respects. That all the data *generated* are used in parameter estimation, rather than merely that up to about $\frac{1}{2}M_\infty$, strengthens the parameter estimates.

It is anticipated that this evaluation will provide a basis for the use of the described models when the use of a physically exact or a general model is contemplated.

REFERENCES

(1) M. H. Jacobs, "Diffusion Processes," Springer-Verlag, New York, N.Y., 1967.

(2) J. Crank, "The Mathematics of Diffusion," Oxford University Press, London, England, 1956.

(3) R. M. Barrer, "Diffusion In and Through Solids," Cambridge University Press, London, England, 1941.

(4) J. Crank, "The Mathematics of Diffusion," Oxford University Press, London, England, 1956, p. 241.

(5) M. H. Jacobs, "Diffusion Processes," Springer-Verlag, New York, N.Y., 1967, p. 49.

(6) *Ibid.*, p. 99.

(7) T. Higuchi, *J. Soc. Cosmet. Chem.*, **11**, 85(1960).

(8) W. I. Higuchi, *J. Pharm. Sci.*, **51**, 802(1962).

(9) J. Ostrenga, C. Steinmetz, and B. Poulsen, *ibid.*, **60**, 1175(1971).

(10) J. Haleblian, R. Runkel, N. Mueller, J. Christopherson, and K. Ng, *ibid.*, **60**, 541(1971).

(11) A. L. Weiss and B. J. Sciarrone, *ibid.*, **58**, 980(1969).

(12) J. Crank, "The Mathematics of Diffusion," Oxford University Press, London, England, 1956, p. 248.

(13) *Ibid.*, p. 248.

(14) T. Koizumi and W. I. Higuchi, *J. Pharm. Sci.*, **57**, 87(1968).

(15) *Ibid.*, **57**, 93(1968).

(16) M. H. Jacobs, "Diffusion Processes," Springer-Verlag, New York, N.Y., 1967, p. 50.

(17) F. T. Wall, P. F. Grieger, and C. W. Childers, *J. Amer. Chem. Soc.*, **74**, 3562(1952).

(18) M. Berman, E. Shahn, and M. F. Weiss, *Biophys. J.*, **2**, 275(1962).

(19) R. M. Barrer, "Diffusion In and Through Solids," Cambridge University Press, London, England, 1941, p. 18.

(20) M. H. Jacobs, "Diffusion Processes," Springer-Verlag, New York, N.Y., 1967, p. 60.

(21) B. J. Poulson, E. Young, V. Coquilla, and M. Katz, *J. Pharm. Sci.*, **57**, 928(1968).

(22) J. Ostrenga, C. Steinmetz, and B. Poulson, *ibid.*, **60**, 1175(1971).

(23) N. F. Billups and N. K. Patel, *Amer. J. Pharm. Educ.*, **34**, 190(1970).

(24) K. C. Patel, G. S. Banker, and H. G. DeKay, *J. Pharm. Sci.*, **50**, 300(1961).

(25) S. Riegelman and W. J. Crowell, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 115(1958).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 16, 1973, from the *Department of Pharmaceutical Science, School of Pharmacy, Oregon State University, Corvallis, OR 97331*

Accepted for publication October 11, 1973.

Supported in part by Grant 30-260-0179 from the Research Council of Oregon State University.

[x] To whom inquiries should be directed. Present address: Department of Pharmacy Administration, Office of the Dean for Educational Development, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612

Substituted Piperazinoquinazolones: Relationship between Selective Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Oxidations and Anticonvulsant Activity

SURENDRA S. PARMAR*‡[x], A. K. CHATURVEDI‡, A. CHAUDHARY‡, and STANLEY J. BRUMLEVE*

Abstract □ Several substituted piperazinoquinazolones were synthesized, characterized, and tested for their ability to affect the respiratory activity of rat brain homogenate. All piperazinoquinazolones were found to inhibit selectively nicotinamide adenine dinucleotide (NAD)-dependent oxidations of pyruvate, citrate, DL-isocitrate, β-hydroxybutyrate, α-ketoglutarate, and NADH while the NAD-independent oxidation of succinate remained unaltered. Inhibition of the oxidation of pyruvic acid by piperazinoquinazolones was concentration dependent, but added NAD, while stimulating the respiratory activity of brain homogenate, reduced the inhibition produced by these compounds. Some of these piperazinoquinazolones possessed anticonvulsant activity; however, this activity was found to be unrelated to their ability to inhibit the respiratory activity of the rat brain homogenate.

