verted to the methiodide salt as above, mp 195–196°. *Anal.* ($C_{15}H_{23}N_2O_2I$) C, H.

1-Methyl-4-(3-thienyl)-4-hydroxypiperidine (6) was prepared in 90% yield according to the method described for **5** using 0.14 mole of freshly prepared 3-thienyllithium and 0.14 mole of **2**. For microanalysis **6** was converted to the corresponding benzyl bromide salt in THF. The solid material was washed several times with absolute EtOH, mp 244–246°. *Anal.* ($C_{17}H_{22}BrNOS$) C, H, N.

1-Methyl-4-(3-thienyl)-4-propionoxypiperidine (8) was obtained by treatment of **6** with 3 equiv of propionyl chloride. For microanalysis **8** was converted to the corresponding benzyl bromide salt. *Anal.* ($C_{20}H_{26}BrNO_2S$) C, H, N.

Biological Data.—Using the mouse hot plate method, **7** had an ED_{50} of 16.0 mg/kg. The onset peak and duration are respectively 3.4, 26.1, and 151.2 min. Compound **8** had an ED_{50} of 3.9 mg/kg as compared with 1.3 for morphine and 7.5 for codeine.

These results suggest that forces other than hydrophobic or van der Waal's are operative.

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The Biochemorphology of Cyclobutanecarboximides

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We have shown several imides of cyclobutanecarboxylic acid to have sedative and hypnotic properties.[1] The effects appear to be structure related since cyclobutanecarboxamide, a variety of small ring imides, and several imides of cyclobutane-1,1-dicarboxylic acid[2] are essentially inactive. To better ascertain the biochemorphology of the cyclobutanecarboximides we have synthesized and evaluated the imides in Table I. They were produced using either the reaction of cyclobutanecarboxamide with excess acetylating agent or amide acylation with cyclobutanecarbonyl chloride in pyridine. The compounds comprise related series. Their biological activity has been correlated with molecular structure, water solubility, and partition coefficients. They have been evaluated for general CNS depressant properties, barbiturate potentiation, myorelaxant, antitremorine, and anticonvulsant potency.

Experimental Section

Chemical Methods.—Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated by elemental symbols only, analytical results obtained for those elements were within ±0.4% of theoretical values.

N-Formylcyclobutanecarboxamide. Method A.—A solution of 0.4 g (0.009 mole) of formamide, and 10 ml of neutral alumina treated and KOH-dried pyridine was cooled in an ice bath. Cyclobutanecarbonyl chloride, 1 g (0.009 mole), was added with stirring. An exothermic reaction ensued. When it subsided the mixture was heated on a steam bath for 1 hr and poured into 100 g of crushed ice. The soluble product was separated by bringing the solution to pH 3 with 1 *N* HCl and extracting with two 50-ml fractions of $CHCl_3$. Evaporation of the solvent after Na_2SO_4 drying gave N-formylcyclobutanecarboxamide which was crystallized from pentane to yield 0.5 g (40%) of product, mp 90°. *Anal.* ($C_6H_9NO_2$) N.

N-Caproylcyclobutanecarboxamide. Method B.—Cyclobutanecarboxamide (0.89 g, 0.009 mole) was dissolved in 8 ml of pyridine treated as above, by heating on a steam bath for 15 min. To this 1.6 g (0.009 mole) of caproyl chloride was added with stirring and cooling. After the vigorous reaction ceased, the mixture was heated for 1 hr on a steam bath and poured over 100 g of crushed ice. The product was filtered, dried, and crystallized from pentane to yield 1.02 g (50%) of imide, mp 65°. *Anal.* ($C_{13}H_{23}NO_2$) N.

Solubility.—An excess of imide was shaken for 2 hr at 25° with 20 ml of distilled H_2O at 200 oscillations/min. The suspensions were filtered and the filtrates were analyzed for imide by uv spectrophotometry using the λ_{max} at 260 mμ.

Partition Coefficients.—The system 1-octanol–glass-distilled H_2O was used. The H_2O phase was presaturated with 1-octanol. The 1-octanol was washed with 6 *N* H_2SO_4, 6 *N* NaOH, and glass-distilled H_2O until the aqueous phase was neutral. The imide (40 mg) was dissolved in 20 ml of 1-octanol, and the solution was mixed with 200 ml of glass-distilled H_2O at 25° and shaken for 1.5 hr as above. The phases were separated and the H_2O layer was centrifuged for 1.5 hr at 2500 rpm. Uv spectroscopy, as above, was used to determine the imide in the H_2O phase; imide content of the 1-octanol layer was determined by difference. Data are expressed as ratios of 1-octanol content/H_2O content.

