TABLE V

| No. | Equation | n[a] | r[b] | s[c] | Log $P_0$[d] (95% confidence interval) |
|---|---|---|---|---|---|
| | CNS stimulants injected in soln | | | | |
| 1 | $\log (1/LD_{50}) = 0.290 \log P + 2.480$ | 12 | 0.564 | 0.423 | |
| 2 | $\log (1/LD_{50}) = -0.288 (\log P)^2 + 0.902 \log P + 2.411$ | 12 | 0.849 | 0.285 | 1.569 (1.20–2.59) |
| 3 | $\log (1/LD_{50}) = 0.202 \log P + 0.305 \mu + 0.980$ | 12 | 0.688 | 0.392 | |
| 4 | $\log (1/LD_{50}) = -0.291 (\log P)^2 + 0.819 \log P + 0.315 \mu + 0.862$ | 12 | 0.942 | 0.193 | 1.406 (1.14–1.83) |
| | CNS stimulants and depressants injected in soln | | | | |
| 5 | $\log (1/LD_{50}) = 0.323 \log P + 2.309$ | 16 | 0.545 | 0.470 | |
| 6 | $\log (1/LD_{50}) = -0.236 (\log P)^2 + 0.829 \log P + 2.236$ | 16 | 0.686 | 0.423 | 1.775 ($\pm\infty$) |
| 7 | $\log (1/LD_{50}) = 0.237 \log P + 0.401 \mu + 0.415$ | 16 | 0.752 | 0.383 | |
| 8 | $\log (1/LD_{50}) = -0.262 (\log P)^2 + 0.792 \log P + 0.429 \mu + 0.197$ | 16 | 0.882 | 0.285 | 1.512 (1.16–2.51) |
| | CNS stimulants and depressants ($R_m$ in BAW) | | | | |
| 9 | $\log (1/LD_{50}) = 0.506 (-R_m) + 2.251$ | 12 | 0.305 | 0.487 | |
| 10 | $\log (1/LD_{50}) = 1.086 (-R_m) + 0.579 \mu - 0.969$ | 12 | 0.877 | 0.259 | |
| | Depressants ($R_m$ in BAW) | | | | |
| 11 | $\log (1/LD_{50}) = 0.821 (-R_m) + 1.547$ | 6 | 0.830 | 0.291 | |
| 12 | $\log (1/LD_{50}) = 0.897 (-R_m) + 2.472 \mu - 8.808$ | 6 | 0.921 | 0.235 | |

[a] $n$ = number of data points used in the analysis. [b] $r$ = the correlation coefficient. [c] $s$ = standard deviation. [d] Log $P_0$ is the ideal log $P$ value for max activity. This can be obtained by setting $[d(\log 1/c)]/[d(\log P)] = 0$.

tant role. This may be due to the anchoring of the drug molecule onto the receptor sites by dipole–dipole interactions or by other type of electrostatic forces.

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# Potential Nonequilibrium Analgetic Receptor Inactivators. Synthesis and Biological Activities of *N*-Acylanileridines[1]

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In a effort to prepare nonequilibrium analgetic receptor inactivators, a variety of *N*-acylanileridines having alkylating capacity were synthesized. The analgetic potencies of this series are reported together with experiments designed to detect receptor blockade. Ethyl *p*-(4-ethoxycarbonyl-4-phenyl-1-piperidinoethyl)fumaranilate (**5**) caused significant blockade of analgetic activity 2 hr after ip administration. Pretreatment with naloxone, a narcotic antagonist, blocked completely the analgetic activity of **5**. Naloxone also protected the analgetic receptors against inactivation. The data suggest that **5** has the capacity to alkylate analgetic receptors selectively.

Strong analgetics are generally believed to exert their effects by interacting with specific receptors located in the CNS. Three main bodies of evidence support this belief; (1) common structural features,[2,3] (2) large potency differences between enantiomers,[4] and (3) the existence of structurally related competitive antagonists.[5,6]

Although structural parameters required for high biological activity have been thoroughly delineated for many classes of strong analgetics and attempts have been made to explain their mechanism of action,[7] the

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(1) This research was supported by National Institutes of Health Grant NS 08738 and GM 15477.