Keyphrases □ Piperazinoquinazolones—synthesis, anticonvulsant activity, and relationship to NAD-dependent oxidations □ Structure-activity relationships—piperazinoquinazolones, anticonvulsant activity, rats □ Inhibition of respiratory activity, NAD-dependent oxidations—synthesis and evaluation of piperazinoquinazolones □ Oxidation of pyruvate, citrate, DL-isocitrate, β-hydroxybutyrate, α-ketoglutarate, NADH, and succinate—effect of piperazinoquinazolones

Piperazinoureas possessing central nervous system (CNS)-depressant activity were recently shown to inhibit the oxidation of pyruvic acid by rat brain homogenate (1). Adrenergic, hypotensive, and antihistaminic activities were also reported for several 2-methyl-3-alkyl or piperazinoalkyl-4-quinazolones (2). Furthermore, selective inhibition of nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvic acid and other substrates of the tricarboxylic acid cycle by 2-methyl-3-*o*-tolyl-4-quinazolone (3–5) possessing hypnotic (6) and anticonvulsant properties (7) led to the synthesis of substituted pipera-

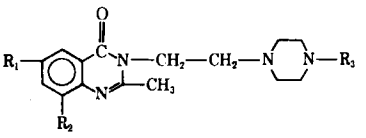
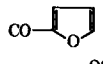
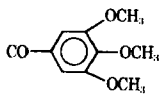
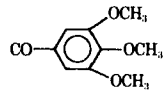


Table I—Substituted Piperazinoquinazolones

Compound	R_1	R_2	R_5	Crystallization Solvent	Yield, %	Melting Point[a]	Molecular Formula	Analysis, % Calc.	Found
I	H	H	H	Absolute ethanol	40	245°	$C_{15}H_{20}N_4O \cdot 2HCl$	C 52.17	52.45
								H 5.79	6.0
								N 16.23	16.20
II	Cl	H	H	Ethanol	75	128°	$C_{15}H_{19}ClN_4O$	C 58.72	59.08
								H 6.19	5.88
								N 18.27	18.12
III	Br	H	H	*n*-Propanol	68	190°	$C_{15}H_{19}BrN_4O$	C 51.28	50.75
								H 5.41	5.67
								N 15.95	15.67
IV	I	H	H	Ethanol	75	210°	$C_{15}H_{19}IN_4O$	C 45.22	45.33
								H 4.77	4.87
								N 14.03	14.25
V	Cl	Cl	H	Benzene–ethanol	70	245°	$C_{15}N_{18}Cl_2N_4O$	C 52.78	52.57
								H 5.27	5.66
								N 16.42	16.30
VI	Br	H	$COCH_3$	Ethyl acetate	60	105°	$C_{17}N_{21}BrN_4O_2$	C 51.90	52.00
								H 5.34	4.90
								N 14.24	13.98
VII	Cl	Cl	$COCH_3$	Methanol	62	190°	$C_{17}H_{20}Cl_2N_4O_2$	C 53.26	52.97
								H 5.22	4.82
								N 14.62	14.26
VIII	Cl	H	COC_6H_5	Acetonitrile	70	255°	$C_{22}N_{23}ClN_4O_2$	C 64.31	64.71
								H 5.60	6.00
								N 11.20	11.54
IX	Br	H	COC_6H_5	Ethyl acetate	60	200°	$C_{22}H_{23}BrN_4O_2$	C 58.02	58.13
								H 5.05	4.95
								N 12.30	12.45
X	Cl	Cl	COC_6H_5	Ethanol	70	270°	$C_{22}H_{22}Cl_2N_4O_2$	C 59.34	59.52
								H 4.94	4.72
								N 12.58	12.78
XI	Cl	Cl		Dioxane	75	270°	$C_{20}H_{20}Cl_2N_4O_3$	C 55.17	55.00
								H 4.59	4.32
								N 12.87	12.60
XII	Br	H		Ethanol	82	144°	$C_{25}H_{29}BrN_4O_5$	C 55.04	54.95
								H 5.32	5.72
								N 10.27	10.57
XIII	Cl	Cl		Ethanol	77	265°	$C_{25}H_{28}Cl_2N_4O_5$	C 56.07	55.97
								H 5.23	5.46
								N 10.46	10.73

