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CNS Activities of Lactam Derivatives

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Abstract □ Data presented show that lactams, which have the resonating structure $\rangle\ddot{N}-C(=A)- \leftrightarrow \rangle\overset{+}{N}=C(-\bar{A})-$ commonly associated with CNS depressants and CNS stimulants, have significant CNS activities. Results of preliminary testing show that lactams of medium ring size and lacking *N*-substitution are fairly potent CNS stimulants. The acute lethal toxicity (log $1/C$) of four CNS stimulants of the lactam type is found to be highly correlated with log P, where P is the 1-octanol–water partition coefficient for the compound. The rate of respiration in mice depressed with sodium pentobarbital is stimulated by 2-azacyclononanone, and the rate and depth of respiration were increased by 2-azacyclooctanone in dogs anesthetized with sodium pentothal. Both 2-azacyclooctanone and 2-azacyclononanone are capable of reducing considerably the sodium pentobarbital sleeping time in mice. At higher doses, the CNS stimulant effects of these drugs are manifested by convulsions. For 2-azacyclooctanone, the convulsant ED_{50} is about 37 mg./kg. and the LD_{50} is about 300 mg./kg. Direct evidence for CNS stimulation by 2-azacyclooctanone was obtained by recording changes in EEG patterns in artificially ventilated dogs paralyzed with *d*-tubocurarine. Diphenylhydantoin is much less effective as an antagonist to the convulsant actions of 2-azacyclooctanone than sodium pentobarbital. This finding, taken together with the tonic-clonic convulsions produced by this drug, strongly suggests that, like nikethamide and pentylenetetrazole, the site of action for its CNS effects is subcortical and supraspinal. The conclusion is drawn that the resonating moiety shown here confers upon many compounds CNS-stimulant activities.

Keyphrases □ Lactams, medium ring derivatives—CNS activities, mice, dogs □ CNS depressants, stimulants—medium ring lactams, mice, dogs □ Tonic-clonic convulsions—lactam derivatives, subcortical, supraspinal CNS effects □ Resonating structure, lactams—CNS activities, mice, dogs

Lien and Kumler (1) pointed out that many CNS depressants and CNS stimulants have a common resonating structure: $\rangle\ddot{N}-C(=A)- \leftrightarrow \rangle\overset{+}{N}=C(-\bar{A})-$, where A = O or S. Since lactams are simple compounds possessing this resonating structure, and since very few studies on the biological activities of these compounds have been reported (2), it was decided to study the physicochemical properties and pharmacological activities of these compounds in the CNS. The purpose of this report is to present results of the preliminary screening of some compounds containing the lactam moiety. γ-Thiobutyrolactone, which has the same resonance structure as lactams, is also included in this study. Also, as general representatives of these drugs, 2-azacyclooctanone and 2-azacyclononanone were subjected to pharmacological investigation.

MATERIALS AND METHODS

γ-Thiobutyrolactone was provided by Kharasch and Langford (3) and was purified before use by distillation under atmospheric pressure (b.p. 198°). δ-Valerolactam (m.p. 35–39°), ε-caprolactam (m.p. 69–70°), 2-azacyclooctanone (m.p. 36°), 2-azacyclononanone (m.p. 77–79°), 1-methyl-2-piperidone (n_D^{20} = 1.4823), and 1-butyl-2-pyrrolidinone (n_D^{20} = 1.4640) were purchased[1] and used without further purification.

N-Ethyl-ε-caprolactam was prepared by alkylation of ε-caprolactam using sodium hydride and ethyl iodide, according to the previously reported procedure for the alkylation of cyclic thiourea (1). The procedure was slightly modified by using a reflux period of 3 rather than 5 hr. after the addition of ethyl iodide. The product was purified by vacuum distillation (b.p. 77–78°/1.3 mm.). Elemental analysis[2] gave the following results.

Anal.—Calc. for C, H, N: C, 68.20; H, 10.63; N, 9.995. Found: C, 68.50; H, 10.82; N, 10.36. IR, 1640 $cm.^{-1}$ (C=O stretching).

Preliminary Test for LD_{50}—Groups of six mice, three males and three females weighing 17–27 g., were injected intraperitoneally with a series of logarithmic doses (30, 100, 300, and 1000 mg./kg., *etc.*). Signs of CNS stimulant activity were observed continuously for 2 hr. and then at regular intervals for 2 days. All drugs were administered in aqueous solutions in a volume of 1 ml. or less. Control animals received saline injections. From the number of animals that did not survive, the LD_{50} was estimated for each drug according to the method of Miller and Tainter (4).

