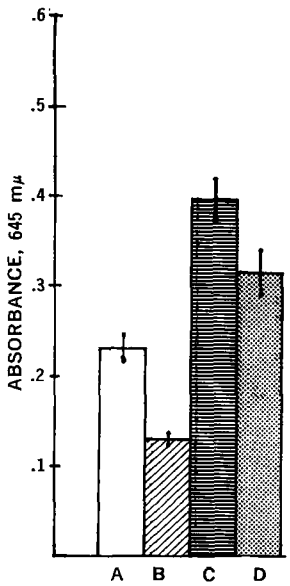


Fig. 6—Effects of phenylbutazone in hypophysectomized-adrenalectomized and sham-operated rats on the levels of serum turbidity (absorbance readings, means ± standard errors). Key: A, sham control (starch); B, sham + phenylbutaz. (100 mg./kg.); C, hypox. + adx. (starch); D, hypox. + adx. + phenylbutaz. (100 mg./kg.).

necessary for the stabilizing action of phenylbutazone.

The results of the present study do not preclude the possibility that endocrines other than the pituitary-adrenal system and/or enzyme systems are involved in the serum-turbidity responses of drugs to heat.

REFERENCES

(1) Piliero, S. J., and Colombo, C., "IIIrd International Congress of Pharmacology," Pergamon Press, New York, N. Y., 1966, p. 153.
(2) Piliero, S. J., and Colombo, C., *J. Clin. Pharmacol.*, **7**, 198(1967).
(3) Mizushima, Y., *Lancet*, **1**, 169(1965).
(4) Mizushima, Y., and Suzuki, H., *Arch. Intern. Pharmacodyn.*, **157**, 115(1965).
(5) Mizushima, Y., and Nakagawa, T., *Rheumatism*, **22**, 16(1966).
(6) Glenn, E. M., and Kooyers, W. M., *Life Sci.*, **5**, 619(1966).
(7) Whitehouse, M. W., and Skidmore, J. F., *J. Pharm. Pharmacol.*, **17**, 668(1965).
(8) Goodwin, J. F., *J. Mich. State Med. Soc.*, **60**, 334 (1964).
(9) Ishisaki, K., and Ishisaki, T., *J. Immunol.*, **85**, 163(1960).
(10) Opie, E. L., *J. Exptl. Med.*, **115**, 597(1962).
(11) Selye, H., "Stress," Acta Inc., Montreal, Canada, 1950.
(12) Sayers, G., *Physiol. Rev.*, **30**, 241(1950).

Keyphrases

Anti-inflammatory activity—screening method
Serum turbidimetric response—heat application
Turbidimetric response, heat—anti-inflammatory agents
Adrenal gland effect—turbidimetric response
Pituitary gland effect—turbidimetric response
Absorbance—analysis, turbidity

Structure-Activity Relationships Among Some Selected Substituted Cyanoacetamides

By HERBERT F. SCHWARTZ, II, ROBERT G. BROWN, EUGENE I. ISAACSON, and JAIME N. DELGADO

Selected *N*-alkyl- and *N,N*-dialkyl-2,2-dialkylcyanoacetamides, including 2-cyanoacyl-, piperidines, pyrrolidines, and morpholines, were synthesized in order to study structure-activity relationships. These were administered to rats and their anticonvulsant activity evaluated by means of the electroshock test. The median effective dose was determined for seven compounds exhibiting protective action, while seven were inactive or displayed convulsant properties. An estimated LD_{50} was determined for the three most effective and least toxic substances. Preliminary pharmacological data have been tabulated and correlations of bioaction with structure are presented.

THE DEMONSTRATION of anticonvulsant activity within a series of 2,2-dialkylcyanoacetamides (1, 2) and in particular, the significant activity of 2-ethyl-2-propylcyanoacetamide and of 2,2-diethylcyanoacetamide against both supramaximal electroshock and subcutaneous pentylenetetrazol led to the consideration of these amides as suitable parent compounds upon which to effect molecular modifications that might serve

Received April 18, 1966, from the Pharmaceutical Chemistry and Pharmacology Laboratories, College of Pharmacy, University of Texas, Austin, TX 78712
Accepted for publication May 15, 1968.

as the basis for the study of structure-activity relationships. The compounds investigated are listed in Table I. The synthetic studies which led to the preparation of these compounds have been summarized in a previous report (2).

