Pharmacological Evaluation of 3,4-Dimethoxyphenylacetamide

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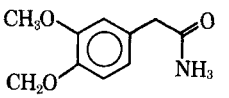
Abstract □ Studies on the pharmacological activity of 3,4-dimethoxyphenylacetamide revealed that the compound produced definite signs of CNS depression. A hypocholesterolemic effect was also demonstrated, plus tendencies toward a reduction in plasma glucose levels.

Keyphrases □ 3,4-Dimethoxyphenylacetamide—pharmacologically evaluated for CNS depression, hypocholesterolemic activity □ Hypocholesterolemic activity—3,4-dimethoxyphenylacetamide from *Catharanthus lanceus* □ CNS depression—3,4-dimethoxyphenylacetamide from *Catharanthus lanceus*

Recent work in these laboratories (1) led to the isolation of a crystalline compound, 3,4-dimethoxyphenylacetamide (I), from leaves of *Catharanthus lanceus* (Apocynaceae). This amide is the first reported example of the occurrence of a dialkoxyarylacetamide-type compound in nature.



I

Data on the pharmacological activity of 3,4-dimethoxyphenylacetamide are completely lacking in the reported literature; however, there are reports on the hypocholesterolemic properties of phenylethylacetic acid and other compounds closely related to 3,4-dimethoxyphenylacetamide in experimental animals and man (2–12). In addition to the hypocholesterolemic effect of these compounds, several investigators have shown: (*a*) that cardiovascular and CNS changes in mice, pigeons, and chickens were noted with phenylmethylacetic and phenylethylacetic acids when administered at extremely high doses (13); (*b*) anti-inflammatory, local anesthetic, and diuretic activity for a series of α,α-disubstituted phenylacetamides (14, 15); and (*c*) anticonvulsant activity for certain amides of trialkoxyphenylacetic acids (16).

Since 3,4-dimethoxyphenylacetamide had not been pharmacologically evaluated and since many related compounds have been reported to be biologically active, it was considered worthwhile to determine the pharmacological profile of 3,4-dimethoxyphenylacetamide.

EXPERIMENTAL

Animals—A total of 234 male albino mice (Swiss-Webster strain, weighing 16–25 g.) and 24 male albino rats (Wistar strain, weighing 250–260 g.) were used[1]. The animals were allowed at least 3 days to acclimatize to the laboratory environment before being used in any experiment.

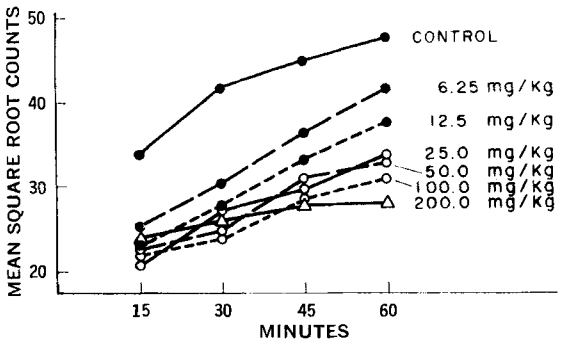


Figure 1—*Effects of 3,4-dimethoxyphenylacetamide on spontaneous activity in mice.*

Drug Preparation and Administration—3,4-Dimethoxyphenylacetamide was synthesized and purified by standard procedures as previously described (1). Since solubility of this compound was incomplete even at a concentration of 1 mg./ml. of distilled water, the compound was suspended in a 0.25% agar solution just prior to administration. Subsequently, drug administration was by the intraperitoneal route in all experiments. Mice received injections in a volume of 0.1 ml./10 g. of body weight, while rats were administered a volume of 0.2 ml./100 g. of body weight; all control animals received an equivalent amount of vehicle.

Determination of LD_{50}—Forty mice were divided into four groups of 10 each. Doses of 3,4-dimethoxyphenylacetamide ranging from 50 to 400 mg./kg. were administered. All groups were kept for 5 days, at which time the total number of dead mice per group was tabulated. This length of time was selected to determine whether the test compound caused any delayed deaths upon acute dosing due to metabolites that might possibly be formed and accumulated.

