

The experimental data in Table II were obtained on randomly selected samples of experimental tablets containing chlorthalidone with and without reserpine. The samples were analyzed in duplicate for chlorthalidone by the HPLC method and the conventional USP procedure. The results from both methods are comparable. However, since the HPLC method is stability indicating with respect to the hydrolysis product, II, this procedure is more reliable than the USP method and more accurately reflects the actual concentration of chlorthalidone per tablet dose.

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# Anticonvulsant Activity of Alkyl-Substituted *N*-Benzylcyanoacetamides

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**Abstract** □ Thirteen new derivatives of 2-alkyl- and 2,2-dialkyl-*N*-benzylcyanoacetamide, a cyano analog of beclamide, were synthesized and tested for anticonvulsant activity. The unsubstituted compound was more active and more toxic than the derivatives. No activity was observed when the alkyl substituents in the symmetrically disubstituted derivatives contained a total of six or more carbon atoms or when benzyl was a substituent. The monosubstituted compounds were more toxic than the disubstituted compounds.

**Keyphrases** □ *N*-Benzylcyanoacetamides, alkyl substituted—synthesized, evaluated for anticonvulsant activity in mice □ Anticonvulsant activity—alkyl-substituted *N*-benzylcyanoacetamides evaluated in mice □ Structure-activity relationships—*N*-benzylcyanoacetamides evaluated for anticonvulsant activity in mice

Although early reports (1-3) of the use of beclamide (I) in the treatment of grand mal and psychomotor seizures were promising, the usefulness of the agent has proved to be disappointing. Beclamide has the characteristic structural arrangement of the common antiepileptics (1), as shown in II. The fact that the  $\alpha$ -carbon atom only bears a single substitution has been suggested as the reason for the variable results obtained clinically.

Schwartz *et al.* (4, 5) reported the anticonvulsant activity of a number of substituted cyanoacetamides. Although several C-monoalkyl cyanoacetamides were prepared as intermediates, only the C-dialkylated products were tested for anticonvulsant activity. Activity against electroshock, sedative activity, and toxicity increased with increasing hydrocarbon substitution on the  $\alpha$ -carbon atom up to dipropyl, which was the most highly substituted compound tested (4). Also, *N*-methylation and *N*-dimethylation caused a significant decrease in potency, while

*N*-propylation and *N*-cyclization (pyrrolidine, piperidine, and morpholine) led to inactive compounds (4).

This report describes the preparation and activity of several substituted *N*-benzylcyanoacetamides as cyano analogs of beclamide. The substituents were chosen to study the effectiveness of substituting a nitrile group for a chloromethyl group in beclamide and the degree of hydrocarbon substitution that would retain activity in this series of cyanoacetamides.

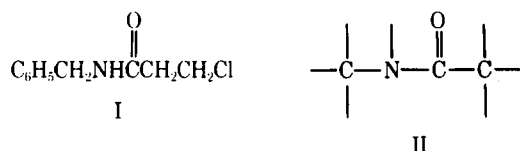
## EXPERIMENTAL<sup>1</sup>

**2-Substituted *N*-Benzylcyanoacetamides (IV-XI and XVI)**—These compounds were synthesized by a modification of the reported method (5). Compound III (6) (17.4 g, 0.10 mole) was added to a solution of potassium hydroxide (5.6 g, 0.10 mole, or 11.2 g, 0.20 mole) in formamide (50-75 ml). The mixture was stirred at ambient temperature for 1 hr, and the appropriate alkyl bromide (0.10 or 0.20 mole) was added.

For the preparation of the monosubstituted compounds, the mixture was stirred at ambient temperature for 3 hr; for the disubstituted derivatives, the mixture was refluxed for 1-3 hr. Then the reaction mixture was poured into ice water with stirring and filtered. The residue was recrystallized from the appropriate solvent (Table I) and activated charcoal.