(2) G. de Stevnes, Ed., "Analgetics," Academic Press, New York, N. Y., 1965.

(3) S. Archer and L. Harris, *Fortschr. Arzneimittelforsch.*, **8**, 262 (1965).

(4) P. S. Portoghese, *J. Pharm. Sci.*, **55**, 865 (1966).

(5) H. L. Fraser and L. S. Harris, *Annu. Rev. Pharmacol.*, **7**, 277 (1967).

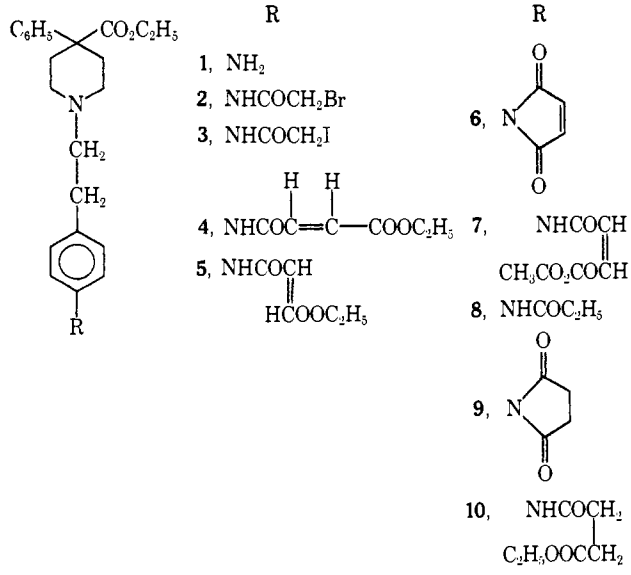
(6) W. R. Martin, *Pharmacol. Rev.*, **19**, 463 (1967).

(7) *Res. Publ. Ass. Res. Nerv. Ment. Dis.*, **46**, (1968).

exact location and the constitution of the receptor components have remained elusive.

In an effort to investigate this problem the method of affinity labeling [8,9] was chosen as a means of selectively forming covalent bonds with analgetic receptors. We describe herein experiments to determine whether or not this approach is feasible. The present evidence suggests that, with a suitable alkylating moiety, nonequilibrium blockade of analgetic receptors can be effected in a selective fashion.

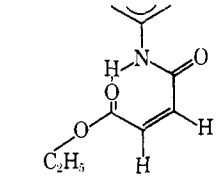
**Chemistry.**—As a preliminary study toward this end, anileridine[10] (**1**), a compound known to possess high analgetic activity, was chemically modified by attachment of various alkylating functions (**2–7**). Compounds containing nonalkylating groups (**8–10**) that are structurally similar to the alkylating moieties were also prepared in order to ascertain whether or not the compounds were penetrating the CNS.




The reactive moieties have the potential for alkylating nucleophilic groups (SH, SMe, $NH_2$, OH, $COO^-$, $PO_3^{3-}$) on or adjacent to the receptor, provided the alkylating moiety is in proper juxtaposition with the nucleophile after a reversible drug–receptor complex has been formed.

The substituted amides (**2–5**, **8**, **10**) were prepared by treating the appropriate acid with anileridine in the presence of DCI or by the interaction of the acid chlorides with **1**. The substituted imides (**6** and **9**) were prepared following the procedure of Cava.[11] Compound **7**, an amidoanhydride derivative of anileridine, was isolated during the synthesis of **6**. Since this compound has 2 reactive sites, it was also tested for receptor blocking activity.

