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Structure–Activity Relationships in Cinnamamides. 1. Synthesis and Pharmacological Evaluation of Some (*E*)- and (*Z*)-*N*-Alkyl-α,β-dimethylcinnamamides

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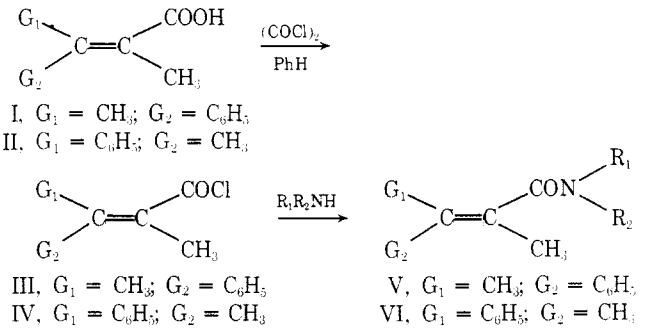
Two series of (*E*)- and (*Z*)-*N*-alkyl-α,β-dimethylcinnamamide derivatives were prepared and the biological activity of these compounds was investigated in a series of pharmacological tests. All compounds tested had clear activity on the CNS; generally, this was depressant with *E* isomers, while *Z* 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,[1-17] muscle-relaxant,[7,18] and anticonvulsant[7,18,19] activities to an antidepressant one;[20-22] *N*-piperazinylcinnamamides are active on the cardiovascular system;[23,24] furthermore, some cinnamamide derivatives are fungicides[25,26] and herbicides.[27] 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 α and β carbon and to compounds monosubstituted either on the α or on the β 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.[20]

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*)-α,β-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*)-α,β-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.[11,28] 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.

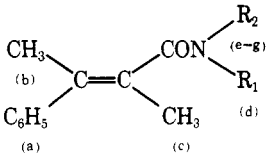
Scheme I



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.[29] However, the possibility of an interconversion of the isomers during the treatment of the acids with oxalyl chloride[28,30] 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,[28] esters,[29] and alcohols,[31] in all cases, the signals of the α-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 NR_1R_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:[32] 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 α- and β-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.[33]

Pharmacology. The following animals were used: mice

Table I. Physical Properties of (*E*)-*N*-Alkyl-α,β-dimethylcinnamamides



No.	R_1	R_2	Crystn solvent[a]	Yield, %	Mp, °C	Formula[b]	NMR spectra parameters[c] δ_a	δ_b	δ_c	δ_d	δ_e	δ_f	δ_g	$J_{b,c}$
1	H	H	F	84	189–190[d]	$C_{11}H_{13}NO$	7.30	2.17	1.80	5.98				1.5
2	H	CH_3	E	82	116–117	$C_{12}H_{15}NO$	7.08	2.04	1.75	6.67			2.88	1.5
3	H	C_2H_5	C	88	54–55	$C_{13}H_{17}NO$	7.18	2.08	1.77	6.42		3.39	1.19	1.5
4	H	n-C_3H_7	A	81	63–64	$C_{14}H_{19}NO$	7.32	2.12	1.80	6.25		3.38 1.56	0.97	1.6
5	H	n-C_4H_9	B	73	46–47	$C_{15}H_{21}NO$	7.37	2.12	1.80	6.04		3.44 1.55	1.00	1.6
6	H	i-C_3H_7	D	74	109–110	$C_{14}H_{19}NO$	7.25	2.09	1.77	5.72	4.21		2.22	1.6
7	H	i-C_4H_9	C	67	117–118	$C_{15}H_{21}NO$	7.34	2.12	1.80	6.07	2.00	3.26	0.99	1.6
8	H	s-C_4H_9	D	69	88–89	$C_{15}H_{21}NO$	7.32	2.10	1.80	5.72	4.11	1.51	1.21 0.97	1.6
9	H	t-C_4H_9	E	67	151–152	$C_{15}H_{21}NO$	7.33	2.10	1.77	5.56			1.45	1.6
10	H	C_3H_5[e]	C	81	93–94	$C_{14}H_{17}NO$	7.35	2.10	1.77	6.35	2.87	0.70		1.6
11	H	C_5H_9[f]	D	68	116–118	$C_{16}H_{21}NO$	7.36	2.11	1.77	6.85	4.40	1.70		1.6
12	H	C_6H_{11}[g]	E	68	159–160	$C_{17}H_{23}NO$	7.35	2.11	1.78	6.65	3.98	1.54		1.6
13	H	$CH_2CH{=}CH_2$	D	74	56–57	$C_{14}H_{17}NO$	7.35	2.11	1.77	6.30	5.82	5.28 4.05		1.6
14	H	$CH_2C{\equiv}CH$	D	65	132–133	$C_{14}H_{15}NO$	7.37	2.15	1.81	6.47	2.30	4.23		1.6
15	CH_3	CH_3	B	65	38–40	$C_{13}H_{17}NO$	7.34	1.97	1.78				3.12 3.07	1.6

