unimolecular layer and would be expected to significantly alter the dose response obtained. It is well to point out here that such interactions would not only manifest themselves in solid dosage forms, as in the case of tablets and capsules, but could also occur in suspensions and ointments, where drug–adjuvant ratios are usually low. It is of significant interest to point out here that one cannot overlook the distinct possibility of the excipient itself existing as a chemisorbed layer covering the surface of the drug resulting in similar alterations of the physical or biochemical behavior of the medicament in dosage forms where the drug–adjuvant ratios are high.

Data presented in this study further illustrate the possibility that donor–acceptor interaction of many varieties play an important role in these drug–adjuvant interactions. This has been illustrated by different magnitudes of spectral shifts observed among aluminum, calcium, magnesium, zinc, and sodium-containing adjuvants as well as nonmetal-containing excipients represented by stearyl alcohol, stearic acid, cetyl alcohol, acacia, tragacanth, tannic acid, and polyethylene glycol. The degree of interaction observed further depends on the nature of the drug and the type of adjuvant used. For example, calcium-containing adjuvants interact strongly with oxytetracycline but show very little interaction tendency for anthracene.

Studies are currently in progress in these laboratories dealing with the effects of such interactions on various aspects of drug dosage formulation and absorption.

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LSD Analogs

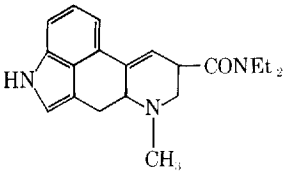
N-Methyl-*N*-*p*-(and *m*-)methoxyphenyl-β-alanine Derivatives

By KENNETH J. LISKA, JAMES L. JOHNSON, JAMES P. MASTRIAN, and MARIE L. STEENBERG

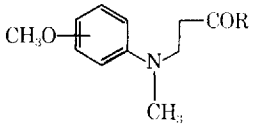
Patterned after a fragment of the LSD molecule, the ethyl esters, simple amides, and *N*,*N*-diethylamides of *N*-methyl-*N*-*p*-(and *m*-)methoxyphenyl-β-alanine were prepared for evaluation as psychotomimetics. Of five compounds tested, three exhibited some degree of antiserotonin activity in the isolated rat fundus preparation. One of these three appeared also to be anticholinergic.

ATTEMPTS HAVE been made to elucidate an active psychotomimetic moiety in the lysergic acid diethylamide (LSD) molecule (1, 2). In the present work, the *N*-methyl-*N*-phenyl-β-alanine fragment of LSD was selected for study; an electron-rich methoxy group *para* or *meta* on the ring was intended to approximate the contribution made by the pyrrole nitrogen.

The ethyl esters (R = OEt in β-alanine moiety



LSD



β-Alanine Moiety

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TABLE I.—EFFECT OF COMPOUNDS I, II, AND VI ON RESPONSE OF ISOLATED RAT FUNDUS TO SEROTONIN

Compd.	Dose, mcg./ml.	No. of Expt.	Inhibition, % Mean ± S.E.
I	78.8	5	94.5 ± 2.3
	53.7	5	79.1 ± 4.5
	35.8	5	53.5 ± 4.7
	17.9	5	39.5 ± 4.4
II	28.4	4	95.4 ± 2.7
	14.4	4	83.6 ± 1.4
	5.7	4	38.9 ± 3.2
	2.8	4	5.0 ± 2.1
VI	7.0	3	95.7 ± 2.1
	2.8	3	78.5 ± 1.7
	1.4	3	20.0 ± 2.5
	0.7	3	0

structure), produced by addition of *N*-methyl-*p*-anisidine or *N*-methyl-*m*-anisidine to ethyl acrylate, were converted into the simple amides (R = NH_2) employing ammonia water at refrigerator temperatures. When the corresponding aminolysis employing the ethyl ester of *N*-methyl-*N*-*p*-methoxyphenyl-β-alanine and diethylamine was attempted at room temperature, at 75° for 72 hr., or at 160° for 22 hr., no amide formation could be detected by infrared spectral examination of the crude reaction products. Only unchanged ester appeared to be recovered. This diethylamine aminolysis was also attempted using catalytic amounts of water (at 110° for 64 hr.), sodium ethoxide (room temperature for 65 hr.), and calcium chloride (112° for 15 hr.). Again, only unchanged ester could be detected by infrared examination of the crude reaction products. Since the *N*,*N*-diethylamide in the *para* series could not be obtained by aminolysis, it and the *N*,*N*-diethylamide in the *meta* series were prepared by treating *N*,*N*-diethyl-3-bromopropionamide with either *N*-methyl-*p*-anisidine or *N*-methyl-*m*-anisidine. As a further proof of structure, the ethyl ester in the *para* series was hydrolyzed to the free acid, which was isolated as the amino acid hydrochloride salt.