The NMR spectra were consistent with the proposed structures. The NMR spectra of IV-XI showed the following common absorption peaks (CDCl<sub>3</sub>):  $\delta$  7.15-7.25 (s, 5H or 10H, ArH) and 4.30-4.40 (d, 2H, NCH<sub>2</sub>Ar) ppm. Additional absorption peaks were noted for: IV, 1.60 (s, 6H, CH<sub>3</sub>) ppm; and V, VII, IX, and XI, 3.30-3.35 (m, 1H, CH) ppm. Depending on the alkyl substituent (V-X), the absorption in the hydrocarbon region ranged from  $\delta$  0.70 to 2.50 ppm with a multiplet splitting pattern. Each spectrum integrated for the correct number of protons. For XVI, the NMR spectrum showed the following absorption peaks (CDCl<sub>3</sub>):  $\delta$  7.20 (s, 15H, ArH), 4.20 (d, 2H, NCH<sub>2</sub>Ar), and 3.20 (q, 4H, CCH<sub>2</sub>Ar) ppm.

***N*-Benzyl-2-benzyl-2-methylcyanoacetamide (XII)**—To a solution of potassium hydroxide (2.80 g, 0.05 mole) and formamide (10 ml) in dimethyl sulfoxide (30 ml) was added XI (13.2 g, 0.05 mole), and the mixture was stirred for 30 min. Potassium iodide (0.1 g) and methyl iodide



<sup>1</sup> Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga. NMR data were recorded on a Varian Associates T-60A spectrophotometer.

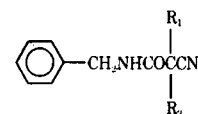


Table I—*N*-Benzylcyanoacetamides

Compound	R <sub>1</sub>	R <sub>2</sub>	Recrystallization Solvent <sup>a</sup>	Yield, %	Melting Point	Formula	Analysis, %		
							Calc.	Found	
III	H	H	A	—	121–124° <sup>b</sup>		—	—	
IV	CH <sub>3</sub>	CH <sub>3</sub>	B	39.1	64–66°	C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> O	C 71.26	71.28	
							H 6.98	6.98	
							N 13.85	13.78	
V	CH <sub>2</sub> CH <sub>3</sub>	H	A	20.0	87–89°	C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> O	C 71.26	71.35	
							H 6.98	7.01	
							N 13.85	13.84	
VI	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	A	32.1	97–99°	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O	C 73.01	73.06	
							H 7.88	7.92	
							N 12.16	12.15	
VII	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	H	A	30.5	83–86°	C <sub>13</sub> H <sub>16</sub> N <sub>2</sub> O	C 72.19	72.20	
							H 7.46	7.51	
							N 12.95	12.82	
VIII	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	A	7.6	77–79°	C <sub>16</sub> H <sub>22</sub> N <sub>2</sub> O	C 74.38	74.44	
							H 8.58	8.56	
							N 10.84	10.90	
IX	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	H	B	15.5	70–72°	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O	C 73.01	73.05	
							H 7.88	7.90	
							N 12.16	12.16	
X	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	A	60.8	76–79°	C <sub>18</sub> H <sub>26</sub> N <sub>2</sub> O	C 75.48	75.61	
							H 9.15	9.21	
							N 9.78	9.73	
XI	H	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	C	12.7	123–125°	C <sub>17</sub> H <sub>16</sub> N <sub>2</sub> O	C 77.25	77.30	
							H 6.10	6.14	
							N 10.60	10.57	
XII	CH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	B	29.4	80–83°	C <sub>18</sub> H <sub>18</sub> N <sub>2</sub> O	C 77.67	77.66	
							H 6.52	6.55	
							N 10.06	9.99	
XIII	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	A	25.5	99–101°	C <sub>19</sub> H <sub>20</sub> N <sub>2</sub> O	C 78.05	78.02	
							H 6.90	6.92	
							N 9.58	9.52	
XIV	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	A	75.6	144–145°	C <sub>20</sub> H <sub>22</sub> N <sub>2</sub> O	C 78.40	78.41	
							H 7.24	7.25	
							N 9.14	9.17	
XV	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	A	49.8	128–130°	C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O	C 78.71	78.67	
							H 7.55	7.66	
							N 8.74	8.68	
XVI	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	A	76.2	141–143°	C <sub>24</sub> H <sub>22</sub> N <sub>2</sub> O	C 81.33	81.27	
							H 6.26	6.28	
							N 7.90	7.92	

<sup>a</sup> A = 95% ethanol, B = isopropyl ether, and C = toluene. <sup>b</sup> Lit. (5) mp 123–124.5°.