Partition Coefficient—The *n*-octanol–water partition coefficients of γ-thiobutyrolactone and *N*-ethyl-ε-caprolactam were measured using a Carey-14 spectrophotometer. γ-Thiobutyrolactone was found to have an absorption maximum ($\lambda_{max.}$) of 2340 Å and a molar absorptivity ($\epsilon_{max.}$) of 4.358×10^3. The partition coefficients of 2-azacyclononanone and *N*-ethyl-ε-caprolactam were measured by GLC[3]. The logarithms of the partition coefficients (log P) are summarized in Table I.

Analeptic Effect of 2-Azacyclononanone in Mice against Sodium Pentobarbital—2-Azacyclononanone (30 mg./kg.) was injected intraperitoneally into a group of 12 mice, which 10 min. earlier had been pretreated with 60 mg./kg. of sodium pentobarbital. Changes in the respiratory rate of control and experimental animals, as well as the mean sleeping times, were compared.

Estimation of ED_{50} and LD_{50} of 2-Azacyclooctanone—Male Swiss albino mice, weighing 20–30 g., were used. The experiments were carried out in a room constantly maintained at 26°. Graded doses of 2-azacyclooctanone dissolved in physiological saline were administered intraperitoneally. For a given experiment,

[1] Aldrich Chemical Co., Inc.
[2] Performed by C. F. Geiger, Ontario, Calif.
[3] Hydrogen-flame detector, column 3% OV-17 on Gas Chrom Q, Applied Science Laboratories.

Table I—CNS Activity of Lactams and γ-Thiobutyrolactone

Compound	*n*	R	Observation	LD_{50} (mg./kg.) C = (mole/kg.)	log 1/C	log P
δ-Valerolactam	4	H	6/6[a] slight sedation[b] at 750 mg./kg.	>1000 (>1.01 × 10^{-2})	<2.00	−0.62[c]
ε-Caprolactam	5	H	6/6 slight sedation[b] at 300 mg./kg. 6/6 Straub tail and clonic and tonic convulsion at 500 mg./kg.	650 (5.74 × 10^{-3})	2.24	−0.19[c]
2-Azacyclooctanone	6	H	6/6 normal at 100 mg./kg. 4/6 Straub tail at 200 mg./kg. 6/6 clonic and tonic convulsions at 300 mg./kg.	270 (2.13 × 10^{-3})	2.67	0.24 ± 0.3[d]
2-Azacyclononanone	7	H	6/6 mild sedation[b] at 100 mg./kg. 5/6 Straub tail at 200 mg./kg. 3/6 clonic and tonic convulsions at 200 mg./kg.	185 (1.31 × 10^{-3})	2.88	0.67 ± 0.01[d]
1-Methyl-2-piperidone	4	CH_3	6/6 mild sedation[b] at 500 mg./kg. 1000 mg./kg.	>1000 (>8.83 × 10^{-3})	—	−0.32[c]
N-Ethyl-ε-caprolactam	5	C_2H_5	6/6 clonic and tonic convulsion at 300 mg./kg.	550 (3.89 × 10^{-3})	2.41	0.65 ± 0.1[d]
1-Butyl-2-pyrrolidinone	3	*n*-C_4H_9	6/6 sedation[b] and ataxia at 300 mg./kg.	500–750[e] (3.54–5.31 × 10^{-3})	—	0.79[c]
2-Azacyclotridecanone	11	H	5/6 side position at 500 mg./kg. 6/6 trembling at 500 mg./kg.	500–750[e] (2.55–3.80 × 10^{-3})	—	1.72[c]
γ-Thiobutyrolactone			5/6 tonic and clonic convulsions at 100 mg./kg.	199 (1.95 × 10^{-3})	2.71	0.60 ± 0.01[d]

[a] Ratio of number of animals responding to number of animals tested. [b] The word "sedation" is used here to indicate diminution of physical activity. Some signs suggested the possibility of a catatonic state. [c] Calculated value taking advantage of the additivity of Hansch's π-constant (see *Reference 5*); π_{-CH_2-} = 0.43 and $\pi_{C_2H_5-(on\ N)}$ = 0.84 obtained in this study were used in the calculations. [d] Standard deviation from three to four separate experimental measurements. [e] Two percent suspension of this drug in 5% acacia solution was used for this injection.

a group of four or five mice received each appropriate dose to be tested. Tonic-clonic convulsions or death within 1 hr. after injection were used to determine the effective convulsant or the lethal doses. Experiments were repeated until 25 animals had been challenged at each dose level. The ED_{50} for convulsion and LD_{50} were estimated by the method of Litchfield and Wilcoxon (6).

