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# Clonidine and Related Analogues. Quantitative Correlations

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Twenty-two structural derivatives of clonidine [2-(2,6-dichlorophenylimino)imidazolidine] have been synthesized and their main physicochemical parameters (log $P$, $\Delta R_M$, p$K_a$) determined. Quantitative correlations between the peripheral $\alpha$-mimetic action (pithed rats) and physicochemical parameters pointed out the critical role of the steric effect in the ortho positions. On the other hand, attempted quantitative correlations between physicochemical parameters and central hypotensive activity were unsuccessful. These results are discussed in the light of the postulated mechanism of action of clonidine.

In view of our interest in the hypotensive agent clonidine [2-(2,6-dichlorophenylimino)imidazolidine],[2] we describe here quantitative structure–activity relationships (QSAR) obtained by the Hansch method[3] in a series of phenyliminoimidazolidines related to clonidine.

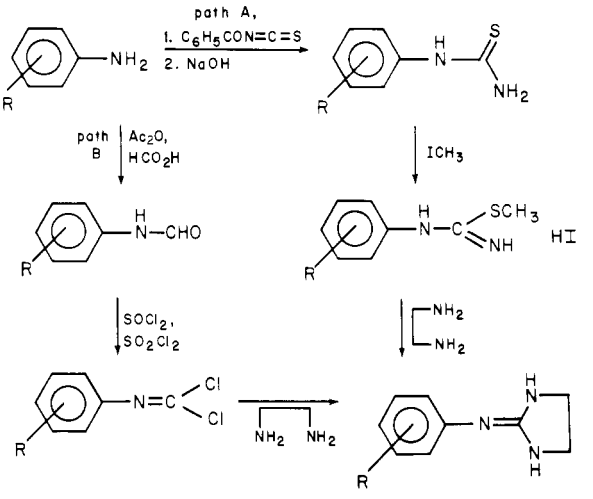
Clonidine is a hypotensive agent widely used therapeutically. It is well known, however, that its overall action on arterial blood pressure is a resultant of peripheral and central effects.[4] Peripheral effects are vasoconstrictive, whereas central effects are hypotensive. The central site of action is located in the medulla oblongata and probably more precisely in the obex[5] or on the ventral surface of the brain stem.[6]

The peripheral mechanism is explained by an $\alpha$-sympathomimetic action. The central mechanism may be similar but one cannot rule out the possibility that clonidine acts by inhibiting adrenergic presynaptic receptors.

Our work is devoted to the synthesis of compounds related to clonidine, to the determination of their physicochemical parameters (log $P$, p$K_a$, $\Delta R_M$), and to their pharmacological evaluation. In particular we have measured the hypertensive effect in the pithed rat (peripheral action). Our aim was to determine the physicochemical parameters which correlate best with the biological activity.

**Synthesis of 2-Aryliminoimidazolidines.** Aryliminoimidazolidines were generally synthesized by the action of ethylenediamine on the *S*-methylisothiouronium salt derivative (Scheme I, path A) and less commonly from the phenyldichloro isocyanide derivative (Scheme I, path B).

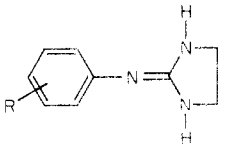
**Scheme I**




Starting materials were generally commercially available. The 2,6-difluoroaniline was obtained according to the method of Burton and Roe.[7] The 2,6-dimethyl-4-methoxyaniline was synthesized by some useful modifications of the method of Saunders and Watson[8] (see Scheme II).

The use of sulfanilic acid instead of aniline facilitated the isolation of the 2,6-dimethyl-4-methoxyaniline. It is also worthwhile mentioning that the original catalytic high-pressure hydrogenation was replaced by a chemical reduction using sodium hydrosulfite. The main physi-