It was noted that the exchange rate of maleamido ethyl ester **4** with $D_2O$ in $CDCl_3$ solution was unusually slow ($t_{1/2} \sim 5$ min) at 37° when compared with fumaramido ethyl ester **5** ($t_{1/2} \ll 1$ min). This can be attributed to the stabilization of the amide proton through H bonding with either the carbonyl O or the ether O of the ester groups as depicted in **11** and **12**. The fumaramido ester is incapable of forming intramolecular H bonds and consequently may exist in conformation **13**. Conformation **14** is less probable because the vinyl proton of the fumarido group would sterically interfere with the ortho aromatic proton. The possible consequences of such conformational differences on the alkylating potential of analgetic receptors will be discussed later in this article.

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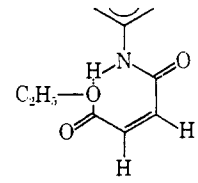


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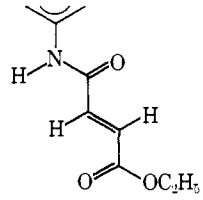


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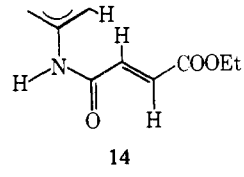


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**Pharmacological Results.**—The $ED_{50}$'s of the compounds are listed in Table I. Morphine and anileridine were employed as standards. Most compounds caused various degrees of CNS stimulation within 5 min after administration followed by sedation in about 1 hr. All compounds produced the Straub tail phenomenon. Severe depression was noted particularly with **2**, **6**, and **7**; death appeared to be due to respiratory failure. In fact, the analgetic activity of **6** and **7** could not be assessed due to toxicity. The analgetic activities of **1**, **3**, and **8** were comparable with that of morphine, while the other compounds tested were 4 to 8 times less active than morphine. In all cases where $ED_{50}$'s were determined, the compounds possessed a very quick onset of action, and the maximum analgetic activity was observed within 20 min after the ip injections. The duration of action was between 60 and 120 min.

TABLE I
ANALGETIC ACTIVITIES OF *N*-ACYL DERIVATIVES OF ANILERIDINE

| Compd | $ED_{50}$ (95% confidence limits), mg/kg |
|---|---|
| 1 | 4.4 (3.1–6.2) |
| 2 | 24.0 (14.6–39.5) |
| 3 | 6.8 (2.8–16.3) |
| 4 | 46.5 (31.0–69.8) |
| 5 | 21.5 (13.4–35.9) |
| 6 | >40[a] |
| 7 | >40[a] |
| 8 | 7.1 (5.6–8.9) |
| 9 | 23.0 (14.8–35.7) |
| 10 | 18.0 (11.4–28.4) |
| Morphine sulfate | 5.5 (3.67–8.25) |

[a] 80–90% of the animals died within 24 hr after a dose of 40 mg/kg. There was no analgetic activity noted with 20 mg/kg of **7** and only 10% of the animals exhibited analgesia with 20 mg/kg of **6**.

In order to determine whether the alkylating agents were capable of blocking analgetic activity, mice were treated with these agents at doses which normally pro-

(8) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.
(9) L. A. Cohen, *Annu. Rev. Biochem.*, **37**, 695 (1968).
(10) Leritine; 1-(*p*-amino-phenethyl)-4-phenylisonipecotic acid ethyl ester.
(11) M. P. Cava, A. A. Deana, K. Muth, and M. J. Mitchell, *Org. Syn.*, **41**, 93 (1961).