Effects of Anticonvulsants on CNS Activity of 2-Azacyclooctanone—A group of 12 mice was injected intraperitoneally with 100 mg./kg. of diphenylhydantoin and a second group of 12 with 40 mg./kg. of sodium pentobarbital. Each group was then challenged with the ED_{50} and the LD_{50} of 2-azacyclooctanone. The number of animals that convulsed or died at each dose was recorded. In a few animals injected with the LD_{50} doses, death was not preceded by frank convulsions. Death in these animals might have been due to toxic effects on a vital organ such as the heart. But in all animals that convulsed, the convulsive event was preceded by signs of CNS stimulation such as rigidity and tremor, staring eyes, and fully extended hindlegs. Since these signs were observed in the few animals that died abruptly, it was presumed that they convulsed also prior to death; these animals were included in the statistical estimation of the protective effects of diphenylhydantoin and sodium pentobarbital against the convulsive effects of the drug.

Activity Measurements in Mice—By using a motility meter[4], activities of mice were compared before and after injection of 2-azacyclooctanone. Six mice were challenged individually with the convulsant ED_{50} (37 mg./kg.) and another group of six with the LD_{50} (300 mg./kg.). The total activity of each animal for 30 min. before injection was used as the control and compared with the activity for 30 min. after the injection of the drug. Values were read directly from the meter at intervals of 5 min.

Electroencephalographic Evidence for CNS-Stimulant Activity of 2-Azacyclooctanone—Three dogs were anesthetized with intravenous doses of 30 mg./kg. sodium pentobarbital. Doses of 2-azacyclooctanone were then injected, and the EEG, ECG, and blood pressure changes were monitored on an E&M physiograph. Three dogs were anesthetized with 15 mg./kg. sodium thiopental slowly injected by the intravenous route. A tracheotomy was rapidly performed and a tube was inserted for artificial ventilation. At the first sign of recovery from surgical anesthesia, an adequate dose of curare (4.5 mg. total) was administered to paralyze the animal, after which enough time was allowed to elapse (usually 30 min.) for full recovery from the anesthesia. This procedure immobilized the animal and enabled changes in EEG to be recorded without interfering signals from associated muscle movements. Doses of 2-azacyclooctanone were injected, and the EEG, ECG, and blood pressure changes were monitored on an E&M physiograph.

Effect of 2-Azacyclooctanone on Sleeping Time—Three groups of 12 mice each were used. The control group received 40 mg./kg. of sodium pentobarbital intraperitoneally, and the experimental group received both pentobarbital and either the ED_{50} or the LD_{50} of 2-azacyclooctanone. The sleeping time was then determined, which is defined as the total time elapsed between the loss and the return of the righting reflex

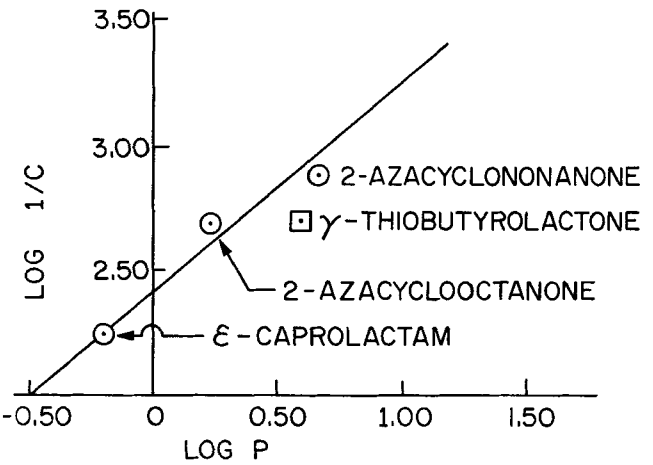

Figure 1—*Plot of 1/C against log* P, *where* C *is concentration expressed in moles per kilogram mouse, and* P *is the partition coefficient (1-octanol–water).*

[4] Motron Produkter, Stockholm, Sweden.

