Substituted 3,4,5-Trimethoxybenzamides: Correlation between Inhibition of Pyruvic Acid Oxidation and Anticonvulsant Activity

A. K. CHATURVEDI, A. CHAUDHARI, and SURENDRA S. PARMAR▲

Abstract □ Several 3,4,5-trimethoxybenzamides were synthesized and evaluated for their inhibitory effects on the oxidation of pyruvic acid by rat brain homogenate. These benzamides were also found to possess an anticonvulsant property which, however, was unrelated to their ability to inhibit the oxidation of pyruvic acid.

Keyphrases □ 3,4,5-Trimethoxybenzamides, substituted—synthesized as pyruvic acid oxidation inhibitors, correlated with anticonvulsant structure–activity relationships □ Structure–activity relationships—pyruvic acid oxidation inhibitors with anticonvulsant activity □ Pyruvic acid oxidation inhibitors—synthesis of 3,4,5-trimethoxybenzamides □ Anticonvulsant activity—3,4,5-trimethoxybenzamides synthesized and screened

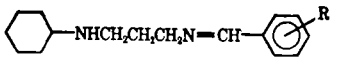
The presence of the 3,4,5-trimethoxybenzene nucleus in reserpine was shown (1-4) to be responsible for its CNS depressant and hypotensive properties. Several trimethoxybenzamides were also reported (5, 6) to have effects on the CNS. Moffett (7) reported that amides had more CNS depressant action than the corresponding esters. The functional role of the 3,4,5-trimethoxyphenyl moiety for CNS activity (8) and the ability of reserpine to lower respiratory activity and the electric shock threshold in mice (9, 10) led to the synthesis of *N*-cyclohexyl-*N*-substituted 3,4,5-trimethoxybenzamides as possible anticonvulsants. Furthermore, selective inhibition of the nicotinamide adenine dinucleotide-dependent oxidation of pyruvic acid and other substrates of the tricarboxylic acid cycle by 2-methyl-3-*ortho*-tolyl-4-quinazolone (11–13) possessing hypnotic (14) and anticonvulsant properties (15) led us to attempt to correlate the ability of 3,4,5-trimethoxybenzamides to inhibit the oxidation of pyruvic acid by rat brain homogenate with their anticonvulsant properties.

CHEMISTRY

The various substituted cyclohexylamines and 3,4,5-trimethoxybenzamides were synthesized according to Scheme I and are summarized in Tables I and II, respectively.