Table I. Physicochemical Parameters of the Aryliminoimidazolidines



| Compd | R | % yield[a] | % yield[b] | Mp, °C[c] | $\Delta R_M$[d] | Log P[e] | p$K_a$[f] | $\Delta$p$K_a$[g] |
|---|---|---|---|---|---|---|---|---|
| 1 | 2,6-Dichloro | 75 | 50 | 305 (*i*-PrOH) | 0.38 | 0.83 | 8.05 | −2.35 |
| 2 | 2,5-Dichloro | 92 | 25 | 266 (*i*-PrOH–EtOAc) | 0.71 | 0.95 | 8.00 | −2.40 |
| 3 | 2,4,5-Trichloro | 36 | 55[h] | 202 (*i*-PrOH) | 1.20 | 1.63 | 7.70 | −2.70 |
| 4 | 2-Chloro-6-methyl | 58 | 50 | 226 (*i*-PrOH–EtOAc) | 0.45 | −0.40 | 9.20 | −1.20 |
| 5 | 2,6-Dimethyl | 90 | 26 | 204 | 0.42 | −1.65 | 10.50 | 0.10 |
| 6 | 2,6-Diethyl | 83 | 40 | 209 | 0.95 | −0.84 | 10.60 | 0.20 |
| 7 | 2,6-Diisopropyl | 60 | 48 | 137 | 1.32 | −0.21 | 10.65 | 0.25 |
| 8 | 2,6-Dibromo | 94 | 20 | 295 | 0.61 | 1.15 | 7.95 | −2.45 |
| 9 | 2-Methyl-4-chloro | 90 | 29 | 201 | 0.75 | −0.58 | 9.75 | −0.65 |
| 10 | 2,4-Dichloro | 90 | 20 | 212 | 0.76 | 0.69 | 8.65 | −1.75 |
| 11 | 2-Methoxy-4-methyl | 91 | 39 | 157 | −0.03 | −1.56 | 10.60 | 0.20 |
| 12 | 5-Chloro-2-methyl | 90 | 37 | 181 | 0.72 | −0.30 | 9.50 | −0.90 |
| 13 | 2,4-Dimethyl | 90 | 42 | 148 | 0.43 | −1.46 | 10.65 | 0.25 |
| 14 | Nonsubstituted | 82[i] | 85[h] | 220 | 0.00 | −1.80 | 10.40 | 0.00 |
| 15 | 2,4,6-Trichloro | 88 | 30 | 270 | 1.00 | −1.38 | 7.60 | −2.80 |
| 16 | 2,5-Dimethoxy | 71 | 34 | 176 | −0.42 | −1.78 | 10.15 | −0.25 |
| 17 | 4-Bromo | 84 | 52 | 208 (*i*-PrOH–$Et_2O$) | 0.58 | −0.50 | 9.65 | −0.75 |
| 18 | 5-Chloro-2,4-dimethoxy | 70 | 16[j] | 211 (*i*-PrOH–$Et_2O$) | −0.07 | −1.37 | 10.15 | −0.25 |
| 19 | 2,6-Difluoro | 53 | 45 | 238 | −0.24 | 0.02 | 8.40 | −2.00 |
| 20 | 2,4,6-Trimethyl | 80 | 35 | 120, 117–121[m] | | | | |
| 21 | 2-Ethyl | 85 | 41 | 120 (EtOAc–MeOH) | | | | |
| 22 | 4-Methoxy-2,6-dimethyl | 88 | 25 | 112 (MeOH–EtOAc) | | | | |
| 23[k] | 4-Hydroxy-2,6-dimethyl | | 90 | 250 (*i*-PrOH) | | | | |
| 24[l] | 2-Trifluoromethyl | | | | | 0.00 | 8.80 | −1.60 |
| 25[l] | 2-Chloro-4-methyl | | | | | −0.28 | 9.55 | −0.85 |
| 26[l] | 2,6-Dichloro-4-hydroxy | | | | | −0.40 | 7.95 | −2.45 |

[a] Yield in thiourea from aniline. [b] Yield in 2-aryliminoimidazolidine, HCl, from *N*-aryl-*S*-methylisothiourea. [c] Melting points and microanalysis of chlorohydrates recrystallized, unless otherwise stated, in *i*-PrOH–EtOAc, followed by addition of $Et_2O$. [d] $\Delta R_M$ of the neutral species (pH 13). [e] Partition coefficient between octan-1-ol and buffered water, pH 7.4. [f] p$K_a$ determined in $H_2O$–EtOH (1:1). [g] $\Delta$p$K_a$ calculated from $\Delta pK_a = pK_{aX} - 10.40$. [h] Compounds prepared from phenyl isocyanide dichloride; see path A, Scheme I. [i] Phenyl isocyanide dichloride obtained by the action of chlorine on phenyl isocyanate. [j] Compounds prepared in refluxing EtOH. [k] Compound prepared by demethylation of 22. [l] Compounds kindly given by C. H. Boehringer Sohn, Ingelheim. [m] See ref 14.

cochemical parameters of our imidazolidines are summarized in Table I.

**Measurement and Calculation of Parameters. Hydrophobic Parameters.** The partition coefficients were obtained by dissolving the imidazolidine salts in the aqueous buffered phase (pH 7.4) and measuring the extinction coefficient by uv spectroscopy. The aqueous phase was shaken under standard conditions with octan-1-ol, and the drug concentrations were again determined in the aqueous phase. In order to compare the partition coefficient of the free bases it would have been necessary to make the measurements at alkaline pH. This was shown to be difficult owing to the poor solubility of the base in the aqueous phase. Therefore the lipophilic characters of our compounds were evaluated by the $\Delta R_M$ in their thin-layer chromatography.