Table II—Influence of 2-Azacyclononanone on Respiratory Rate and Sleeping Time of Mice Pretreated with Sodium Pentobarbital[a]

Minutes after Injection of Pentobarbital	Respiratory Rate/Minute—Sodium Pentobarbital (60 mg./kg.)	Sodium Pentobarbital (60 mg./kg.) and 2-Azacyclononanone (30 mg./kg.)	Percent Increase	$N = 6, df = 5, t (t_{0.95} = 2.02)$
20	53 ± 13	81 ± 18	53	2.86
30	47 ± 14	65 ± 13	40	2.22
40	42 ± 18	74 ± 24	76	2.42
50	47 ± 14	81 ± 28	71	2.42
Mean sleeping time	86 ± 11	68 ± 16	−20	2.03

[a] 2-Azacyclononanone injected 10 min. after sodium pentobarbital.

RESULTS AND DISCUSSION

Results of the preliminary screening for activity and the partition coefficients of eight lactams and the structurally related γ-thiobutyrolactone are shown in Table I. From this table, it can be seen that most of the lactams studied had CNS-stimulating activity, although a few had CNS-depressant components. For the lactams with small ring size (n = 5, 6, 7, 11; R = H, C_6H_5), CNS stimulation appeared to be dominant. This is especially true for n = 6, which did not show any sedative effect. This finding is similar to what was observed in the case of cyclic thioureas. *N,N'*-Dimethyltrimethylenethiourea was found to be the most potent respiratory stimulant among the homologs reported (1). This finding suggests that 2-azacyclooctanone and 2-azacyclononanone may be potential analeptic agents with fairly low toxicity. γ-Thiobutyrolactone, which has a similar resonating structure: —S—C=O↔ —$\overset{+}{S}$=C—$\overset{-}{O}$, is a potent convulsant while γ-butyrolactone is known to be a CNS depressant (7–9).

The plot of log $1/C$ against log P in Fig. 1 shows that there is a high degree of correlation between the acute lethal toxicity and the lipophilic–hydrophilic character of the *N*-unsubstituted lactams: 2-azacyclononanone, 2-azacyclooctanone, and ε-caprolactam. This is true for γ-thiobutyrolactone also. The following regression is obtained by the method of least squares: $\log 1/C = 0.655 \log P + 2.409$, $n = 4$, $r = 0.949$, $s = 0.106$, where n is the number of the data points, r is the correlation coefficient, and s is the standard deviation. This correlation is significant at the 90 percentile level ($F_{1,2} = 17$; $F_{1,2_{0.90}} = 8.5$).

The toxicity of *N*-alkylated lactams is appreciably lower as compared with that of the nonalkylated lactams with the same log P value. The reason why the *N*-alkylated lactams are less toxic than the nonalkylated ones may be due to the interference of dipole–dipole interactions at the receptor sites by the *N*-alkyl group.

In estimating the ED_{50} of 2-azacyclooctanone, 25 mice were used at each dose and the doses ranged from 10 to 200 mg./kg. In estimating the LD_{50} of 2-azacyclooctanone, 25 mice were also used at each dose, but the doses ranged from 50 to 5000 mg./kg. The convulsant ED_{50} was estimated to be 37 mg./kg. with 95% confidence limits of 24–59 mg./kg., and the LD_{50} was estimated to be 300 mg./kg. with 95% confidence limits of 240–375 mg./kg. This gives a ratio of LD_{50} to convulsant ED_{50} of about 8.

Eighty percent of the mice pretreated with diphenylhydantoin and only 20% of the animals treated with sodium pentobarbital convulsed when challenged with the ED_{50} dose of 2-azacyclooctanone. Since diphenylhydantoin does not protect against midbrain and medullary convulsions, the findings suggest that 2-azacyclooctanone has the midbrain and medulla oblongata as the main sites of action. In this respect, the drug is similar to nikethamide and pentylenetetrazole. Although the other CNS-stimulant drugs discussed in this report were not tested as extensively as 2-azacyclooctanone, the results of the preliminary screening and the physicochemical and molecular considerations led to the suspicion that

