

# Structure-Activity Relationships in Reactivators of Organophosphorus-Inhibited Acetylcholinesterase. 9. N-Heterocyclic Acraldioximes Methiodides<sup>1</sup>

Palmarisa Franchetti, Mario Grifantini,\* and Sante Martelli

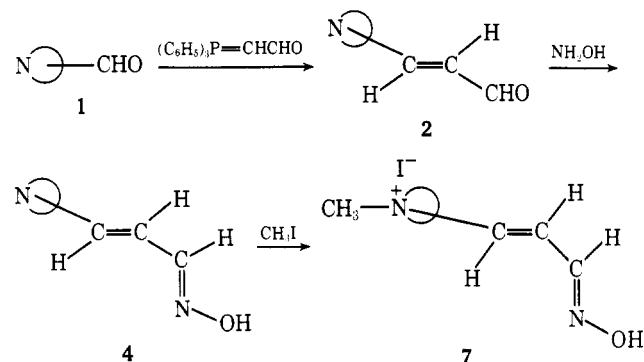
Institute of Pharmaceutical and Organic Chemistry, University of Camerino, Camerino, Italy. Received January 2, 1975

N-Heterocyclic acraldioximes methiodides, where the heterocyclic residues are 2-, 3-, and 4-pyridyl, 2-(1-methyl)imidazolyl or 4-pyrimidyl, were prepared and tested for their reactivating potency on acetylcholinesterase inhibited from diisopropylphosphorofluoridate (DFP). The *in vitro* testing revealed that the new compounds are good reactivators of the phosphorylated electric eel cholinesterase. The structure-activity relationships are briefly discussed.

Quaternary salts of some N-heterocyclic aldioximes are effective reactivators of organophosphorus-inhibited acetylcholinesterase (AChE).<sup>2-7</sup> Among these the methiodides of pyridinaldioximes, 1-methylimidazole-2-aldoxime, and 4-pyrimidinaldoxime are particularly active. As part of our continuing studies to investigate further the relationships between molecular structure and antidotic ability of reactivators of phosphorylated AChE, we have synthesized vinyl homologs of the above-mentioned aldioximes.

**Chemistry.** The acraldioximes **4** substituted at the  $\beta$  position with a pyridyl, 2-(1-methyl)imidazolyl, or 4-pyrimidyl residue were prepared by refluxing the corresponding aldehydes **1** with a MeOH solution of  $\text{NH}_2\text{OH}\cdot\text{HCl}$ . The acraldehydes **2** were prepared in high yields by the classical Wittig synthesis from the formyl derivatives **1** and formyl-methylenetriphosphorane (Scheme I, Table I).<sup>8</sup> In the case of formyl derivative **1e** also the 5-(pyrimidin-4-yl)penta-2,4-dienal **3** was obtained. The 2- and 3-H signals appear in the NMR spectra of acraldehydes in  $\text{CDCl}_3$  as a pair of doublets and a doublet, respectively, with a mutual trans coupling of 16 Hz.<sup>9</sup>

Scheme I

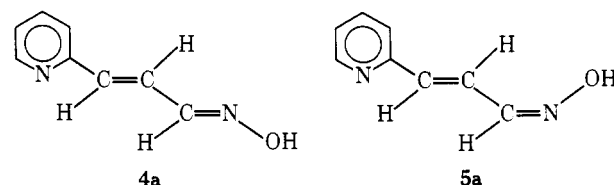


- a, N = 2-pyridyl
- b, N = 3-pyridyl
- c, N = 4-pyridyl
- d, N = 2-(1-methyl)imidazolyl
- e, N = 4-pyrimidyl