TABLE II
PRETREATMENT STUDIES

| Pretreatment[a] | | Test | | % of animals showing analgesia | Increase in reaction[b] time, sec m ± S.E. | Total analgesia,[b] min-sec m ± S.E. |
|---|---|---|---|---|---|---|
| Compd | Dose, mg/kg | Compd | Dose, mg/kg | | | |
| None | | 8 | 10 | 90 | 15.1 ± 2.5 | 762.7 ± 136.6 |
| 2 | 40 | 8 | 10 | *c* | *c* | *c* |
| 3 | 40 | 8 | 10 | 90 | 15.9 ± 2.0 | 1203.7 ± 162.4 |
| None | | Ms | 10 | 90 | 14.4 ± 2.3 | 843 ± 173.5 |
| 2 | 40 | Ms | 10 | *c* | *c* | *c* |
| 3 | 40 | Ms | 10 | 80 | 13.6 ± 2.7 | 1131.0 ± 22.0 |
| None | | 10 | 40 | 100 | 18.4 ± 1.6 | 883.2 ± 116.7 |
| 5 | 50 | 10 | 40 | 60 | 10.6 ± 2.5[d] | 746.6 ± 171.4 |
| 4 | 100 | 10 | 40 | 90 | 14.3 ± 1.5 | 775.4 ± 201.0 |
| None | | Ms | 11 | 80 | 13.2 ± 2.1 | 711.0 ± 135.5 |
| 5 | 40 | Ms | 11 | 30 | 5.8 ± 1.7[d] | 309.0 ± 96.8[d] |
| None | | 9 | 50 | 90 | 10.7 ± 2.4 | 404.3 ± 86.7 |
| 7 | 20 | 9 | 50 | 90 | 12.6 ± 3.1 | 656.2 ± 166.0 |
| None | | 9 | 50 | 90 | 11.1 ± 2.4 | 553.0 ± 95.0 |
| 6 | 20 | 9 | 50 | 60 | 9.4 ± 2.8 | 435.3 ± 128.6 |

[a] Animals were pretreated ip 2 hr before the test compound. The analgetic effect of the pretreatment drugs had disappeared by 2 hr. [b] Mean ± S.E. of 10 mice. [c] All animals died within 2 hr after the test doses. [d] Values significantly different ($P < 0.05$) from those observed in animal which were not pretreated; Ms, morphine sulfate.

duce analgesia in 80 to 100% of the animals. The analgetic effect of the compounds had disappeared after 2 hrs and the animals were then administered the structurally similar nonalkylating analgetic to see whether or not analgesia could still be elicited. For these studies the compounds were divided into 3 groups. In the first group, mice were pretreated with the alkylating compounds **2** or **3**, and then either **8** or morphine was tested for analgetic activity. In the second group, mice were pretreated with the alkylating compounds **4** or **5**, and then either **10** or morphine was tested for analgetic activity. In the third group, mice were pretreated with the alkylating compounds **6** or **7**, and **9** was tested for analgetic activity.

The results of the above experiments are presented in Table II. All animals pretreated with **2** died within 2 hr after a test dose of either **8** or morphine. Pretreatment with **3** did not alter the analgetic effect of morphine or **8**. Similarly, pretreatment with either **6** or **7** did not block the analgetic activity of **9**. On the other hand, pretreatment with **5**, the fumaramido ethyl ester of anileridine, appeared to have some blocking action on the analgetic activities of **10** and morphine. The mean reaction times of the animals due to both **10** and morphine were significantly decreased by the pretreatment, while a decrease in the total analgetic effect was observed only with morphine. This is graphically illustrated in Figure 1. If the data are expressed on a quantal basis, pretreatment with **5** did not significantly decrease the percentage of animals exhibiting analgesia due to **10**. The inhibition of morphine analgesia by pretreatment with **5**, however, was of borderline significance.

Since **5** exhibited some analgetic blocking property, its presumed action on analgetic receptors was studied further. Mice were pretreated with the narcotic antagonist, naloxone,[5] before the administration of **5**. Analgetic activity was measured for 2 hr at 20-min intervals. After 2 hr, when the effect of naloxone is no longer evident,[12] the analgetic activity of morphine was assessed in the same animals. If naloxone could antagonize the analgetic effects of **5**, it would then be expected to protect the analgetic receptor from alkylation by **5**. Results in Table III show that naloxone did

