the drug and its metabolites were extracted into the acid by shaking the mixture for 20 min.

The aqueous layer was separated using a 50-ml separator, and its fluorescence was measured in a fluorescence spectrophotometer[3]. The fluorescence and excitation wavelengths were 310 and 280 nm, respectively, and the slit dials were set to give a bandpass of 8 nm for excitation and of 5 nm for fluorescence. The concentrations of the drug and its metabolites in the sample were read from a calibration curve whose validity was determined by including three or four known concentrations of dextrorphan added to normal human plasma with each series of unknowns.

Bioavailability Studies in Humans—Six healthy male volunteers, 27–32 years old, received 30 mg po of dextromethorphan hydrobromide in a syrup (10 ml) after an overnight fast. Blood samples were drawn from the antecubetal vein into heparinized tubes immediately prior to and at 15 and 30 min and 1, 2, and 4 hr after drug administration. Plasma was separated by centrifuging at 3000 rpm for 20 min, and an aliquot was assayed by the described procedure.

RESULTS AND DISCUSSION

A calibration curve was constructed by plotting the fluorescence intensity against the concentration of dextrorphan added to drug-free plasma. Regression analysis of the least-squares line fitting the data points provided an equation for the curve as $y = 0.2658x + 3.728$ ($r = 0.9956$), where y represents fluorescence units and x is nanograms of dextrorphan per milliliter of plasma. The recovery of known amounts of dextrorphan added to human plasma was 70%. To determine the precision of the method, eight plasma samples spiked with 400 ng of dextrorphan/ml and four samples containing 600 ng of dextrorphan/ml were analyzed in duplicate over 6 months. The coefficients of variation at these two concentrations were 8 and 4%, respectively.

The fluorescence characteristics of dextrorphan and dextromethorphan in 1.0 *N* hydrochloric acid are rather similar. The fluorescence and excitation wavelengths of dextrorphan are 310 and 280 nm, respectively; for dextromethorphan, they are 305 and 270 nm, respectively.

The fluorescence spectrum of dextrorphan extracted from plasma was identical with that of dextrorphan in 1.0 *N* HCl. Some interference by the unmetabolized drug and possibly other minor metabolites could be expected in the measurement of dextrorphan levels. However, the contribution to fluorescence by the unmetabolized drug would be negligible in this method because extracts from plasma of humans administered dextromethorphan hydrobromide exhibited fluorescence spectra characteristic of dextrorphan but not dextromethorphan. Furthermore, since dextromethorphan is metabolized rapidly and extensively upon oral administration, the amount present in the extracts would be very low.

Plasma Levels in Humans—The average plasma levels ($\pm SE$) of dextrorphan and its conjugates following 30 mg po of dextromethorphan hydrobromide to six volunteers at 15, 30, and 60 min were 21.3 ± 6.7, 107.3 ± 44.6, and 368.0 ± 71.2 ng/ml, respectively. The peak level was 381.3 ± 56.9 ng/ml at 2 hr and it declined thereafter, reaching 262.7 ± 43.3 ng/ml at 4 hr. Almost all of the dextrorphan was present in the form of conjugates. This finding was confirmed by analyzing the plasma samples from one of the six volunteers, with and without enzymatic hydrolysis. Whereas hydrolysis gave rise to concentrations ranging from 26 to 380 ng/ml, none of the unhydrolyzed samples contained measurable levels of the unconjugated drug or metabolites.

It may be concluded that this method is applicable to the determination of the bioavailability of dextromethorphan hydrobromide in humans.

[3] Perkin-Elmer model MPF-3.

REFERENCES

(1) J. A. F. de Silva and L. D'Arconte, *J. Forensic Sci.*, **14**, 184 (1969).

(2) J. Axelrod, *J. Pharmacol. Exp. Ther.*, **117**, 322 (1956).

(3) C. Elison and H. Elliott, *ibid.*, **144**, 265 (1964).

(4) J. Kamm, A. Taddeo, and E. Van Loon, *ibid.*, **158**, 437 (1967).

(5) A. Brossi, O. Haffliger, and O. Schneider, *Arzneim.-Forsch.*, **5**, 62 (1955).