Pharmacological testing for serotonin inhibitory activity was performed utilizing the isolated rat fundus. Five compounds were tested: the ethyl esters in both series, the simple amide in the *para* series, and the *N*,*N*-diethylamides in both series (compounds I, II, III, V, and VI. See under *Experimental*). Compounds I, II, and VI antagonized the action of serotonin on the isolated rat fundus. Compound VI appeared to be the most potent antagonist tested, showing inhibition at concentrations as low as 1.4 mcg./ml. and an ED_{50} of 2.2 mcg./ml. Compounds II and I were effective antagonists of serotonin, exhibiting approximately 50% inhibition at concentrations of 7.4 and 28.2 mcg./ml., respectively. Compound II also antagonized acetylcholine-induced contractions; 0.14 mg./ml. produced a 50% inhibition. Compounds I, II, and VI appear as relatively weak serotonin antagonists when their activities are compared to the activity of LSD on the mouse uterus (3). The most active compound (VI) appears to have a dual mechanism, antiserotonin and anticholinergic. These results are summarized in Table I. Compounds III and V were inactive.

EXPERIMENTAL[1]

N-Methyl-p-anisidine.—This was prepared from commercial *p*-anisidine according to the method of King and Tonkin (4), b.p. 139–141° (27 mm.). HCl salt, m.p. 121–123°. Major I.R. spectral peaks in $cm.^{-1}$: 3405 (m), 1506 (s), 1230 (s), 1030 (s), 815 (s).

N-Methyl-m-anisidine.—This was prepared from commercial *m*-anisidine following the directions of King and Tonkin (4) for the *para* compound, b.p. 119–120° (11 mm.), n_D^{24} 1.5670; picrate from alcohol, m.p. 147.5–149.5°. Major I.R. spectral peaks in $cm.^{-1}$: 3405 (m), 1600 (s), 1490 (s), 1204 (s), 1155 (s), 820 (m), 750 (m), and 680 (m).

N,N-Diethyl-3-bromopropionamide.—This was prepared according to the method of Gearien and Liska (5), b.p. 82–100° (0.5 to 1.0 mm.).

Ethyl 3-(N-Methyl-N-p-methoxyphenylamino)-propionate (I).—To a mixture of 52.0 Gm. (0.38 mole) of *N*-methyl-*p*-anisidine and 57.0 Gm. (0.57 mole) of ethyl acrylate was added 2 ml. of glacial acetic acid, and the mixture refluxed for 7 hr., then allowed to stand overnight. The product was taken up in ether, washed once with saturated sodium bicarbonate solution, once with water, dried over anhydrous sodium carbonate, the ether evaporated, and distilled. Discarding a forerun, the authors collected the fraction, b.p. 144–148° (0.1 mm.). The yield was 60.4 Gm. (67%) of pale yellow oil. Ester CO (str) 1720 $cm.^{-1}$.

Anal.—Calcd. for $C_{13}H_{19}NO_3$: C, 65.82; H, 8.02; N, 5.90. Found: C, 66.01; H, 8.10; N, 6.00.

The HCl salt could not be obtained in a solid form. Picrate, from alcohol, m.p. 126–129°.

Ethyl 3-(N-Methyl-N-m-methoxyphenylamino)-propionate (II).—A mixture of 12.2 Gm. (0.089 mole) of *N*-methyl-*m*-anisidine, 9.0 Gm. (0.089 mole) of ethyl acrylate, and 1 ml. of glacial acetic acid was heated under reflux for 5 hr. and then allowed to stand overnight. The workup was the same as for the *para* compound. Obtained was 12.0 Gm. (57%) of a pale yellow oil, b.p. 145–150° (0.1 mm.). Ester CO (str) 1720 $cm.^{-1}$.

Anal.—Calcd. for $C_{13}H_{19}NO_3$: C, 65.82; H, 8.02; N, 5.90. Found: C, 66.05; H, 8.16; N, 6.10.

The HCl salt could not be obtained in a solid form. Picrate, from alcohol, m.p. 104–106°.

3-(N-Methyl-N-p-methoxyphenylamino)-propionamide (III).—Ammonia water (25 ml. of 29% ammonia) was enclosed with 8.8 Gm. (0.037

[1] Melting points and boiling points are uncorrected. Infrared data from a Beckman IR-8. Analyses by Galbraith Laboratories and Weiler & Strauss.

mole) of ethyl 3-(*N*-methyl-*N*-*p*-methoxyphenylamino)propionate and stored with occasional shaking in a refrigerator for 10 days. The supernatant liquid was then decanted from the semisolid mass, a fresh 25-ml. portion of ammonia added, and the mixture allowed to stand an additional 3 days. The supernatants upon chilling to 0° yielded crude amide which was combined with additional amide obtained by cold ether extraction of the semisolid mass. Recrystallization from ligroin yielded fluffy white needles, m.p. 110–112°. Obtained 1.0 Gm. (13%). Amide CO (str) 1670 cm.$^{-1}$.