[a] Melting points were taken in open capillary tubes and are uncorrected.

zinoquinazolones and to an investigation of their ability to inhibit respiratory activity of rat brain homogenate, with a view to studying their biochemical mechanism of action. In the present study, attempts also were made to correlate the anticonvulsant activity possessed by these piperazinoquinazolones with their enzyme inhibitory effectiveness.

CHEMISTRY

The various piperazinoquinazolones (Table I) were synthesized by following the methods outlined in Scheme I. Acetanthranils (II) were synthesized by refluxing 1 mole of the appropriate anthranilic acid (I) with 2 moles of acetic anhydride. Piperazinoquinazolones (IV) were synthesized by heating equimolar quantities of appropriate acetanthranils (II) and *N*-(2-aminoethyl)piperazine (III).

EXPERIMENTAL[1]

Anthranilic Acids (I)—The method of Endicott *et al.* (8) was used for the synthesis of 5-chloro- and 3,5-dichloroanthranilic acids. 5-Bromoanthranilic acid was prepared by the method of Wheeler and Oats (9), while the method of Klemme and Hunter (10) was used for the synthesis of 5-iodoanthranilic acid.

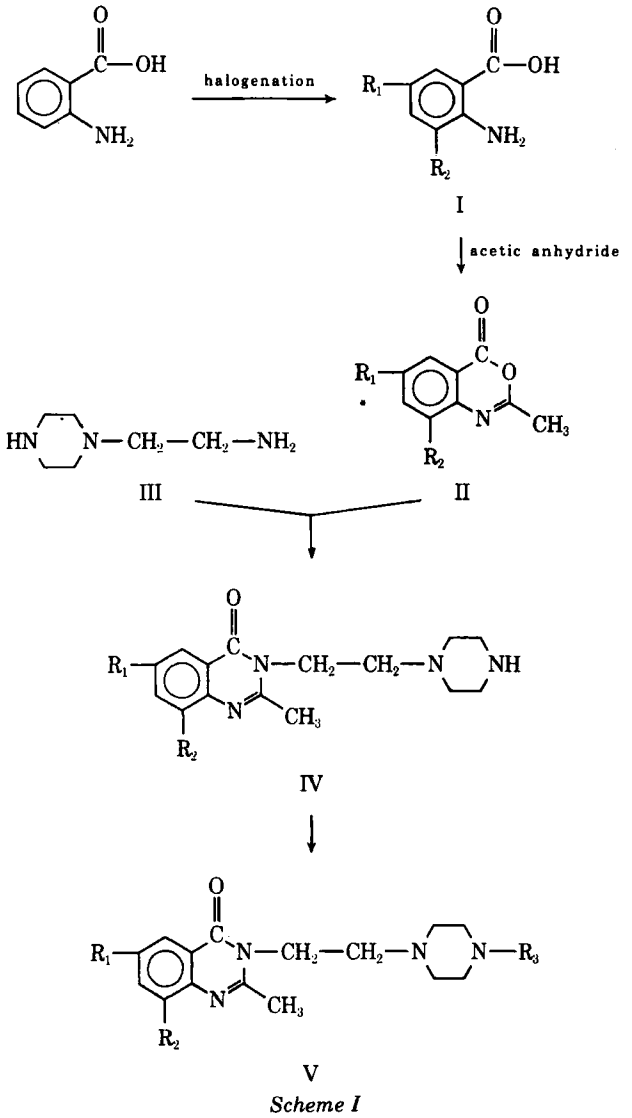
Acetanthranils (II)—The appropriate anthranilic acid (1 mole) was refluxed with acetic anhydride (2 moles) for 1 hr. After excess acetic anhydride was distilled off, the acetanthranil that separated out as a solid mass was dried quickly and used without further purification (II).