(6) K. Willner, *ibid.*, **13**, 20 (1963).

(7) H. Kaiser and H. Jori, *Arch. Pharm.*, **287**, 224 (1954).

(8) P. A. Shore, J. Axelrod, C. A. M. Hogben, and B. B. Brodie, *J. Pharmacol. Exp. Ther.* **113**, 192 (1955).

(9) K. D. Wuepper, S. Y. Yeh, and L. A. Woods, *Proc. Soc. Exp. Biol. Med.*, **124**, 1146 (1967).

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Effects of 3,4-Dimethoxyphenethylamine Derivatives on Monoamine Oxidase

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Abstract □ The cactus alkaloid 3,4-dimethoxyphenethylamine and its naturally occurring *N*-methylated homologs inhibited the deamination of tyramine and tryptamine by rat brain monoamine oxidase. In contrast, the β-hydroxylated derivatives of this series failed to inhibit the action of monoamine oxidase on both tyramine and tryptamine.

Keyphrases □ 3,4-Dimethoxyphenethylamine and *N*-methyl homologs—effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine □ Monoamine oxidase—deamination of tyramine and tryptamine, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Tyramine—monoamine oxidase-catalyzed oxidative deamination, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Tryptamine—monoamine oxidase-catalyzed deamination, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Enzymes—monoamine oxidase, deamination of tyramine and tryptamine, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Structure–activity relationships—3,4-dimethoxyphenethylamine and *N*-methyl homologs, effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine

The present study was directed at observing the effects of 3,4-dimethoxyphenethylamine (I) and its *N*-methyl homologs on rat brain monoamine oxidase, using both tyramine and tryptamine as substrates. The results of these experiments were contrasted with those obtained with the β-hydroxy derivatives of the same series. The al-

Table I—Inhibiting Effects of Compounds on Oxidation of Tyramine Hydrochloride and Tryptamine Hydrochloride by Rat Brain Mitochondria [a]

Compound	Inhibition, %	Tyramine Hydrochloride Significance	Tyramine Hydrochloride SD	Tryptamine Hydrochloride Inhibition, %	Tryptamine Hydrochloride Significance	Tryptamine Hydrochloride SD
I	32	+	1.3	27	+	0.7
II	49	+	1.3	27	+	1.3
III	33	+	1.1	37	+	1.3
IV	7	−	2.6	19	−	2.3
V	7	−	2.6	19	−	3.2
VI	0	−	2.8	16	−	2.9
Isocarboxazid [b]	100	+	1.5	67	+	1.0

[a] Each value represents the results obtained using three or more flasks per compound for each substrate. Percent inhibition was calculated by comparing control and experimental values for each compound with each substrate. [b] Reference.

leged mind-altering effects produced by ingesting cacti containing one or more of the test compounds could be partially explained by data generated from this investigation.

BACKGROUND

Many members of the Cactaceae produce and accumulate various derivatives of I. Compound I was detected in several species of *Trichocereus* (1–3), *Echinocereus merkeri* Hildm. (4), *Pelecyphora aselliformis* Ehrenberg (5), *Stetsonia coryne* (SD.)-Br. and R. (3), and *Lophophora williamsii* (Lem.) Coult. (6). *N*-Methyl-3,4-dimethoxyphenethylamine (II) was identified as a natural product (2) and was isolated from numerous cacti (4, 5, 7–15). The *N,N*-dimethyl derivative of I has a more restricted distribution in nature, having been originally isolated from extracts of *E. merkeri* (4) and subsequently found to be present in *Coryphantha greenwoodii* H. Bravo (14) and *Ariocarpus scapharostrus* Bodeker (15).

N-Methylated homologs of 3,4-dimethoxy-β-hydroxyphenethylamine are particularly abundant in the cactus genus *Coryphantha*. Macromerine [2-hydroxy-2-(3,4-dimethoxyphenyl)-*N,N*-dimethylethylamine] occurs naturally in *C. macromeris* (Engelm.) Lem. (16) and several other *Coryphantha* species (10, 11, 17).