[a] A, Petroleum ether (bp 30–50°); B, petroleum ether (bp 40–60°); C, hexane; D, ligroine (bp 60–80°); E, ligroine (bp 80–100°); F, benzene–hexane. [b] All compounds were analyzed for C, H, and N. [c] e, methine protons; f, methylene protons; g, methyl protons. [d] Lit.[28] mp 190–191°. [e] Cyclopropyl. [f] Cyclopentyl. [g] Cyclohexyl.

Icem-CET (SPF Caw) weighing 17–22 g and rats Icem-CER (SPF Caw) weighing 130–150 g, fasted for 9 hr. All compounds were administered orally as suspension in 0.5% Methocel 90° HG 400 cP; the volume of gavage was 0.1–0.2 ml/10 g of body weight in mice and 0.25 ml/100 g of body weight in rats.

Observation Assessment of Mouse Behavior.[34,35] The compounds were administered orally at six dose levels (from 25 to 800 mg/kg) using suspensions at different concentrations. Two male and two female mice for each dose were kept under observation. In order to check the maximum of intensity and the persistence of the symptoms, the animals were examined 0.5, 1, 2, 4, and 6 hr after administration. The symptomatology was checked again 24 hr later. The approximate LD_{50} values were determined by graphical interpolation from the dose–response curves of the data plotted on logarithmic–probability paper after 7 days of observation.

Anticonvulsant Activity in Mice. This activity was checked by testing the ability of the compounds to antagonize a dose of pentylenetetrazole (130 mg/kg ip) that induces maximal extensor seizures in 100% of the control animals. The compounds were administered orally at 50 mg/kg to groups of ten male mice 30 min before the pentylenetetrazole. The compounds which proved active at this dose (protection ≥30% of animals) were further tested, on groups of 20 male mice per dose level, to define the ED_{50}, which was calculated by probit analysis.[36]

Other Activities. The following activities were also investigated: antagonism of reserpine-induced blepharospasm and hypothermia in the mouse,[37,38] prevention or enhancement of central stimulation, hyperthermia and autonomic effects induced by amphetamine in the mouse,[39–41] inhibition of phenylquinone-induced writhing in the mouse[42] and carrageenin-induced edema in the rat hind paw;[43] anticholinergic, antihistaminic, and antibarium chloride activity were also tested on isolated ilea from guinea pigs and rats.

Results and Discussion

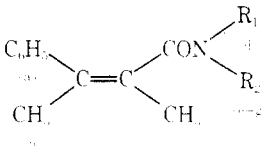
Pharmacological data on (*E*)- and (*Z*)-amides are summarized in Table III. The main activities observed were on the CNS.

Data from Irwin's test indicate that (*E*)-cinnamamides (V) caused CNS depression, evident in the reduced locomotor activity and positional passivity. Ataxia, loss of righting reflex, hypothermia, and, at larger doses, muscle relaxation also occurred. This symptomatology reached its maximum between 30 and 60 min after administration and had largely regressed after 3 hr, and all the surviving animals appeared normal after 24 hr. The CNS depressant activity does not show any obvious structure–activity relationship and, moreover, does not correlate with the toxicity. Some of these (*E*)-cinnamamides demonstrated an antagonistic effect against maximal extensor seizures induced by pentylenetetrazole. This activity does not parallel with the depressant action. The aminopropargyl derivative **14** is the most potent but also the most toxic compound.

The *Z* isomers (VI), with the exception of one derivative (**16**), caused pronounced CNS excitation. Most drugs induced tremors and clonic convulsions and compounds **19** and **29** also induced tonic extensor convulsions. This symptomatology appeared within a few minutes and did not last more than 90 min; the tremors were the longer lasting symptom.

