Potential Inhibitors of Polyamine Biosynthesis. 2. α-Alkyl- and Benzyl-(±)-ornithine

Mahmoud M. Abdel-Monem,* Nancy E. Newton, Bartholomeus C. Ho, and Charles E. Weeks

College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455. *Received August 16, 1974*

α-Methyl-(±)-ornithine hydrochloride was not a substrate for ornithine decarboxylase from rat prostate glands. It produced equal inhibition of ornithine decarboxylase obtained from rat prostate glands, spleens of mice inoculated with L1210 leukemic cells, and regenerating rat liver indicating its lack of selectivity for any of these tissues. In these three tissues the inhibition was competitive with L-ornithine. A number of α-alkyl- and α-aralkyl-substituted analogs of (±)-ornithine were synthesized and evaluated in vitro as inhibitors of the enzyme L-ornithine decarboxylase obtained from prostate glands of rats. These compounds were obtained by the reaction of alkyl iodide or benzyl bromide with the anion obtained by treatment of 3-(benzalimino)piperidin-2-one with sodium hydride. The following α-substituted analogs of (±)-ornithine were obtained: ethyl, *n*-propyl, *n*-butyl, *n*-hexyl, *n*-octyl, and benzyl. The synthesized compounds were found to be much less active than α-methyl-(±)-ornithine as competitive inhibitors of ornithine decarboxylase in vitro. The most active compound in the series was α-*n*-octyl-(±)-ornithine which was 60-fold less active than α-methyl-(±)-ornithine and the least active analog was α-*n*-butyl-(±)-ornithine which was 270-fold less active than the α-methyl-(±)-ornithine.

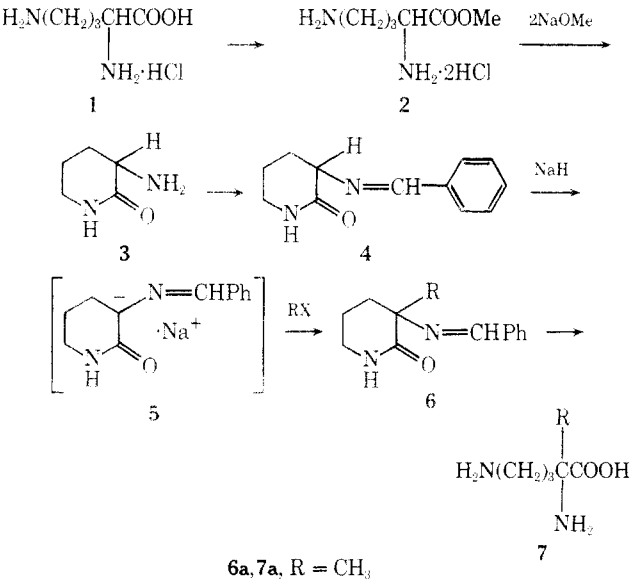
Numerous investigations have shown that activation of biosynthesis and accumulation of polyamines in tissues accompany both normal and neoplastic cell growth and that the biosynthesis of polyamines in normal resting tissues is very slow.[1] It is not known, however, if the increase in polyamine levels in these tissues mediates the elevated rate of cellular growth or if growth itself produces the increase in polyamine levels. One way of elucidating the role of polyamines in proliferating tissue would be to block their biosynthesis and to determine if this inhibits any phase of cellular proliferation. A likely way to produce this blockade is to inhibit the enzyme ornithine decarboxylase since the decarboxylation of L-ornithine appears to be the rate-limiting step in polyamine synthesis.[2]

Several inhibitors of ornithine decarboxylase have been studied. We reported the synthesis of α-methyl-(±)-ornithine which was found to be a potent competitive inhibitor, in vitro, of ornithine decarboxylase from the prostate glands of rats.[3] α-Methyl-(±)-ornithine also was synthesized by Honigberg et al.[4] and inhibited, in vitro, ornithine decarboxylase from regenerating rat liver.[1] Furthermore, other investigators have found that L-canaline[5] and α-hydrazinoornithine[6] are inhibitors of ornithine decarboxylase and that the latter blocks the accumulation of putrescine in regenerating liver as well as in cultured rat hepatoma cells.[6]

This communication describes the inhibition of ornithine decarboxylase by α-methyl-(±)-ornithine in several types of proliferating tissue as well as studies which indicate that α-methyl-(±)-ornithine is a competitive inhibitor of this enzyme and not an alternate substrate. We also describe the synthesis of a number of α-alkyl- and α-benzyl-(±)-ornithines and the evaluation of their inhibitory effect on mammalian ornithine decarboxylase. These analogs were studied on the premise that since the substitution of the α-hydrogen in the ornithine molecule with a methyl group provided a potent competitive inhibitor of ornithine decarboxylase, the replacement of the α-hydrogen with other alkyl or aralkyl groups might also provide potent inhibitors of this enzyme. Furthermore, the evaluation of the inhibitory activity of these analogs on ornithine decarboxylase would provide valuable information as to the nature and steric requirements of the portion of the enzyme surface occupied by the α-hydrogen of the substrate.