Normacromerine [2-hydroxy-2-(3,4-dimethoxyphenyl)-*N*-methylethylamine] was isolated from *C. macromeris* (Engelm.) Br. and R. var. *runyonii* L. Benson (18) and two other *Coryphantha* species (13, 14), *Dolichothele longimamma* (DC.) Br. and R. (19), and a legume, *Desmodium tiliaefolium* G. Don. (20). The parent compound of this series is not a natural product.

L. williamsii and the various *Trichocereus* species are hallucinogenic because of their mescaline content. The other previously mentioned cacti are suspected of containing psychoactive substances because of various folkloric tales. Recently, the monoamine oxidase inhibitory activity of 4-methoxylated phenethylamines was used in an attempt to explain the purported pharmacological activity of cactus plants containing these compounds (21). The hypothesis was expanded when a series of β-hydroxylated 4-methoxyphenethylamines was observed to inhibit selectively enzymatic oxidative deamination of tyramine but not tryptamine (22).

EXPERIMENTAL

Synthesis—Compound I hydrochloride[1] was available commercially and served as the starting point for the *N*-methylation reactions that yielded the other two members of this series. Compound II hydrochloride, mp 132–134° [lit. (4) mp 134–136°], was synthesized by reacting I with 36% formaldehyde[2] followed by a sodium borohydride reduction of the Schiff-base intermediate. Refluxing equimolar quantities of II, 36% formaldehyde, and 88% formic acid[3] gave *N,N*-dimethyl-3,4-dimethoxyphenethylamine (III) hydrochloride, mp 193–194° [lit. (4) mp 193–196°].

Racemic 3,4-dimethoxy-β-hydroxyphenethylamine (IV) hydrochloride, mp 170–171° [lit. (23) mp 170–171°], *N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine (V) hydrochloride, mp 115–116° [lit. (18) mp 115–117°], and *N,N*-dimethyl-3,4-dimethoxy-β-hydroxyphenethylamine (VI) hydrochloride, mp 161–162° [lit. (23) mp 161–162°], were produced by a Houben–Hoesch condensation of veratrole[3] with aminoacetonitrile hydrochloride[4], *N*-methylaminoacetonitrile hydrochloride[4], and *N,N*-dimethylaminoacetonitrile hydrochloride[4], respectively, followed by a reduction of the ketone intermediate with sodium borohydride.

Warburg Studies—As an evaluation of possible monoamine oxidase inhibition, the effects of the compounds on the oxidation of tyramine and tryptamine hydrochlorides by rat brain mitochondria were investigated. Brain mitochondria (containing monoamine oxidase) were prepared from albino Wistar female rats, 150–200 g, according to the method of Brody and Bain (24). Monoamine oxidase activity was determined in the presence and absence of the respective compounds by conventional manometric techniques (25)[5].

In the manometric determinations, the main compartment of the Warburg flasks contained 0.8 ml of a 0.1 *M* buffered solution of the compound studied (or buffer), 1 ml of mitochondrial suspension (representing 500 mg wet weight of original brain tissue), and sufficient 0.01 *M* phosphate buffer (pH 7.4) to make a total volume of 2.7 ml. All aliquots for a given run were taken from the same mitochondrial preparation. The side arm of each flask contained 0.3 ml of 0.1 *M* tyramine or tryptamine hydrochloride. After the flasks were allowed to equilibrate for 15 min at 37°, the manometer valves were closed and the side arm contents of each were tipped in.

Manometers were set at 150 mm, and readings were taken at 15-min intervals for 90 min. Values for the 90-min reaction period were obtained for each flask and were multiplied by the respective flask constants after adjusting for changes in the thermobarometer. The total microliters of oxygen uptake for each flask was thus obtained.

Reference flasks, using 0.05 *M* isocarboxazid (0.8 ml) as the monoamine oxidase inhibitor, were employed as already described and were used for comparison.

Statistical Analysis—The mean and standard error were determined for each control or treated group. Values thus obtained were compared, using the difference between the means and the standard error of the difference between the means for the respective control-treated pairs of groups. The Student *t* test was applied to the results, and the probability was determined (p values of less than 0.05 were considered significant).