Piperazinoquinazolones (IV)—Equimolar quantities (1 mole) of the appropriate acetanthranil and *N*-(2-aminoethyl)piperazine were heated directly to yield the desired piperazinoquinazolones (11), which were recrystallized from suitable solvents and characterized by their sharp melting points and elemental analyses (Table I, Compounds I–V).

Substituted 3-[2-*N*-(*N'*-Acetylpiperazino)ethyl]quinazolones (V)—Appropriate piperazinoquinazolones (0.01 mole) were refluxed with acetic anhydride (0.02 mole) and anhydrous sodium acetate (0.02 mole) for 2 hr. The oily products thus obtained were added slowly into crushed ice, and the mixture was stirred for 1 hr. The crude products that separated were filtered, washed, dried, and recrystallized from suitable solvents. The various piperazinoquinazolone derivatives were characterized by their sharp melting points and elemental analyses (Table I, Compounds VI and VII).

Substituted 3-[2-*N*-(*N'*-Benzoyl/2-furoyl/3,4,5-trimethoxybenzoyl/piperazino)ethyl]quinazolones (V)—Piperazinoquinazolones (1 mole) and appropriate acid chloride (1 mole) were re-

[1] Melting points were taken in open capillary tubes and are uncorrected.



Scheme I

fluxed in dry benzene on a steam bath for 6–8 hr. The mixture was cooled and washed with 5% HCl, 5% Na_2CO_3 solution, and finally water. After distilling off the excess of benzene under reduced pressure, the solid mass that separated was filtered and recrystallized from suitable solvent. The various piperazinoquinazolone derivatives were characterized by their sharp melting points and elemental analyses (Table I, Compounds VIII–XIII).

BIOCHEMICAL[2] AND PHARMACOLOGICAL

Determination of Respiratory Activity of Rat Brain Homogenate—Male albino rats, kept on *ad libitum* diet, were used in all experiments. Rat brains isolated from decapitated animals were immediately homogenized in ice-cold 0.25 *M* sucrose in a homogenizer (Potter-Elvehjem) in a ratio of 1:9 (w/v). All incubations were carried out at 37°, and the oxygen uptake was measured by the conventional Warburg manometric technique with air as the gas phase (5). Fresh rat brain homogenate (1 ml) equivalent to 100 mg wet weight was added to chilled Warburg vessels containing 6.7 m*M* magnesium sulfate, 20 m*M* sodium hydrogen phosphate buffer solution (pH 7.4), 1 m*M* adenosine monophosphate (sodium salt), 33 m*M* potassium chloride, and 500 μg of cytochrome c in a final volume of 3 ml unless otherwise stated. The central well contained 0.2 ml of 20% KOH solution. Pyruvate, succinate, α-ketoglutarate, DL-isocitrate, β-hydroxybutyrate, and citrate were used at a final concentration of 10 m*M*. NADH and NAD were used at a final concentration of 0.5 m*M*. It was presumed that the endogenous NAD, present in this homogenate, was sufficient for these oxidative processes. All compounds under assay were dissolved in propylene glycol (100%), and an equal volume of the solvent was added to the control vessels.

Table II—Inhibition of Oxidation of Pyruvic Acid in Rat Brain Homogenate by Substituted Piperazinoquinazolones

Compound	Percent Inhibition[a] Without Added NAD, 1 m*M*[b]	Without Added NAD, 2 m*M*[b]	With Added NAD, 2 m*M*[b]
I	14.3 ± 1.2	28.0 ± 1.2	22.9 ± 1.4
II	25.1 ± 2.2	37.6 ± 1.2	34.6 ± 0.9
III	27.2 ± 1.9	55.3 ± 2.4	47.8 ± 1.1
IV	30.5 ± 2.0	68.2 ± 1.4	56.6 ± 0.4
V	40.4 ± 2.3	77.6 ± 2.6	68.0 ± 2.4
VI	27.7 ± 2.6	51.2 ± 1.3	43.4 ± 1.3
VII	12.4 ± 2.0	28.4 ± 2.0	22.8 ± 1.9
VIII	16.1 ± 3.8	42.7 ± 1.0	35.2 ± 1.5
IX	27.3 ± 3.4	44.8 ± 1.0	39.9 ± 0.9
X	21.3 ± 1.0	37.0 ± 2.0	29.6 ± 1.5
XI	17.0 ± 0.9	26.7 ± 1.5	11.9 ± 2.0
XII	25.0 ± 3.4	43.1 ± 2.0	37.3 ± 1.0
XIII	9.3 ± 0.8	22.5 ± 1.0	13.3 ± 1.8