(7.10 g, 0.05 mole) were added, and the mixture was stirred at ambient temperature for 22 hr. The mixture was poured into ice water, and the water layer was decanted. The semisolid residue was washed with water, air dried, and recrystallized from isopropyl ether (Table I).

The NMR spectrum was consistent with the proposed structure; NMR (CDCl<sub>3</sub>): δ 7.20 (s, 10H, ArH), 4.30 (d, 2H, NCH<sub>2</sub>Ar), 3.05 (q, 2H, CCH<sub>2</sub>Ar), and 1.60 (s, 3H, CH<sub>3</sub>) ppm.

***N*-Benzyl-2-alkyl-2-benzylcyanoacetamides (XIII–XV)**—To a solution of sodium metal (0.89 g, 0.0387 mole) in absolute ethanol (50 ml) was added the appropriate monosubstituted *N*-benzylcyanoacetamide (V, VII, or IX) (0.035 mole). After heating at reflux for 30 min, benzyl chloride (4.90 g, 0.0387 mole) was added dropwise, and the mixture was heated at reflux for 2 hr. After cooling, water (50 ml) was added, and the solution was extracted with chloroform (3 × 60 ml). The combined

chloroform extracts were dried (anhydrous magnesium sulfate) and concentrated *in vacuo*. The residue was recrystallized from 95% ethanol and activated charcoal (Table I).

The NMR data agreed with the proposed structures. The NMR spectra exhibited the following common absorption peaks (CDCl<sub>3</sub>): δ 7.20–7.25 (s, 10H, ArH), 4.30 (d, 2H, NCH<sub>2</sub>Ar), and 3.05–3.10 (q, 2H, CCH<sub>2</sub>Ar) ppm. Absorption in the hydrocarbon region (δ 0.70–2.20 ppm) exhibited a multiplet splitting pattern and integrated for the correct number of protons assigned to each structure.

**Anticonvulsant Testing**—All compounds were tested for anticonvulsant activity<sup>2</sup> using the Anticonvulsant Screening Project test systems (7). Three tests were performed in male Carworth Farms No. 1 mice: the maximal electroshock seizure test (MES), the subcutaneous pentylenetetrazol seizure threshold test (scMet), and the rotorod test to evaluate neurotoxicity.

All compounds were tested at three dose levels (30, 100, and 300 mg/kg) at 30 min and 4 hr after their intraperitoneal administration. Typically, four animals were injected with each dose, solubilized or suspended in 30% polyethylene glycol 400, and examined 30 min later for toxicity in the rotorod test. Anticonvulsant activity was evaluated immediately thereafter by subjecting one mouse to the maximal electroshock seizure test and another to the subcutaneous pentylenetetrazol seizure threshold test. The same tests were repeated after 4 hr on the two remaining mice.

Anticonvulsant activity in the maximal electroshock seizure test is defined as abolition of the limb tonic extensor component of the maximal

Table II—Anticonvulsant and Toxic Effects

Compound	MES Activity <sup>a</sup>		scMet Activity <sup>a</sup>		Toxicity <sup>a</sup>	
	0.5 hr	4 hr	0.5 hr	4 hr	0.5 hr	4 hr
I <sup>b</sup>	+	—	+	—	—	—
III	+	+	++	—	++	+
IV	+	—	—	—	—	—
V	+	—	+	—	++	—
VI	—	—	+	—	—	—
VII	+	—	+	+	+	—
IX	+	—	+	—	+	—

<sup>a</sup> Activity and toxicity at 30, 100, or 300 mg/kg are represented by +, ++, or +, respectively; — denotes no activity or toxicity observed at 300 mg/kg. Compounds VIII and X–XVI demonstrated no activity or toxicity at 300 mg/kg. <sup>b</sup> The reported (6) values for MES ED<sub>50</sub>, scMet ED<sub>50</sub>, and median toxic dose in the rotorod test are 183 (161–208) mg/kg, 175 (132–233) mg/kg, and 273 (246–303) mg/kg, respectively.

<sup>2</sup> Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20014.

electroshock seizure elicited in mice with a 60-Hz alternating current of 50 mamp delivered for 0.2 sec *via* corneal electrodes. Activity in the subcutaneous pentylenetetrazol seizure threshold test is defined as failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 sec in duration) at a dose of 85 mg/kg.