In the reaction between the acraldehydes and hydroxylamine hydrochloride a single product was usually obtained. Only in the case of the 2-pyridyl derivative did the oximation reaction give geometrical isomers which were separated by chromatography on silica gel. Comparison of the NMR spectra in deuterioacetone at 90 MHz revealed that in the low-melting isomer **4a** the proton ( $\text{H}_a$ ) linked to the carbon of the oxime group gave a signal (doublet) downfield relative to that of the corresponding proton of the high-melting isomer **5a**. On the other hand, the signal of the proton next to the oxime ( $\text{H}_b$ ) in the case of isomer **5a** could be clearly seen as a pair of doublets at 0.60 ppm downfield relative to the corresponding signal for isomer **4a**. These chemical shift differences allow the assignment of the *Z* configuration to the oxime group of the high-melt-

ing isomer and the *E* configuration to the other.<sup>10</sup> The NMR spectra of the oximes obtained from the other acraldehydes showed for the protons  $\text{H}_a$  and  $\text{H}_b$  two signals with chemical shifts similar to those of the corresponding protons of the *E* isomer **4a**. For this reason we have assigned the same configuration to the oxime group of these compounds. The coupling constants values (16 Hz) of the  $\text{H}_b$  and  $\text{H}_c$  protons of compounds **4a-e** and **5a** indicated a trans (*E*) arrangement about the 2,3 double bond. The identity of chemical shift of the  $\text{H}_c$  protons of the compounds **4a-e** and **5a** gives evidence to the absence of any deshielding effect of the oxime group and suggests a trans-transoid conformation for these compounds (Table II).

The oximes **4a-e** and the oxime **6** obtained from the aldehyde **3**, by reaction with MeI in acetone at room temperature, gave the respective quaternary salts **7a-e** and **8** (Table III).



Because of the greater complexity of NMR spectra of quaternized compounds we have not been able to ascertain if, in the case of methiodides **7a,b,e**, configurational equilibration or conversion could have occurred during quaternization. However, since in the NMR spectra of compounds **7c** and **7d** the signals of the protons in the side chain, although slightly shifted, have features similar to those of the relative protons of acraldioximes **4c,d**, we think that these methiodides have the same configuration.

## Results and Discussion

From the results of the biological testing tabulated in Table IV it may be seen that compounds **7a-e** exhibit a reactivating activity ranging from 0.35 to 0.61 times that of 2-pyridinaldoxime methiodide (2-PAM). The most active of them appears to be the  $\beta$ -(4-pyrimidyl)acraldoxime (**7e**); the insertion of a second vinyl group in the side chain of **7e** (compound **8**) causes a diminution of the activity. It is interesting to note that compound **7b** with the acraldoxime group in the 3 position is equally active as a reactivator as the isomers **7a** and **7c**. This is in contrast to the behavior of the methiodide of pyridinaldioxime isomers, where the nicotinic isomer is very less active,<sup>3</sup> and confirms that, when the oxime group is distant from the ring, the three pyridine isomers show nearly equivalent activity.<sup>13</sup> The fact that 2-PAM does not inhibit the enzyme at the concentration used while the acraldioximes methiodides **7a-e** do inhibit the enzyme may be rationalized as due to lower bond strength with the enzyme surface resulting from van der Waals attractions and hydrophobic bonds; this was confirmed by the finding that the compound **8**, with a longer chain of carbon atoms and with an additional double bond,







**Table I. Acraldehydes**

		$  \begin{array}{c}  \text{R} \quad \text{H}_b \\  \diagdown \quad \diagup \\  \text{C}=\text{C} \\  \diagup \quad \diagdown \\  \text{H}_c \quad \text{CH}_3\text{O}  \end{array}  $									Formula <sup>b</sup>	
		Chemical shifts <sup>a</sup> (τ) and coupling constant (Hz)										
Compd	R	Yield, %	Mp, °C	Recrystn solvent	H <sub>a</sub>	H <sub>b</sub>	H <sub>c</sub>	J <sub>ab</sub>	J <sub>bc</sub>	J <sub>ac</sub>		
2a <sup>c</sup>	2-Pyridyl	71			0.35 <sup>e</sup> (dd)	2.92 <sup>e</sup> (dd)	2.57 <sup>e</sup> (dd)	7	16	1.8		
2b <sup>d</sup>	3-Pyridyl	60			0.34 (d)	3.23 (dd)	2.50 (d)	7	16			
2c <sup>d</sup>	4-Pyridyl	74	39–40	CHCl <sub>3</sub>	0.28 (d)	3.25 (dd)	2.59 (d)	7	16			
2d	2-(1-Methyl)imidazolyl	46	105–108	C <sub>6</sub> H <sub>6</sub>	0.36 <sup>e</sup> (d)	3.34 <sup>e</sup> (dd)	2.34 <sup>e</sup> (d)	8	16	C <sub>7</sub> H <sub>8</sub> N <sub>2</sub> O		
2e	4-Pyrimidyl	63	106–107	CHCl <sub>3</sub>	0.38 (dd)	2.92 (dd)	2.57 (dd)	7	16	2.0 C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> O		