[a] Vessel contents and assay procedures were as described in the text. Each experiment was done in duplicate, and the values are the mean values of three separate experiments with ± standard error of the mean. The oxygen uptake was measured at 5-min intervals during a 1-hr incubation. The percentage inhibition was calculated from the decrease in the oxygen uptake per 100 mg wet brain weight. The final concentrations of pyruvic acid and NAD were 10 and 0.5 m*M*, respectively. [b] Indicates final concentration of substituted piperazinoquinazolones.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in albino mice, 20–25 g, of either sex (12). The mice were divided into groups of 10, keeping the group weights as near the same as possible. Each piperazinoquinazolone was suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compound was injected intraperitoneally, 100 mg/kg, in a group of 10 animals. Four hours after the administration of the test compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol was shown to produce convulsions in almost all untreated mice, and the mice were also found to exhibit 100% mortality during 24 hr. On the other hand, no mortality was observed during 24 hr in animals treated with 100 mg/kg of the test compounds alone.

The mice were observed 60 min for the occurrence of seizures. An episode of clonic spasm persisting for at least 5 sec was considered a threshold convulsion. Transient intermittent jerks of tremulousness were not counted. Animals devoid of threshold convulsions during the 60-min period were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these piperazinoquinazolones was represented as percent protection. The animals were then observed for 24 hr and their mortality was recorded.

RESULTS AND DISCUSSION

Inhibition of certain metabolic processes in the brain was shown to be the mechanism of various CNS depressants (12, 13) where a parallelism exists between *in vitro* and *in vivo* effects, since greater hypnotic activity of some agents was reflected by greater *in vitro* inhibition of respiration (14, 15).

The inhibitory effects of piperazinoquinazolones on the oxidation of pyruvic acid by rat brain homogenate are presented in Table II. All piperazinoquinazolones inhibited oxidation of pyruvic acid, and this inhibition was concentration dependent since an increase in inhibition was observed with a simultaneous increase in concentration. The presence of added NAD was found not only to increase the respiratory activity of rat brain homogenate but also to decrease the inhibitory effectiveness of these piperazinoquinazolones. These results thus provide evidence for a possible competition between the piperazinoquinazolones and

[2] Commercial chemicals were used. Sodium pyruvate, adenosine monophosphate (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from British Drug House, Bombay, India.

Table III—Inhibition of Respiratory Activity of Rat Brain Homogenate by Substituted Piperazinoquinazolones