Chemistry. The target compounds **7b–g** were obtained from ornithine (**1**) using a synthetic scheme similar to that used for the synthesis of α-methyl-(±)-ornithine (**7a**).[3] Ornithine methyl ester dihydrochloride (**2** 2HCl) was obtained from ornithine using standard methods in good yields. Treatment of **2** 2HCl with 2 molar equiv of sodium methoxide produced crude 3-amino-2-piperidone (**3**). The latter was identified by its spectral characteristics and was not purified. Crude **3** was treated with benzaldehyde with concomitant removal of water to provide the Schiff base **4**. Treatment of a solution of **4** with 1–2 molar equiv of a strong base resulted in the formation of the anion **5**. A number of bases were investigated and sodium hydride was found to be the most convenient. The anion **5** was treated with alkyl iodide or benzyl bromide to provide compounds **6b–g** which were crystallized from the concentrated reaction mixtures (Table I). Hydrolysis of compounds **6b–g** with 2 *N* hydrochloric acid provided the α-substituted ornithines **7b–g** as the dihydrochloride salts. The overall yields of these compounds, based on the amount of **4** used, were 23–83% (Table II). The products **7b–g** were optically inactive (Scheme I).

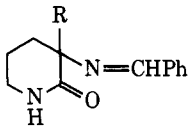
Scheme I



6a,7a, R = CH_3
6b,7b, R = C_2H_5
6c,7c, R = *n*-C_3H_7
6d,7d, R = *n*-C_4H_9
6e,7e, R = *n*-C_6H_{13}
6f,7f, R = *n*-C_8H_{17}
6g,7g, R = CH_2Ph

α-Methyl-(±)-ornithine-*1*-^{14}C was obtained using $K^{14}CN$ as described in a previously published synthesis.[3]

Table I. Properties of 3-Substituted 3-(Benzalimino)piperidin-2-one



Compd no.	R	Formula[a]	% yield	Mp, °C
6b	C_2H_5	$C_{14}H_{18}N_2O$	48	101
6c	n-C_3H_7	$C_{15}H_{20}N_2O$	40	102
6d	n-C_4H_9	$C_{16}H_{22}N_2O$	48	123
6e	n-C_6H_{13}	$C_{18}H_{26}N_2O$	48	
6g	CH_2Ph	$C_{19}H_{20}N_2O$	86	117

[a]Satisfactory analyses were obtained for C, H, and N for all compounds.

The identity of the product was established by comparison of its melting point, ir, and TLC mobility with those of an authentic sample of **7a**. The isotopic purity of **7a**-*1*-^{14}C was established using three TLC systems. In all systems used, the radioactivity was confined to the position of the ninhydrin-positive spot corresponding to authentic **7a**.

Results and Discussion

The inhibition by **7** of the enzymatic decarboxylation of L-ornithine was measured, in vitro, using cell-free extracts from mammalian tissues as the source of the enzyme ornithine decarboxylase. The ornithine decarboxylase activity was measured by determining the amount of $^{14}CO_2$ released from (±)-ornithine-*1*-^{14}C in the presence of pyridoxal phosphate. As we have previously reported[3] the activity of ornithine is highly dependent on pyridoxal phosphate concentration. Consequently, all the experiments discussed in the present study were carried out using a dialyzed preparation of the enzyme in the presence of 2×10^{-4} *M* pyridoxal phosphate. For each tissue studied the decarboxylation of ornithine was found to be linear with time and concentration of enzyme used. Also, as has been reported numerous times[7,8] we found that 4 hr after partial hepatectomy in rats, there was a large (25-fold) increase in the ornithine decarboxylase activity in the remaining liver lobes as compared to the activity in the same lobes of sham operated animals. We also confirmed the report[9] that inoculation of mice with L1210 leukemic cells produces an increase (fourfold) in ornithine decarboxylase activity in the spleen.