TABLE III
ANALGETIC RECEPTOR PROTECTION STUDY WITH NALOXONE

| Treatment of animals | No. of animals | % of animals exhibiting analgesia | Mean increase in reaction time ± S.E., sec |
|---|---|---|---|
| 50 mg/kg of **5** ip | 10 | 80 | 14.7 ± 2.4 |
| Pretreated with 1 mg/kg of naloxone sc 10 min before injection of 50 mg/kg of **5** | 10 | 0 | 0.4 ± 0.8 |
| 11 mg/kg of morphine sulfate ip to the above mice 2 hr after treatment with naloxone and **5** | 10 | 80 | 10.3 ± 2.3 |
| 11 mg/kg of morphine sulfate ip | 10 | 80 | 11.4 ± 2.3 |

block the analgetic effects of **5**. Furthermore, this receptor protection is evident from the fact that when morphine was administered after the effect of naloxone had disappeared, the full analgetic activity of morphine was observed.

## Discussion

At the outset of our studies we had anticipated that the anileridine analogs would not possess optimal capacity to alkylate analgetic receptors. This was expected because 4-phenylpiperidine-type analgetics are less potent than morphine in terms of their brain levels.[13] Hence the analgetic receptors are likely to have less affinity for the former compounds. Although this appeared to be a major drawback, the commercial availability of anileridine and the ability of the $NH_2$ group to be derivatized, prompted us to utilize this analgetic for our preliminary studies.

(12) S. E. Smits and A. E. Takemori, *Brit. J. Pharmacol.*, **39**, 627 (1970).

(13) E. L. Way and T. K. Adler, *Bull. W. H. O.* **25**, 227 (1961); **26**, 51, 261 (1962); **27**, 359 (1962).

The fact that the fumaramido ester **5** was not overly toxic and was the only alkylating agent in the series to exhibit significant blockade of analgetic activity suggests that this compound has some action on analgetic receptors. Moreover the toxicity of the maleimide **6** and the amidoanhydride **7** indicates that these compounds are involved in nonspecific alkylation. The ability of naloxone pretreatment to block analgetic activity of **5** leads us to believe that this alkylating drug has some specificity for analgetic receptors. This is further supported by the observation that morphine possessed full potency after naloxone and **5** pretreatments.

It is noteworthy that **5** produces an analgetic effect while presumably being capable of alkylating the receptors. This can be rationalized by assuming that reversible complex formation gives rise to analgesia while subsequent alkylation of the receptors results in inactivation.

Interestingly, the maleamido ester **4** does not appear to alkylate analgetic receptors. This could be related to the conformation of the alkylating groups. Conceivably, the maleamido ester moiety is not in proper juxtaposition with the nucleophilic groups on or adjacent to the receptor because of intramolecular H bonding which constrains the alkylating moiety in a cyclic conformation (**11** or **12**). On the other hand, extended conformation **13** of the fumaramido ester **5** might allow the nucleophilic groups to be in closer proximity so that alkylation could occur.

To our knowledge the only other attempt to selectively alkylate analgetic receptors was reported by May, *et al.*[14] In their study, *N*-2-bromoethyl and *N*-2-bromopropylbenzomorphinan derivatives produced prolonged CNS depression together with a low order of analgetic activity. However, the authors could not conclude whether this effect was caused by alkylation of certain sites in the CNS.

Our success in obtaining compounds which can presumably alkylate analgetic receptors selectively has prompted us to investigate structures related to morphine in an attempt to obtain more selective receptor inactivators. Studies on these compounds are in progress.

## Experimental Section[15]

**Pharmacology.**—Male Swiss–Webster mice (15–20 g) were used in all experiments. The test compounds were dissolved in 40% propylene glycol, and the final concentration was made so that 10 ml/kg was injected at each dose level. All drugs were administered ip. It was determined that 40% propylene glycol did not have any influence in the analgetic assay used in this study. Analgetic activity was estimated by using the hot-plate method of Eddy and Leimbach.[16] Three to four groups of 10 mice were used to assay each compound. Duration of action as well as the increase in reaction times was noted. The responses