Compound	Percent Inhibition[a]					
	Citrate	DL-Isocitrate	β-Hydroxybutyrate	α-Ketoglutarate	NADH	Succinate
I	21.2 ± 1.4	11.3 ± 0.8	34.4 ± 1.2	53.1 ± 1.3	10.1 ± 0.9	Nil
II	36.4 ± 1.0	12.5 ± 0.9	37.2 ± 1.4	45.1 ± 2.0	21.2 ± 1.0	Nil
III	57.6 ± 1.2	59.5 ± 1.2	61.3 ± 1.5	78.8 ± 4.3	62.3 ± 2.3	Nil
IV	44.1 ± 1.1	12.7 ± 1.0	37.4 ± 1.3	47.0 ± 2.0	41.1 ± 1.0	Nil
V	89.4 ± 3.5	72.0 ± 2.3	73.8 ± 2.9	79.4 ± 3.0	72.0 ± 3.3	Nil
VI	31.5 ± 1.0	59.9 ± 2.3	37.2 ± 1.1	66.3 ± 3.0	42.1 ± 2.1	Nil
VII	57.1 ± 1.7	36.0 ± 1.0	45.9 ± 1.2	67.7 ± 2.0	13.2 ± 1.7	Nil
VIII	38.1 ± 1.3	51.3 ± 1.7	41.0 ± 1.3	61.0 ± 1.9	44.1 ± 2.3	Nil
IX	43.3 ± 1.7	35.1 ± 1.6	41.9 ± 2.0	48.0 ± 1.0	13.6 ± 2.1	Nil
X	53.0 ± 1.3	51.4 ± 1.3	54.2 ± 1.5	70.8 ± 2.1	29.6 ± 0.8	Nil
XI	59.5 ± 2.3	45.6 ± 1.2	46.3 ± 1.3	54.2 ± 1.8	28.9 ± 1.2	Nil
XII	57.6 ± 3.1	17.4 ± 1.1	30.3 ± 1.7	44.9 ± 1.6	40.0 ± 1.4	Nil
XIII	57.7 ± 1.2	50.2 ± 1.4	49.3 ± 1.7	58.9 ± 1.3	15.8 ± 1.3	Nil

[a] Vessel contents and assay procedures were as described in the text and in Table II. The final concentrations of the various substrates, NADH, and substituted piperazinoquinazolones were 10, 0.5, and 2 m*M*, respectively.

Table IV—Anticonvulsant Activity of Substituted Piperazinoquinazolones

Compound	Anticonvulsant Activity[a], % Protection	Percent Pentylenetetrazol Mortality
I	Nil	100
II	70	10
III	50	50
IV	30	30
V	30	80
VI	10	80
VII	50	20
VIII	Nil	80
IX	Nil	80
X	40	60
XI	20	50
XII	30	20
XIII	20	30

[a] Screening procedures for the determination of anticonvulsant activity were described in the text. Substituted piperazinoquinazolones were administered intraperitoneally (100 mg/kg) 4 hr before the administration of pentylenetetrazol (90 mg/kg) subcutaneously. Mortality in pentylenetetrazol-treated animals was observed for 24 hr.

NAD for the active site(s) on the enzyme molecule. This was further supported by the ability of the piperazinoquinazolones to inhibit the oxidation of NADH. As is evident from Table III, all piperazinoquinazolones selectively inhibited NAD-dependent oxidations of citrate, DL-isocitrate, β-hydroxybutyrate, α-ketoglutarate, and NADH while the NAD-independent oxidation of succinate remained unaltered. These results exhibiting selective inhibition of NAD-dependent oxidations are in agreement with earlier studies with 2-methyl-3-*o*-tolyl-4-quinazolone (4, 5). Inhibition of the oxidation of NADH has indicated possible inactivation of the electron-transport chain by piperazinoquinazolones, presumably at the site of the transfer of the electrons from NADH to flavine adenine dinucleotide.

Inhibitory effects of piperazinoquinazolones were not found to be related to their chemical structure, so a definite structure–activity relationship was not exhibited. Introduction of the halogen substituents at the 6-position or the 6- and 8-positions caused a significant increase in their inhibitory effects. A decrease in the effectiveness of these compounds to inhibit oxidation of pyruvic acid because of the attachment of an acetyl, benzoyl, 2-furoyl, or 3,4,5-trimethoxybenzoyl substituent at the R_3-position indicated that the presence of a free amine group at the piperazino nucleus is essential for the inhibitory effects of piperazinoquinazolones. In the present study, maximum inhibition was observed with 6,8-dichloro-2-methyl-3-(2-*N*-piperazinoethyl)-4-quinazolone (Compound V), having chloro substituents at both the 6- and 8-positions.