Previously, we reported that **7a** HCl is a competitive inhibitor of the decarboxylation of L-ornithine by ornithine decarboxylase in cell-free extracts of the prostate glands of rats. We wished, also, to determine if **7a** HCl would produce similar inhibition of the enzymes obtained from other proliferating tissues. Therefore, we studied the effects of α-methyl-(±)-ornithine on ornithine decarboxylase from spleens of mice inoculated with L1210 leukemic cells and remnants of liver from partially hepatectomized rats.

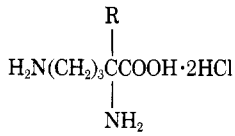
In the presence of 2×10^{-4} *M* pyridoxal phosphate, ornithine decarboxylase from spleens of mice inoculated 6 days earlier with L1210 leukemic cells had an apparent K_m for L-ornithine of 9.0×10^{-5} *M* and a V_{max} of 0.09 nmol of CO_2/mg of wet weight tissue/hr. The addition of α-methyl-(±)-ornithine hydrochloride in concentrations of 8.7×10^{-5}, 2.7×10^{-5}, and 8.7×10^{-6} *M* resulted in the supression of the production of $^{14}CO_2$. It appeared from the Lineweaver–Burk plots of these data that **7a** HCl was a competitive inhibitor of decarboxylation of L-ornithine by ornithine decarboxylase. The K_i for α-methyl-(±)-ornithine was 2.6×10^{-5} *M*.

Ornithine decarboxylase from regenerating rat liver was likewise inhibited by **7a** HCl. The K_i determined at 2×10^{-4} *M* pyridoxal phosphate was 8.0×10^{-5} *M* and the K_m for L-ornithine was 1.0×10^{-4} *M*. The Lineweaver–Burk plots of these data indicated that the inhibition was competitive with ornithine.

Thus, the degree of inhibition is similar for the enzyme from the prostate gland ($K_i = 4 \times 10^{-5}$ *M*) and the spleens ($K_i = 2.6 \times 10^{-5}$ *M*) but is somewhat less for the liver ($K_i = 8 \times 10^{-5}$ *M*). There is, however, no great selectivity and it was decided that any of these tissues will be appropriate to use in further in vitro studies.

Incubation of α-methyl-(±)-ornithine-*1*-^{14}C (sp act. 0.53 mCi/mmol) in 10^{-4}, 10^{-3}, and 10^{-2} *M* concentrations with ornithine decarboxylase from rat prostate glands in the presence of 2×10^{-4} *M* pyridoxal phosphate did not result in a measurable production of $^{14}CO_2$. The incubation of L-ornithine-*1*-^{14}C in 5.0×10^{-5} and 5.0×10^{-4} *M* concentrations under the same conditions resulted in the decarboxylation of 4.8 and 1.2% of the substrate, respectively. The

Table II. Properties of α-Substituted (±)-Ornithine Dihydrochloride



Compd no.	R	Formula[a]	Yield, %[b]	Mp, °C	R_m	K_i[c]
7a	CH_3				0.52	4.0×10^{-5}
7b	C_2H_5	$C_7H_{16}N_2O_2 \cdot 2HCl \cdot 0.5\ H_2O$	93	140–146	0.42	5.3×10^{-3}
7c	n-C_3H_7	$C_8H_{18}N_2O_2 \cdot 2HCl$	57	204–210	0.29	7.8×10^{-3}
7d	n-C_4H_9	$C_9H_{20}N_2O_2 \cdot 2HCl$	68	210–216	0.195	1.1×10^{-2}
7e	n-C_6H_{13}	$C_{11}H_{24}N_2O_2 \cdot 2HCl$	75	224–229	0.10	4.7×10^{-3}
7f	n-C_8H_{17}	$C_{13}H_{28}N_2O_2 \cdot 2HCl$	16[d]	223–228	0.025	2.3×10^{-3}
7g	CH_2Ph	$C_{12}H_{18}N_2O_2 \cdot 2HCl$	97	Dec	0.225	5.3×10^{-3}
Ornithine	H				0.63	

[a]Satisfactory analyses were obtained for C, H, and N for all new compounds. [b]Yield is based on the amount of 3-substituted 3-benzalimino)piperidin-2-one (**6**) used. [c]The reactions were carried out in the presence of 1.6×10^{-5}–1.02×10^{-4} *M* L-ornithine and 2×10^{-4} *M* pyridoxal phosphate. The enzyme had an apparent K_m for L-ornithine of 1.3×10^{-4} *M* and the V_{max} was 0.40 nmol of CO_2/mg of wet weight tissue/hr. The concentration of inhibitors used was 4.7×10^{-3} *M*. [d]Yield is based on the amount of 3-(benzalimino)piperidin-2-one (**4**).