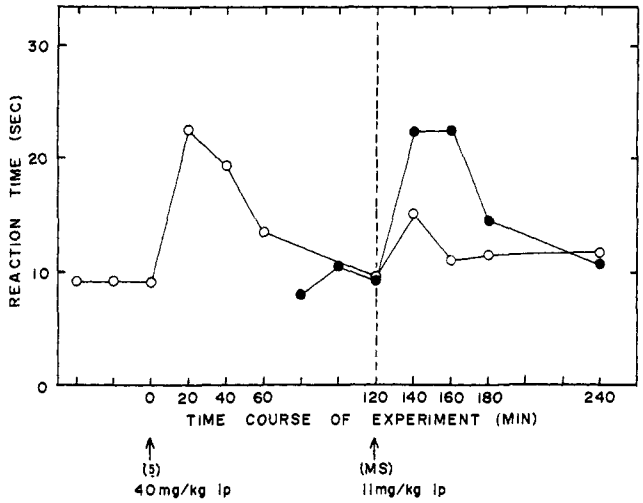


Figure 1.—Each circle represents mean reaction times of 10 mice at the times indicated on the abscissa. The open circle represents a group of mice which had been injected with 40 mg/kg of **5** (left of dotted line) and, after the analgetic effect had disappeared, injected with 11 mg/kg of morphine sulfate (MS) (right of dotted line). The closed circles represent a control group of mice injected with 11 mg/kg of morphine sulfate.

were considered analgetically positive if the reaction times after drug administration were greater than 2 standard deviations of the mean reaction time of all mice of the particular group being tested. Dose–response curves were plotted for analgetic responses occurring at 20 min after administration of the drugs and the $ED_{50}$'s with 95% confidence limits were estimated by the method of Litchfield and Wilcoxon.[17] The total analgetic effect was estimated by the method of Winter and Flataker.[18] The values which express the total analgetic effect of the test compounds include the duration of action as well as the increase in reaction time of the animals.

Measured data were analyzed by the Student *t* test and quantal data were analyzed by the $\chi$-square test.

**Ethyl 1-(*p*-Bromoacetamidophenethyl)-4-phenyl-4-piperidinecarboxylate·HBr (2).**—To a cold soln of 0.176 g (0.0005 mole) of **1** in 5 ml of $Me_2CO$ was added 0.126 g (0.0005 mole) of $BrCH_2COBr$, and the soln was stirred vigorously for 0.5 hr at room temp. The reaction mixture was filtered, and the residue was washed several times with cold $Et_2O$ and then dried to give 0.249 g (90%) of **2** (recrystd from EtOH–$Et_2O$), mp 205–206°. *Anal.* ($C_{24}H_{29}BrN_2O_3$·HBr) C, H, N.

**Ethyl 1-(*p*-Iodoacetamidophenethyl)-4-phenyl-4-piperidinecarboxylate·HCl (3).**—To a soln of 0.58 g (0.0015 mole) of anileridine·HCl and 0.26 g (0.0015 mole) of DCI in 60 ml of dry MeCN was added 0.278 g (0.0015 mole) of $ICH_2CO_2H$, and the soln was stirred for 18 hr at room temp. After the reaction mixture was filtered, the solvent was evapd *in vacuo* to give a solid which was recrystd (MeOH–$Et_2O$) to give 0.31 g (55.8%) of the product, mp 190–192° dec. *Anal.* ($C_{24}H_{29}IN_2O_3$·HCl) C, H, N.

**Ethyl *p*-(4-Ethoxycarbonyl-4-phenyl-1-piperidinoethyl)maleanilate (4).**—Anileridine (0.352 g, 0.001 mole) was treated with 0.144 g (0.001 mole) of maleic acid monoethyl ester in $CH_2Cl_2$ under conditions similar to those described for **3**. Removal of the solvent resulted in an oil which was purified by column chromatography (EtOAc–silica gel). Recrystn from EtOH–hexane gave 0.35 g (68%) of product, mp 124–125°. *Anal.* ($C_{28}H_{34}N_2O_5$) C, H.