**Chromatographic $\Delta R_M$ Values.** The $R_f$ values of the molecules were determined by means of a reverse thin-layer chromatography technique. The mobile phase chosen was sufficiently alkaline (pH ~13) to keep the molecules in their neutral form. The measured $R_f$ values were expressed according to the equation of Bate-Smith.[9]

$$R_M = \log (1/R_f - 1)$$

Boyce and Milborow[10] had shown that this lipophilic constant was analogous to the partition coefficient. The difference in $R_M$ between the substituted and the unsubstituted compound, respectively, is noted as $\Delta R_M$, this constant being similar to the hydrophobicity constant ($\pi$) defined by Hansch.

$$\Delta R_M = R_{M(X)} - R_{M(H)}$$
$$\pi_X = \log P_{(X)} - \log P_{(H)}$$

We have tried to measure the $\Delta R_M$ of our imidazolidines using a pH 7.4 buffered aqueous phase but the observed separations were too small to allow a clear differentiation of the compounds. The use of a polyamide stationary phase according to Draber et al.[11] and of a mobile phase (buffered water, pH 7.4) containing variable quantities of acetone or dioxane gave no better separation.

**Electronic Parameters.** The influence of the aromatic substituents was expressed by the p$K_a$ values of the imidazolidines or more precisely by the term $\Delta pK_a = pK_{aX} - pK_{aH}$, where $pK_{aX}$ and $pK_{aH}$ are the p$K_a$ values of the substituted and unsubstituted compounds, respectively.

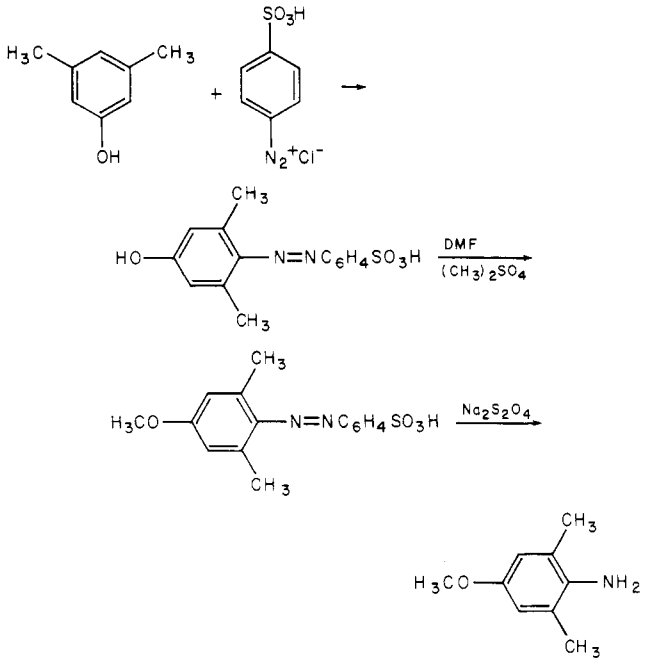
**Steric Parameters.**[12,16] We have chosen the value $E_s = 0$ for hydrogen, the steric constants of other groups being obtained by subtracting 1.24 from the tabulated values. As regards $\pi$ and $E_s$ parameters, we have employed either the sum of all the values of the phenyl ring substituents or a partial sum limited to a certain position, e.g., $E_{s_{2+6}}$. Arbitrarily the $E_{s_2}$ value was attributed to the smaller

Table II. Biological and Physicochemical Properties of the 2-Aryliminoimidazolidines

| Compd | $E_{s_2}$[a] | $E_{s_{2+6}}$[a] | $E_{s_3}$[a] | $\Sigma\pi$[b] | $\mathcal{F}$[c] | $\mathcal{R}$[c] | BP[d] | $pD_2^*$ ± SEM[e] Obsd | Calcd[f] | $\Delta pD_2^*$[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | −1.71 | −3.42 | 0 | 2.80 | −0.18 | −0.24 | 0 | 5.79 ± 0.03 (25) | 5.87 | 0.08 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.84 ± 0.04 (49) | 6.17 | 0.33 |
| 17 | 0 | 0 | 0 | 1.02 | 0.41 | −0.18 | 0 | 6.09 ± 0.07 (35) | 6.01 | −0.08 |
| 24 | 0 | −2.40 | 0 | 0.88 | 0.35 | 0.19 | 0 | 6.13 ± 0.04 (32) | 6.23 | 0.10 |
| 11 | 0 | −0.25 | 0 | 0.19 | 0.41 | −0.64 | 0 | 6.58 ± 0.04 (44) | 6.45 | 0.13 |
| 18 | 0 | −0.25 | −1.06 | 0.39 | 1.08 | −1.04 | 0 | 6.68 ± 0.06 (42) | 6.94 | 0.26 |
| 10 | 0 | −1.06 | 0 | 1.29 | 1.13 | −0.32 | ++ | 6.71 ± 0.03 (34) | 6.92 | 0.21 |
| 16 | 0 | −0.25 | −0.25 | −0.21 | 0.69 | −0.64 | 0 | 6.98 ± 0.03 (34) | 6.52 | −0.46 |
| 25 | 0 | −1.06 | 0 | 1.11 | 0.71 | −0.30 | ++ | 7.08 ± 0.06 (35) | 7.08 | 0.00 |
| 9 | 0 | −1.24 | 0 | 1.38 | 0.33 | −0.30 | 0 | 7.11 ± 0.04 (32) | 7.27 | 0.16 |
| 15 | −1.06 | −2.12 | 0 | 1.88 | 1.87 | −0.48 | ++ | 7.13 ± 0.04 (41) | 7.35 | 0.22 |
| 19 | −0.75 | −1.50 | 0 | 0.02 | 1.52 | −0.68 | 0 | 7.23 ± 0.05 (38) | 7.65 | 0.42 |
| 8 | −1.24 | −2.48 | 0 | 1.50 | 1.56 | −0.36 | ++ | 7.28 ± 0.05 (33) | 7.10 | −0.18 |
| 26 | −1.06 | −2.12 | 0 | 0.57 | 1.75 | −0.96 | 0 | 7.37 ± 0.05 (37) | 7.40 | 0.03 |
| 6 | −1.31 | −2.62 | 0 | 2.44 | −0.14 | −0.22 | − | 7.61 ± 0.04 (35) | 7.55 | −0.06 |
| 12 | 0 | −1.24 | −1.06 | 1.44 | 0.35 | −0.18 | 0 | 7.64 ± 0.03 (34) | 7.99 | 0.35 |
| 2 | 0 | −1.06 | −1.06 | 1.35 | 1.15 | −0.20 | 0 | 7.65 ± 0.07 (38) | 7.65 | 0.00 |
| 1 | −1.06 | −2.12 | 0 | 1.18 | 1.48 | −0.32 | ++ | 7.66 ± 0.04 (35) | 7.50 | −0.16 |
| 13 | 0 | −1.24 | 0 | 1.20 | −0.09 | −0.28 | − | 7.74 ± 0.05 (34) | 7.41 | −0.33 |
| 4 | −1.06 | −2.30 | 0 | 1.27 | 0.68 | −0.30 | ++ | 7.74 ± 0.04 (35) | 7.54 | −0.20 |
| 5 | −1.24 | −2.48 | 0 | 1.36 | −0.12 | −0.28 | 0 | 7.74 ± 0.04 (34) | 7.74 | 0.00 |