<sup>a</sup>In CDCl<sub>3</sub>. <sup>b</sup>All compounds were analyzed for C, H, and N. <sup>c</sup>See ref 11. <sup>d</sup>See ref 12. <sup>e</sup>In DMSO-*d*<sub>6</sub>.**Table II. Acraldoximes**

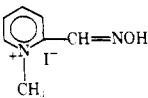
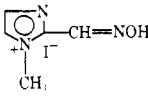
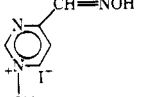
<div style="text-align: center;"> <math display="block">  \begin{array}{c}  \text{R} \qquad \text{H}_b \\  \diagdown \quad \diagup \\  \text{C}=\text{C} \\  \diagup \quad \diagdown \\  \text{H}_c \qquad \text{CH}_a=\text{NOH}  \end{array}  </math> </div>												
Chemical shifts <sup>a</sup> and coupling constants (Hz)												
Compd	R	Con-fig	Yield, %	Mp, °C	Recrystn solvent	H <sub>a</sub>	H <sub>b</sub>	H <sub>c</sub>	J <sub>ab</sub>	J <sub>bc</sub>	J <sub>ac</sub>	Formula <sup>b</sup>
5a	2-Pyridyl	<i>Z</i>	6	123–125	C <sub>6</sub> H <sub>6</sub>	2.70 <sup>c</sup> (d)	2.19 <sup>c</sup> (dd)	3.20 <sup>c</sup> (d)	8	16		C <sub>8</sub> H <sub>8</sub> N <sub>2</sub> O
4a	2-Pyridyl	<i>E</i>	51	113–115	C <sub>6</sub> H <sub>6</sub>	2.13 <sup>c</sup> (d)	2.79 <sup>c</sup> (dd)	3.20 <sup>c</sup> (d)	8	16		C <sub>8</sub> H <sub>8</sub> N <sub>2</sub> O
4b	3-Pyridyl	<i>E</i>	67	147–149	EtOAc	2.10 (d)	2.73 (dd)	3.25 (d)	8	16		C <sub>8</sub> H <sub>8</sub> N <sub>2</sub> O
4c	4-Pyridyl	<i>E</i>	63	149–151	EtOAc	2.11 (d)	3.10 (dd)	3.27 (d)	8	16		C <sub>8</sub> H <sub>8</sub> N <sub>2</sub> O
4d	2-(1-Methyl)imidazolyl	<i>E</i>	55	215–217	MeOH	2.15 (dd)	2.68 (dd)	3.27 (dd)	8	16	1.8	C <sub>7</sub> H <sub>9</sub> N <sub>3</sub> O
4e	4-Pyrimidyl	<i>E</i>	58	136–138	EtOAc	2.08 (dd)	2.51 (dd)	3.20 (dd)	8	16	2.0	C <sub>7</sub> H <sub>7</sub> N <sub>3</sub> O

<sup>a</sup>At 60 MHz in a mixture of (CD<sub>3</sub>)<sub>2</sub>CO-DMSO-*d*<sub>6</sub> (80:20). <sup>b</sup>See footnote b, Table I. <sup>c</sup>At 90 MHz.**Table III. Methiodides of Oximes 4a–e and 6**

