Biochemorphology of Cyclobutanecarbonylureas

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Abstract □ Ten urea derivatives of cyclobutanecarboxylic acid were synthesized and examined for general CNS depressant properties, barbiturate potentiation, and myorelaxant, antitremorine, and anticonvulsant potencies. Although water solubility plays an important role in the activity of these compounds, other factors also appear to be involved. 1-Cyclobutanecarbonyl-3,3-dimethylurea appears to be the most active CNS depressant, whereas 1-cyclobutanecarbonyl-3-(α-naphthyl)thiourea is the most active barbiturate potentiator. 1-Cyclobutanecarbonyl-3,3-dimethylurea, 1-cyclobutanecarbonyl-3-phenylurea, and 1-cyclobutanecarbonyl-3-(2,6-xylyl)urea appear to be the most active myorelaxants, while 1-cyclobutanecarbonyl-3-propylurea, 1-cyclobutanecarbonyl-3-*tert*-butylurea, 1-cyclobutanecarbonyl-3-allylurea, and 1-cyclobutanecarbonyl-3-phenylurea apparently are the most active against pentylenetetrazol-induced convulsions. 1-Cyclobutanecarbonyl-3-*tert*-butylurea, 1-cyclobutanecarbonyl-3,3-dimethylurea, and 1-cyclobutanecarbonyl-3-phenylurea are also slightly active tremorine antagonists.

Keyphrases □ Cyclobutanecarbonylureas—synthesis, CNS activity, correlated with molecular structure and aqueous solubility □ CNS activity—synthesis and screening of cyclobutanecarbonylureas, biological activity correlated with molecular structure and aqueous solubility

In continuing research on the synthesis of new derivatives of certain cyclobutanecarboxylic acids (1–6) with central nervous system (CNS) depressant activity, 10 urea derivatives of cyclobutanecarboxylic acid were prepared by interaction of cyclobutanecarbonyl chloride with the corresponding ureas and pharmacological data were compiled. The compounds were tested for general CNS depressant properties, barbiturate potentiation, and myorelaxant, antitremorine, and anticonvulsant potencies. Furthermore, the biological activity of the compounds was correlated with molecular structure and aqueous solubility.

EXPERIMENTAL

The compounds were prepared by conventional routes involving acylation of a urea with cyclobutanecarbonyl chloride in either pyridine or dimethylformamide (3) (Table I).

White Swiss, Charles River strain mice were used once in these studies. They were previously untreated with any drug and were permitted to feed *ad libitum*.

Bioassay for Sedative and Hypnotic Properties—The depressant activity of the compounds was determined by observing their effects on the spontaneous activity and righting reflex of virgin female mice weighing 20–25 g. The drugs were given orally either as a solution or a suspension in 0.2–0.4 ml of 10% gum acacia, using a blunted and bent 18-gauge hypodermic needle feeding tube. A reduction in spontaneous activity was subjectively graded and compared with that of the control animals given the drug vehicle only.

The effect of the test drug was described in the following terms: when 100% of the animals equaled the control animals in spontaneous activity, the effect was considered as nil. When 50% or more of the test population showed a slight decrease in activity, the effect was considered slight. When 50% or more of the test population showed an intermediate decrease, the effect was considered intermediate. When 50% or more of the test animals showed a loss of righting reflex, the loss of spontaneous activity was considered marked. For each dose, five control and five test mice were used.

Barbiturate Potentiation—Female mice, 20–30 g, were used, and the test substances were administered orally as in the previous experiment. Pentobarbital sodium (50 mg/kg) was administered 30 min after the test drug. For each test and each control experiment, five mice were used. Generally, all animals lost the righting reflex within 10 min after pentobarbital injection. The animals were placed on their backs until spontaneous righting occurred. They were again placed on their backs until righting was effected within 5 sec, at which time the animals were judged to have regained the righting reflex. The measure of potentiation used was the ratio $(\text{drug} + \text{barbiturate}_{\text{sleep time}})/(\text{barbiturate}_{\text{sleep time}} + \text{drug}_{\text{sleep time}})$.

Myorelaxant Activity—Male mice, 20–22 g, were used. The test compounds were given orally as in the prior experiments, and strychnine nitrate (1.6 mg/kg ip) in distilled water was administered 30 min after the test substance. The strychnine dosage was 100% lethal in controls which died within 10–12 min. The ability of a drug to protect against strychnine-induced lethality was assessed by survival of the animals after 30 min and 24 hr. Survival for 30 min was judged as partial protection; survival for 24 hr was judged as complete protection.