[a] From ref 12 and 16. [b] From ref 13 and 14. [c] From ref 15. [d] Variation of mean blood pressure in the intact rat: ++, decrease of at least 15%; 0, no significant variation (less than 10%); −, increase of more than 10%. [e] $pD_2^*$ ± standard error of the mean with the number of experimental values in parentheses. [f] Calculated using eq 4. [g] Difference between observed and calculated log $1/C$.

Scheme II




substituent in the ortho position, while $E_{s_6}$ was for the bulky one. The values are summarized in Table II.

**Biological Response.** The α-mimetic activity measured on the pithed rat was expressed by the $pD_2$, that is, the negative logarithm of the molar concentration of an agonist drug which causes a response equal to 50% of the maximum response of that drug.

Compounds were injected intravenously and the variations in blood pressure were measured by means of a cannula inserted into the carotid artery connected to a strain gauge transducer. The central action was obtained by intraventricular injection in the rat.

**Interparametric Correlations.** As our lipophilic parameters were determined by using two different techniques at different pH, it was of interest to ensure their validity by intercorrelating them with each other.

**Relationship between $\Delta R_M$ and $\pi$.** In the series of imidazolidines studied, the $pK_a$ values range from 7.6 to 10.6; in other words, it was necessary to correct the $R_M$ value for the ionization at pH 7.4. Using the $\pi$ values from the phenoxyacetic series a linear relationship between $\pi$ and $\Delta R_M$ values was obtained.

$$\Delta R_M = 0.60\ (\pm 0.10)\ \Sigma\pi \text{ phenoxy} - 0.21$$
$$n = 21;\ r = 0.94;\ s = 0.16;\ F = 149;\ p < 0.005$$

It was noted that highly significant relationships between $\Delta R_M$ and $\pi$ had been established by several authors.[17-20] The correlation we obtained showed that groups in the ortho position have the expected values; in other words, there was no marked influence of the neighboring imidazolidine function.

**Relationship between Log $P$, $\Delta R_M$, and $\Delta pK_a$.** Taking into account the $pK_a$ of the phenyliminoimidazolidines it should be possible to find a relationship between the $\Delta R_M$ measured in alkaline medium and the log $P$ obtained at pH 7.4. With 19 compounds we obtained the following equation.

$$\log P_{pH\ 7.4} = 0.90\ (\pm 0.22)\ \Delta R_M - 0.82\ (\pm 0.09)\ \Delta pK_a - 1.60$$
$$n = 19;\ r = 0.99;\ s = 0.20;\ F = 276;\ p < 0.005$$

Introduction of the term log $[K_a/(K_a + H^+)]$ in the equation led to

$$\log P_{pH\ 7.4} = 0.92\ \Delta R_M + 0.86 \log [K_a/(K_a + H^+)] + 0.96$$
$$n = 19;\ r = 0.99;\ s = 0.20;\ F = 286;\ p < 0.005$$

Bearing in mind that the partition coefficient of the base is a linear function of $\Delta R_M$, the equation is similar to that used by Cymerman-Craig and Diamantis.[21]

$$\log P_{pH} = \log P_{base} + \log [K_a/(K_a + H^+)]$$

**Relationship between $\Delta pK_a$, $\mathcal{F}$, and $\mathcal{R}$.** Close correlations were found between $pK_a$ and the tabulated electronic parameters $\sigma_{DB}$,[22] $\sigma^+$,[23] $\mathcal{F}$, and $\mathcal{R}$.[15,24]