		$  \begin{array}{c}  \text{R} \quad \text{H} \\  \diagdown \quad \diagup \\  \text{C}=\text{C} \\  \diagup \quad \diagdown \\  \text{H} \quad \text{CH}=\text{NOH}  \end{array}  $					Formula <sup>a</sup>
Compd	R	Yield, %	Mp, °C	Recrystn solvent	λ <sub>max</sub> (EtOH), nm		
7a		82	254–255	MeOH	322	C <sub>9</sub> H <sub>11</sub> IN <sub>2</sub> O	
7b		75	208–210	MeOH	285	C <sub>9</sub> H <sub>11</sub> IN <sub>2</sub> O	
7c		85	249–250	MeOH	327	C <sub>9</sub> H <sub>11</sub> IN <sub>2</sub> O	
7d		62	284–286	MeOH	290	C <sub>8</sub> H <sub>12</sub> IN <sub>3</sub> O	
7e		45	185–186	MeOH	335	C <sub>7</sub> H <sub>10</sub> IN <sub>3</sub> O	
8		53	193–195	MeOH	380	C <sub>9</sub> H <sub>12</sub> IN <sub>3</sub> O	

<sup>a</sup>See footnote b, Table I.

Table IV. Activities of Methiodides 7a-e and 8 on AChE and on DFP-Inhibited AChE

Compd	pK <sub>a</sub> <sup>a</sup>	Rel potency as reactivator of oxime, 1 × 10 <sup>-3</sup> M		% inhibn by oxime, 1 × 10 <sup>-3</sup> M	I <sub>50</sub> , <sup>d</sup>
		A <sup>b</sup>	B <sup>c</sup>		
	7.85	1	1	0	3.8 × 10 <sup>-3</sup>
	8.30	0.55 ± 0.04	0.70	11	1.8 × 10 <sup>-3</sup>
	7.30	0.79 ± 0.06 <sup>e</sup>	0.79	0	3.8 × 10 <sup>-3</sup>
7a	9.00	0.39 ± 0.04	0.75	48	2.2 × 10 <sup>-3</sup>
7b	9.35	0.38 ± 0.05	0.75	49	2.1 × 10 <sup>-3</sup>
7c	9.10	0.35 ± 0.03	0.83	59	1.6 × 10 <sup>-3</sup>
7d	9.05	0.46 ± 0.03	0.89	47	2.3 × 10 <sup>-3</sup>
7e	8.95	0.61 ± 0.06	1.20	51	2.1 × 10 <sup>-3</sup>
8	9.60	0.21 ± 0.03	0.55	62	1.1 × 10 <sup>-3</sup>

<sup>a</sup>pK<sub>a</sub> values were obtained by potentiometric titration. <sup>b</sup>The A values are the mean of four experiments. <sup>c</sup>Corrected for anti-AChE activity of the oximes. <sup>d</sup>Concentration for 50% inhibition. <sup>e</sup>SD.

is the greater enzyme inhibitor in this series of compounds.

## Experimental Section

Melting points were determined in a capillary using a Buchi apparatus and are uncorrected. The uv spectra were recorded on a Unicam SP-800 spectrophotometer in EtOH solution; the NMR spectra were measured on a Jeol 60-HL spectrometer and on a Hitachi Perkin-Elmer R12 A spectrometer using Me<sub>4</sub>Si as internal standard. Uv, ir, and NMR spectra were in accord with the proposed structures. When analyses are indicated only by symbols of the elements, the analytical results obtained for these elements were within 0.4% of the theoretical values.

**Acraldehydes 2.** The formyl derivative 1a (0.02 mol) and formylmethylenetriphenylphosphorane (0.02 mol) in benzene (200 ml) were heated under reflux for several hours. The solution was extracted two times with 30 ml of 7% HCl and the aqueous HCl solution, after basification to pH 8 with Na<sub>2</sub>CO<sub>3</sub>, was extracted several times with EtOAc. Removal of the solvent under reduced pressure followed by column chromatography [silica gel, EtOAc-C<sub>6</sub>H<sub>6</sub> (70:30)] yielded compounds 2a-e.