With all these compounds a subordinate locomotor activ-

Table II. Physical Properties of (*Z*)-*N*-Alkyl-α,β-dimethylcinnamamides



No.	R_1	R_2	Crystn solvent[a]	Yield, %	Mp, °C	Formula[b]	NMR spectra parameters[c,d] δ_a	$\delta_{b,c}$	δ_d	δ_e	δ_f	δ_g
16	H	H	F	65	172–173[e]	$C_{11}H_{13}NO$	7.31	2.02	5.30			
17	H	CH_3	C	63	79–80	$C_{12}H_{15}NO$	7.37	2.08	5.25			2.49
18	H	C_2H_5	C	72	65–66	$C_{13}H_{17}NO$	7.20	2.04	5.12		2.93	0.61
19	H	*n*-C_3H_7	A	72	52–53	$C_{14}H_{19}NO$	7.35	2.05	5.12		2.94 0.96	0.56
20	H	*n*-C_4H_9		68	160 (8 mm)[f]	$C_{15}H_{21}NO$	7.37	2.06	5.50		2.99 0.82	0.75
21	H	*i*-C_3H_7	D	78	99–100	$C_{14}H_{19}NO$	7.34	2.05	4.79	3.87		0.68
22	H	*i*-C_4H_9	A	65	64–65	$C_{15}H_{21}NO$	7.37	2.07	5.10	1.32	2.81	0.56
23	H	*s*-C_4H_9	E	70	95–96	$C_{15}H_{21}NO$	7.37	2.06	4.78	3.70	1.01	0.65 0.57
24	H	*t*-C_4H_9	B	72	94–95	$C_{15}H_{21}NO$	7.35	2.05	4.80			0.96
25	H	C_3H_5[g]	C	77	85–86	$C_{14}H_{17}NO$	7.33	2.05	5.12	2.42	0.49 −0.12	
26	H	C_5H_9[h]	D	70	107–108	$C_{16}H_{21}NO$	7.35	2.05	4.93	4.03	1.35	
27	H	C_6H_{11}[i]	C	67	106–107	$C_{17}H_{23}NO$	7.35	2.05	4.90	3.55	1.36	
28	H	$CH_2CH{=}CH_2$	A	77	54–55	$C_{14}H_{17}NO$	7.36	2.07	4.90	5.26	4.90 3.60	
29	H	$CH_2C{\equiv}CH$	D	75	89–91	$C_{14}H_{15}NO$	7.37	2.06	5.27	2.06	3.77	
30	CH_3	CH_3	A	69	73–74	$C_{13}H_{17}NO$	7.16	2.03[j] 1.96[k]	2.61			2.48

[a–c] See corresponding footnotes in Table I. [d] Overlapping of the α- and β-methyl protons does not allow identification of the exact chemical shifts of these protons and therefore of their coupling constants; α- and β-methyl protons are sufficiently separated only in the case of compound **30**. [e] Lit.[28] mp 174–175°. [f] Boiling point; n^{20}D 1.5310. [g] Cyclopropyl. [h] Cyclopentyl. [i] Cyclohexyl. [j] δ_c, $J_{b,c}$ = 0.6 Hz. [k] δ_b.

ity reduction appeared at doses inducing tremor and convulsions. None of these amides showed anticonvulsant activity.

As far as the structure–activity relationship is concerned, pharmacological results clearly indicate that the *N*-monoalkyl-substituted (*E*)- and (*Z*)-α,β-dimethylcinnamamides display a different action on the CNS, the *E* derivatives showing CNS depressant and, some of them, anticonvulsant activity, and the *Z* isomers possessing CNS stimulant properties. A comparative study on the biological activity of (*E*)- and (*Z*)-α,β-unsubstituted cinnamamides[20] has shown only quantitative differences between the two configurations; in this case, the *E* are much more active than the *Z* isomers, especially as antidepressants. As regards the N-unsubstituted and *N,N*-dimethyl derivatives, the *E* isomers **1** and **15** exhibited CNS-depressant activity, in accordance with that shown by the other compounds of this series; on the other hand, the *Z* isomer **16**, as previously pointed out, showed activity different from that found in all the other *Z* derivatives examined.