The best one correlates the field effect $\mathcal{F}$ and the resonance effect $\mathcal{R}$ both defined by Swain and Lupton[24] with $\Delta pK_a$.

$$\Delta pK_a = -1.84\ (\pm 0.19)\ \mathfrak{F} - 1.83\ (\pm 0.46)\ \mathfrak{R} - 0.40$$
$$n = 22;\ r = 0.98;\ s = 0.24;\ F = 202;\ p < 0.005$$

**Peripheral α-Mimetic Action. Structure–Activity Correlations.** For the correlations, we considered that the fixation on the receptor is obtained with the protonated molecule, and we use instead of the $pD_2$ (of the peripheral α-mimetic action) values the $pD_2^* = \log 1/C^+$ where $C^+$ represents the concentration expressed in mol/kg of molecules which are protonated at pH 7.4 [$\log 1/C^+ = \log 1/C + \log [(K_a + H^+)/K_a]$]. The equations using $pD_2^*$ were slightly improved with respect to those using $pD_2$. We found the following relations.

$$pD_2^* = -1.70\ (\pm 0.78)\ E_{s_{2+6}} - 0.49\ (\pm 0.24)\ [E_{s_{2+6}}]^2 + 5.95 \quad (1)$$
$$n = 17;\ r = 0.78;\ s = 0.45;\ F = 11.1;\ p < 0.005$$
$$pD_2^* = -1.57\ (\pm 0.62)\ E_{s_{2+6}} - 0.59\ (\pm 0.20)\ [E_{s_{2+6}}]^2 - 0.84\ (\pm 0.58)\ E_{s_2} + 6.03 \quad (2)$$
$$n = 17;\ r = 0.88;\ s = 0.36;\ F = 15;\ p < 0.005$$
$$pD_2^* = -2.08\ (\pm 0.45)\ E_{s_{2+6}} - 0.82\ (\pm 0.17)\ [E_{s_{2+6}}]^2 - 1.28\ (\pm 0.42)\ E_{s_2} - 0.48\ (\pm 0.22)\ \mathfrak{F} + 6.11 \quad (3)$$
$$n = 17;\ r = 0.96;\ s = 0.22;\ F = 35.8;\ p < 0.005$$
$$pD_2^* = -1.93\ (\pm 0.50)\ E_{s_{2+6}} - 0.77\ (\pm 0.20)\ [E_{s_{2+6}}]^2 - 1.19\ (\pm 0.52)\ E_{s_2} - 0.70\ (\pm 0.26)\ E_{s_3} - 0.38\ (\pm 0.36)\ \mathfrak{F} + 6.17 \quad (4)$$
$$n = 22;\ r = 0.93;\ s = 0.29;\ F = 19.3;\ p < 0.005$$

Equation 1 reveals the importance of the steric factors in the ortho position. Equation 1 was improved by the addition of the term $E_{s_2}$ to give eq 2. Finally the best correlation contains in addition the field effect $\mathfrak{F}$ defined by Swain and Lupton (eq 3). In eq 2 and 3 the replacement of the $E_{s_2}$ term by the $E_{s_6}$ term leads to an equation of the same significance (identical $r$, $s$, and $p$ values). This suggests that the $E_{s_2}$ or $E_{s_6}$ terms simply differentiate the ortho-disubstituted compounds from the ortho-monosubstituted ones. In other words the $pD_2$ value of the 2,6-disubstituted 2-aryliminoimidazolidines not only depends on the steric hindrance in the ortho position but also on the distribution of the substituents on both the ortho positions.

Addition of a term taking into account the lipophilic or the steric hindrance in the 4 position did not improve the correlation. These parameters in the 4 position are probably not playing a role in the molecule–receptor interaction.

A slightly decreased correlation ($F_{5,16} = 19.3$) for the 22 compounds (including the meta-substituted derivatives) is given by eq 4. Comparison of eq 3 and 4 shows that the supplementary parameter $E_{s_3}$ does not entirely explain the influence of the meta substituent. Table II contains the data of calculated and observed activity of the 22 compounds used in eq 4.