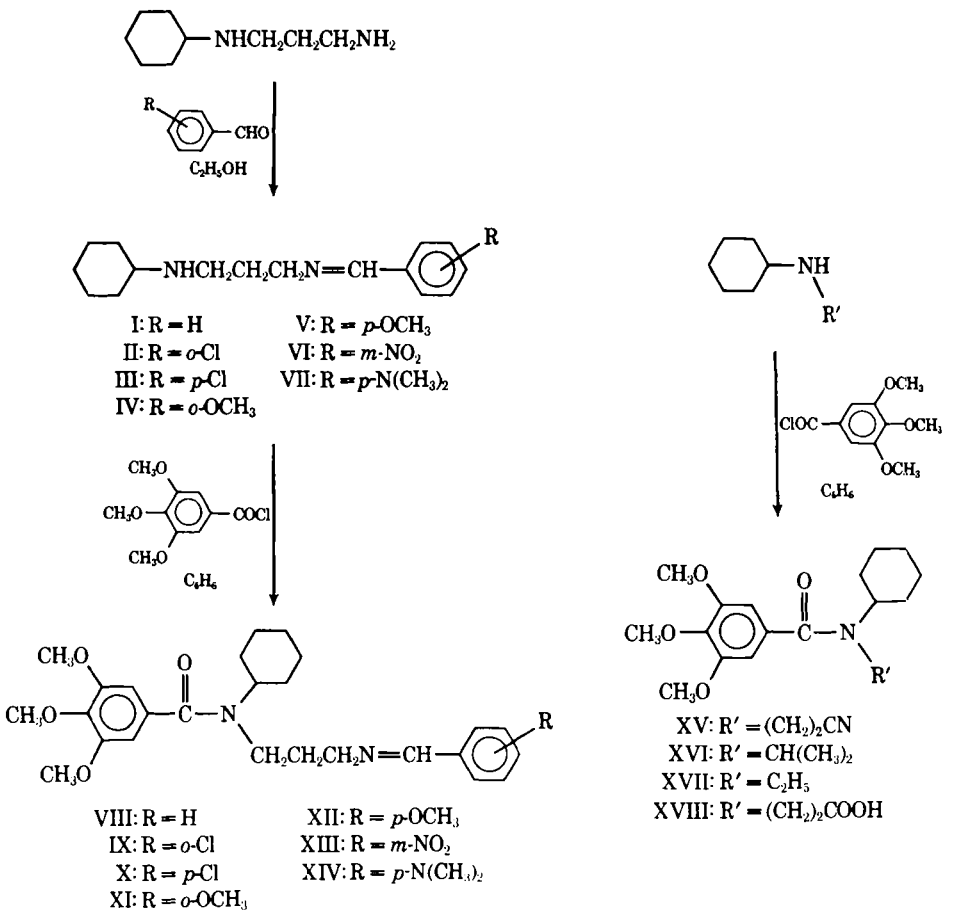
Various derivatives of cyclohexylamines (I–VII) were obtained by condensation of *N*-(3-aminopropyl)cyclohexylamine with suitable aromatic aldehydes. Compounds VIII–XVII (Table II) were prepared by the reaction of 3,4,5-trimethoxybenzoyl chloride with suitable cyclohexylamine derivatives. Compound XVIII was also obtained by the reaction of 3,4,5-trimethoxybenzoyl chloride with *N*-cyclohexyl-β-alanine at room temperature. Compounds VIII–XVIII showed their characteristic absorptions for —CON<. Their

Table I—Physical Constants of *N*-(Substituted Benzylideneaminopropyl)cyclohexylamines



Compound	R	Yield, %	Melting Point	Recrystallization Solvent[a]	Molecular Formula	Analysis, % Calc.	Found
I	H	60	260°	A	$C_{16}H_{24}N_2 \cdot HCl$	C 68.44	68.52
						H 8.91	9.00
						N 9.98	10.03
II	*o*-Cl	55	68°	B	$C_{16}H_{23}ClN_2$	C 68.94	68.53
						H 8.25	8.72
						N 10.05	9.92
III	*p*-Cl	65	177°	C	$C_{16}H_{23}ClN_2 \cdot HCl$	C 60.95	61.22
						H 7.61	7.32
						N 8.88	8.43
IV	*o*-OCH_3	55	90°	D	$C_{17}H_{26}N_2O$	C 74.45	74.70
						H 9.48	9.78
						N 10.21	9.98
V	*p*-OCH_3	60	161°	A	$C_{17}H_{26}N_2O$	C 74.45	74.68
						H 9.48	9.52
						N 10.21	10.00
VI	*m*-NO_2	60	249°	A	$C_{16}H_{23}N_3O_2 \cdot HCl$	C 58.98	59.03
						H 7.37	7.73
						N 12.90	12.54
VII	*p*-$N(CH_3)_2$	60	242–244°	A	$C_{18}H_{29}N_3$	C 75.26	75.43
						H 10.10	10.21
						N 14.63	15.04

[a] A = ethanol, B = chloroform–petroleum ether (60–80°), C = benzene, and D = benzene–petroleum ether (60–80°).



Scheme I

corresponding starting materials do not possess this type of functional group.

EXPERIMENTAL[1]

***N*-(Substituted Benzylideneaminopropyl)cyclohexylamines (I–VII)** – These compounds were synthesized by refluxing equimolar proportions of *N*-(3-aminopropyl)cyclohexylamine with the appropriate aromatic aldehyde in a minimum amount of absolute ethanol on a steam bath for 2 hr. The reaction mixture was kept in a refrigerator for 24 hr. After removing the solvent under reduced pressure, the desired cyclohexylamines thus obtained were filtered, dried, and recrystallized from suitable solvents. They were characterized by their sharp melting points and elemental analyses (Table I).