Experimental Section

Melting points were determined on a Kofler hot-stage and are uncorrected. Elemental analyses were performed by the microanalytical laboratory of the Institute of Pharmaceutical Chemistry. All analytical samples gave combustion values within 0.4% of theoretical values. Ir spectra were taken with a Perkin-Elmer Infracord Model 137 as Nujol mulls in the case of solid compounds or as liquid film in the case of liquids. NMR spectra were recorded in ca. 10% solutions in $CDCl_3$ on a JEOL C-60 HL spectrometer using TMS as internal standard. Chemical shifts (δ, ppm) were measured directly from the spectra determined at a sweep width of 540 Hz. The recorded *J* values (Hz) were measured using a sweep width of 108 Hz.

(*E*)- (I) and (*Z*)-α,β-dimethylcinnamic acids (II) were prepared according to the procedure of Jackman and Lown[29] from ethyl 2-methyl-3-phenyl-3-hydroxybutyrate and separated through fractional crystallization from petroleum ether: I, mp 109–110° (lit.[29] 109–110°); II, mp 111–112° (lit.[29] 112–113°).

Preparation of (*E*)- (V) and (*Z*)-*N*-Alkyl-α,β-dimethylcinnamamides (VI). A solution of I or II (3.52 g, 0.02 mol) and dimethylformamide (0.4 ml) in anhydrous benzene (120 ml) was treated dropwise with a solution of oxalyl chloride (5.08 g, 0.04 mol) in anhydrous benzene (60 ml). After completion of the addition, the reaction mixture was left at room temperature for 24 hr and then evaporated in vacuo (rotating evaporator) to dryness. In order to remove completely the unreacted oxalyl chloride, anhydrous benzene (30 ml) was added to the residue and the solution was evaporated again to dryness under reduced pressure; this operation was repeated twice. The residue of (*E*)-(III) [ir 1750 and 1780 cm^{-1} (C=O) (lit.[28] 1758 and 1791 cm^{-1})] or (*Z*)-α,β-dimethylcinnamoyl chloride [ir 1780 cm^{-1} (C=O) (lit.[28] 1770 cm^{-1})] was dissolved in anhydrous benzene (60 ml) and treated dropwise with a solution of the amine (0.04 mol) in anhydrous benzene (60 ml). In the case of ammonia or gaseous amines, the gas was bubbled into the benzene solution of the acid chloride for 20 min. After the addition of the base, the reaction mixture was left at room temperature for 12 hr with occasional stirring, extracted with dilute aqueous HCl and saturated aqueous $NaHCO_3$, and evaporated to dryness to give the amide which was crystallized or distilled. Crude amides were shown to be configurationally homogeneous by their ir spectra.

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Table III. Results Obtained in Pharmacological Screening Tests

	Observational assessment of mouse behavior					
	Behavioral		Neurologic			
No.[a]	Locomotory act., reduction,[b] mg/kg po	Positional passivity,[b] mg/kg po	Tremors,[b] mg/kg po	Clonic-type convulsions, MED,[c] mg/kg po	Approximate LD_{50},[d] mg/kg po	Anticonvulsant act., ED_{50},[e] mg/kg po
1	500	450	*f*		>800	60.9 (49.6–74.8)
2	200	400			600	63.0 (44.5–121.7)
3	200	300			600	
4	100	300			>800	
5	200	500	*g*		>800	
6	200	600	*h*		>800	
10	100	250			500	55.9 (47.0–66.4)
12	400	800	*h*		>800	
13	130	300			550	67.0 (58.4–76.9)
14	150	400			300	45.3 (37.4–54.9)
15	300	350			600	
16	400	800			>800	
17	250		200	400	600	
18	400		150	400	600	
19	250		400	400	450	
20	800		400	400	750	
21	250		400	200	380	
25	400		300	400	600	
27	>800		800	800	>800	
28	550		400	400	600	
29	150		100	200	600	
30	400		200	400	600	

[a] Compounds 7–9, 11, 22–24, and 26 were not tested because of insufficient material. [b] Dose, causing 50% of the maximal effect according to Irwin,[34,35] assessed by graphical interpolation from the log dose/peak effect function. [c] Minimum effective dose. [d] See text. [e] In parentheses the fiducial limits for $p = 0.05$. [f] Inactive at the screening doses. [g] Tense appearance at 200 mg/kg. [h] Tense appearance at 400 mg/kg.