The present work was undertaken with the hope of delineating structure-activity relationships among these cyanoacetamides. Such structure-activity studies are fundamental to further structural alteration and drug design.

Members of this series include compounds possessing different N-substituents: acyclic or cyclic groups. Such modifications were effected in order to study the influence of N-substitution on anticonvulsant properties. Acyclic *N*-alkyl substituents affect the lipid-water solubility ratio and the electron distribution of the amide function, and these physicochemical factors should influence the bioactivity. The inclusion of heterocyclic compounds provides additional alteration of physicochemical properties. Specifically, the morpholino compound has a greater degree of water solubility than the piperidino analog because of the presence of the ether-oxygen atom. It is useful to speculate that at the molecular level these agents act through a mechanism proposed by Johnson and Eyring (3) to account for the action of certain structurally nonspecific CNS depressants, *e.g.*, urethan and phenobarbital. According to this proposal the depressant reversibly unfolds or conformationally alters a central catalytic protein, probably an enzyme, thus reducing the catalytic ability of the protein, the ultimate manifestation being depression of central activity. A major driving force for the drug–protein interaction and consequent inactivation was demonstrated to be hydrophobic forces. Because hydrogen bonds are important in maintaining native protein structure, Johnson and Eyring theorized that agents that hydrogen bond could also inactivate catalytic proteins through hydrogen bonding (3).

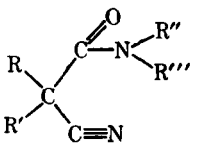
It is interesting to note that many anticonvulsants, *e.g.*, phenobarbital, have a nonpolar hydrocarbon moiety attached to a polar moiety. The latter is often characterized by the presence of an imide or amide function. An imide or amide moiety could conceivably hydrogen bond through the media of the high electron density of the carbonyl oxygen with a partial proton donor present in or on bioactive molecules. The amide or imide function, when the nitrogen bears a hydrogen atom could hydrogen bond through the hydrogen atom. Additionally, when the nitrogen atom does not have an available hydrogen atom there exists the possibility at least in the case of imides, that an electrostatic interaction involving the partial positive charge on the nitrogen atom could take place.

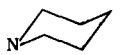
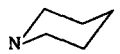
It has been found that *N*-methylated anticonvulsants undergo metabolic demethylation with ease and it has been suggested that the demethylated products are responsible for the action (4–11).

If *N*-dealkylation is important for bioactivity this tends to lead to the conclusion that the presence of the >N—H moiety is important and with respect to the mode of binding of the amide or imide moiety, hydrogen bonding involving the hydrogen of the imide or amide is important.

An objective of this preliminary investigation was to examine the role and possible mode(s) of binding of the polar amide function of a series of anticonvulsant alpha-cyanoacetamides.

Table I—Cyanoacetamides



Compd.[a] No.	R	R′	R″	R‴
1	C_2H_5	C_3H_7	H	H
2	C_2H_5	C_3H_7	H	CH_3
3	C_2H_5	C_3H_7	CH_3	CH_3
4	C_2H_5	C_3H_7	H	C_3H_7
5	C_2H_5	C_3H_7		
6	C_2H_5	C_3H_7		
7	C_3H_7	C_3H_7	H	CH_3
8	C_2H_5	C_2H_5	H	H
9	C_2H_5	C_2H_5	H	CH_3
10	C_2H_5	C_2H_5	CH_3	CH_3
11	C_2H_5	C_2H_5	H	C_3H_7
12	C_2H_5	C_2H_5		
13	C_2H_5	C_2H_5		
14	C_2H_5	C_2H_5		

[a] Compounds 1,8, Schwartz, H. F., and Doerge, R. F., *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 80(1955); and 9, Conrad, M., and Zart, A., *Ann.*, **340**, 329(1905) have been previously synthesized.