Results presented in Table IV represent anticonvulsant activity exhibited by some piperazinoquinazolones against pentylenetetrazol-induced seizures. Anticonvulsant activity of these piperazinoquinazolones ranged from 10 to 70%; 6-chloro-2-methyl-3-(2-*N*-piperazinoethyl)-4-quinazolone (Compound II) was found to exhibit maximum protection in a dose of 100 mg/kg. Two compounds (Compounds VIII and IX) were found to be devoid of anticonvulsant activity. These studies failed to provide any definite correlation between the anticonvulsant property exhibited by these piperazinoquinazolones and their ability to inhibit the respiratory activity of the rat brain homogenate as reflected by the inhibition of NAD-dependent oxidations. Data on anticonvulsant activity of these compounds and 24-hr pentylenetetrazol-induced mortality (Table IV) did not indicate an association between increased protection from convulsions and decreased pentylenetetrazol mortality in experimental animals. None of these piperazinoquinazolones exhibited any appreciable sedative or CNS-depressant effect or 24-hr mortality in the dose of 100 mg/kg used in the present investigation. It is hoped that detailed pharmacological and toxicological studies and investigations of the effects of these piperazinoquinazolones on other enzyme systems may reflect a biochemical basis for their anticonvulsant activity.

REFERENCES

(1) V. K. Agarwal, K. C. Sah, S. Nagar, and S. S. Parmar, *J. Prakt. Chem.*, **312**, 964(1970).

(2) S. Hayao, H. J. Havera, W. G. Strycker, and E. Hong, *J. Med. Chem.*, **12**, 936(1969).

(3) P. K. Seth, S. S. Parmar, and K. Kishor, *Biochem. Pharmacol.*, **13**, 1362(1964).

(4) S. S. Parmar and P. K. Seth, *Can. J. Biochem.*, **43**, 1179(1965).

(5) P. K. Seth and S. S. Parmar, *Can. J. Physiol. Pharmacol.*, **43**, 1019(1965).

(6) M. L. Gujral, R. P. Kohli, and P. N. Saxena, *J. Ass. Physicians, India*, **2**, 29(1965).

(7) M. L. Gujral, P. N. Saxena, and R. P. Kohli, *Indian J. Med. Res.*, **45**, 207(1951).

(8) M. M. Endicott, B. B. Alden, and M. L. Sherill, *J. Amer. Chem. Soc.*, **68**, 1303(1946).

(9) S. Wheeler and W. H. Oats, *ibid.*, **32**, 770(1910).

(10) J. Klemme and J. H. Hunter, *J. Org. Chem.*, **5**, 227(1940).

(11) S. S. Parmar, A. K. Chaturvedi, and B. Ali, *J. Prakt. Chem.*, **312**, 950(1970).

(12) J. H. Quastel, *Physiol. Rev.*, **19**, 135(1939).

(13) J. H. Quastel, *Trans. Faraday Soc.*, **39**, 348(1943).

(14) J. H. Quastel and A. H. M. Wheatley, *Proc. Roy. Soc., London*, **112**, 60(1932).

(15) F. A. Furham and J. Field, *J. Pharmacol. Exp. Ther.*, **77**, 392(1943).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 23, 1973, from the *Department of Physiology and Pharmacology, School of Medicine, University of North

Dakota, Grand Forks, ND 58201, and the ‡*Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow 3, India.*

Accepted for publication September 24, 1973.

Supported by the School of Medicine, University of North Dakota; National Institutes of Health, U. S. Public Health Service, General Research Support Grant 5 S01 RRO 5407; and the Council of Scientific and Industrial Research, New Delhi, India, and the Indian National Science Academy, New Delhi, India, in the form of a Postdoctoral Research Fellowship and Senior Research Fellowship to A. K. Chaturvedi and A. Chaudhary, respectively.

The authors thank Professor K. P. Bhargava and Dr. J. P. Barthwal for their advice and encouragement. Grateful acknowledgment is made to the National Science Foundation for providing a Senior Foreign Visiting Scientist award to S. S. Parmar.

ˣ To whom inquiries should be directed (at the University of North Dakota).

Determination of Clindamycin 2-Palmitate in Clinical Human Serum Samples

T. F. BRODASKY ˣ and F. F. SUN

Abstract □ Clindamycin 2-palmitate, used in pediatric formulations, is readily hydrolyzed to clindamycin. During clinical investigations of this antibiotic, it was necessary to determine if unhydrolyzed clindamycin palmitate was present in the serum of clinical subjects. Chemical and enzymatic hydrolyses were unsuitable and instrumental techniques were ultimately developed. Two methods are described, one based on a combination of GLC and single-ion focused mass spectroscopy and a second using TLC and GLC. Examination of human serum from subjects receiving 300–600 mg clindamycin base equivalents of clindamycin palmitate hydrochloride revealed no statistically significant quantities of unhydrolyzed clindamycin palmitate.