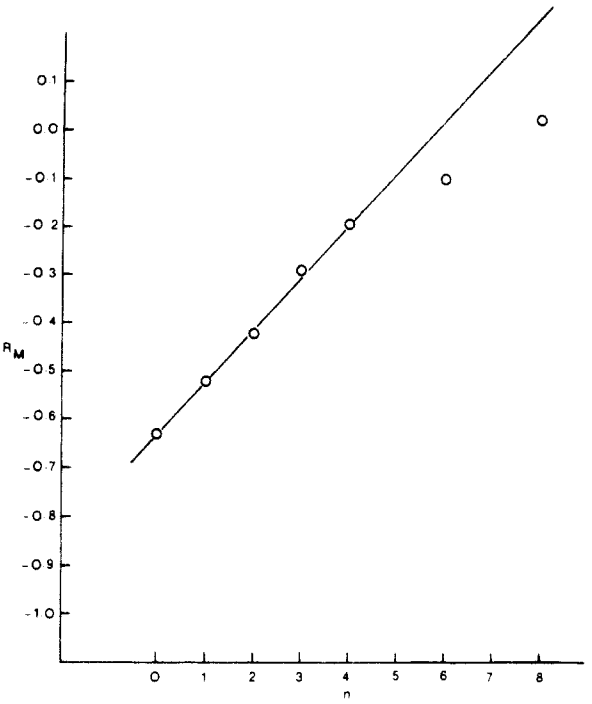


Figure 1. Plot of the R_m values vs. the number of methylene units in the α-substituent in the series of α-alkyl-(±)-ornithine. n = number of methylene units in the α-substituent.

specific activity of **7a**-*1*-^{14}C is high enough that even 0.01% decarboxylation at a concentration of 1.0×10^{-4} M would produce about 50 cpm of $^{14}CO_2$ which should have been detected. Thus, α-methyl-(±)-ornithine could have only very little activity as a substrate for ornithine decarboxylase, if any, and is indeed primarily an inhibitor of this enzyme.

At a concentration of 2×10^{-4} M pyridoxal phosphate the ornithine decarboxylase from rat prostate had an apparent K_m for L-ornithine of 1.3×10^{-4} M and the V_{max} was 0.40 nmol of CO_2/mg of wet weight of tissue/hr. The addition of any of the α-substituted ornithine derivatives **7b–g** in concentrations varying from 10^{-4} to 4.7×10^{-3} M resulted in a slight suppression of the production of $^{14}CO_2$. This effect was very weak compared to the effect of α-methyl-(±)-ornithine (**7a**). The Lineweaver–Burk plots of the effect of 7 2HCl on the rate of L-ornithine decarboxylation by ornithine decarboxylase indicated that all these compounds were apparently competitive inhibitors of the decarboxylation of L-ornithine by ornithine decarboxylase. The K_i's for these compounds were calculated from the Lineweaver–Burk plots and are presented in Table II.

The substitution of the α-hydrogen in ornithine with a methyl group in α-methyl-(±)-ornithine produced a potent competitive inhibitor of ornithine decarboxylase obtained from the prostate glands of rats. However, the replacement of the α-methyl group in **7a** with an α-ethyl group resulted in a dramatic loss of the inhibitory activity. The α-ethyl-(±)-ornithine was 130-fold less active than the α-methyl-(±)-ornithine. The increase in chain length of the α-substituent from α-ethyl to α-butyl caused a further small progressive decrease in activity.

Surprisingly, an increase in chain length of the α-substituent to the α-hexyl- and α-octyl-(±)-ornithine produced a small enhancement of activity and the α-octyl-(±)-ornithine, the most active compound in this series, was 60-fold less active than the α-methyl-(±)-ornithine. α-Benzyl-(±)-ornithine had the same inhibitory potency as the α-ethyl-(±)-ornithine. It is important to recognize that all these compounds were very poor inhibitors of the enzyme ornithine decarboxylase. It is not known whether these compounds are substrates for ornithine decarboxylase and, hence, produce their apparent inhibitory effect by acting as alternative substrates which would mimic the effects of a competitive inhibitor in the Lineweaver–Burk plots. However, α-methyl-(±)-ornithine is not a substrate for ornithine decarboxylase and, hence, it is reasonable to believe that these compounds, likewise, are not substrates for ornithine decarboxylase.