***N*-Cyclohexyl-*N*-substituted 3,4,5-Trimethoxybenzamides (VIII–XVII)**—Various trimethoxybenzamides were prepared by refluxing 3,4,5-trimethoxybenzoyl chloride with the appropriate *N*-substituted cyclohexylamine (1:2 molar ratio) in dry benzene on a steam bath for 1–4 hr. The mixture was cooled and then washed with 5% HCl, 5% Na_2CO_3 solution, and finally water. The solvent was removed by distillation under reduced pressure. The solid mass which separated out was filtered and recrystallized from a suitable solvent. The amides thus obtained were characterized by their sharp melting points, elemental analyses, and IR spectra (Table II).

***N*-Cyclohexyl-*N*-(β-propionic acid)-3,4,5-trimethoxybenzamide (XVIII)**—A solution of 3,4,5-trimethoxybenzoyl chloride (0.01 mole) in 25 ml. of dry benzene was added slowly to the cold solution of *N*-cyclohexyl-β-alanine (0.02 mole) in 25 ml. of dry benzene. The reaction mixture was stirred at room temperature for 1 hr. and then refluxed for 1 hr. on a steam bath. On evaporation of the solvent in a vacuum, the solid residue thus obtained was washed with 5% HCl to remove unused *N*-cyclohexyl-β-alanine and dissolved in sodium carbonate solution. On acidification with dilute hydrochloric acid, the solution yielded a precipitate which was filtered, washed with water, dried, and recrystallized from petroleum ether (60–80°), m.p. 80°; yield 45%; IR: 1635 cm.$^{-1}$ (—CON<).

Anal.—Calc. for $C_{19}H_{27}NO_6$: C, 62.46; H, 7.39; N, 3.83. Found: C, 62.74; H, 7.62; N, 3.52.

Assay of Pyruvic Acid Oxidation by Rat Brain Homogenate—Male albino rats kept on an *ad libitum* diet were used for these experiments. Rat brains isolated from decapitated animals were immediately homogenized in ice-cold 0.25 *M* sucrose in a homogenizer (Potter–Elvehjem) in the ratio of 1:9 (w/v). All incubations were carried out at 37°, and the oxygen uptake was measured at 10-min. intervals, using air as the gas phase (13). The reaction mixture, in a total volume of 3.0 ml., contained 6.7 m*M* $MgSO_4$, 20 m*M* Na_2HPO_4 in a buffer solution of pH 7.4, 1 m*M* adenylic acid (sodium salt), 33 m*M* KCl, and 500 mcg. of cytochrome c. The central well contained 0.2 ml. of 20% KOH. Pyruvate was used at the final concentration of 10 m*M*. The compounds were dissolved in propylene glycol (100%), and an equal volume of the solvent was added to the control vessels.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in albino mice as reported earlier (16). The percentage protection offered by the drug against the convulsions produced by administration of pentylenetetrazol was taken as the anticonvulsant activity.

DISCUSSION

Results summarized in Table III indicate the ability of *N*-cyclohexyl-*N*-substituted 3,4,5-trimethoxybenzamides to inhibit oxidation of pyruvic acid by rat brain homogenate. Inhibition of pyruvic acid oxidation was found to increase, with a simultaneous increase in the concentration of these trimethoxybenzamides. Among these compounds, XI was found to show maximum inhibition. In addition, all 3,4,5-trimethoxybenzamides were found to possess anti-

[1] Melting points were taken in open capillary tubes and were corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137 equipped with NaCl optics in KBr films in the range of 700–3500 cm.$^{-1}$.