**5-(Pyrimidin-4-yl)penta-2,4-dienal (3).** From the reaction between 4-formylpyrimidine and formylmethylenetriphenylphosphorane a second product was isolated by column chromatography [silica gel, EtOAc-C<sub>6</sub>H<sub>6</sub> (70:30)]. It was recrystallized from CHCl<sub>3</sub>: mp 124-126° (the yield in this compound increases with the time of reaction and becomes high after 8 hr); λ<sub>max</sub> (EtOH) 310 nm; ν<sub>max</sub> (Nujol) 1663 (C=O); τ (CDCl<sub>3</sub>, 60 MHz) 0.50 (1 H, d, J = 7.5 Hz), 0.97 (1 H, pseudo s), 1.45 (1 H, J = 5 Hz), 2.20-2.90 (3 H, m), 3.22 (1 H, d, J = 15 Hz), 3.75 (1 H, q, J = 7.5 and 15 Hz). Anal. (C<sub>9</sub>H<sub>8</sub>N<sub>2</sub>O) C, H, N.

**Acraldoximes 4a-e and 5a.** A mixture of 0.05 mol of acraldehyde and 0.15 mol of NH<sub>2</sub>OH·HCl in 100 ml of MeOH was refluxed for 30 min. Evaporation of the solvent gave a residue which was dissolved in H<sub>2</sub>O. The solution was made basic with dilute aqueous Na<sub>2</sub>CO<sub>3</sub> and extracted with EtOAc. Concentration of the organic extract left a residue which was recrystallized. In the case of the oxime of 3-(2-pyridyl)acraldehyde the residue obtained was found to consist of a two-component mixture, which was separated by column chromatography with EtOAc-C<sub>6</sub>H<sub>6</sub> (50:50) as eluent. Evaporation of the first fraction of eluate gave the E isomer 4a. The Z isomer 5a was obtained by evaporation of the second fraction of eluate.

**5-(4-Pyrimidyl)penta-2,4-dienal Oxime (6).** Conditions similar to those used to form the acraldoximes 4 were used to synthesize 6 from the aldehyde 3. The residue from the EtOAc extracts was recrystallized from EtOAc: mp 158-160°; τ [(CD<sub>3</sub>)<sub>2</sub>CO-DMSO-d<sub>6</sub> (90:10)] (60 MHz) 1.10 (1 H, br s), 1.00 (1 H, pseudo s), 1.40 (1

H, d, J = 5.5 Hz), 2.05-3.50 (6 H, m). Anal. (C<sub>8</sub>H<sub>8</sub>N<sub>3</sub>O) C, H, N.

**Acraldoximes Methiodides 7a-e and Methiodide of Oxime 6 (8).** To 0.01 mol of oxime dissolved in 100 ml of Me<sub>2</sub>CO, 2.5 ml (0.04 mol) of CH<sub>3</sub>I was added. The solution was allowed to stand at room temperature for 3 days. The quaternary salt, which separated as crystals, was filtered and recrystallized: τ for compound 7c (DMSO-d<sub>6</sub>) 1.20 (2 H, d, J = 6 Hz, α-pyridinic protons), 1.73 (2 H, d, J = 6 Hz, β-pyridinic protons), 2.20 (1 H, d, J = 8 Hz, -CH=NOH), 3.00 (1 H, d, J = 16 Hz, -CH=CH-); τ for compound 7d (DMSO-d<sub>6</sub>) 1.80 (1 H, dd, J = 8 and 2 Hz, -CH=NOH), 2.28 (2 H, pseudo s, heterocyclic protons), 2.70 (1 H, dd, J = 16 and 8 Hz, =CHCH=NOH), 3.00 (1 H, dd, J = 16 and 2 Hz, -CH=CH-).