The effect of the modification of the α-substituent on the partition coefficient of these compounds was determined by measuring their R_m values.[10] The chromatographic system used was silica gel plates impregnated with 1-octanol as the stationary phase and pH 7.4 phosphate buffer (0.025 M) as the mobile phase. As would be expected the increase in the size of the α-substituent resulted in an increase in the retention of the compound in the 1-octanol stationary phase (Table II). The α-benzyl-(±)-ornithine (**7g**) showed partition characteristics between the α-propyl- and α-butyl-(±)-ornithine. There was a linear relationship between the R_m values and the number of methylene units in the α-substituent up to the α-butyl-(±)-ornithine[10] (Figure 1). α-Hexyl- and α-octyl-(±)-ornithine had R_m values which were significantly lower than the calculated values. Since the R_m values measured in our system reflect both the degree of ionization of the amino acid at the pH of the aqueous buffer (pH 7.4) and its relative solubility in the two phases used, the abnormal behavior of the analogs **7e** and **7f** could be attributed to abnormal changes in either property. It is noteworthy that the two analogs in this series which showed R_m values lower than the calculated values are the same analogs which showed increased inhibition of ornithine decarboxylase.

Conclusions

α-Methyl-(±)-ornithine hydrochloride was not a substrate for ornithine decarboxylase obtained from rat prostate gland. It inhibited equally the ornithine decarboxylase obtained from rat prostate gland, spleens of mice inoculated with L1210 leukemic cells, and regenerating rat liver. In these three tissues the inhibition appeared to be competitive with L-ornithine. There appear to be certain steric requirements for binding to the active site of the enzyme ornithine decarboxylase at the region where the α-hydrogen of the substrate ornithine binds. The replacement of the α-hydrogen of the substrate with a methyl group produced a potent competitive inhibitor; however, further increases in the size of the α-substituent resulted in a dramatic decrease in the inhibitory activity.

Experimental Section

The melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. Elemental analysis was performed by M-H-W Laboratories, Garden City, Mich. The ir spectra were obtained with a Perkin-Elmer 237B or Beckman IR-9 spectrophotometer. The NMR spectra were taken on a Varian A-60D in $CDCl_3$ or D_2O with Me_4Si or DSS as internal standards, respectively. Mass spectral analyses were performed on AEI MS-30 at 70 eV and 200° chamber temperature. Optical rotation measurements were obtained on a Perkin-Elmer 141 polarimeter. All spectral data were consistent with the proposed structures. Radioactivity was measured using a Beckman LS-150 liquid scintillation counter.

5-(3-Phthalimidopropyl)-5-methylhydantoin-*4*-^{14}C. A solution of 1-phthalimidopentan-4-one[3] (0.231 g, 0.001 mol) in EtOH (6 ml) was treated dropwise with a solution of $NaHSO_3$ (0.1044 g, 0.001 mol) in H_2O (1.5 ml) and the mixture was stirred for 30 min. The reaction mixture was treated with a solution of $K^{14}CN$ (0.13 mg, 0.83 mCi, sp act. 51.23 mCi/mmol) in H_2O (1.0 ml), stirred at room temperature for 1 hr, treated with a solution of KCN (0.0647 g, 0.001 mol) in H_2O (1.0 ml), and stirred for 30 min. The reaction mixture was treated with a solution of $(NH_4)_2CO_3$ (0.4558 g, 0.004

mol) and KCN (0.0653 g, 0.001 mol) in H_2O (2.2 ml) and heated under reflux at 60° for 16 hr and at 100° for additional 90 min to decompose the excess $(NH_4)_2CO_3$. The mixture was evaporated to dryness, and the residue was dissolved in water (50 ml) and extracted with $CHCl_3$ (3 × 50 ml). The water phase was evaporated to dryness; the residue was treated with anhydrous MeOH (20 ml), heated, stirred, and filtered. The filtrate was evaporated to dryness to provide the title compound with some inorganic salts.