EXPERIMENTAL

Method—Young adult female albino rats, weighing initially 100–120 g.,[1] maintained on commercial laboratory feed[2] and permitted free access to food and water except during actual test periods were administered the drugs. No animal was dosed

[1] Holtzman Co., Madison, Wisc.
[2] Purina laboratory chow.

more frequently than once weekly and use of the animal was discontinued when its weight reached about 250 g. Acute toxicity studies were accomplished on older, heavier animals, thus the LD_{50} values obtained can be considered only approximations.

Pharmacodynamics[3]—Anticonvulsant activity was determined by the supramaximal electroshock seizure test (MES), essentially that described by Toman, Swinyard, and Goodman (12), using ear-clip electrodes and prior application of an acacia-sodium chloride paste. The apparatus used was the Electroshock stimulator model D,[4] adjusted to deliver 150 ma. for 0.2 sec.

Each group of rats was screened for positive response (extensor thrust of the hind limbs) to the electric shock and nonconformers removed from the test group. Checking of the undosed animals was repeated frequently throughout the entire period of evaluation.

The median effective dose (ED_{50}) of each active drug was determined from the dose-response curve obtained by plotting the effect of at least 3 dose levels on log-probability graph paper (13). Groups of at least 10 rats were given various doses until the required points were obtained.

All doses (mg./kg. body weight) were given intraperitoneally, using appropriate concentrations in propylene glycol. Most of the drugs were sufficiently soluble in the solvent to be given in solution, but a few had to be warmed slightly to maintain a homogeneous solution. *N*-Methyl-2,2-dipropylcyanoacetamide and *N*-methyl-2,2-diethylcyanoacetamide were injected as suspensions in propylene glycol only when the concentration of the solution being used was 100 mg./ml. and 200 mg./ml., respectively. Since the volume of solvent was limited to not more than 1.5 ml./kg. of total body weight (14), there exists a wide margin of safety over the toxic dose of propylene glycol as reported by Spiegel and Noseworthy (15).

Initial observations of the action of every compound in intact animals indicated a rather long period of action. These observations were confirmed in a later experiment utilizing ED_{99} doses in animals and checking for effect at 30-min. intervals up to 4 hr. after administration. The time-course studies indicated an optimal effect at the 3-hr. interval. Varying degrees of rapidly developing gross sedation were noted early in the time course of the drug effects. Since it was not desirable to shock the animals more than once, a single 3-hr. time interval was selected between administration and the test for anticonvulsant activity. This is meaningful in terms of a single finite time interval in the intact animal reflecting all processes of distribution and approach to the site of action.

RESULTS

The experimental results are summarized in Tables II and III. Several drugs were active and protected against electroshock. Some were convulsive, whereas others were sedative in action. Only those drugs active against electroshock were quantified. Although convulsive tendencies and/or lack of activity prevented the quantification of others in the series, qualitative observations of drug action were made and are recorded in the tables.

The compounds listed in Table II possess protective action against electroshock. One of the most effective drugs was 2-ethyl-2-propylcyanoacetamide. No toxic symptoms were observed at protective dose levels. *N*-Methyl-2,2-dipropylcyanoacetamide was as active as 2-ethyl-2-propylcyanoacetamide but displayed toxic symptoms at a relatively low dose. 2,2-Diethylcyanoacetamide showed no toxic symptoms in doses required to provide protection. Therapeutic indexes were calculated only for the best compounds (highest activity, least toxic effect).

Dose-effect curves are presented in Figs. 1 and 2 for the active anticonvulsant compounds. Figure 3 shows the comparison of dose-effect curves for anticonvulsant activity and lethality for 2-ethyl-2-propylcyanoacetamide. The Therapeutic Index (TI) was 8.1 and the certain safety factor (LD_1/ED_{99}) 1.7; the latter figure detracting from the favorable TI.

Table III lists those drugs which were inactive or whose activity was considered not useful due to predominantly undesirable actions. Pertinent remarks concerning qualitative observations are also included.

Structure-Activity Relationships—The preliminary pharmacologic studies have provided sufficient information to demonstrate trends in biological activity.