Pharmacological Methods.—In all of the following, mice were used once. They were previously untreated with any drug and permitted to feed *ad libidum*.

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, Swiss–Webster mice weighing 18–22 g. When the righting reflex was lost a sleeping time determination was made. On oral administration, the compounds were given either as a solution or suspension in 0.2–0.4 ml of 1% gum tragacanth using a blunted and bent 18-gauge hypodermic needle feeding tube. On intraperitoneal administration the compounds were given as solutions or suspensions in 0.25% methylcellulose sterile vehicle. The volume of administered solution was 0.2–0.4 ml. In all experiments the control animals received vehicle. For each dose four control and four test mice were used.

Barbiturate Potentiation.—Mice, as above, weighing 18–30 g were used and the test substances were administered orally and intraperitoneally as above. Pentobarbital sodium (50 mg/kg) was administered 30 min after the test drug. All solutions were adjusted so that 0.2–0.4 ml was used. For each test and each control experiment five mice were used. One-way analysis of variance tests were run to determine the significance of differences between test group mean sleeping times and their respective controls. For all experiments with probabilities <0.01, the data were further analyzed using Duncans multiple range test. 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 (drug + barbiturate$_{\text{sleep time}}$)/(barbiturate$_{\text{sleep time}}$ + drug$_{\text{sleep time}}$).

Myorelaxant Activity.—Male Swiss–Webster mice weighing 20–22 g were used. The test compounds were given orally as above. Strychnine sulfate (2 mg/kg) in 0.25% methylcellulose sterile vehicle was administered intraperitoneally 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 convulsions was assessed by survival of 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.

Antitremorine Activity.—Male Swiss–Webster mice weighing 18–24 g were used. The test compounds were administered orally as above and at a dose of 1000 mg/kg. Tremorine (20 mg/kg) in 0.25% methylcellulose sterile vehicle was given intraperitoneally 30 min after the test substance. In controls this dose produced centrally mediated tremors plus signs of parasympathetic stimulation including salivation, lachrymation, diarrhea, and urination. Subjective grading was used to establish the degree of protection

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(2) K. A. Zirvi and C. H. Jarboe, *ibid.*, **11**, 183 (1968).

TABLE I
IMIDES OF CYCLOBUTANECARBOXYLIC ACID
CONHCOR

No.	R	Method	Yield, %	Mp, °C[a]	Cryst solvent[b]	Formula	Analyses	Soly[c]	PC[d]
1	H	A	40	90	F	$C_6H_9NO_2$	N	36.9	0.2
2	CH_3[1]	A				$C_7H_{11}NO_2$		35.0	0.4
3	C_2H_5	A	55	138	F	$C_8H_{13}NO_2$	N	4.2	0.9
4	n-C_3H_7[1]	A				$C_9H_{15}NO_2$		5.2	2.6
5	n-C_4H_9	A	60	109	A	$C_{10}H_{17}NO_2$	C, H		5.6
6	n-C_5H_{11}	A	45	85	F	$C_{11}H_{19}NO_2$	N		7.7
7	n-C_6H_{13}	B	50	58	F	$C_{12}H_{21}NO_2$	N		
8	n-C_7H_{15}	B	50	65	F	$C_{13}H_{23}NO_2$	N	0.8	7.0
9	n-C_8H_{17}	A	45	75	F	$C_{14}H_{25}NO_2$	C, H		
10	n-$C_{11}H_{23}$	B	45	72	A	$C_{17}H_{31}NO_2$	C, H, N	0.8	19.0
11	i-C_3H_7	A	35	156	F	$C_9H_{15}NO_2$	N	1.2	1.8
12	i-C_4H_9	A	40	122	A	$C_{10}H_{17}NO_2$	C, H	1.0	3.0
13	t-C_4H_9	A	40	168	P	$C_{10}H_{17}NO_2$	N		3.4

[a] Corrected. [b] A, Me_2CO; F, C_5H_{12}; P, petroleum ether (bp 60–80°). [c] Water solubility at 25° expressed as mg of solute/ml. [d] Partition coefficient at 25° using 1-Octanol–H_2O.

against tremorine. To judge protection, the animals were suspended by the tail. When tremor was the same as for 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 and no tremor was rated as complete protection. For each drug and each control five mice were used.