α-Methyl-(±)-ornithine-*1*-^{14}C Hydrochloride (7a-*1*-^{14}C HCl). 5-(3-Phthalimidopropyl)-5-methylhydantoin-*1*-^{14}C obtained from above was added to a suspension of $Ba(OH)_2 \cdot 8H_2O$ (0.6322 g, 0.002 mol) in H_2O (25 ml). The mixture was heated in a pressure bottle at 160° for 3 hr. The mixture was cooled, filtered, treated with $(NH_4)_2CO_3$ until no further precipitation occurred, heated to boiling, and filtered. The filtrate was evaporated to dryness to provide an oil which was treated with 6 *N* HCl (5.0 ml); the mixture was boiled under reflux for 20 hr and cooled. The precipitate formed was filtered off and identified as *o*-phthalic acid (mp 205–208°). The filtrate was evaporated to an oil and the residue was treated with acetone; the mixture was stirred and cooled. The white solid which separated was filtered and washed with cold acetone. The precipitate was dissolved in water and the solution was adjusted to pH 5–6 by the addition of NH_4OH. The solution was treated with Amberlite IR-120CP ion-exchange resin (4.0 ml) and the mixture was stirred for 2 hr. The mixture was filtered and the resin was washed with water until the washings were neutral. The resin was added to 5 *N* NH_4OH (15 ml), the mixture was stirred for 30 min and filtered, and the resin was washed with water (20 ml). The filtrate was evaporated to dryness in vacuo, and the residue was titrated with 0.1 *N* HCl to produce the monohydrochloride salt. The solution was evaporated to dryness to give **7a-*1*-^{14}C** HCl (0.1669 g, yield 91%). Three recrystallizations from H_2O–acetone provided **7a-*1*-^{14}C** HCl H_2O (0.0976 g, sp act. 0.53 mCi/mmol). The ir and melting point were identical with those of an authentic sample of **7a** HCl H_2O. The radiochemical purity was determined using Cellulose powder TLC plates (Eastment Kodak No. 6065) and the following three solvent systems: MeOH–pyridine–1.25 *N* HCl (8:1:2); MeOH–pyridine–H_2O–acetic acid (6:6:4:1), and *n*-BuOH–acetic acid–H_2O (25:4:10).

L-Ornithine Methyl Ester Dihydrochloride (2). The title compound was prepared using the method of Yamada et al.[11] in 97.0–98.0% yields, mp 188.5–190° (lit.[12] mp 192–194°).

3-(Benzalimino)piperidin-2-one (4). A solution of compound **2** (6.8 g, 0.03 mol) in dry CH_3OH (400 ml) was treated with a solution of Na (1.55 g, 0.067 g-atom) in dry CH_3OH (50 ml). The mixture was concentrated under reduced pressure to remove all the solvent, the residue was dissolved in $CHCl_3$ (150 ml), and the mixture was stirred for 30 min and filtered. The filtrate was concentrated under reduced pressure to remove the solvent, the residue was dissolved in PhH (200 ml) by the aid of gentle heating, and the solution was filtered from any traces of insoluble matter. The benzene solution was treated with benzaldehyde (3.7 g, 0.035 mol) in a flask connected to a Dean-Stark distillation receiver and the reaction mixture was heated under reflux until no more water was collected in the distillation receiver. The reaction mixture was concentrated to a volume of about 150 ml and allowed to cool slowly to provide 4.87 g (80%) of the title compound, mp 143–145°. Anal. ($C_{12}H_{14}N_2O$) C, H, N.

3-Substituted 3-(Benzalimino)piperidin-2-one. The following syntheses were carried out under dry conditions in N_2 atmosphere. NaH (650 mg of 50% dispersion in oil, 0.014 mol) was added to 50 ml of THF freshly distilled off $LiAlH_4$. The mixture was treated dropwise with a solution of **4** (2.0 g, 0.01 mol) in dry THF (100 ml). The mixture was heated under reflux for 2 hr, cooled to room temperature and treated with the alkyl iodide or benzyl bromide (0.011 mol), and then heated for additional 2 hr in the case of **6d–f.** The mixture was stirred overnight (about 16 hr) and filtered and the solvent was removed under reduced pressure. The residue was crystallized from benzene–petroleum ether to provide **6b, 6c,** and **6g.** Alternatively, the residue was treated with a pH 6.5 phosphate buffer (20 ml) and extracted with ether. The combined ether extract was washed with brine, dried (anhydrous $MgSO_4$), and filtered and the solvent was removed under reduced pressure. The residue was crystallized from a benzene–petroleum ether mixture to provide **6d** and **6e.** For the preparation of **6f,** the residue obtained after the evaporation of ether failed to crystallize and the crude residue was purified by chromatography on a silica gel column using chloroform–2-propanol (9:1) as a solvent. The product obtained was characterized by its spectral properties and was not purified further.