Gross Observations—Gross observations were made in clear plastic cages. Groups of two mice each were placed in these cages 30 min. prior to drug administration for general gross observations. This period was designated as the control phase of the experiment. Subsequently, these same groups were given either 50, 100, or 200 mg./kg. i.p. of 3,4-dimethoxyphenylacetamide; gross behavioral and physiological changes were recorded at 15, 30, 60, and 120 min. following injection.

Spontaneous Motor Activity—Sixty mice were tested, using three actophotometers[2], 15 min. after the administration of doses of 3,4-dimethoxyphenylacetamide ranging from 6.25 to 200 mg./kg. i.p. according to the method described by Dews (17). The cumulative number of counts for each group was converted into square roots, and statistical differences among them were determined using the paired *t*-test of the means.

Hexobarbital Sleeping Time—Forty mice, divided into four groups of 10 mice each, were used. One group received only the vehicle, while the three remaining groups received 25, 50, and 100 mg./kg. i.p. of 3,4-dimethoxyphenylacetamide, respectively. Thirty minutes later, all groups were administered 100 mg./kg. i.p. of hexobarbital sodium. The time to complete loss of the righting reflex (induction time) and the time at which each animal regained its righting reflex were recorded, and statistical evaluation of the data was made using Student's *t* test.

Anticonvulsant Activity—Forty mice were divided into four groups of 10 mice each. Three groups were given 25, 50, and 100 mg./kg. i.p. of 3,4-dimethoxyphenylacetamide, while the last group received vehicle. Thirty minutes after drug administration, all mice were subjected to maximal electroshock, *i.e.*, 50 ma. of current for

[1] All animals were obtained from Hilltop Lab Animals, Inc., Scottdale, Pa.

[2] Metro Industries.

Table I—Effects of 3,4-Dimethoxyphenylacetamide on Plasma Glucose and Cholesterol Levels in Rats

Minutes following Injection	Glucose, mg. %		Cholesterol, mg. %	
	Control	Drug	Control	Drug
30	70.4 ± 2.15	70.7 ± 1.96	70.1 ± 3.39	62.4 ± 3.86
60	74.3 ± 1.22	70.7 ± 1.87	65.2 ± 3.06	51.2 ± 3.40[a]

[a] Significantly different from control at $p > 0.01$.

0.2-sec. duration *via* corneal electrodes as described by Swinyard *et al.* (18). Anticonvulsant activity was considered when protection against the tonic extension of both front and hindlimbs was afforded the animals.

Anticholinergic Activity—Four groups of six mice each were administered vehicle or 50, 100, or 200 mg./kg. i.p. of 3,4-dimethoxyphenylacetamide 30 min. prior to 1 mg./kg. i.p. oxotremorine. For a 30-min. period following oxotremorine injection, the mice were observed for the occurrence of tremors and salivation.

Antidepressant Activity—The reversal of hypothermia and the incidence of ptosis induced by reserpine were used as measures of antidepressant activity for 3,4-dimethoxyphenylacetamide according to a modification of the method described by Vernier *et al.* (19). A total of 24 mice (four groups of six each) was used. After assignment to groups, the degree of ptosis and rectal body temperature were measured. Ptosis was qualitatively evaluated, and the degree of severity was given the following scores: 0 = none, 1 = partial, and 2 = complete. Immediately following this initial testing, all mice were given 5 mg./kg. i.p. of reserpine. Twenty-four hours later, the degree of ptosis and rectal temperatures of all mice were again recorded. Immediately after the second temperature reading, each group received either 50, 100, or 200 mg./kg. i.p. of 3,4-dimethoxyphenylacetamide or its vehicle. All mice were again graded as to their degree of ptosis and rectal temperature was taken 30, 60, and 120 min. later. At each testing interval, ptosis was graded before body temperature was taken to minimize the effects of handling on the former. Antidepressant activity was considered when ptosis was either partially or completely reversed and rectal temperatures showed a statistically (Student's *t* test) significant elevation.