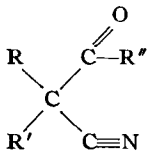
Although the effect of variation of alkyl groups at C2 was not a primary objective of the investigation, experimental results indicate the activity against electroshock to decrease: dipropyl > ethyl, propyl > diethyl for this series of mono-*N*-methyl compounds. The order ethyl, propyl > diethyl is seen also in the unsubstituted amides and in the di-*N*-methyl compounds. This decrease in activity appears to be in accord with current interpretations of distribution phenomena (16) by providing a decreasing lipid solubility in the order given. Among the compounds described, increasing hydrocarbon substitution at C_2 apparently provides a more favorable ratio of lipid-water solubility characteristics, possibly resulting in more effective distribution of drug to the site of action. A comparison of dose levels required to produce a similar degree of sedative effect among compounds differing only in their C2 substituents gave an order of decreasing sedative activity identical to that for decreasing activity against electroshock. The effect of *C*-alkylation on convulsive action could not be evaluated in this series.

N-Alkyl Modifications—(Tables II and III and Fig. 4)—With the exception of compound 8 *versus* 10, which will probably fit the generalization if the confidence limits are lowered to 90%, there is a significant decrease in potency by *N*-monomethylation. There is no further significant decrease in potency with *N*-dimethylation. *N*-Monopropylation and cyclization decreased the potency sufficiently so as to prevent quantification (Table III). The production of convulsions and lack of protection in the cyclic series suggested a complete investigation of dose-response effects would be pointless.

In those compounds having N-atom substitution, simultaneous sedative and convulsive tendencies

[3] The authors acknowledge the technical assistance of Mr. Richard G. Hendrick.
[4] M. L. Wahlquist, Salt Lake City, Utah.

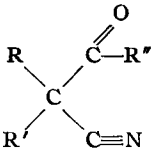
TABLE II—PHARMACOLOGICAL DATA, ACTIVE SUBSTITUTED CYANOACETAMIDES



Designation	R	R′	R″	Dose, mg./kg.	Electroshock, Protection, %	Remarks[a]	ED_{50}, mg./kg.	LD_{50} (Estim.),[b] mg./kg.	TI (Estim.)[c]
7	Propyl	Propyl	NH-Methyl	50	10(1/10)		70(60–82)	450	6.4
				75	60(6/10)				
				85	70(7/10)	[d,e]			
1	Ethyl	Propyl	NH_2	50	25(5/20)		71(60–84)	575	8.1
				75	50(10/20)				
				100	79(15/19)				
2	Ethyl	Propyl	NH-Methyl	75	17(3/18)		98(88–109)		
				100	65(11/17)	[f] (brief)			
				125	61(11/18)	[d,g]			
				150	100(10/10)[h]	[e,d] (brief),[g]			
3	Ethyl	Propyl	*N*-Dimethyl	75	18(2/11)		102(86–120)		
				100	50(5/10)	[f,e]			
				150	82(9/11)	[d,e,i,g]			
				200	100(10/10)[h]	[d,e,i,g]			
8	Ethyl	Ethyl	NH_2	100	0(0/10)[h]		170(152–197)	1000	5.9
				150	40(8/20)				
				190	70(7/10)				
				225	80(8/10)				
				300	90(9/10)				
9	Ethyl	Ethyl	NH-Methyl	190	33(5/15)	[f] (brief)	218(200–238)		
				225	44(7/16)	[f] (brief)			
				250	87(13/15)	[d] (righting ref. greater than 3 hr.)			
10	Ethyl	Ethyl	*N*-Dimethyl	150	6(1/16)	[d,g] (brief)	225(192–263)		
				200	38(6/16)	[d,g]			
				250	60(9/15)	[d,g,e] (brief, one death)			

[a] Qualitative evaluation. [b] Death within 24 hr. [c] LD_{50}/ED_{50} therapeutic index. [d] Depression, loss of righting reflex within 2–5 min. [e] General tenseness or slight muscular tremor, facial muscle twitching, provoked into spasm or convulsion by noise or touch. [f] Mild sedation, no loss of righting reflex, ataxia. [g] Recovery of righting reflex less than 3 hr. [h] Zero and 100% effect corrected according to the method of Litchfield and Wilcoxon (13). [i] Convulsive.