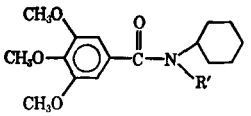


Table II—Physical Constants of *N*-Cyclohexyl-*N*-substituted 3,4,5-Trimethoxybenzamides

Compound	R′	Yield, %	Melting Point	Recrystallization Solvent[a]	Molecular Formula	IR, cm.$^{-1}$, —CON<	Analysis, % Calc.	Found
VIII	$(CH_2)_3N{=}CHC_6H_5$	60	105°	A	$C_{26}H_{34}N_2O_4$	1645	C 71.23	71.50
							H 7.76	7.43
							N 6.39	6.66
IX	$(CH_2)_3N{=}CH\text{-}o\text{-}C_6H_4Cl$	40	80°	B	$C_{26}H_{33}ClN_2O_4$	1655	C 66.03	66.45
							H 6.98	6.49
							N 5.92	5.70
X	$(CH_2)_3N{=}CH\text{-}p\text{-}C_6H_4Cl$	50	73°	B	$C_{26}H_{33}ClN_2O_4$	1645	C 66.03	66.21
							H 6.98	6.71
							N 5.92	5.81
XI	$(CH_2)_3N{=}CH\text{-}o\text{-}C_6H_4OCH_3$	50	75°	B	$C_{27}H_{36}N_2O_5$	1640	C 69.23	69.30
							H 7.69	7.52
							N 5.98	6.02
XII	$(CH_2)_3N{=}CH\text{-}p\text{-}C_6H_4OCH_3$	55	150°	B	$C_{27}H_{36}N_2O_5$	1650	C 69.23	69.48
							H 7.69	7.47
							N 5.98	6.00
XIII	$(CH_2)_3N{=}CH\text{-}m\text{-}C_6H_4NO_2$	45	177°	C	$C_{26}H_{33}N_3O_6$	1630	C 64.59	65.01
							H 6.83	7.00
							N 8.69	8.44
XIV	$(CH_2)_3N{=}CH\text{-}p\text{-}C_6H_4N(CH_3)_2$	50	52°	A	$C_{28}H_{39}N_3O_4$	1665	C 69.85	70.00
							H 8.10	8.52
							N 8.72	8.54
XV	$(CH_2)_2CN$	80	112°	D	$C_{19}H_{26}N_2O_4$	1645	C 65.89	66.13
							H 7.51	7.89
							N 8.09	8.43
XVI	$CH(CH_3)_2$	75	98°	A	$C_{19}H_{29}NO_4$	1645	C 68.05	67.98
							H 8.65	9.00
							N 4.17	4.53
XVII	C_2H_5	85	92°	A	$C_{18}H_{27}NO_4$	1635	C 67.28	67.40
							H 8.41	8.79
							N 4.36	4.85

[a] A = petroleum ether (60–80°), B = benzene–petroleum ether (60–80°), C = methanol, and D = cyclohexane.

convulsant properties, where maximum protection of 60% was observed with Compound XVIII against pentylenetetrazol-induced convulsions. Substitution at position R′ of the trimethoxybenzamide nucleus with $(CH_2)_3N{=}CHC_6H_5$ or $(CH_2)_3N{=}CH\text{-}o\text{-}C_6H_4Cl$ was found to bring down the degree of protection to only 10%. Compounds X and XII were found to exhibit a higher degree of protection than their corresponding *ortho*-isomers. Almost all trimethoxybenzamides produced mortality during 24 hr. Four derivatives, including Compound XV which possesses a toxic cyano group in its molecular structure, were found to exhibit 100% mortality. Such anticonvulsant effects, however, were found to be in no way related to the ability of these trimethoxybenzamides to inhibit the oxidation of pyruvic acid. These results are in agreement with the inhibitory effects of quinazolone allyl ethers and allyl phenols (17). It is hoped that further studies may attribute suitable pharmacological properties of these trimethoxybenzamides

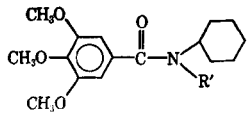


Table III—Effect of *N*-Cyclohexyl-*N*-substituted 3,4,5-Trimethoxybenzamides on Pyruvic Acid Oxidation by Rat Brain Homogenate and Their Anticonvulsant Properties