Pentylenetetrazole Antagonism.—Mice and drugs as above were used. Pentylenetetrazole (100 mg/kg) in 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 control animals. Drug-treated animals were observed individually for complete protection in the 30 min following injection of pentylenetetrazole. For each drug and each control four mice were used.

Discussion

When tested for general depressant activity it was apparent that only compounds **1** and **2** were reasonably active. Further, they were equally active whether given intraperitoneally or orally. There was, however, a marked depression of spontaneous activity in mice treated with **3**, a noticeable slowing in the case of **4**, and some loss of activity with **11**. The results suggested the effect to be dependent upon structure but in a way not ordinarily seen.[3] A sleeping time dose–response study was made using **1** and **2**. At 100 mg/kg **1** produced a marked depression of spontaneous activity but no righting reflex loss. At higher doses the mean sleeping times were 47 min (250 mg/kg), 127 min (500 mg/kg), and 374 min (1000 mg/kg). The potency of **2** was less and no dose lower than 400 mg/kg produced loss of the righting reflex although 250 mg/kg did slow the animals. For **2** the various sleeping times and doses were 18 min (400 mg/kg), 50 min (600 mg/kg), 84 min (800 mg/kg), and 218 min (1000 mg/kg). Plots of the data showed nonlinear but roughly parallel lines, indicating the validity of potency comparisons. On the basis of plot comparisons **1** was over three times as potent as **2**.

In view of the loss in activity as carbon is added to the acyl chain of the imides both the aqueous solubility and the 1-octanol–H_2O partition coefficients[4] were measured (Table I). The water solubility changed little for NHCHO (**1**) and $NHCOCH_3$ (**2**) but dropped dramatically when further additions were made to the acyl chain. The much decreased solubility paralleled loss of depressant activity. The partition coefficients showed small changes, as increments of carbon were added in the first members of the series and any addition increased 1-octanol solubility. In the higher members great increases resulted from the addition of a single CH_2. The change in both physical properties suggests activity to be limited by aqueous solubility.

Barbiturate potentiation has been recognized as a means of ascertaining CNS activity in compounds with no discernible central effects.[5,6] The possibility that undetected central activity existed in compounds other than **1-4** and **11** prompted testing of the series for pentobarbital potentiating effects. Since certain members were hypnotic the selected measure of potentiation was a sleeping time ratio that expressed potentiation rather than additivity; it was (drug + barbiturate$_{\text{sleep time}}$)/(barbiturate$_{\text{sleep time}}$ + drug$_{\text{sleep time}}$). At 1000 mg/kg (oral and ip) the potentiation factor for **1** was 8.3, and for **2**, **3**, and **4** it was 4.9, 2.3, and 1.8, respectively. The change in activity as molecular weight increases clearly differs from the former experiments. This is especially apparent where potencies may be compared. The hypnotic effect of **1** is over three times that of **2** while its barbiturate potentiation is only 1.7 times that of **2**. The potentiation by **3** and **4** is greater than their directly measured depressant activity and **11** is inactive as a potentiator. Since changes in the two effects are not parallel these data suggest the locus for barbiturate potentiation to be functionally different from the sites involved in the direct depressant effect.

The pentobarbital potentiation of **1** and **2** was studied as a function of intraperitoneal dose. At 100 mg/kg **1** was inactive; at 250 and 500 mg/kg the potentiation factors were 1.7 and 2.9, respectively. At 250 mg/kg **2** was inactive; at 400-, 600-, and 800-mg/kg doses the potentiation factors were 1.3, 1.7, and 2.5, respectively. Plots were nonlinear and roughly parallel.

Meprobamate-like myorelaxant activity is studied conveniently by ascertaining whether a compound an-

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(4) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1967).

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(6) C. H. Holton and V. Larson, *Acta Pharmacol. Toxicol.*, **12**, 346 (1956).

tagonizes strychnine lethality.[7-9] In the present series myorelaxant activity was present in those compounds with depressant activity. At 100 mg/kg (oral and ip) **1** and **2** completely protected[10] against strychnine-induced convulsions while **3**, **4**, and **5** protected only one of four experimental animals. At 500 mg/kg (ip) **1** and **2** partially protected all and completely protected three of four test animals. Although **1** and **2** were shown by comparison experiments to have the same order of potency as meprobamate, the sedative properties of the former compounds preclude any pointed consideration of their muscle relaxant properties.