## Discussion

All of our equations contain the term $(E_{s_{2+6}})^2$ implying the existence of an ideal steric hindrance in the ortho position. The term $E_{s_2}$ is implicitly included in the term $E_{s_{2+6}}$; this is the reason why we have considered two cases—firstly, that of monosubstituted compounds and, secondly, that of symmetrically disubstituted compounds. In the first case $E_{s_2}$ = H and therefore, according to our convention, equal to zero. Equation 3 can be written

$$pD_2^* = -2.08\ E_{s_6} - 0.82\ (E_{s_6})^2 - 0.48\ \mathfrak{F} + 6.11$$

Table III. Comparison of Observed and Calculated α-Adrenergic Activities from Eq 3

| Compd | Structure | $pD_2^*$ ± SEM[a] Obsd | Calcd |
|---|---|---|---|
| **20** | 2,4,6-Trimethyl | 7.80 ± 0.05 (36) | 7.82 |
| **23** | 2,6-Dimethyl-4-hydroxy | 7.51 ± 0.08 (29) | 7.60 |
| **21** | 2-Ethyl | 7.55 ± 0.09 (18) | 7.45 |

[a] $pD_2^*$ ± standard error of the mean with the number of experimental values in parentheses.

from which one finds the ideal value of $E_{s_6} = -1.27$. In the second case $E_{s_2} = E_{s_6}$ or $E_{2+6} = 2E_{s_2}$ and eq 3 can be written

$$pD_2^* = -2.08 \times 2E_{s_6} - 0.82\ (2E_{s_6})^2 - 1.28\ E_{s_6} - 0.48\ \mathfrak{F} + 6.11$$

the ideal value of $E_{s_6}$ being −0.83.

According to eq 3 and following our convention $E_{s_2} \leq E_{s_6}$ the more active compounds will be those disubstituted in the ortho position by atoms of the same size. For a given value of $E_{s_{2+6}}$ the higher in absolute value is the term $E_{s_2}$, the higher the activity will be.

The synthesis of 2-aryliminoimidazolidines having high $pD_2$ values, e.g., strong hypertensive properties, requires the 2,6-substituent with steric hindrance near −0.83. The tabulated steric hindrance of chlorine atom (−1.06) is close to the ideal value, but its electronic influence is unfavorable. It seems that the best compromise between steric and electronic effects is obtained with methyl groups ($E_{s_2} = -1.24$). Since it is known that the para position does not intervene in the hypertensive action, this position becomes free for the introduction of substituents improving the α-mimetic activity.

In connection with this idea, we have synthesized the following derivatives: **20**, having a methyl group in position 4; **23**, having a hydroxyl group in the 4 position; and the monosubstituted derivative **21**, the tabulated $E_{s_2}$ value for the ethyl group (−1.31) being very close to the ideal value (−1.27).

The observed and calculated α-adrenergic activities of these compounds not included in eq 4 are summarized in Table III.

The *p*-methoxy derivative **22** was not included owing to its high toxicity ($LD_{50}$ of 54 μM/kg against 172 μM/kg for clonidine). Taking into account the accuracy of the biological results (SD ≤0.25) and the standard deviation of eq 3 ($s = 0.22$), calculated and observed values are in good agreement, therefore supporting the validity of the equations and their physicochemical parameters.

**Mechanism of Action of Clonidine.** We have not been able to correlate the hypotensive action of the imidazolidine derivatives, the hypotensive action having been assessed intravenously or intracerebrally. This failure was probably due to the small number of hypotensive drugs (7 out of 22), but this is not necessarily the only one. Indeed, if all of the 22 analogues are potent α-mimetics, some of them, with a partition coefficient and a $pK_a$ value consistent with a central action, are devoid of hypotensive effect; e.g., the 2,5-dichlorophenyliminoimidazolidine **2**, whose $pD_2$, $pK_a$, and log *P* are similar to those of clonidine, has no hypotensive effect at all, whatever the administration and dosage.

Such discrepancies again pose the problem of the central mechanism of action of clonidine. One might simply admit that central and peripheral adrenergic receptors are dif-

ferent; possibly also, clonidine might have a central mechanism of action different from that so far accepted. It is known that all noradrenomimetic molecules show a negative feedback action on presynaptic relay concomitant with their action on postsynaptic receptors. Recently Starke et al.[25] have shown that in the rabbit artery pulmonary, clonidine has a higher affinity for the presynaptic $\alpha$-receptor when compared to the postsynaptic $\alpha$-receptor; in other words, inhibitory action might be preponderant. Lastly, the nonspecific action of clonidine on presynaptic receptors cannot be excluded.

## Experimental Section

**Pharamacology. Peripheral $\alpha$-Adrenergic Activity.** The experiments were performed in the pithed rats with Shipley and Tilden's method[26] which has been somewhat modified; the animals were anesthetized with pentobarbitone sodium (50 mg/kg) and were not dosed with atropine. Mean blood pressure (BP) was recorded (Gilson MSP) by means of a transducer (Statham P23 Db) connected to the carotid artery. For intravenous injections a jugular vein was cannulated and cummulative doses of each product were administered to eight rats. The $\alpha$-adrenergic vasopressive activity is expressed in terms of $pD_2^*$ values (negative logarithm of the molar concentration, corrected for ionization, of an agonist which causes a response equal to 50% of the maximum response of that agonist).

**Centrally Mediated Hypotensive Action.** Intact anesthetized rats were used for intravenous and intracerebral injections. In the latter case the skull was fixed in a stereotaxic instrument; the products were injected (5 $\mu$l of saline) into the right lateral ventricle. Injection of Evans blue and dissection of the brain enabled verification of injection localization. Anesthesia and blood pressure measurement techniques were similar to the above.