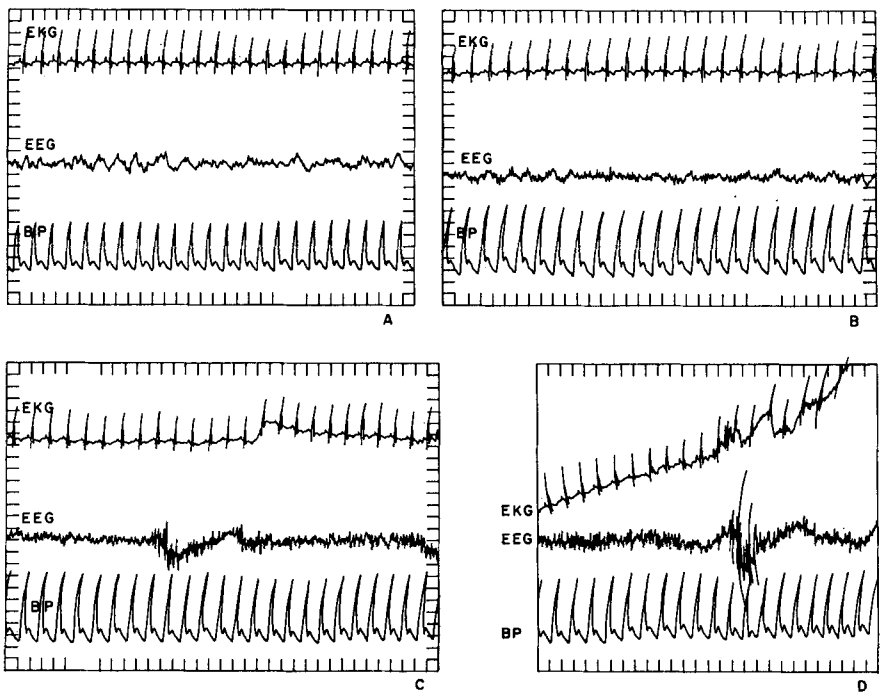

Figure 2—*EEG, ECG (lead I), and blood pressure tracings (right femoral artery) of a male dog 40 min. after the intravenous injection of 30 mg./kg. sodium pentobarbital (A); EEG, ECG (lead I), and blood pressure tracings (femoral artery) 40 min. after the recordings of A were taken (B) and immediately after injection of a total of 1600 mg. of 2-azacyclooctanone. The tracings of C were taken 3 min. after B; the tracings of D were taken 4 min. after B. Note the gradual appearance of an alerted EEG culminating in full-blown convulsion.*

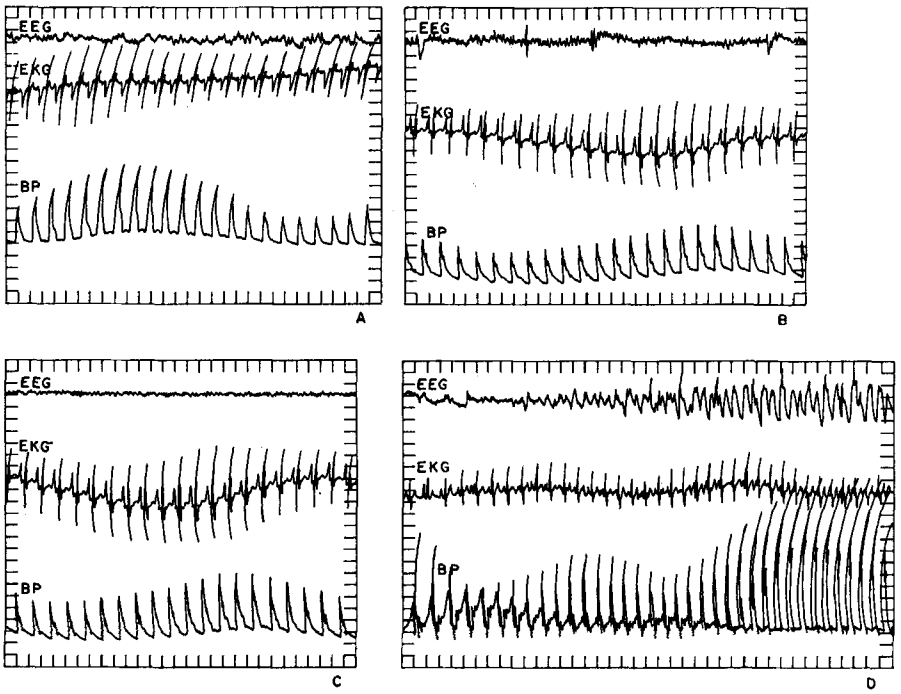

Figure 3—*ECG (lead I), EEG, and blood pressure tracings (right femoral artery) of a male dog anesthetized with 15 mg./kg. thiopental, artificially ventilated through a tracheotomy, paralyzed with* d-*tubocurarine, and injected intravenously with 1600 mg. of 2-azacyclooctanone (A). After 2 min., the record of B was seen showing spikes in the EEG. After 4 min., the record of C was seen showing the high frequency, low amplitude waves of an alert EEG. D shows evidence of a full-blown convulsion.*

they too have the same neuroanatomical areas as their sites of action.