Pentylenetetrazol Antagonism—Male mice, 20–25 g, were used. The test compounds were given orally as before, and pentylenetetrazol (100 mg/kg) in a 0.25% methylcellulose sterile vehicle was injected subcutaneously 30 min after the test compound. The dose was convulsive to 100% within 30 min and 99% lethal in the control animals. Drug-treated animals were observed individually for complete protection in the 30 min following injection of pentylenetetrazol. For each drug and each control, five mice were used.

Antitremorine Activity—Male mice, 20–30 g, were used. The test compounds were administered orally as before and at a dose of 1000 mg/kg. Tremorine (20 mg/kg ip) in a 0.25% methylcellulose sterile vehicle was given 30 min after the test substance. In controls, this dose produced centrally mediated tremors plus signs of parasympathetic stimulation including salivation, lacrimation, diarrhea, and urination. Subjective grading was used to establish the degree of protection against tremorine.

To judge protection, animals were suspended by the tail. When the tremor was the same as for the controls, protection was rated as nil. For a slight reduction in tremor intensity, the degree of protection was judged slight; for a slight tremor, protection was moderate; no tremor was rated as complete protection. For each drug and each control, five mice were used.

RESULTS AND DISCUSSION

To elucidate the biochemorphology of the cyclobutanecarbonylureas, the compounds in Table II were arranged according to their structural and physicochemical properties. When tested for general depressant activity, it was observed that the straight chain derivatives, Compounds 1–4, although insoluble in water, showed slight CNS depressant activity. Furthermore, 1-cyclobutanecarbonyl-3,3-dimethylurea, Compound 5, in contrast to 1-cyclobutanecarbonyl-3-methylurea, Compound 1, was highly water soluble and highly active in that long-term loss of the righting reflex was observed. This finding suggested that water solubility plays an important role in the activity of these compounds.

Introduction of a double bond into the aliphatic chain of 1-cyclobutanecarbonyl-3-propylurea, Compound 3, gave 1-cyclobutanecarbonyl-3-allylurea, Compound 6, which was identical to the parent compound in water solubility and activity. However, the allylthiourea derivative, although insoluble in water, was more active than the allylurea derivative. It caused intermediate loss of spontaneous motor activity. Similarly, the α-naphthylthiourea derivative, although insoluble in water, produced marked reduction in spontaneous motor activity, whereas the other two aromatic derivatives, Compounds 8 and 9, were insoluble in water and inactive as CNS depressants. The data suggest that, although water solubility

Table I—Cyclobutanecarbonylureas

Compound Number	Compound Name	Yield, %	Melting[a] Point	Recrystallization Solvent[b]	Molecular Formula	Analysis, % Calc.	Analysis, % Found
1	1-Cyclobutanecarbonyl-3-methylurea	80	155–158°	P/MC	$C_7H_{12}N_2O_2$	C 53.84 H 7.69 N 17.94	C 53.49 H 7.87 N 18.01
2	1-Cyclobutanecarbonyl-3-ethylurea	45	110°	A	$C_8H_{14}N_2O_2$	N 16.49	N 16.47
3	1-Cyclobutanecarbonyl-3-propylurea	70	86–88°	W/A	$C_9H_{16}N_2O_2$	C 58.69 H 8.69 N 15.21	C 58.75 H 8.90 N 15.13
4	1-Cyclobutanecarbonyl-3-*tert*-butylurea	75	137–139°	W/A	$C_{10}H_{18}N_2O_2$	C 60.60 H 9.09 N 14.14	C 60.62 H 9.17 N 14.14
5	1-Cyclobutanecarbonyl-3,3-dimethylurea	35	180°	P/MC	$C_8H_{14}N_2O_2$	N 16.47	N 17.01
6	1-Cyclobutanecarbonyl-3-allylurea	60	82°	P	$C_9H_{14}N_2O_2$	N 15.38	N 15.34
7	1-Cyclobutanecarbonyl-3-allylthiourea	80	50°	P	$C_9H_{14}N_2OS$	C 54.54 H 7.07 N 14.14	C 54.44 H 7.16 N 13.92
8	1-Cyclobutanecarbonyl-3-phenylurea	75	145°	P/MC	$C_{12}H_{14}N_2O_2$	C 66.05 H 6.42 N 12.84	C 66.35 H 6.53 N 13.00
9	1-Cyclobutanecarbonyl-3-(2,6-xylyl)urea	80	180–182°	P/MC	$C_{14}H_{18}N_2O_2$	C 68.29 H 7.23 N 11.38	C 68.28 H 7.46 N 11.33
10	1-Cyclobutanecarbonyl-3-(α-naphthyl)thiourea	85	165°	A/M	$C_{16}H_{16}N_2O$	C 67.60 H 5.63 N 9.85	C 67.49 H 5.93 N 9.58