## RESULTS AND DISCUSSION

The results of the anticonvulsant activity tests (Table II) suggest that the substitution of a nitrile group for a chloromethyl group in beclamide leads to an increase in toxicity with a retention in activity against maximal electroshock and pentylenetetrazol-induced seizures. Compound III, having no carbon substitution, showed activity at a comparable or lower dose than beclamide (I). However, additional testing is required to determine whether the differences in potencies and toxicity are significant.

Of the four monosubstituted compounds tested, V, VII, and IX exhibited weak activity against maximal electroshock and pentylenetetrazol-induced seizures and neurotoxicity as evaluated by the rotorod test. Compound V appeared to be more toxic than VII or IX. Whether higher monosubstituted homologs would be active and/or toxic was not determined. No activity or toxicity was observed for the benzyl-substi-

tuted derivative (XI).

No activity was observed when the alkyl substituents in the symmetrically disubstituted derivatives contained six or more carbon atoms. Unlike the monosubstituted compounds, the active disubstituted derivatives (IV and VI) exhibited no toxicity at the dose levels tested.

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# Direct Spectrophotometric Assay of Quaternary Ammonium Compounds Using Bromthymol Blue

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Received August 9, 1976, from Flow Pharmaceuticals, Inc., Palo Alto, CA 94303. Cooper Laboratories, Inc., Mountain View, CA 94043.

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**Abstract** □ Benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate were assayed quantitatively by a direct spectrophotometric method with bromthymol blue buffered at pH 7.5. The method shows good results at concentrations of 0–300 µg/ml and in the presence of epinephrine bitartrate, phenylephrine hydrochloride, pilocarpine hydrochloride, and polyvinyl alcohol.

**Keyphrases** □ Quaternary ammonium compounds, various—spectrophotometric analyses in prepared and commercial solutions □ Spectrophotometry—analyses, various quaternary ammonium compounds in prepared and commercial solutions □ Bromthymol blue—used in spectrophotometric analyses of various quaternary ammonium compounds in prepared and commercial solutions □ Preservatives—various quaternary ammonium compounds, spectrophotometric analyses in prepared and commercial solutions

Low concentrations of quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate, which are used in clear ophthalmic solutions, are difficult to determine quantitatively and reproducibly. Most methods rely on complexing the quaternary ammonium compound with an acid dye such as methyl orange or bromphenol blue and extracting the complex with a chlorinated hydrocarbon solvent (1, 2). These methods have difficulties and inaccuracies arising from incomplete extraction or the emulsions formed with the hydrocarbon solvent and the quaternary ammonium compound-containing solution.

In response to the problems resulting from extraction of the dye complex, a direct method was developed using bromthymol blue buffered at pH 7.5; the reduction in ab-

**Table I—Standard Curves for Benzalkonium Chloride, Benzethonium Chloride, and Chlorhexidine Gluconate**

Concentration, µg/ml	Absorbance		
	Benzalkonium Chloride	Benzethonium Chloride	Chlorhexidine Gluconate
0	0.804	0.804	0.804
100	0.593	0.659	0.667
200	0.370	0.471	0.538
300	0.225	0.325	0.438

sorbance was measured at 610 nm. The method was tested with benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate.

## EXPERIMENTAL

**Apparatus**—A pH meter and a spectrophotometer with 1-cm cells were used<sup>1</sup>.

**Reagents**—Benzalkonium chloride USP, benzethonium chloride USP, epinephrine hydrochloride USP, polyvinyl alcohol, pilocarpine hydrochloride USP, phenylephrine hydrochloride USP, chlorhexidine gluconate BP, and hydroxyethylcellulose<sup>2</sup> were used as received. All other chemicals were reagent grade.

**Buffer Solutions**—Stock solutions of 0.05 and 0.25 M dibasic potassium phosphate and monobasic potassium phosphate were used in buffer preparation. A buffer of pH 7.5 was prepared by mixing either the 0.05 M solutions together or the 0.25 M solutions together until a pH of 7.5 was obtained.

<sup>1</sup> Beckman DU spectrophotometer.

<sup>2</sup> The 250 MR grade, Hercules Inc., Wilmington, Del.