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Potential Central Nervous System Antitumor Agents. Hydantoin Derivatives

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Hydantoin derivatives of varying lipophilic character were prepared as nitrogen mustard carriers for CNS antitumor evaluation. Activity was studied in the murine ependymoblastoma brain tumor system. Multiple cures were observed for three of the four analogs examined. The compounds were also active in the intraperitoneal leukemia L1210 and P388 systems as well as in B16 melanoma and Lewis lung carcinoma.

The design of drugs which may be useful in the chemotherapy of tumors of the central nervous system contains numerous challenges. In addition to possessing antitumor activity, a compound should have structural features which allow it to circumvent natural defense mechanisms, such as the blood-brain barrier (BBB). There appear to be significant differences in the anatomical structure of brain tumors and their surrounding areas compared to normal brain.[1] While the neoplasm appears to alter the BBB in such a way that drug penetration is sometimes enhanced,[2-4] the changes are not constant among different types of brain tumors. This indicates that the permeability properties are not altered to the same extent[5] and the BBB is a factor which must be considered. The situation is complex and in seeking new antitumor drugs, one should be concerned with the type of structure which (a) penetrates the BBB,[6] (b) does so in significant concentrations, and (c) has antitumor activity.

The principle of using a carrier for an antitumor active functional group, e.g., a nitrogen mustard, is not new, and phenylalanine mustard (sarcolysin) is an example of this application. In a recent review of CNS antitumor agents,[7] Broder and Rall concluded that new drug emphasis should be placed on alkylating agents which are able to cross the BBB. The reports that 5,5-diphenylhydantoin (DPH) penetrated the BBB in significant concentrations[8] and localized in brain tumors relative to surrounding normal brain tissue,[9] but had no antitumor activity, prompted a study of hydantoins as carriers for nitrogen mustard groups in an attempt to prepare agents which might have utility as drugs for CNS and brain tumors. Mauger and Ross have previously used the hydantoin ring as a carrier in a series of active bis(2-chloroethyl)aminoarylhydantoins which were tested against the Walker tumor system.[10]

The importance of the partition coefficient as a parameter in CNS drug activity has been shown and quantitated.[11] DPH has a log P value of 2.47 in the octanol–water system.[12] This value approximates an optimum value (log P_0) of about 2.0 found to be characteristic of many CNS agents.[11] The addition of an alkylating group to DPH to yield the DPH mustard **16** should significantly alter the partition coefficient of the compound relative to DPH. An approximation of the log P of **16** as the neutral molecule can be made as seen in eq 1. The modification made to con-

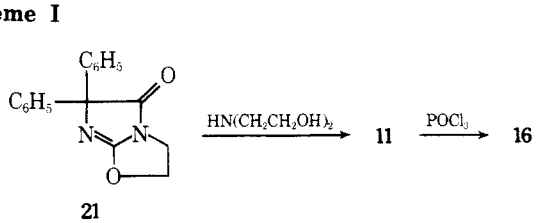
$$\log P(\text{DPH}) + \log P(\text{Et}_3\text{N}) + 2\pi(\text{Cl}) = \log P(\mathbf{16}) \quad (1)$$

$$2.47 + 1.44 + 2(0.39) = 4.69$$

fer antitumor activity on DPH might, therefore, alter its log P value in such a way as to minimize its CNS properties. Because of the large increase in lipophilic character caused by the addition of the $CH_2CH_2N(CH_2CH_2Cl)_2$ group, the carrier hydantoin was modified in order to compensate. An additional four derivatives (**17–20**) were chosen for synthesis based on their estimated log P values (1.5–2.7). After **16–20** were synthesized, attempts were made to measure the partition coefficients of several of these molecules by the method of Hansch.[12,13] These experiments, however, were not successful because of solution decomposition problems. This is often the case with bis(2-chloroethyl) derivatives.

Chemistry. The initial member of the series, **16**, was prepared by two procedures. An earlier hydantoin study[14] had produced the bicyclic compound **21**. During an investigation of the alkylating properties of **21**, it was allowed to react with diethanolamine to produce **11** which was subsequently converted to **16** (Scheme I).

Scheme I



21

A more general method employed a 3-(β-chloroethyl)hydantoin (Scheme II). Physical and chemical data are shown in Table I. Several of the intermediate products were oils. In those instances, yields were computed based on the first crystalline material isolated.