Compound	R′	Pyruvic Acid Oxidation Inhibition[a], % 1×10^{-3} *M*	Pyruvic Acid Oxidation Inhibition[a], % 2×10^{-3} *M*	Anticonvulsant Activity[b], % Protection	Anticonvulsant Activity[b], % 24-hr. Mortality
VIII	$(CH_2)_3N{=}CHC_6H_5$	29.00 ± 0.97	40.70 ± 1.24	10	80
IX	$(CH_2)_3N{=}CH\text{-}o\text{-}C_6H_4Cl$	24.90 ± 1.00	55.60 ± 1.11	10	90
X	$(CH_2)_3N{=}CH\text{-}p\text{-}C_6H_4Cl$	24.97 ± 0.78	54.02 ± 1.01	20	60
XI	$(CH_2)_3N{=}CH\text{-}o\text{-}C_6H_4OCH_3$	53.71 ± 1.24	92.41 ± 0.79	30	70
XII	$(CH_2)_3N{=}CH\text{-}p\text{-}C_6H_4OCH_3$	27.40 ± 0.97	39.01 ± 1.24	40	100
XIII	$(CH_2)_3N{=}CH\text{-}m\text{-}C_6H_4NO_2$	34.61 ± 1.04	38.04 ± 0.88	30	100
XIV	$(CH_2)_3N{=}CH\text{-}p\text{-}C_6H_4N(CH_3)_2$	19.88 ± 1.24	35.41 ± 0.79	20	40
XV	$(CH_2)_2CN$	18.93 ± 0.78	31.55 ± 1.00	40	100
XVI	$CH(CH_3)_2$	22.54 ± 0.78	65.67 ± 0.89	30	80
XVII	C_2H_5	23.78 ± 0.89	29.62 ± 0.89	20	100
XVIII	$(CH_2)_2COOH$	28.94 ± 1.02	45.41 ± 1.00	60	70

[a] Vessel contents and the assay procedure are as described in the text. All values are the mean of four duplicate experiments. The percentage inhibition and the standard errors were calculated for the decrease in the oxygen uptake per hour per 150 mg. of wet weight of tissue. The mean oxygen uptake observed was found to be 145.16 μl. during the oxidation. [b] The screening procedure is as indicated in the text. Compounds were tested at the dose of 100 mg./kg.

to their ability to inhibit the oxidation of other substrates of the tricarboxylic acid cycle.

REFERENCES

(1) H. J. Bein, *Pharmacol. Rev.*, **8**, 435(1956).
(2) H. J. Bein, *Experientia*, **9**, 107(1953).
(3) J. Tripod, H. J. Bein, and R. Weier, *Arch. Int. Pharmacodyn. Ther.*, **36**, 406(1954).
(4) H. J. Bein, F. Gross, J. Tripod, and R. Meier, *Schweiz. Med. Wochnschr.*, **83**, 1007(1953).
(5) K. Borsy, B. Dumbovich, L. Vargha, and L. Farkas, Hung. pat. 147,687(1960).
(6) P. C. Dandiya, P. K. Sharma, and M. K. Menon, *Indian J. Med. Res.*, **50**, 750(1962).
(7) R. B. Moffett, *J. Med. Chem.*, **7**, 319(1964).
(8) R. B. Petigara, C. V. Deliwala, S. S. Mandrekar, N. K. Dadkar, and U. K. Sheth, *ibid.*, **12**, 865(1969).
(9) J. C. Gupta, B. S. Kahali, and A. Dutta, *Indian J. Med. Res.*, **32**, 183(1944).
(10) A. J. Plummer, W. E. Barrett, G. Wagle, and F. F. Yonkman, *Fed. Proc.*, **12**, 357(1953).
(11) P. K. Seth, S. S. Parmar, and K. Kishor, *Biochem. Pharmacol.*, **13**, 1362(1964).
(12) S. S. Parmar and P. K. Seth, *Can. J. Biochem.*, **43**, 1179 (1965).
(13) P. K. Seth and S. S. Parmar, *Can. J. Physiol. Pharmacol.*, **43**, 1019(1965).
(14) M. L. Gujral, R. P. Kohli, and P. N. Saxena, *J. Ass. Physicians, India*, **II**, 29(1955).
(15) M. L. Gujral, P. N. Saxena, and R. P. Kohli, *Indian J. Med. Res.*, **45**, 207(1957).
(16) S. S. Parmar, A. K. Chaturvedi, A. Chaudhari, and R. S. Misra, *J. Pharm. Sci.*, **61**, 78(1972).
(17) S. S. Parmar, V. K. Rastogi, T. K. Gupta, and R. C. Arora, *Jap. J. Pharmacol.*, **20**, 325(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 11, 1971, from the *Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow-3, India.*