α-Substituted (±)-Ornithine Dihydrochloride (7b–g 2HCl). A solution of **6** (1 mmol) in THF (10 ml) was heated to 85–90° and treated dropwise with 2 *N* HCl (20 ml). The mixture was heated under reflux for 24 hr and then cooled to room temperature. The mixture was extracted with ether (2 × 50 ml) and then $CHCl_3$ (2 × 50 ml), and the organic solvent extracts were discarded. The aqueous acid layer was concentrated in vacuo to provide the crude title compounds. These products were crystallized from ethanol–acetone (Table II).

Determination of the R_m Values. Silica gel plates (20 × 20, 250 μ, Analtech, Inc.) were impregnated by allowing a 5% solution of 1-octanol in chloroform (v/v) to run to the top of the plate. The plates were air-dried at room temperature for 4 hr. A 100-μl (12.2 m*M*) solution of the compounds in methanol was applied along a line 2 cm from one edge of the plate. Each compound was applied at two different spots and the R_f for each compound was the average of the two values. The compounds were applied to predetermined random positions on the plate. The plates were developed in a preequilibrated tank with the mobile phase consisting of pH 7.4 phosphate buffer (0.025 *M*) saturated with 1-octanol until the solvent front had advanced 16 cm from the origin. The plates were dried at 110° for 10 min, sprayed with ninhydrin reagent (2% in ethanol), and heated on a hot plate for 5 min. The R_f values were calculated for each compound. The R_m was calculated from the formula,[7] $R_m = \log (1/R_f - 1)$.

Animals. Adult male Sprague–Dawley rats (200–275 g) were used as the source of either prostate glands or regenerating liver. L1210 leukemic cells were carried in DBA/2 mice and all experiments were done with spleens from BDF_1 mice. The animals were fed a diet of Purina Chow and water ad libitum.

Materials and Solutions. These were the same as previously described.[3]

Tissue Extracts. Rats were decapitated and their prostate glands were carefully removed, cleaned of adhering tissue, and placed in ice-cold homogenization solution. The glands were blotted, weighed, and homogenized in 3 vol of homogenization solution with a hand homogenizer. The homogenates were centrifuged at 10,000g for 10 min at 2° and the supernatants were then centrifuged at 100,000g for 90 min at 2°. These high-speed supernatants were dialyzed overnight against 100 vol of homogenization solution and were used for the determination of enzymatic activity.

Lymphoid leukemia L1210 was carried in DBA/2 mice by weekly ip passages. A suspension containing 1.0 × 10^6 of L1210 ascites leukemic cells obtained from DBA/2 mice was inoculated ip in each recipient BDF_1 mouse. The mice were sacrificed 7 days after inoculation and the spleens were removed, placed in ice-cold homogenization solution, blotted, weighed, and homogenized in 3 vol of homogenization solution with a hand homogenizer. The homogenate was treated in exactly the same way as the homogenate from prostate glands.

Partial hepatectomy was carried out under light ether anesthesia[13] and the liver remnants were later removed also under ether anesthesia. Liver lobes were homogenized in 1 vol of a twofold concentrated homogenization solution and centrifuged at 20,000g for 20 min at 2°. The supernatants were then centrifuged at 100,000g for 90 min at 2° and these high-speed supernatants were dialyzed overnight against 100 vol of homogenization solution and were used for the determination of enzymatic activity.

Assay of Ornithine Decarboxylase Activity. This was carried out using the method described in ref 3.

Acknowledgments. We gratefully acknowledge support of this work by U.S. Public Health Service Grant CA 14238.

References and Notes

(1) D. H. Russell in "Polyamines in Normal and Neoplastic Growth", D. H. Russell, Ed., Raven Press, New York, N.Y., 1973, p 1.
(2) A. Raina, J. Tamne, P. Hannonem, and F. Holtta, *Ann. N.Y. Acad. Sci.*, **171**, 697 (1970).
(3) M. M. Abdel-Monem, N. E. Newton, and C. E. Weeks, *J. Med. Chem.*, **17**, 447 (1974).
(4) J. J. Ellington and I. L. Honigberg, *J. Org. Chem.*, **39**, 104 (1974).
(5) E. L. Rahiala, M. Kekomaki, J. Janne, A. Raina, and N. C. Raiha, *Biochem. Biophys. Acta*, **227**, 337 (1971).
(6) S. I. Harik and S. H. Snyder, *Biochem. Biophys. Acta*, **327**, 501 (1973).
(7) S. S. Cohen in "Introduction to the Polyamines", Prentice-

Hall, Englewood Cliffs, N.J., 1971.
(8) D. H. Russell and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **60**, 1420 (1968).
(9) D. H. Russell, *Cancer Res.*, **32**, 2459 (1972).
(10) E. C. Bate-Smith and R. G. Westall, *Biochem. Biophys. Acta*, **4**, 427 (1950).
(11) S. Yamada, K. Koga, and H. Matsuo, *Chem. Pharm. Bull.*, **11**, 1140 (1963).
(12) K. Golankiewicz and M. Wiewiorowski, *Acta Biochim. Pol.*, **10**, 443 (1963).
(13) G. M. Higgins and R. M. Anderson, *Arch. Pathol.*, **12**, 186 (1931).