**Enzymatic Assays.** The in vitro reactivating potency of the products 7a-e and 8 was determined on electric eel AChE (Sigma Chemical Co.) inhibited by diisopropylphosphorofluoridate (DFP) according to the procedure previously described.<sup>14</sup> Since the effectiveness of an AChE reactivator is limited by its ability to inhibit AChE, the inhibitor potency of compounds 7a-e and 8 was determined under the same experimental condition. The results were compared to the activity of methiodides of 2-pyridinaldoxime (2-PAM), 2-(1-methyl)imidazolidoxime, and 4-(1-methyl)pyrimidinaldoxime (Table IV). The concentration of 2-PAM which we have used (1.10<sup>-3</sup> M) is a little greater than the lowest which is effective against DFP-inhibited AChE. We have preferred to use this concentration since the same concentration was used by us and by other authors to test several reactivators of DFP-inhibited AChE; reactivation data relative to different reactivators are therefore comparable.<sup>1,14,15</sup>

**Acknowledgment.** We are indebted to Professor M. L. Stein for stimulating discussion of these results.

## References and Notes

- (a) This work was supported by the Italian National Research Council. (b) P. Franchetti, M. Grifantini, S. Martelli, and M. L. Stein, *Farmacol. Ed. Sci.*, **29**, 309 (1974) (paper 8).
- I. B. Wilson and S. Ginsburg, *Biochim. Biophys. Acta*, **18**, 168 (1955).
- F. Hobbiger, D. G. O'Sullivan, and P. W. Sadler, *Nature (London)*, **182**, 1498 (1958).
- I. B. Wilson, *Biochem. Pharmacol.*, **1**, 200 (1959).
- Y. Ashani, H. Edery, J. Zahavy, W. Kuhnberg, and S. Cohen, *Isr. J. Chem.*, **3**, 133 (1965).
- H. P. Benschoop, A. M. Van Oosten, D. H. J. M. Platenburg,

- and C. Van Hooideonk, *J. Med. Chem.*, **13**, 1208 (1970).
- (7) M. Grifantini, S. Martelli, and M. L. Stein, *J. Pharm. Sci.*, **61**, 631 (1972).
- (8) S. Trippet and D. M. Walker, *J. Chem. Soc.*, 1226 (1961).
- (9) L. M. Jackman and S. Sternahall, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon, Oxford, 1969, p 301.
- (10) Reference 9, p 226.
- (11) M. Ohta and Y. Isowa, *Nippon Kagaku Zasshi*, **80**, 1198 (1959) [*Chem. Abstr.*, **55**, 4504 (1961)].
- (12) L. S. Davies and G. Jones, *J. Chem. Soc. C*, 2572 (1971).
- (13) P. Franchetti, M. Grifantini, and M. L. Stein, *J. Pharm. Sci.*, **59**, 710 (1970).
- (14) P. Franchetti, M. Grifantini, S. Martelli, and M. L. Stein, *J. Med. Chem.*, **17**, 18 (1974).
- (15) Y. Ashani and S. Cohen, *Isr. J. Chem.*, **5**, 59 (1967).

## Structure-Activity Relationships in Cinnamamides. 1. Synthesis and Pharmacological Evaluation of Some (*E*)- and (*Z*)-*N*-Alkyl- $\alpha,\beta$ -dimethylcinnamamides

A. Balsamo, P. L. Barili, P. Crotti, B. Macchia,\* F. Macchia, A. Pecchia,

*Istituti di Chimica farmaceutica e di Chimica organica dell'Università di Pisa, 56100 Pisa, Italy*

A. Cuttica, and N. Passerini

*Istituto Carlo Erba per Ricerche terapeutiche, 20159 Milano, Italy. Received January 29, 1975*

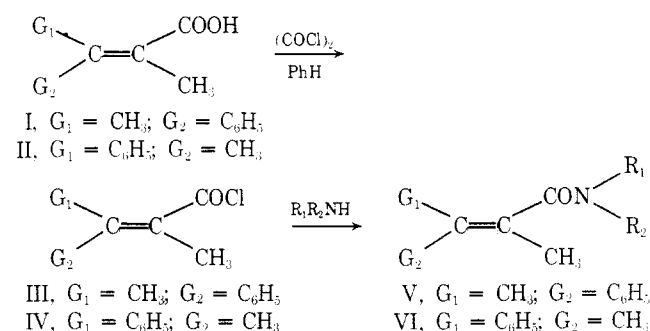
Two series of (*E*)- and (*Z*)-*N*-alkyl- $\alpha,\beta$ -dimethylcinnamamide derivatives were prepared and the biological activity of these compounds was investigated in a series of pharmacological tests. All compounds tested had clear activity on the CNS; generally, this was depressant with *E* isomers, while *Z* isomers always caused marked stimulation (tremors and convulsions). Some of the *E* isomers also had a clear-cut anticonvulsant activity as shown by the antagonistic effect on pentylenetetrazole-induced seizures in the mouse. The NMR spectra of these compounds, which confirm their configurations, are discussed.