Accepted for publication March 7, 1972.

Supported by grants from the Council of Scientific and Industrial Research, New Delhi, India, and the State Council of Scientific and Industrial Research, Lucknow, India, in the form of Junior Research Fellowships (A. K. Chaturvedi and A. Chaudhari).

The authors express their appreciation to Professor K. P. Bhargava and Professor D. Dayal for their advice and encouragement and to Dr. M. L. Dhar and Dr. Nitya Nand of the Central Drug Research Institute, Lucknow, India, for providing facilities for microanalysis. Grateful acknowledgment is also made to Abbott Laboratories, North Chicago, Ill., and Strasenburgh Laboratories, Rochester, N. Y., for generous gifts of research chemicals.

▲ To whom inquiries should be directed.

Decomposition of Aspirin in Polyethylene Glycols

H. W. JUN▲, C. W. WHITWORTH, and L. A. LUZZI

Abstract □ Decomposition of aspirin in polyethylene glycols was studied at four temperatures. The decomposition proceeded as a pseudo-first-order reaction at these temperatures. Different molecular weights of polyethylene glycol did not affect the reaction rate. It is shown that decomposition of aspirin in polyethylene glycols is due to a transesterification reaction. The effect of temperature on decomposition rate was found to be significant, and an Arrhenius plot is shown.

Keyphrases □ Aspirin—decomposition in polyethylene glycols at various temperatures □ Polyethylene glycols—effect of temperature on aspirin decomposition □ Decomposition—aspirin in polyethylene glycols, effect of temperature

Decomposition of aspirin has been encountered in various pharmaceutical dosage forms (1, 2), notably in liquid dosage forms. Because of the use of aspirin in suppository bases in which polyethylene glycols may be incorporated, the rate of decomposition of aspirin in four polyethylene glycols with different molecular weights was studied at four temperatures.

Preliminary studies indicated that a significant amount of aspirin was decomposed in polyethylene glycol bases in the apparent absence of water. Although the rate of degradation of aspirin in polyethylene glycols was considerably decreased in the absence of water compared to that in the presence of added water, it was a significant factor when the shelflife of polyethylene glycol–aspirin products was involved.

This report shows that aspirin degrades in polyethylene glycols by transesterification in the absence of added water, and that the resultant products of this degradation are salicylic acid and acetylated polyethylene glycol.

EXPERIMENTAL

Materials—Aspirin USP[1] and polyethylene glycols[2] 400, 1540, 4000, and 6000 were used as received. Chloroform[3] was spectroscopic grade; all other chemicals were reagent grade.

Analytical Method—Spectrophotofluorometric analysis[4], as reported by Miles and Schenk (3), was employed to measure aspirin and salicylic acid. Uncorrected excitation and emission maxima for aspirin and salicylic acid were 280 and 350 nm. and 312 and 450 nm., respectively. Calibration curves were obtained by dissolving known amount of aspirin and/or salicylic acid in a 1% acetic acid–chloroform solution.

Procedure—Ten percent aspirin was incorporated in each polyethylene glycol base at elevated temperature. Preparations were kept in airtight amber containers and stored in a desiccator at tempera-

[1] Merck & Co., Inc., Rahway, N. J.
[2] Matheson, Coleman & Bell, Norwood, Ohio.
[3] J. T. Baker Chemical Co., Phillipsburg, N. J.
[4] The instrument used was the Aminco-Bowman spectrophotofluorometer with 150-w. xenon lamp.