Keyphrases □ Clindamycin 2-palmitate—determination in human serum using GLC and GLC–mass spectroscopy □ GLC—determination, clindamycin 2-palmitate in human serum □ GLC–mass spectroscopy—determination, clindamycin 2-palmitate in human serum

Clindamycin palmitate hydrochloride[1] (II) is the derivative of clindamycin used in pediatric formulations. The presence of the palmitate group markedly improves the taste characteristics of the derivative compared to the parent compound. Although the ester is not biologically active, it is readily hydrolyzed *in vivo* to clindamycin (I). During clinical studies, the question arose as to the presence of unhydrolyzed clindamycin 2-palmitate in human serum. Initially, attempts were made to hydrolyze the palmitate using specific esterases; although this approach was successful in urine samples, it failed in serum. Chemical hydrolysis in serum was unsuitable since the reaction, in addition to hydrolyzing clindamycin 2-palmitate, destroyed other biologically active metabolites of clindamycin (1). In some cases, this led to less biological activity after hydrolysis than was observed before.

At this point, studies were initiated which ultimately led to instrumental methods of detecting clindamycin 2-palmitate. Although the techniques do not require hydrolysis of the palmitate ester, it is necessary to prepare trimethylsilyl ether derivatives to impart volatility. Two independent studies will be described, one based on a GLC–mass spectroscopy technique and a subsequent method based on GLC only. Because of the need to extract the ester from serum samples prior to derivatization, poorer quantitation was achieved than had been expected. It was possible, however, to demonstrate the absence of unhydrolyzed clindamycin palmitate in clinical blood samples.

EXPERIMENTAL

GLC–Mass Spectroscopy Method—*Clindamycin 2-Palmitate Trimethylsilyl Ether (IV)*—A stock solution of II containing 10 μg/ml in analytical reagent grade pyridine (dried over sodium hydroxide pellets) was prepared. Then 100 μl of the II stock solution and 900 μl of the trimethylsilylation reagent[2] (V) [trimethylsilylimidazole–bis(trimethylsilyl)acetamide–trimethylchlorosilane (3:3:2)] were mixed in a dry, evacuated, 1-ml tube[3] and heated on a hot plate for 5 min at 75°. These solutions were used for initial GLC and GLC–mass spectroscopy studies. Solutions for structural correlation work were prepared in a similar manner from 20 mg of solid II and 1 ml of V.

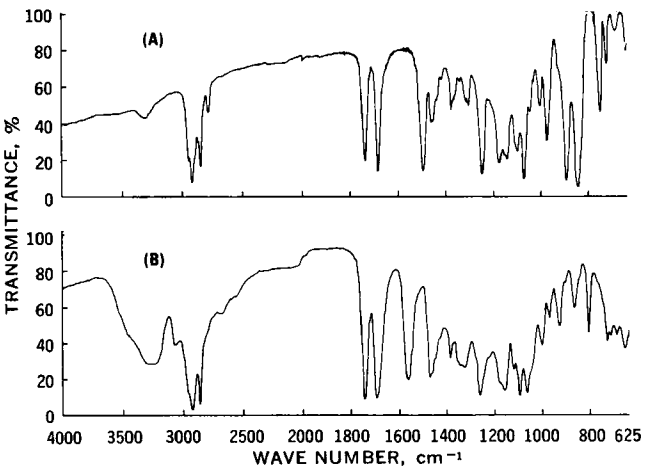


Figure 1—*Total reflectance IR spectra of II (B) and IV (A).*

[1] Cleocin Pediatric is The Upjohn Co. trademark for a product containing clindamycin palmitate hydrochloride (clindamycin 2-palmitate hydrochloride).

[2] Pierce Chemical Co.

[3] Reactivial, Supelco Co.