**Ethyl *p*-(4-Ethoxycarbonyl-4-phenyl-1-piperidinoethyl)fumaranilate·HCl (5).**—A soln of 0.24 g (0.0015 mole) of fumaryl chloride monoethyl ester in 2 ml of $CHCl_3$ was added dropwise to a soln of 0.53 g (0.0015 mole) of **1** in 5 ml of $CHCl_3$. After 2 hr of reflux the pptd HCl salt was filtered and washed several times with dry $Et_2O$. Two recrystns (MeOH–$Et_2O$) gave 0.58 g (75%) of product, mp 227–228°. *Anal.* ($C_{28}H_{34}N_2O_5$·HCl) C, H, N.

**Ethyl 1-(*p*-Maleimidophenethyl)-4-piperidinecarboxylate·HCl (6).**—Compd **7a** (0.45 g, 0.001 mole) was heated with 0.3 g of $Ac_2O$ and 0.041 g of NaOAc at 100° for 10 hr. After adding

(14) M. May, L. Czoncha, D. R. Garrison, and D. J. Triggle, *J. Pharm. Sci.*, **57**, 884 (1968).

(15) Melting points were determined in open capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Microanalytical Laboratory, Department of Chemistry, University of Minnesota, Minneapolis, Minn. and in part by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. The ir and nmr spectra were recorded using Perkin-Elmer 237 B spectrophotometer and Varian A-60D spectrometer. All spectra were consistent with the expected structures.

(16) N. B. Eddy and D. G. Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953).

(17) J. T. Litchfield Jr. and F. Wilcoxon, *ibid.*, **96**, 99 (1949).

(18) C. A. Winter and L. Flataker, *ibid.*, **98**, 305 (1950).

the reaction mixture to ice water, the aq layer was extd with $CHCl_3$, washed with 8% $NaHCO_3$ soln, and dried ($MgSO_4$). Removal of solvent afforded a brown oil which after drying *in vacuo* was redissolved in $CHCl_3$ and decolorized. Addn of dry HCl followed by evapn of solvent resulted in an oily residue which solidified when triturated with $Et_2O$. The yield of cryst (MeOH–$Et_2O$) product, mp 198–200° dec, was 0.26 g (55%). *Anal.* ($C_{26}H_{28}N_2O_4 \cdot HCl$) C, H, N.

**p-(4-Ethoxycarbonyl-4-phenyl-1-piperidinoethyl)maleanilic Acid (7a).**—This was obtained in 89% yield following the procedure of Cava[11] for the prepn of maleanilic acid; recrystd from THF–$Et_2O$, mp 236–238 dec. *Anal.* ($C_{26}H_{30}N_2O_5$) C, H, N.

**Acetic N-(p-Ethoxycarbonyl-4-phenyl-1-piperidinophenethyl)-maleamic Anhydride·HCl (7).**—When **7a** was treated with $Ac_2O$ and NaOAc as per the procedure of Cava[11] described for maleimide, an oily residue was obtd which was purified on column (silica gel) using EtOAc. It was dissolved in $CHCl_3$ and treated with dry HCl. Evapn of the solvent left an oily residue which solidified on washing with $Et_2O$. Two recrystns (MeOH–$Et_2O$) gave 0.31 g (63%) of the product as hydrochloride, mp 222–223°. *Anal.* ($C_{28}H_{32}N_2O_6 \cdot HCl$) C, H, N.

**Ethyl 1-(p-propionamidophenethyl)-4-phenyl-4-piperidinecarboxylate·HCl (8)** was prepared in 79% yield by treating anileridine·HCl with $EtCO_2H$ as described for **3**; recrystd (MeOH–$Et_2O$) mp 191–192°. *Anal.* ($C_{25}H_{32}N_2O_3 \cdot HCl$) C, H, N.