**Chemistry.** NMR spectra were determined on a Perkin-Elmer R 12A instrument with $CDCl_3$ solutions and $Me_4Si$ internal standard. Melting points were determined on a Kofler block and were uncorrected. The reactions were monitored routinely on TLC using Merck $F_{254}$ silica gel impregnated plates which were developed in a mixture hexane–EtOAc containing 5% diethylamine.

All compounds described in the present work gave C, H, and N analyses within 0.4% of theoretical values.

**General Procedure (as Illustrated by the Synthesis of Clonidine).[27] Path A.** 2,6-Dichlorophenylthiourea was prepared according to Kinoshita.[28]

***N*-(2,6-Dichlorophenyl)-*S*-methylisothiourea Hydroiodide.** Methyl iodide (14 ml, 0.22 mol) was added to a solution of 2,-6-dichlorophenylthiourea (49.8 g, 0.225 mol) in MeOH (600 ml). The solution was refluxed for 2 h, cooled, and evaporated to dryness under reduced pressure. The crystalline material was washed several times with $Et_2O$ to yield the expected salt (64 g), mp 173°.

**2-(2,6-Dichlorophenylimino-2)imidazolidine (ST 155).** A mixture of *S*-methylisothiourea (17.52 g, 0.06 mol), ethylenediamine (12 ml, 0.18 mol), and EtOH (150 ml) was heated at 120° for 15 h. The mixture was cooled, whereupon $CH_3SH$ was released, and evaporated to dryness under reduced pressure. The residue was dissolved in acidic water (pH 5) and washed with $Et_2O$ to eliminate the neutral products. The aqueous solution was alkalinized, extracted with EtOAc, dried over $MgSO_4$, and evaporated to dryness under reduced pressure. The imidazolidine derivatives prepared by this method (see Table I) were purified by recrystallization or column chromatography.

**Path B.** The following method given for clonidine has also been used for the synthesis of **3** and **14** (see Table I).

**2,6-Dichloroformanilide.** The acetic–formic anhydride was prepared by heating $Ac_2O$ (50 ml, 0.5 mol) with $HCO_2H$ (21.5 ml, 0.52 mol) at 50° for 15 min and cooled immediately to 0°. To this solution was added 2,6-dichloroaniline (40.5 g, 0.25 mol); the mixture was heated to 50° for 5 h and allowed to stand overnight at ambient temperature, evaporated to dryness, and recrystallized from benzene: yield 80%; mp 178° (lit.[29] mp 175–178°).

**2,6-Dichlorophenyl Isocyanide Dichloride.** $SOCl_2$ (175 g, 1.5 mol) and $SO_2Cl_2$ (27.5 g, 0.2 mol) were introduced into a round-bottomed flask and followed by 2,6-dichloroformanilide (37 g, 0.2 mol). The reaction mixture was heated to 50° with stirring for 12 h. The excess $SOCl_2$ was removed under reduced pressure and the residual oil purified by vacuum distillation: yield 71%; bp 80° (0.1 mm).

**2-(2,6-Dichlorophenylimino-2)imidazolidine.** The previous isocyanide (4.01 g, 16.5 mmol) in EtOAc (4.5 ml) and ethylenediamine (1.98 g, 33 mmol) in EtOAc (4.5 ml) were simultaneously added dropwise to anhydrous triethylamine (12.5 ml, 0.09 mol) in EtOAc (10 ml). The mixture was stirred for 10 h, diluted with water, and extracted into EtOAc. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure to give 1.70 g (45%) of crude clonidine, mp 136°.

**2,6-Dimethyl-4-methoxyaniline.** $Na_2CO_3$ (21.2 g, 0.2 mol) was added to a stirred suspension of sulfanilic acid (69.2 g, 0.4 mol) in water (400 ml) and the mixture heated until dissolution. The solution was cooled to 15° and $NaNO_2$ (29.6 g, 0.43 mol) dissolved in water (80 ml) was added. After 5 min this solution was rapidly poured onto crushed ice (480 g) and HCl (85 ml) and stirred for 15 min. This was then added to a mixture of 3,5-dimethylphenol (48.8 g, 0.4 mol) in alkaline water (88 g, 2.2 mol of NaOH in 480 ml) and ice (320 g).

The mixture was stirred for 1 h and the crystalline (2,6-dimethyl-4-hydroxyphenylazo)benzenesulfonic acid filtered off.

This crude sulfonic acid was suspended in a vigourously stirred alkaline solution (4.8 g of NaOH in 150 ml of water and 50 ml of DMF) and $Me_2SO_4$ (46 g, 0.36 mol) was carefully added.

Cooling and dilution with water (400 ml) precipitated the methylated product (acidification of the mother liquor precipitated about 15 g of starting material).

This crude material was suspended in NaOH (3.32 g) in about 400 ml of water. The mixture was treated at 80–90° and $Na_2S_2O_4$ (150 g) added until decolorization (if necessary some NaOH pellets were added).

