *J. Natl. Cancer Inst.*, **16**, 541 (1956).
(15) H. Kahler and W. B. Robertson, *J. Natl. Cancer Inst.*, **3**, 495 (1943).
(16) J. R. Dimmock, N. W. Hamon, E. W. K. Chow, D. L. Kirkpatrick, L. M. Smith, and M. G. Prior, *Can. J. Pharm. Sci.*, **15**, 84 (1980).
(17) J. H. Burchenal, E. A. D. Holmberg, J. J. Fox, S. C. Hemphill, and J. A. Reppert, *Cancer Res.*, **19**, 494 (1959).
(18) J. R. Dimmock and W. G. Taylor, *J. Pharm. Sci.*, **63**, 69 (1974).
(19) P. N. Gordon, J. D. Johnston, and A. R. English, in "Antimicrobial Agents and Chemotherapy," G. L. Hobby, Ed., American Society for Microbiology, Ann Arbor, Mich., 1965, p. 165.
(20) H. Schönenberger, T. Bastug, L. Bindl, A. Adam, D. Adam, A. Petter, and W. Zwez, *Pharm. Acta Helv.*, **44**, 691 (1969).
(21) J. G. Topliss, *J. Med. Chem.*, **20**, 463 (1977).
(22) W. C. J. Ross, "Biological Alkylating Agents," Butterworths and Co. Ltd., London, 1962, p. 107.
(23) E. J. Ariens in "Drug Design," Vol. 1, E. J. Ariens, Ed., Academic, New York, N.Y., 1971, p. 42 seq.
(24) C. E. Maxwell, *Org. Syn.*, **23**, 30 (1943).
(25) Italian pat. 637,371, (March 29, 1962); through *Chem. Abstr.*, **60**, 479e (1964).
(26) D. W. Adamson and J. W. Billinghurst, *J. Chem. Soc.*, **1950**, 1039.
(27) D. L. Deardoff in "Remington's Pharmaceutical Sciences," 14th ed., Mack Publishing Company, Easton, Pa. 1970, p. 1554.
(28) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.* (Part 3), **3**, (Sept., 1972).

## ACKNOWLEDGMENTS

The authors thank the Medical Research Council of Canada for the award of an operating grant (MA-5538) to J. R. Dimmock and for providing a Summer Undergraduate Research Scholarship to D. J. Harwood. The receipt of Graduate Scholarships from the University of Saskatchewan to K. Shyam and S. K. Raghavan is acknowledged gratefully.

Appreciation is accorded to Dr. S. M. Wallace of the College of Pharmacy, University of Saskatchewan, for assistance in the statistical evaluation of some of the results obtained from experimentation with mitochondria and also to the National Cancer Institute, Bethesda, Md., which undertook the antineoplastic evaluation of some of the compounds reported in this investigation.

# Pharmacokinetics of Piperacillin and Gentamicin Following Intravenous Administration to Dogs

**VIJAY K. BATRA ˣ, JOHN A. MORRISON, and THOMAS R. HOFFMAN**

Received February 5, 1982, from the *Pharmacodynamics Department and Statistical Design and Analysis Department, Medical Research Division, American Cyanamid Co., Pearl River, NY 10965.* Accepted for publication July 30, 1982.

**Abstract** □ Piperacillin sodium was administered intravenously to dogs, alone or in combination with gentamicin, twice a day (~5 hr apart) for 36–37 days. The pharmacokinetics of neither drug changed in the presence of the other; however, the percentage of the gentamicin dose recovered in the urine decreased significantly when coadministered with piperacillin. The data demonstrate that interaction between the two drugs in urine is feasible.

**Keyphrases** □ Piperacillin—pharmacokinetics in the dog, effect of concomitant administration of gentamicin □ Gentamicin—pharmacokinetics in the dog, effect of concomitant administration of piperacillin □ Pharmacokinetics—of piperacillin and gentamicin in the dog, effect of concomitant administration

Piperacillin[1], sodium (2*S*, 5*R*, 6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, is a novel semisynthetic penicillin that possesses broad spectrum antibacterial activity against Gram-negative and Gram-positive pathogenic bacteria, including anaerobes. Results of *in vitro* studies (1) have shown piperacillin to be superior to ampicillin, carbenicillin, and cephalosporins against Gram-negative bacteria, particularly *Klebsiella*, *Proteus*, and *Serratia* species and *Pseudomonas aeruginosa*. In certain cases, a piperacillin and gentamicin combination would be preferred to obtain a synergistic effect. To evaluate the toxicity of these two drugs when administered alone or in combination, a 1-month study was undertaken in dogs. Since aminoglycosides can interact with β-lactam antibiotics (2–4), the study was designed to allow the serum concentrations to be analyzed pharmacokinetically. This paper describes the pharmacokinetics of piperacillin and gentamicin when given alone or in combination.

## EXPERIMENTAL

**Animal Studies**—Six groups of 18–20-month-old beagle dogs[2] (two males and two females in each group) were utilized for the study. The weight range was 9.4–12.3 kg for the males and 7.9–9.6 kg for the females. The dogs were assigned to groups using a table of random numbers. They were housed individually in a room maintained at 21–24°, with a 12-hr on/off light cycle. Food[3] (250–300 g) was offered to each dog daily, ~30 min after the last dose; water was available *ad libitum*.

Drug solutions, made prior to each dose, were administered twice daily (~5 hr apart) over a 5-min period with an infusion pump[4] calibrated using the specific syringes, solutions, and tubing employed. Doses, adjusted to the body weight twice a week, were administered according to the schedule shown in Table I. The concentration of the piperacillin solution in sterile water for injection, expressed as free acid, was 250 mg/ml. The gentamicin solution was made in concentrations of 1 and 2 mg/ml (expressed as base equivalent activity) using sterile isotonic saline. For dogs

[1] Pipracil; American Cyanamid Co.

[2] Marshall Research Animals, North Rose, N.Y.
[3] Respond 2000, Country Foods Div., Agway, Hauppauge, N.Y.
[4] Model 355, Sage Instrument, Cambridge, Mass.



*0022-3549/83/0800-0894$01.00/0*