RESULTS AND DISCUSSION

Data from Warburg studies revealed that I–III produced significant ($p < 0.05$) inhibition of rat brain monoamine oxidase when tyramine and tryptamine were used as substrates. Conversely, the β-hydroxylated derivatives of this series (IV–VI) had little effect on the enzymatic oxidative deamination of tyramine and tryptamine. These results were validated by the activity exhibited by reference isocarboxazid (Table I).

Substrate selectivity was observed with an *N*-methylated series of 4-methoxy-β-hydroxyphenethylamines, with each member inhibiting the action of monoamine oxidase on tyramine but not on tryptamine (22). The same series of compounds without the β-hydroxy group inhibited monoamine oxidase with respect to both tyramine and tryptamine. Johnston (26) found that there are at least two forms of monoamine oxidase in rat brain, while Tabakoff *et al.* (27) suggested that separate interacting sites exist in brain monoamine oxidase for the binding of phenethylamines and for compounds possessing an indole nucleus. With this information, it was proposed that the presence of a β-hydroxy group in the 4-methoxyphenethylamine moiety interferes with the binding of

[1] Calbiochem.
[2] Fisher Scientific Co.
[3] Aldrich Chemical Co.
[4] K and K Laboratories.
[5] Precision Warburg apparatus, Precision Scientific Co.

these derivatives with the active site and/or isozyme responsible for the oxidation of tryptamine (22). From these suppositions, it appears that a 3,4-dimethoxyphenethylamine nucleus with a β-hydroxy group cannot properly bind with the active site and/or isozyme associated with both tyramine and tryptamine oxidation.

The monoamine oxidase inhibitory activity of 4-methoxyphenethylamine derivatives has been used to explain the folkloric uses of *D. longimamma* as a psychoactive "peyote" cactus (19). Macromerine and normacromerine are the major alkaloids in *C. macromeris* var. *runyonii* (11), and this cactus was reported to be psychoactive (16, 28). Vogel *et al.* (29) concluded that macromerine and normacromerine were not responsible for the psychoactivity, since they produced no effects on the conditioned avoidance response in rats. Since macromerine and normacromerine are devoid of monoamine oxidase inhibitory activity, there appears to be no correlation between the presence of these compounds and the purported psychoactivity of *C. macromeris* var. *runyonii*.

Harley-Mason (30) predicted that an abnormal methylation of dopamine and/or norepinephrine *in vivo* could produce I or IV. Of these compounds, I received wide attention because of its detection in the urine of schizophrenics (31). Later studies revealed that urinary excretion of I is not unique to schizophrenia (32) and that administration of this compound to humans produces no psychological changes (33, 34). However, the ingestion of cacti containing I and/or its *N*-methyl derivatives together with indirectly acting amines such as synephrine could alter behavior by inhibiting monoamine oxidase and thus potentiating the effects of the indirectly acting amines. This may be the case with the supposedly psychoactive *C. macromeris* var. *runyonii*. Although the major alkaloids do not inhibit monoamine oxidase, this cactus does contain two known monoamine oxidase inhibitors, *N*-methyl-4-methoxyphenethylamine and II, together with several indirectly acting amines (11). This situation may also contribute to the well-documented hallucinogenic activity of the mescaline-containing cacti.

REFERENCES

(1) S. Agurell, *Lloydia*, **32**, 40 (1969).

(2) *Ibid.*, **32**, 206 (1969).

(3) S. Agurell, J. G. Bruhn, J. Lundstrom, and U. Svensson, *Lloydia*, **34**, 183 (1971).

(4) S. Agurell, J. Lundstrom, and A. Masoud, *J. Pharm. Sci.*, **58**, 1413 (1969).

(5) J. M. Neal, P. T. Sato, W. N. Howald, and J. L. McLaughlin, *Science*, **176**, 1131 (1972).

(6) J. Lundstrom and S. Agurell, *J. Chromatogr.*, **36**, 105 (1968).

(7) D. G. Norquist and J. L. McLaughlin, *J. Pharm. Sci.*, **59**, 1840 (1970).

(8) W. W. Speir, V. Mihranian, and J. L. McLaughlin, *Lloydia*, **33**, 15 (1970).

(9) J. M. Neal and J. L. McLaughlin, *ibid.*, **33**, 395 (1970).