Plasma Glucose and Cholesterol Levels—Four groups of six rats each were used in this study; two groups served as controls, receiving vehicle only, and two groups were given 100 mg./kg. 3,4-dimethoxyphenylacetamide. This dose of 3,4-dimethoxyphenylacetamide was chosen since data from the LD_{50} study indicated that this compound was relatively nontoxic. In addition, the 100-mg./kg. dose of 3,4-dimethoxyphenylacetamide is in the dose range exhibited by closely related compounds (2–12) for hypocholesterolemic activity. A group of control rats and one group of drug-treated animals were studied at 30-min. postinjection as were two separate groups at 60 min. At the preset absorption time, each animal was sacrificed by decapitation and blood samples (2–4 ml.) were collected in heparinized beakers. Immediately following the collections, each blood sample was centrifuged at $2000 \times g$ for 20 min. Plasma samples were taken and frozen until glucose and cholesterol determinations could be made using the Dow Diagnostest colorimetric technique[3]. Statistical analyses of the data were done using Student's *t* test.

RESULTS AND DISCUSSION

3,4-Dimethoxyphenylacetamide, in a dose range of 50–400 mg./kg., caused no deaths in mice for a period of up to 5 days following a single intraperitoneal injection. Therefore, it can be calculated that the LD_{50} is greater than 400 mg./kg.

Definite signs of CNS depression were observed in the gross observation studies: decreased motor activity, ataxia, passivity, and decreases in limb tone, body tone, and grip strength. The severity of these symptoms appeared to be dose dependent, with an onset of action of 15–30 min. and a duration of at least 60 min. Moreover, the autonomic nervous system (ANS) appeared to be slightly affected. Blockade of the sympathetic division of the ANS might have been manifested since signs of flushing, a slight degree of ptosis, and a slight amount of diarrhea were evident, indicating dominance of the cholinergic division. Compounds related to 3,4-dimethoxyphenylacetamide possessed similar effects on the cardiovascular system (13); however, no diuretic or local anesthetic effect was observed with 3,4-dimethoxyphenylacetamide (14, 15).

Data obtained in the spontaneous motor activity studies indicated a dose-related depressant effect through 25 mg./kg. of 3,4-dimethoxyphenylacetamide (Fig. 1). Above this dose, *i.e.*, 50, 100, and 200 mg./kg., there was no statistically reliable difference ($p > 0.1$) among the doses. However, whenever the lowest dose (6.25 mg./kg.) was compared with the control responses, the depressant action was markedly significant ($p < 0.001$); that is, differences were significant at each 15-min. interval. Both the 6.25- and 12.5-mg./kg. doses exhibited a significantly lesser amount of depression when compared with all the higher doses tested.

The onset and duration of action of hexobarbital sleeping time were unaffected by 25–100-mg./kg. doses of 3,4-dimethoxyphenylacetamide, since the effects among the vehicle and drug-treated groups did not differ significantly from one another ($p > 0.10$). In addition, 3,4-dimethoxyphenylacetamide did not display anticonvulsant activity in doses of 25–100 mg./kg. when tested against maximal electroshock-induced seizures. Likewise, 3,4-dimethoxyphenylacetamide did not exhibit either anticholinergic or antidepressant activity in doses of 50–200 mg./kg.

Table I summarizes the effects of 100 mg./kg. 3,4-dimethoxyphenylacetamide on plasma glucose and cholesterol levels. There was no statistically significant effect of 3,4-dimethoxyphenylacetamide on plasma glucose levels either 30 or 60 min. following drug administration; however, at 60 min., there was a tendency toward a decrease. Cholesterol levels were reduced at both testing times, with a significant reduction ($p < 0.01$) occurring only at the 60-min. postinjection time.

3,4-Dimethoxyphenylacetamide appears to have selective hypocholesterolemic activity; however, the compound also exhibited definite signs of CNS depression. Additional studies are underway in these laboratories to explore further the selectivity and potency of the cholesterol-reducing properties of 3,4-dimethoxyphenylacetamide.

[3] The Dow Chemical Co., Diagnostic Products, Indianapolis, Ind.

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Structure and Activity in Molluscicides III: Enzymatic Peroxidation of the Molluscicidal Agent Pentachlorophenol

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Abstract □ Pentachlorophenol, a widely used molluscicidal and herbicidal agent, is biologically oxidized through the catalytic effect of the protoporphyrin enzyme peroxidase, detected within the snail's body, to give 2,2′,3,3′,5,5′,6,6′-octachlorobiphenylquinone. This compound showed potent molluscicidal activity. The reaction goes through the symmetrical pairing of the initially formed chlorophenoxy radical, accompanied by the rupture of the *para*- (C—Cl) link. The eliminated chlorine ion was determined in the reaction medium.