[a] Uncorrected. [b] A = acetone, MC = methylene chloride, M = methanol, P = petroleum ether, and W = water.

plays an important role in the activity of these compounds, other factors also play a part as activity determinants.

To test the previously reported phenomenon (3) that the locus for barbiturate potentiation appeared to be functionally different from the sites involved in the directly measured depressant effect, a comparison was made of the barbiturate potentiation and general depressant effect of these compounds. It was presumed that if there were two functionally different loci for these activities, it should be possible to observe three different modes of CNS action, *i.e.*, CNS depression only (Category A), potentiation only (Category B), and both CNS depression and potentiation (Category C).

When analyzed for pentobarbital potentiation activity at a dose of 500 mg/kg, a sleep prolongation factor, R (Table II), was used as the criterion for effect. For compounds not causing loss of the righting reflex, R is a measure of true potentiation and becomes significant when greater than 1.4. As apparent from Table II, most compounds with hypnotic and sedative activity also prolonged pentobarbital sleeping time significantly and thus appear to belong to Category C. However, Compounds 1 and 2, active as hypnotics and sedatives, were found to be inactive as potentiators and belong to Category A. On the other hand, Compounds 8 and 9, found to be inactive during gross screening, potentiated the sleep-

Table II—Biological Data for Cyclobutanecarbonylureas[a]

Compound Number	Compound Name	Gross[b] Effects	Barbiturate[c] Potentiation, R	CNS Activity[d] (Category)	Strychnine Lethality Test[e] Partial	Strychnine Lethality Test[e] Complete	Pentylenetetrazol Test[f]
1	1-Cyclobutanecarbonyl-3-methylurea	S	1.4	A	3/5	1/5	0/5
2	1-Cyclobutanecarbonyl-3-ethylurea	S	0.9	A	3/5	3/5	1/5
3	1-Cyclobutanecarbonyl-3-propylurea	S	1.6	C	3/5	1/5	5/5
4	1-Cyclobutanecarbonyl-3-*tert*-butylurea	S	3.5	C	5/5	1/5	5/5
5	1-Cyclobutanecarbonyl-3,3-dimethylurea	M[g]	1.9	C	5/5	5/5	0/5
6	1-Cyclobutanecarbonyl-3-allylurea	S	1.8	C	3/5	1/5	2/5
7	1-Cyclobutanecarbonyl-3-allylthiourea	I	1.5	C	5/5	1/5	5/5
8	1-Cyclobutanecarbonyl-3-phenylurea	N	4.1	B	5/5	5/5	5/5
9	1-Cyclobutanecarbonyl-3-(2,6-xylyl)urea	N	1.8	B	5/5	5/5	3/5
10	1-Cyclobutanecarbonyl-3-(α-naphthyl)thiourea	M	5.2	C	5/5	2/5	0/5

[a] In each test the dose used was 1000 mg/kg po except where indicated. [b] M, I, S, and N represent marked, intermediate, slight, and no reduction in the spontaneous motor activity, respectively. [c] Pentobarbital sodium given intraperitoneally at a dose of 50 mg/kg; R is the ratio (drug + barbiturate$_{\text{sleep time}}$)/(drug$_{\text{sleep time}}$ + barbiturate$_{\text{sleep time}}$); the drugs were given orally at a dose of 500 mg/kg. [d] A = CNS depressant ability only, B = barbiturate potentiation only, and C = both CNS depressant and barbiturate potentiation ability. [e] Strychnine nitrate, 1.6 mg/kg ip. This dose is 100% lethal. The mice die within 10–12 min. Partial = animals protected longer than 30 min from death/animals tested. Complete = animals completely protected from death/animals tested. [f] Pentylenetetrazol, 100 mg/kg sc; animals protected/animals tested. [g] Dose of 500 mg/kg po.

ing time significantly and can be associated with Category B. With Compound 8 the potentiation was extraordinary; it potentiated the sleeping time four times.