As can be seen from the results in Table II, the respiratory rate of the animals receiving a combination of 2-azacyclononanone and pentobarbital increased by 40–76% and the sleeping time decreased by about 20% when compared with the animals receiving pentobarbital alone. Using the same standard *t*-test, these differences were found to be significant at the 95% level.

Figure 2A shows the EEG, ECG, and blood pressure recordings from a dog anesthetized with 30 mg./kg. sodium pentobarbital 40 min. after injection. The EEG shows the low frequency, high amplitude waves of surgical anesthesia of approximately Stage III Plane III (10). Figure 2B shows the EEG obtained 40 min. after injection of 2-azacyclooctanone to a total dose of 1600 mg. The appearance of the high frequency, low amplitude waves is consistent with an alerted CNS. The ability of 2-azacyclooctanone to produce changes in the EEG from a sleeping to an alerted pattern is taken to mean that the compound has a strong CNS-stimulant effect. No significant changes in the ECG and the blood pressure tracings were noted. Both depth and rate of respiration were increased. Figure 2C shows the recording obtained 43 min. after the injection of a total dose of 1600 mg. 2-azacyclooctanone. Convulsive waves began to appear, and 1 min. later a full-blown convulsion set in, as shown in Fig. 2D. Except for an increase in pulse pressure, no significant changes in blood pressure were seen.

Figure 3 shows EEG, ECG, and blood pressure recordings obtained from a dog which was paralyzed with *d*-tubocurarine and artificially ventilated. This dog was anesthetized with just sufficient thiopental to produce anesthesia to enable performance of tracheostomy and insertion of a cannula for the artificial ventilation; the effects of the anesthetic were then allowed to wear off. Upon injection of 2-azacyclooctanone in several doses to this alert, paralyzed animal, the gradual stimulation of the CNS was noted until a total dose of 1600 mg. was given and a full-blown convulsion was seen (Figs. 3A–D). In spite of the marked CNS activity, again as before, no significant changes in blood pressure were noted and the ECG patterns appeared normal.

The effect of 2-azacyclooctanone on the time elapsed between loss and return of the righting reflex (sleeping time) in mice treated with 40 mg./kg. of sodium pentobarbital is shown in Table III. The convulsant ED_{50} caused a reduction of greater than 50%. This effect is also reflected in corresponding changes in the ranges of sleeping time. The findings provide further evidence for the CNS-stimulant activity of this compound.

The activity measurements with the motility meter showed a peculiar course which was not in accord with predictions based on the known activities of the compound, namely CNS stimulation. Injection of effective doses of the drug to mice produced a diminution of activity which may terminate in recovery, convulsions, or convulsions and death. Prior to these end-points, at no time did the animals show any increase in activity above the control levels. But, even when the activities appeared depressed, the animals appeared somewhat "stunned," with some tremor and a posture as if faced with impending danger. Their behavior may be a manifestation of a catatonic state rather than a true CNS depression. Respiratory depth and rate were considerably higher. Convulsions set in abruptly and may continue until recovery or death. In spite of this unexplainable effect of the drug, sufficient CNS analeptic stimulant activity has been demonstrated by other experiments to conclude that 2-azacyclooctanone is a CNS stimulant.

The experimental findings and observations presented in this report confirm to a large degree the prediction, based on the earlier observations of Lien and Kumler (1), that many CNS stimulants and depressants contain the common resonating structures:

Table III—Effect of 2-Azacyclooctanone on Sleeping Time of Pentobarbital-Treated Mice (40 mg./kg.)