Keyphrases □ Pentachlorophenol—enzymatic peroxidation, structure–activity relationships of molluscicides □ Molluscicidal agents —pentachlorophenol enzymatic peroxidation, structure–activity relationships □ Structure–activity relationships—molluscicidal agents

A previous communication (1) explained the structure–activity relationship in molluscicides of the phenolic type. Biological findings reported by various investigators (2, 3) showed the existence of the structural specificity that governs the biological activity as molluscicides within these types of compounds. The activity depends on the nature of substituents on the aromatic ring. Those bearing bulky groups were of least activity, while those carrying smaller substituents were of significant activity. This specificity in structure with relation to the biological activity parallels the ability of the phenoxy radical, the intermediate stage in the chemical oxidation of phenols, to undergo subsequent transformation to the corresponding quinoid system, which is similarly dependent on the nature of the substituent on the ring. Compounds belonging to this system are biologically active as molluscicides.

Previous studies revealed the presence of the protoporphyrin enzyme, peroxidase, in the snail's body. The biochemical assay for this enzyme was determined in some species of snails of economic importance (4).

MECHANISM

The effect of the enzyme peroxidase on some types of phenols and amines has been studied. Of these, 2,6-dimethylphenol could be oxidized by this system to give 3,5,3′,5′-tetramethyldipheno-4,4′-quinone; 2,6-dimethoxyphenol could be similarly changed to the corresponding diphenoquinone: 3,5,3′,5′-tetramethoxy-4,4′-quinone (5). Oxidation may also proceed to give a mesomeric quinoid structure as 2-hydroxy-1,3,5-trimethylbenzene, with which the reaction gives 2,6-dimethyl-1,4-benzoquinone (6, 7). With polysubstituted alkylphenols such as 1-hydroxy-2,3,5,6-tetramethylbenzene, the corresponding 2,3,5,6-tetramethyl-1,4-benzoquinone was obtained. With *para*-halogenated amines, such as *p*-chloroaniline, elimination of the chlorine ion takes place, thus causing a drop in the pH of the reaction medium and resulting in the formation of 2-amino-5-(*p*-chloroanilino)-benzoquinone-di-*p*-chloranil (8). Other aromatic amines with halogens as *para*-substituents could be oxidized in a similar fashion to give quininoid systems with the displacement of the halogen atom at the *para*-position. In the case of fluorine, the reaction is toxified through the hydrofluoride which acts as an enzyme poison.

Mechanistically, it has been suggested that the oxidation through peroxidase involves free radical formation either by direct loss of a hydrogen atom or by an electron removal followed by a loss of a proton; this may be followed by the symmetrical pairing of two such radicals (9).

The phenoxy radical is a monovalent oxygen radical species, which can be formed through the homolysis of the O—H bond. The delocalization of the unpaired electron over the aromatic ring and its substituents was proved (10, 11); thus the radical is stabilized by resonance. For the radical to exist other than as a transient intermediate, it is necessary that the ring be substituted with bulky groups, which would give steric hindrance to block further decomposition of the radical by slow dimerization or disproportionation. The polyhalophenoxy radical usually undergoes dimer formation readily. The stability of these radicals is due to the mesomeric contribution of halogen on the ring. The pentachlorophenoxy radical has been studied (12, 13); carbon–carbon dimerization of the radical may proceed with the loss of the *para*-substituent. The formation of such dimers was reported for a large number of these phenols (14, 15).

In this work, the catalytic oxidation of the enzyme peroxidase and hydrogen peroxide on pentachlorophenol (I), a widely used molluscicide and herbicide, was investigated. It was submitted, as its sodium salt, to the action of the enzyme in a water–phosphate buffer solution at pH 7 and 37°. The hydrogen peroxide solution was added to the sodium pentachlorophenate in the phosphate buffer, followed by the addition of the enzyme peroxidase solution; the temperature of the mixture was kept constant. The isolated product was identified as 2,2′,3,3′,5,5′,6,6′-octachlorobiphenylquinone (II). IR spectral analysis showed an absorption band at 1610 cm.$^{-1}$ referring to the quinoidal structure (16). Oxidation of II