Physicochemical–Activity Relations in Practice. 1. A Rational and Self-Consistent Data Bank

Franklin E. Norrington,* Richard M. Hyde, Stanley G. Williams, and Raymond Wootton

Department of Biophysics & Biochemistry, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, England.
Received November 5, 1974

A data bank of substituent constants for 26 ortho and 34 meta and para benzenoid substituents is presented for use in physicochemical–activity relations (PAR) studies. The distributive parameters π and π_-, a bulk parameter based on molar refraction, and positionally weighted electronic parameters F and R are listed for the three substituent positions. There are no gaps in the table caused by missing values and the interparameter correlations are low.

When a new, biologically active molecule is discovered, the common practice among medicinal chemists is to modify its structure in order to determine the effect of these changes on its potency. This often involves the introduction of different substituents into a molecule and Hansch has provided a method of correlating the differences in activity for the resulting series of compounds with the changes in their physicochemical properties which result from altering the substitution pattern.[1] The term "quantitative structure–activity relationships" (QSAR) has been used to describe the method[2] but this is a wide-ranging term which also embraces the Free and Wilson approach and the various quantum mechanical methods. It is therefore suggested that "physicochemical–activity relationships" (PAR) should be used to describe the original method of Hansch[1] and subsequent modifications by other workers.[3,4] The aim of PAR, then, is to explain the interactions between organic molecules and a biological system in terms of a few quantitative parameters which describe physicochemical properties of the organic molecules.

Since there is no rigorous way of selecting the most appropriate parameters to use and no unique analytical strategy, biased or equivocal conclusions may well be drawn unless these points are settled in advance.[4] Such an analytical strategy will be described in a forthcoming paper,[5] while the present paper gives the numerical values for a rationally selected and self-consistent set of physicochemical parameters, suitable for studying a wide range of benzenoid compounds.

In order that the problem shall be of manageable size, it is customary to study a congeneric series of compounds in which a parent molecule is modified by the presence of one or more substituents. Implicit in this approach is the assumption that all members of the series act on the biological system by the same mechanism and only their quantitative potency is modified by the substituents. The appearance over the last 10 years of a large number of successful PAR correlations in the literature supports this assumption.

As a further simplification, only cases in which the substitution occurs in a benzene ring will be considered at present. This covers a wide range of potential drug molecules. The data bank therefore comprises a set of "substituent constants" which define the relative magnitude of each property between compounds in the series, while the absolute value of the properties for any compound need not be known.

Selection of Substituents and Parameters. The data bank was restricted to stable, chemically accessible, and useful substituents having as wide a range of properties as possible. It was considered unsatisfactory to present a data bank having missing values where the parameters in question had not been measured and these considerations restricted the number of substituents to 34. Eight of these were not considered for the ortho position since they are bulky and it was expected that they would interact sterically with the side chain, thus rendering their parameter values invalid when applied to other systems. In compiling this data bank no attempt has been made to extend the list by including a large number of substituents of doubtful value.

The physicochemical parameters were required to satisfy the four following criteria. Firstly, each parameter must describe a likely interaction between a small molecule and its biological environment. Secondly, it must be possible to obtain parameters from the literature, measure them in a reproducible in vitro system using model compounds, or reliably deduce them from related values. Thirdly, since the ability to predict parameters and hence biological activities is central to the overall aim, a parameter, once measured in a model compound, must be applicable to the same substituent in another benzenoid compound. Finally, parameters must describe distinct physicochemical properties which are essentially uncorrelated with one another; the dangers in using highly correlated parameters together have been pointed out.[4,6]

In view of these criteria, the final selection comprised the distributive parameters π and π_-, the electronic parameters F and R, and the bulk parameter MR, as described below.

Distributive Parameters. π has been firmly established as the parameter of choice for correlating both binding to biological macromolecules and transport through a biological system.[7-10] Ideally, its use is restricted to molecules where comparatively little perturbing effect is exerted on the electrons of the benzene ring. In the case of compounds having electron-donating side chains, the partition coefficient is better described using π_-, a similar parameter