**Ethyl p-(4-Ethoxycarbonyl-4-phenyl-1-piperidinoethyl)succinanilate·HCl (9).**—Succinyl chloride monoethyl ester was treated with **1** according to the procedure described for **5** with the exception that the reaction mixture was heated for 4 hr. Two recrystns (MeOH–$Et_2O$) yielded **9** (78%), mp 176–177°. *Anal.* ($C_{28}H_{36}N_2O_5 \cdot HCl$) C, H, N.

**Ethyl 1-(p-succinimidophenethyl)-4-phenyl-4-piperidinecarboxylate·HCl (10)** was obtained in 75% yield following the procedure of Fieser.[19] The product melted at 239–240°. *Anal.* ($C_{26}H_{30}N_2O_5 \cdot HCl$) C, H, N.

**Acknowledgments.**—We thank Dr. A. A. Patchett, Research Laboratories, Merck and Co., Rahway, N. J., for the generous supply of anileridine and Dr. Ralph Jacobsen, Endo Laboratories, Garden City, N. Y., for the suply of naloxone used in these studies.

(19) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed, D. C. Heath and Company, Boston, Mass., 1955, pp 105–106.

# Structure–Activity Study of Phenethylamines as Substrates of Biosynthetic Enzymes of Sympathetic Transmitters[1]

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Regression analyses by a modification of the Free–Wilson technique were applied to the phenethylamines as substrates of enzymes associated with the biosynthetic pathway of sympathetic transmitters, such as dopamine β-hydroxylase and phenylethanolamine *N*-methyltransferase. The mathematical contribution of substituents to the activity of unsubstituted phenethylamine was found to be additive with the use of logarithmic activity data. It was also shown that the contribution values can be related to more fundamental physicochemical substituent parameters such as the Hammett σ and the hydrophobic constant, π, in certain cases.

Recently, we have reported an example of analyses of structure–activity relationship of a series of sympathomimetic amines using the Free–Wilson technique.[2,3] The inhibitory effect of the amines of variously modified structures on the norepinephrine uptake into isolated rat heart has been nicely correlated with the mathematical sum of contributions of structural fragments to the total activity of the molecule [3]

In this paper we wish to extend this mathematical method to the structure–activity analysis of phenethylamines as substrates of enzymes associated with the biosynthetic pathway of sympathetic transmitters such as dopamine β-hydroxylase and phenylethanolamine *N*-methyltransferase.

## Method

The method used in this paper is a modification of the Free–Wilson technique. In the same manner as in our previous analysis, we have used the log of activity data, since the log of activity is considered to be a free energy related parameter which is additive. Assuming that the effect of a certain substituent at a certain position on the activity of the phenethylamine molecule is constant and additive, we can derive a linear equation for each compound in the form of eq 1, where $A$ and $A_0$ represent the

$$\log \frac{A}{A_0} = \Sigma G_i X_i \qquad (1)$$

magnitude of the activity of substituted and unsubstituted phenethylamine, respectively, $G_i$ is the log activity contribution or the log activity enhancement factor of the $i$th substituent relative to that of H and $X_i$ is a parameter which takes a value of 1 or 0 according to the presence or absence of the $i$th substituent.

For a set of structure–activity analysis, $\log A_0$ is a constant. Thus, eq 1 is modified to eq 2, where $c$ is a constant. Substitution of the values of $X_i$, for substituents at various positions, into eq 2 yields simultaneous equations, the number of which is equal

$$\log A = \Sigma G_i X_i + c \qquad (2)$$

to the number of compounds in the set. The activities of the compounds, for example, are given as shown in eq 3–6, where the notations in parenthesis represent the substituent and its position.

$$\text{phenethylamine: } \log A = c \qquad (3)$$

$$\text{dopamine: } \log A = G(p\text{-OH}) + G(m\text{-OH}) + c \qquad (4)$$

$$\text{3,5-dimethoxy-}p\text{-tyramine: } \log A = G(p\text{-OH}) + G(m\text{-OCH}_3) + G(m'\text{-OCH}_3) + c \qquad (5)$$

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