The colorless aqueous phase was extracted several times with $Et_2O$. The $Et_2O$ was dried over $MgSO_4$ and evaporated under reduced pressure to give 25.3 g of 2,6-dimethyl-4-methoxyaniline (overall yield of 42%), mp 40° (lit.[8] mp 41°).

**Correlations.** Correlation between hypertensive activity ($pD_2$) and physicochemical parameters of the substituted imidazolidines was searched for as described by Hansch.[3a] The constants used to characterize the substituent R carried by the aromatic nucleus were the hydrophobic constant,[13,14] the field effect $\mathcal{F}$ defined by Swain and Lupton[15,24] and $E_s$, the Taft steric factor.[12]

The regression analyses were performed on a Univac 1110 computer; $r$ is the correlation coefficient and $s$ the standard deviation; the test $F$ was calculated according to ref 30. Each equation has its 95% confidence intervals in parentheses. Unless otherwise stated the significance level of the variables is $p < 0.005$.

**Partition Coefficients.** The octan-1-ol (1 l., Merck Rein Art. 991) was purified by washing with 4 N $H_2SO_4$ (3 × 100 ml), $H_2O$ (2 × 100 ml), 2 N NaOH (3 × 100 ml), and $H_2O$ (4 × 100 ml) and distilled under reduced pressure. The aqueous phase was prepared by mixing aqueous M/15 $KH_2PO_4$ (19.6 ml) with M/15 $Na_2HPO_4$ (80.4 ml). The buffer thus obtained was saturated with the purified octan-1-ol.

The imidazolidine hydrochloride (10–20 mg) was dissolved in the buffered aqueous solution (50 ml) saturated with octan-1-ol. The aqueous solution was then shaken with an equal volume of octan-1-ol saturated with water until the equilibrium was reached.

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# (−)-3-Isothujone, a Small Nonnitrogenous Molecule with Antinociceptive Activity in Mice

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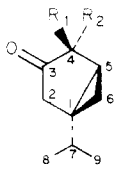
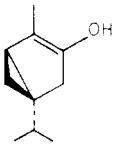
(−)-3-Isothujone and (+)-3-thujone were examined for antinociceptive activity using the hot-plate and Nilsen tests. In the hot-plate test (−)-3-isothujone ($ED_{50}$ = 6.5 mg/kg) was found to be codeine-like and equipotent with (−)-$\Delta^9$-tetrahydrocannabinol while the racemic material was essentially half as potent as the levorotatory isomer. (+)-3-Thujone was inactive in both antinociceptive tests as were several structural analogues of the 3-thujones. As with the THC's less antinociceptive activity was observed in the Nilsen test than in the hot-plate assay. Acute toxicities for the 3-thujones were determined and vastly improved synthetic procedures have been developed for two long-known but difficultly accessible 3-thujanols.

The widely occurring natural products (+)-3-thujone (**1**) and (−)-3-isothujone (**2**)[1,2] are ketonic constituents of the essential oils of the two[2] *Artemisia* (family Compositae) species (*A. absinthium* L. and *A. pontica* L.) from which the alcoholic drink absinthe was prepared in France before its prohibition in 1915. In a recent report del Castillo et al.[3] compared the structure of a 3-thujone[4] and the $\Delta^{3,4}$-enol (**3**) with (−)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC, **4**), the major psychoactive component of marihuana. One of the ketones (**1** or **2**) was credited[3] with being responsible for certain CNS effects of absinthe and based upon certain geometrical similarities in their structures, del Castillo et al.[3] suggested that this ketone or the $\Delta^{3,4}$-enol (**3**) and the THC's (or their biologically active 11-hydroxy metabolites) act at a common receptor in the CNS.




**1**, $R_1 = CH_3$; $R_2 = H$ (+)
**2**, $R_1 = H$; $R_2 = CH_3$ (−)




**3**

In a search for novel nonaddicting analgesics, we have examined the tetrahydrocannabinols, many of their metabolites,[5] and a considerable number of synthetic analogues.[6] Therefore it was of interest to examine **1**, **2**, and related compounds for possible antinociceptive activities.

As our initial evaluation of a commercial mixture of **1** and **2** revealed significant antinociceptive activity, determined as described below, a detailed search of the literature was made in order to determine how to best purify each of these two ketones. During this examination of the literature, we reviewed the careful work by Norin in which the relative[7] and absolute[8] stereochemistry of the 3-thujones and 3-thujanols was assigned. Later work,[9-11] including an x-ray crystal study,[12] has confirmed the assignments by Norin who showed that the absolute stereochemistry of natural (−)-3-isothujone is represented by **2** and that of its epimer, (+)-3-thujone, by **1**. The $\Delta^{3,4}$-enol of these ketones must therefore have structure **5**. Since this structure is enantiomeric with that of **3** which del Castillo et al. compared with natural (−)-$\Delta^9$-THC, known[13] to have the absolute stereochemistry shown in **4**, it follows that the topological comparison of these authors was made between unnatural $\Delta^{3,4}$-thujone enol (**3**) and natural $\Delta^9$-THC (**4**). Because of this situation and the