TABLE III—PHARMACOLOGICAL DATA, INACTIVE SUBSTITUTED CYANOACETAMIDES



No.	R	R′	R″	Dose, mg./kg.	Electroshock, % Protection	Remarks[a]
4	Ethyl	Propyl	NH-propyl	25	10(1/10)	
				50	5(1/20)	
				75	14(2/15)	
				100	17(1/6)	
				125	0(0/3)	[b,c]
				150	0(0/1)	[b,c] (72 hr.)
				200	75(3/4)	[b] (3 deaths in 24 hr.)
11	Ethyl	Ethyl	NH-propyl	100	10(1/10)	[b,c]
6	Ethyl	Propyl	Piperidine	100	0(0/9)	[b] (brief)
13	Ethyl	Ethyl	Piperidine	75	15(3/20)	
				150	15(3/20)	
				175	40(2/5)	[b]
				200	0(0/15)	[b]
5	Ethyl	Propyl	Pyrrolidine	200	0(0/10)	[b]
12	Ethyl	Ethyl	Pyrrolidine	200	10(1/10)	[b,d]
14	Ethyl	Ethyl	Morpholine	100	0(0/7)	[c]
				200	0(0/3)	[b] (mild)[c]

[a] Qualitative evaluation. [b] Convulsive. [c] General tenseness or slight muscular tremor, facial muscle twitching, provoked into a spasm or convulsion by noise or touch. [d] Mild sedation, no loss of righting reflex, ataxia.

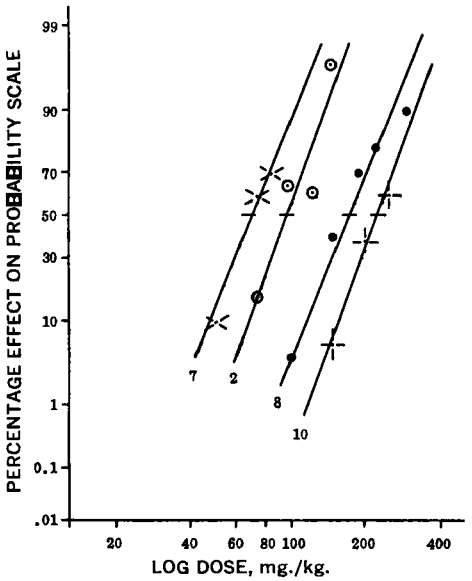


Fig. 1—Dose-response curve of active substituted cyanoacetamides. Key: 7, dipropyl-N-methylcyanoacetamide; 2, ethylpropyl-N-methylcyanoacetamide; 8, diethylcyanoacetamide; 10, diethyl-N-dimethylcyanoacetamide.

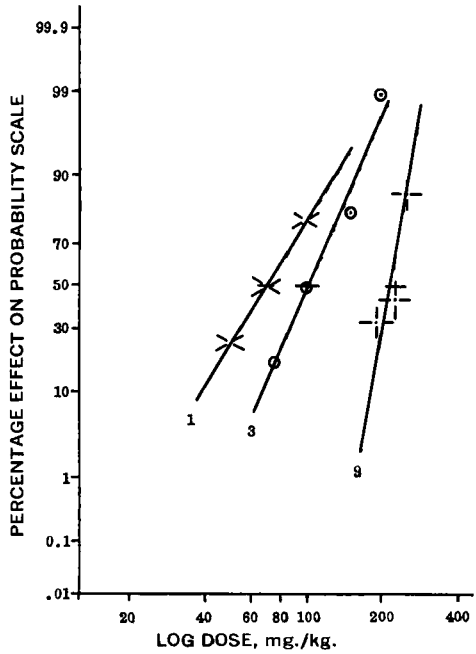


Fig. 2—Dose-response curve of active substituted cyanoacetamides. Key: 1, ethylpropylcyanoacetamide; 3, ethylpropyl-N-dimethylcyanoacetamide; 9, diethyl-N-methylcyanoacetamide.