	Sodium Pentobarbital Alone, min.	*Sodium* Pentobarbital and Convulsant ED_{50} of 2-Azacyclooctanone, min.	*Sodium* Pentobarbital and LD_{50} of 2-Azacyclooctanone, min.
Mean sleeping time	25	11.8	0.5
Range of sleeping times	5–47	2–22	0–5
Number of animals	12	12	12

$\rangle\ddot{N}-C(=O)- \leftrightarrow \rangle\overset{+}{N}=C(-\overset{-}{O})-$. In general, of the compounds studied, the lactams possess predominantly CNS-stimulant activities. This effect is especially pronounced with 2-azacyclooctanone and 2-azacyclononanone. While many compounds were studied in order to make a generalization with regard to the effects of lipophilic–hydrophilic character and alkyl substitutions on the general biological behavior of these drugs, the majority of the report converges on the most active stimulants, namely 2-azacyclooctanone and 2-azacyclononanone. By various methodologies, sufficient data and information were obtained to conclude that they are CNS stimulants with the predominant site of action located in the midbrain and the medulla, that is, supraspinal and subcortical.

Nikethamide and pentylenetetrazole are two clinically useful stimulant drugs thought to exert their effects also in the midbrain and medulla. Moreover, all penicillins are irritating to the CNS. EEG abnormalities and convulsions have followed the application of penicillin directly to the human cerebral cortex (10). The administration of high doses of penicillin intravenously has produced convulsions in some patients, and convulsions and death have occurred following intraarterial administration of large doses of penicillin to patients with impaired renal function (11). The penicillins, like nikethamide and pentylenetetrazole, also contain moieties that have similar resonating structures to the compounds discussed in this report. These observations, together with the results presented in this report, particularly those obtained with 2-azacyclooctanone and 2-azacyclononanone, demonstrate that the resonating moiety referred to confers upon many substances CNS-stimulant activities.

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GLC Determination of *dl*-2-(3-Phenoxyphenyl)propionic Acid (Fenoprofen) in Human Plasma

J. F. NASH, R. J. BOPP, and A. RUBIN

Abstract □ The quantitative determination of *dl*-2-(3-phenoxyphenyl)propionic acid (fenoprofen) in plasma is described. Fenoprofen, extracted with hexane from 1 ml. of acidified plasma, is converted to the silyl ester and then measured by GLC utilizing a flame-ionization detector. Favorable quantitation is achieved by using *dl*-2-(4-phenoxyphenyl)valeric acid as a mass internal standard. The assay is quantitative above 0.25 mcg./ml. Overall, precision and accuracy of the assay are approximately ±10% (*RSD*) and ±5% (*RE*) in the range of 3–8 mcg./ml. Plasma data are presented to demonstrate the clinical utility of the method.

Keyphrases □ *dl*-2-(3-Phenoxyphenyl)propionic acid—GLC analysis in human plasma □ Fenoprofen—GLC analysis in human plasma □ Plasma levels—fenoprofen, GLC analysis □ GLC—analysis, *dl*-2-(3-phenoxyphenyl)propionic acid (fenoprofen) in human plasma

Since Northover (1) showed that aryl- and alkyl-substituted phenoxyacetic acids exhibit anti-inflammatory properties, several structurally related chemicals have been synthesized and marketed. These include ibufenac or 2-(4-isobutylphenyl)acetic acid (2), ibuprofen or 2-(4-isobutylphenyl)propionic acid, and namoxyrate or 2-dimethylaminoethanol salt of 2-(4-biphenyl)butyric acid (3). In general, sensitive, simple, specific, and reproducible methods have not been readily available to determine quantitatively this class of chemical compounds in biological fluids. To determine blood levels of ibufenac, Adams and Cliffe (2) developed a method based upon paper chromatographic separation and reaction of the drug with bromcresol purple. The method was quantitative above 7.5 mcg./ml. serum, with a precision of ±15%; unfortunately, the analysis required 48 hr. Bergen *et al.* (3) used ^{14}C-namoxyrate to study the absorption, distribution, and metabolism of the drug.

Nickander *et al.* (4) reported on the synthesis and pharmacologic effects of a series of structurally related chemicals, one of which is fenoprofen, *dl*-2-(3-phenoxyphenyl)propionic acid. A procedure is described herein for its quantitative determination in plasma using GLC. This method should be useful in quantitating other structurally related compounds in biological media.

EXPERIMENTAL

Reagents—Sodium fenoprofen dihydrate and *dl*-2-(4-phenoxyphenyl)valeric acid were synthesized[1]. The latter compound served as a mass internal standard (MIS). All solvents were analytical reagent quality, except spectroquality hexane, which was used at the concentration stage of the procedure. Diatoport-S (80–100 mesh)

[1] At Eli Lilly and Co., Indianapolis, Ind.