Several amides of cinnamic acid or of its derivatives display a variety of pharmacological properties, ranging from CNS depressant,<sup>1-17</sup> muscle-relaxant,<sup>7,18</sup> and anticonvulsant<sup>7,18,19</sup> activities to an antidepressant one;<sup>20-22</sup> *N*-piperazincinnamamides are active on the cardiovascular system;<sup>23,24</sup> furthermore, some cinnamamide derivatives are fungicides<sup>25,26</sup> and herbicides.<sup>27</sup> The lack of specificity is attributed to the different type of substituents present on the double bond carbon atoms, on the phenyl group, or on the amidic nitrogen of these drug molecules. Although this class of compounds has been studied fairly extensively, mostly in the patent literature, attention has been paid only to derivatives unsubstituted on the  $\alpha$  and  $\beta$  carbon and to compounds monosubstituted either on the  $\alpha$  or on the  $\beta$  carbon. Moreover, although some approaches have been attempted to correlate structure with the pharmacological activity, only in one case has a comparison been made between pharmacological activity of *E* and *Z* isomers.<sup>20</sup>

As part of a program to examine the influence of structural modification and configuration on the biological properties of cinnamamides, two series of *N*-monoalkyl-substituted (*E*)- and (*Z*)- $\alpha,\beta$ -dimethylcinnamamides were prepared and tested pharmacologically. For comparison, the *N*-unsubstituted and *N,N*-dimethyl derivatives were also studied. The results of this investigation are described in this paper.

**Chemistry.** Amides (Tables I and II) were synthesized according to Scheme I. (*E*)- (I) and (*Z*)- $\alpha,\beta$ -dimethylcinnamic acids (II) were transformed into their corresponding acid chlorides (III and IV) by treatment with oxalyl chloride in benzene; use of thionyl chloride is unsuccessful in this case, because both I and II are transformed almost quantitatively by this reagent to 2,3-dimethylindenone.<sup>11,28</sup> Acid chlorides III and IV were converted without purification into the (*E*)- and (*Z*)-amides V and VI by treatment with the appropriate base in benzene.

### Scheme I



At first sight, the configurations of amides V and VI could be directly derived from those of the corresponding acids (I and II) of known stereochemistry.<sup>29</sup> However, the possibility of an interconversion of the isomers during the treatment of the acids with oxalyl chloride<sup>28,30</sup> renders necessary confirmatory evidence of the amide configurations. This can be provided by the NMR spectral data summarized in Tables I and II. As for the corresponding acids,<sup>28</sup> esters,<sup>29</sup> and alcohols,<sup>31</sup> in all cases, the signals of the  $\alpha$ -methyl protons are at a higher field in the spectra of the *E* series in which they are *cis* to the phenyl group. Analogously, the protons of the  $\text{NR}_1\text{R}_2$  group of the *Z* compounds resonate at a higher field than the same protons of the *E* series. Another effect which might be used to distinguish the two series is the anisotropy of the carbonyl group;<sup>32</sup> we shall report in a separate paper on the lack of this effect in our case. A further noteworthy point in the NMR spectra is the value of the long-range coupling constant between the two  $\alpha$ - and  $\beta$ -methyl groups in the *E* series ( $J = 1.6$  Hz), which is in excellent agreement with the values found for a *trans* homoallylic coupling constant.<sup>33</sup>

**Pharmacology.** The following animals were used: mice