None of the compounds in this series antagonized the parkinson-like tremors induced by tremorine-[11,12] or pentylenetetrazole-induced convulsions.

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Isolation and Identification of Cephalexin from Human Urine

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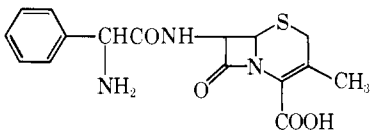
Cephalexin, 7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid is a broad-spectrum cephalosporin antibiotic. The oral administration of



doses of cephalexin in mice[1] as well as in human volunteers[2] demonstrated nearly complete absorption, high serum and urine concentration, and antibacterial activity. Experiments conducted on cephalexin-^{14}C in rats and mice[3] indicated that unchanged cephalexin-^{14}C was the only radioactive substance appearing in urine or bile. Absorption and excretion at the administered dose level was also essentially quantitative. Human urine after single cephalexin doses was shown *via* paper chromatography–bioautography techniques[4] to contain only one substance active against *Sarcina lutea* with an R_f value identical with that of cephalexin. Since the criteria of the chromatography–bioautography are not necessarily specific, it was desirable to secure definitive evidence that the antibiotic was excreted unchanged. This paper reports the direct isolation and complete identification of cephalexin from urine after single cephalexin doses.

The urine samples were obtained from a group of ten normal volunteers, who after fasting overnight were given an oral dose of 500 mg of cephalexin. Urine specimens were then collected during 0–6 hr after drug administration, the total volumes were measured, and all aliquots were frozen for microbiological assay *via* the cup–plate method using *Sarcina lutea* to establish the cephalexin concentration and the total cephalexin contained in urine as shown in Table I.

TABLE I[a]

	Urine		
Volunteer no.	Volume, ml	Cephalexin, μg/ml	Total cephalexin, mg[b]
1	190	2700	533
2	370	1620	598
3	450	1225	551
4	195	1260	209
5	420	1340	563
6	295	150	44
7	600	917	550
8	290	1995	578
9	285	1890	539
10	360	1480	533
Average		1458	470

[a] Many groups of normal volunteers' urine samples had been tested; this table was used as a typical example. The oral dose was 500 mg. [b] Actual dose of cephalexin in the 250-mg capsule was 265 ±11 mg.

To isolate the cephalexin excreted in the urine and establish its identity, ion-exchange chromatography was employed. The cephalexin in urine can be isolated *via* its anion using AG2-X8 resin in acetate form or *via* its cation using Dowex 50 W-X2 resin in sodium form. However, the anionic resin was better than the cationic resin since no complication by salt formation was encountered. The eluted fractions from the column were followed by means of the ninhydrin test, uv absorption maximum at 262 mμ,[5] paper chromatography–bioautography, and microbiological assays. Only those fractions with a positive ninhydrin test and highest absorbance (*A*) were combined and used for crystallization. Two crystalline forms of cephalexin, namely, cephalexin hydrochloride and cephalexin anhydrate (acetonitrile solvated) were obtained, and their identity was established by ir, uv, and nmr spectra and by direct comparison of X-ray diffraction patterns with respective authentic samples. In addition, tlc also revealed that it had the same R_f value as cephalexin, and bioautography also indicated that their activities against *Sarcina lutea* were comparable.[6] The fractions with lower absorbance at 262 mμ were not used for crystallization of cephalexin. Instead they were assayed to establish the total content of cephalexin in all the fractions from the column as shown in Table II. The total content of

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(2) R. L. Perkins, H. N. Carlisle, and S. Saslaw, *Amer. J. Med. Sci.*, **256**, 122 (1968).
(3) Personal communication from H. R. Sullivan, Lilly Research Laboratories.
(4) Personal communication from W. E. Wick, Lilly Research Laboratories.
(5) R. R. Chauvette, *et al.*, *J. Am. Chem. Soc.*, **84**, 3401 (1962), showed that the ultraviolet maximum near 260 mμ was attributed to the C=CNC=C chromophore of the ring.
(6) The microbiological assay of the isolated cephalexin anhydrate using the cup–plate method had an average value of 1045 μg/mg, compared with standard sample of cephalexin at 1000 μg/mg.