were present. Both effects were more obvious at higher doses. Although it was not possible to evaluate contributions of *N*-alkylation to sedative effect, it was noted that convulsive tendencies increased with an increase in number of substituents or with an increase in the size or cyclization of

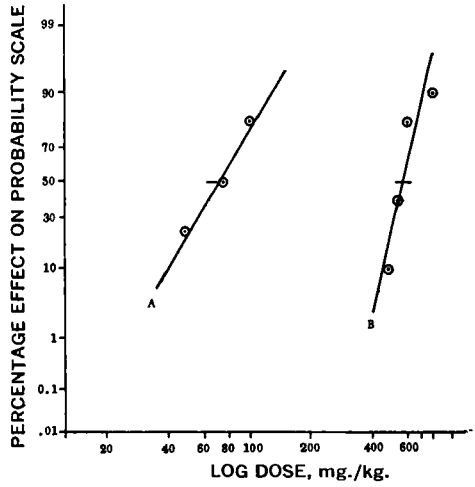


Fig. 3—Comparison of dose effect curves for anticonvulsant activity (A) and lethality (B) compound 1 (ethylpropylcyanoacetamide).

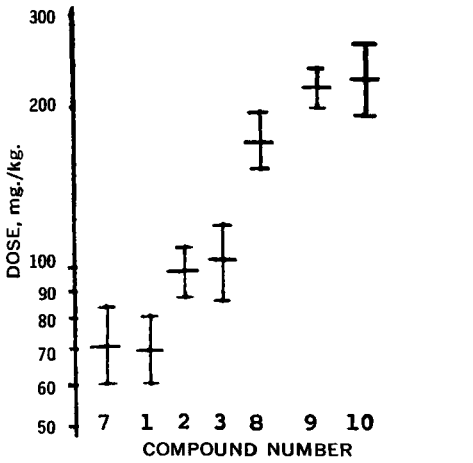


Fig. 4—Comparison of 95% confidence limits for the individual active drugs.

these substituents. The heterocyclic compounds and the *N*-propyl congeners were convulsive by the qualitative evaluation as indicated in Table III.

In this series of amides, mono-*N*-alkylation decreases activity significantly, this may be rationalized in the following ways: (*a*) the amide function is now sterically protected making any type of interaction, including hydrogen bonding *via* the amide N-H moeity, with a bioactive macromolecule less likely, (*b*) the hydrogen atom is now less acidic and consequently will not hydrogen bond as readily, (*c*) a combination of *a* and *b*.

The effect of di-*N*-alkylation now may be considered: there is no significant loss of activity relative to mono-*N*-alkylation. This appears to dispute rationalization *b* above, for this rationalization demands the presence of a hydrogen atom attached to the nitrogen atom. Rationalization *a* is also apparently disputed, for if one alkyl substituent sterically protects the amide function, then two

substituents should protect better and more effectively preclude interaction with a bioactive macromolecule. Rationalization *c*, because it is a combination of *a* and *b*, also is apparently disputed.

The fact that the *N,N*-dialkylamides are as potent as the *N*-mono-alkylamides could be rationalized as follows: (*a*) better distribution to the receptor because of the increased hydrocarbon content, (*b*) a substantial amount of *N*-dealkylation, (*c*) increased hydrophobic interactions. Upon examination however, rationalizations *a* and *c* must be discarded, for on the basis of these, *N*-monoalkylation should not have decreased activity. It thus appears that *N*-dealkylation may be a factor. If this is the case the three rationalizations previously set forth with respect to the effect upon activity of mono-*N*-alkylation may be valid. These rationalizations are consonant with a sterically accessible amide function interacting with the bioactive macromolecule. The interaction possibly involves hydrogen bonding through the media of an amide hydrogen atom.