Moreover, during gross screening, Compound 5 appeared to be the most active CNS depressant. However, in the pentobarbital potentiation analysis, Compounds 4, 8, and 10 seemed to be more active than 5; of these, Compound 10 was the most active. It prolonged the sleeping time about three times more than 1-cyclobutanecarbonyl-3,3-dimethylurea. These observations lend further support to the hypothesis that two mechanistically separate modes of action exist for these compounds: one for barbiturate potentiation and the other for depressant ability. However, the data are not sufficient to allow a definite conclusion regarding the mechanism of barbiturate potentiation. The possibility that the compounds potentiate pentobarbital sleeping time *via* the inhibition of the liver microsomal enzymatic system cannot be ruled out.

Myorelaxant activity of the meprobamate type is conveniently studied by ascertaining whether a compound antagonizes strychnine lethality (7–9). In the present series of 10 compounds, all compounds showed myorelaxant activity; in the gross screening and barbiturate potentiation test, only eight were active. This finding suggests that the compounds in question are predominantly myorelaxants and that hypnotic and sedative activity is a secondary effect. Furthermore, these compounds can be classified into two categories. Some are only myorelaxant (Compounds 8 and 9) and belong to a class of compounds exemplified by mephenesin. Others show slight depressant effects along with myorelaxant activity (Compounds 1–7 and 10) and belong to a class of compounds exemplified by meprobamate. At a dose of 1000 mg/kg po, Compounds 5, 8, and 9 protected 100% and Compound 2 protected 60% of the test animals completely from strychnine lethality. Compounds 1, 3, 4, 6, 7, and 10 protected only 20–40% of the population tested. However, as far as partial protection from strychnine lethality is concerned, Compounds 4, 5, and 7–10 protected 100% of the test animals; Compounds 1–3 and 6 protected 60% of the population tested.

All of these compounds were also tested for pentylenetetrazol antagonism (10). At a dose of 1000 mg/kg po, Compounds 3, 4, 7, and 8 protected 100% and Compounds 2, 6, and 9 protected 20–60% of the test animals completely against pentylenetetrazol-induced convulsions. Compounds 1, 5, and 10 did not show antagonism to pentylenetetrazol.

All compounds were tested for an ability to antagonize tremorine-induced, peripheral parasympathetic stimulation and centrally originating parkinsonian-like tremors (11, 12). At a dose of 1000 mg/kg po, the antitremor activity was limited to Compounds 1, 4, 5, 8, and 10. Compounds 1 and 10 protected 20–80% of the test animals moderately, but none was completely protected. However, Compounds 4, 5, and 8 protected 20–80% of the test animals completely from tremors. These compounds were classified as slightly active since a highly active compound should be able to protect 100% of the test animals completely at a dose much lower than used in this test. Standard agents such as atropine, scopolamine, or trihexyphenidyl block tremorine effects in doses of 5–10 mg/kg in mice. The compounds showed no protection against tremorine-induced, peripheral parasympathetic stimulation.

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Fluorescence Characteristics of Benzodiazepines in Strong Acid

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Abstract □ The fluorescence characteristics of 10 substituted 1,4-benzodiazepines in strong acid solution were investigated. The compounds that fluoresce in the Hammett acidity region possess, or can form by enolization, an azomethine linkage in the 1,2- or 4,5-position. All benzodiazepines that fluoresce in strong acid show increases in fluorescence intensity with corresponding blue shifts as acidity increases. Two pKa's in the Hammett acidity region were observed for both the fluorometric and absorptiometric titrations of the benzodiazepines possessing a carbonyl in the 2-position. No evidence of excited state prototropism was observed.

Keyphrases □ Benzodiazepines—fluorescence characteristics in strong acid □ Fluorescence characteristics—10 substituted 1,4-benzodiazepines in strong acid, fluorometric and absorptiometric titrations

Of the spectroscopic methods utilized routinely in pharmaceutical analysis, fluorescence techniques are by far the most sensitive. Fluorescence methods are often discarded as analytical methods because many compounds do not appear to possess active fluorescence properties and those that do often provide as-