(10) K. M. K. Hornemann, J. M. Neal, and J. L. McLaughlin, *J. Pharm. Sci.*, **61**, 41 (1972).

(11) W. J. Keller, J. L. McLaughlin, and L. R. Brady, *ibid.*, **62**, 408 (1973).

(12) J. G. Bruhn and C. Bruhn, *Econ. Bot.*, **27**, 241 (1973).

(13) J. G. Bruhn and S. Agurell, *J. Pharm. Sci.*, **63**, 574 (1974).

(14) J. G. Bruhn, S. Agurell, and J. E. Lindgren, *Acta Pharm. Suec.*, **12**, 199 (1975).

(15) J. G. Bruhn, *Phytochemistry*, **14**, 2509 (1975).

(16) J. E. Hodgkins, S. D. Brown, and J. L. Massingill, *Tetrahedron Lett.*, **14**, 1321 (1967).

(17) L. E. Below, A. Y. Leung, J. L. McLaughlin, and A. G. Paul, *J. Pharm. Sci.*, **57**, 515 (1968).

(18) W. J. Keller and J. L. McLaughlin, *ibid.*, **61**, 147 (1972).

(19) R. L. Ranieri and J. L. McLaughlin, *J. Org. Chem.*, **41**, 319 (1976).

(20) S. Ghosal and R. S. Srivastava, *Phytochemistry*, **12**, 193 (1973).

(21) G. G. Ferguson and W. J. Keller, *J. Pharm. Sci.*, **64**, 1431 (1975).

(22) W. J. Keller and G. G. Ferguson, *ibid.*, **65**, 1539 (1976).

(23) W. J. Keller, Ph.D. dissertation, University of Washington, Seattle, Wash., 1972.

(24) T. M. Brody and J. A. Bain, *J. Biol. Chem.*, **195**, 685 (1952).

(25) W. W. Umbreit, R. H. Burris, and J. F. Stauffer, "Manometric Techniques," 4th ed., Burgess, Minneapolis, Minn., 1964, pp. 61, 76.

(26) J. P. Johnston, *Biochem. Pharmacol.*, **17**, 1285 (1968).

(27) B. Tabakoff, L. R. Meyerson, and S. G. A. Alivisatos, *Brain Res.*, **66**, 491 (1974).

(28) M. J. Superweed, "Herbal Highs," Stone Kingdom Syndicate, San Francisco, Calif., 1970, p. 5.

(29) W. H. Vogel, B. D. Evans, E. M. Bonnem, J. F. Fischer, and J. L. McLaughlin, *Psychopharmacologia*, **30**, 145 (1973).

(30) J. Harley-Mason, *J. Ment. Sci.*, **98**, 313 (1952).

(31) A. J. Friedhoff and E. Van Winkle, *Nature*, **194**, 897 (1962).

(32) A. A. Boulton, *Nature*, **231**, 22 (1971).

(33) L. E. Hollister and A. J. Friedhoff, *ibid.*, **210**, 1377 (1966).

(34) A. T. Shulgin, T. Sargent, and C. Naranjo, *ibid.*, **212**, 1606 (1966).

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Alkaloids of *Papaver orientale* L.

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Abstract □ According to the alkaloid profiles, five different chemotypes (A, B, C, D, and E) were classified in *Papaver orientale* L. with haploid chromosome number $n = 14$. Chemotype A had only oripavine; chemotype B contained oripavine and thebaine; chemotype C had isothebaine in addition to oripavine; chemotype D contained oripavine and alpinigenine; and chemotype E had oripavine, thebaine, and alpinigenine. In all chemotypes, oripavine was either the sole alkaloid or the single major alkaloid.

Keyphrases □ *Papaver orientale* L.—chloroform extract of seed capsules, alkaloids isolated and identified, five chemotypes classified □ Alkaloids—isolated and identified, chloroform extract of seed capsules of *Papaver orientale* L., five chemotypes classified

In continuation of a broad study of the Iranian wild Papaveraceae (1–5), alkaloids of *Papaver pseudo-orientale* Fedde. and preliminary results on the alkaloids of *P. orientale* L. were reported (4). In this work, qualitative and