CONCLUSIONS

The following compounds: 2-ethyl-2-propylcyanoacetamide[5]; *N*-methyl-2,2-dipropylcyanoacetamide; *N*-methyl-2-ethyl-2-propylcyanoacetamide; *N*-dimethyl-2-ethyl-2-propylcyanoacetamide; 2,2-diethylcyanoacetamide[5]; *N*-methyl-2,2-diethylcyanoacetamide[5]; and *N*-dimethyl-2,2-diethylcyanoacetamide have been found active when subjected to testing by an electroshock method using rats. The median effective dose (ED_{50}, mg./kg.) of each active drug was determined and reported. The least toxic and most active compounds were ($ED_{50}LD_{50}$,[6] TI, respectively): *N*-methyl-2,2-dipropylcyanoacetamide, 70, 450, 6.4; 2-ethyl-2-propylcyanoacetamide, 71, 575, 8.1; 2,2-diethylcyanoacetamide, 170, 1,000, 5.9.

[5] See Footnote *a*, Table I.
[6] Approximate.

Convulsive tendencies and/or lack of activity prevented quantification of the seven drugs: *N*-propyl-2-ethyl-2-propylcyanoacetamide; *N*-propyl-2,2-diethylcyanoacetamide; 1-(2-cyano-2-ethylbutyryl)-piperidine; 1-(2-cyano-2-ethylvaleryl)-piperidine; 1-(2-cyano-2-ethylbutyryl)-pyrrolidine; 1-(2-cyanoethylvaleryl)-pyrrolidine; and 4-(2-cyano-2-ethylbutyryl)-morpholine. Qualitative observations were made and recorded.

REFERENCES

(1) Cunningham, R. W., personal communication.
(2) Schwartz, H. F., Worrell, L. P., and Delgado, J. N., *J. Pharm. Sci.*, **56**, 80(1967).
(3) Johnson, F. H., Eyring, H., and Polissar, M. J., "The Kinetic Basis of Molecular Biology," Wiley, New York, N. Y., 1954, pp. 253, 260, 334, 437, 447.
(4) Close, W. J., and Spielman, M. A., "Medicinal Chemistry," vol. V, Wiley New York, N. Y., 1961, p. 37.
(5) Butler, T. C., *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 367(1955).
(6) Butler, T. C., *J. Pharmacol. Expll. Therap.*, **104**, 229 (1952).
(7) *Ibid.*, **108**, 11(1953).
(8) *Ibid.*, **108**, 474(1953).
(9) *Ibid.*, **109**, 340(1953).
(10) *Ibid.*, **113**, 178(1955).
(11) Butler, T. C., and Waddell, W. J., *Neurology*, **8**, 106 (1958).
(12) Toman, J. E. P., Swinyard, E. A., and Goodman, L. S., *J. Neurophysiol.*, **9**, 231(1946).
(13) Litchfield, J. T., and Wilcoxon, F., *J. Pharmacol. Expll. Therap.*, **96**, 99(1949).
(14) Swinyard, E. A., *J. Am. Pharm. Assoc., Sci. Ed.*, **38**, 201(1949).
(15) Spiegel, A. J., and Noseworthy, M. M., *J. Pharm. Sci.*, **52**, 917(1963).
(16) Albert, A., "Selective Toxicity," 3rd ed., Wiley, New York, N. Y., 1965, pp. 42–67.

Keyphrases

Cyanoacetamides—synthesis
Structure-activity relationships—cyanoacetamides
Anticonvulsant activity—supramaximal electroshock seizure test
ED_{50} values—cyanoacetamides

Development of a Sustained-Release Aspirin Tablet

By EDWARD H. WISEMAN and N. J. FEDERICI*

Several sustained-release aspirin tablets, some of which are capable of attaining and maintaining plasma salicylate concentrations of about 30 mcg./ml. for 8 hr. after ingestion from a total dose of 975 mg. aspirin are described. The design of the final tablet characteristics was guided by analysis of plasma salicylate concentrations *in vivo*, and the rate of aspirin release *in vitro*.

A SUSTAINED-RELEASE product has been defined as "one in which a drug is initially made available to the body in an amount sufficient to cause the desired pharmacological response as rapidly as is consistent with the properties of the drug determining its intrinsic availability for absorption; and one which provides for maintenance

Received April 12, 1968, from the Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, CT 06340
Accepted for publication July 10, 1968.
* Thomas Leeming & Co./Pacquin Divisions, Chas. Pfizer & Co., Inc., Parsippany, N. J.