Anal.—Calcd. for $C_{11}H_{16}N_2O_2$: C, 63.46; H, 7.69; N, 13.46. Found: C, 63.24; H, 7.76; N, 13.23.

3 - (N - Methyl - N - m - methoxyphenylamino)-propionamide (IV).—This simple amide was prepared in 14% yield following approximately the same procedure used for the *para* isomer. Recrystallized from water, m.p. 94.5–96°. Amide CO (str) 1670 cm.$^{-1}$.

Anal.—Calcd. for $C_{11}H_{16}N_2O_2$: C, 63.46; H, 7.69; N, 13.46. Found: C, 63.66; H, 7.80; N, 13.27.

3 - (N - Methyl - N - p - methoxyphenylamino)-propionic Acid Hydrochloride.—The hydrolysis of ethyl 3-(*N*-methyl-*N*-*p*-methoxyphenylamino)propionate (10.0 Gm., 0.042 mole) was accomplished by refluxing with 50 ml. of 10% HCl for 4 hr. The volume of water was reduced to about one-fifth. The solution was allowed to stand overnight, whereupon 3.2 Gm. of pure, highly crystalline amino acid hydrochloride, m.p. 159–160°, was obtained. Evaporation of the remainder of the water and trituration of the solids with anhydrous ether yielded an additional 2.7 Gm. of pure product for a total yield of 57%. The salt recrystallizes well from absolute alcohol.

Anal.—Calcd. for $C_{11}H_{16}ClNO_3$: Cl, 14.43. Found: Cl, 14.19.

3 - (N - Methyl - N - p - methoxyphenylamino)-N′,N′-diethylpropionamide (V).—A solution of 10.2 Gm. (0.049 mole) of *N*,*N*-diethyl-3-bromopropionamide, 6.9 Gm. (0.049 mole) of *N*-methyl-*p*-anisidine, 5.3 Gm. (0.05 mole) of anhydrous sodium carbonate, and 15 ml. of benzene was heated at gentle reflux for 7.5 hr., and then allowed to stand overnight. The mixture was filtered, the solids washed with ether, the combined filtrates freed of solvents, and distilled. After a large forerun, the fraction, b.p. 183–186° (0.09 mm.), was collected as a yellow oil, amounting to 3.9 Gm. (30%). Tertiary amide CO (str) 1625 cm.$^{-1}$. n_D^{23} 1.5396.

Anal.—Calcd. for $C_{15}H_{24}N_2O_2$: C, 68.18; H, 9.09; N, 10.61. Found: C, 68.35; H, 9.20; N, 10.80.

The HCl salt was prepared crystalline but was very hygroscopic. The picrate could not be obobtained in a solid form. Methiodide, recrystallized from absolute ethanol, m.p. 121–124°, slightly hygroscopic.

3 - (N - Methyl - N - m - methoxyphenylamino)-N′,N′-diethylpropionamide (VI).—A mixture of 6.0 Gm. (0.044 mole) of *N*-methyl-*m*-anisidine, 9.1 Gm. (0.044 mole) of *N*,*N*-diethyl-3-bromopropionamide, 4.6 Gm. (0.044 mole) of anhydrous sodium carbonate, and 15 ml. of benzene was heated at gentle reflux for 5 hr. The mixture was cooled, filtered, the freed solids washed with benzene, and the combined benzene filtrates dried over sodium sulfate. After removal of the solvent, the residue was distilled and the fraction collected, b.p. 173–181° (0.09 mm.). Obtained 4.3 Gm. (37%) of a yellow oil, n_D^{23} 1.5448. Tertiary amide CO (str) 1629 cm.$^{-1}$.

Anal.—Calcd. for $C_{15}H_{24}N_2O_2$: C, 68.18; H, 9.09; N, 10.61. Found: C, 68.03; H, 8.98; N, 10.71.

The HCl salt could not be prepared in a solid state. The picrate was crystallized, after 5 days standing, from ethanol, m.p. 125–127°. Methiodide from absolute ethanol, m.p. 108–111°, hygroscopic.

PHARMACOLOGY

Wistar rats of both sexes (100–150 Gm.) were fasted for 24 hr., killed by a blow on the head, and the stomach removed. The fundus was removed and cut into a strip by opening the tissue along the lesser curvature and cutting to preserve the longitudinal muscle as described by Vane (6). The fundus strip was suspended in a 10-ml. muscle bath containing modified Ringer solution (7) (Gm./L.: NaCl, 9.0; KCl, 0.42; $CaCl_2 \cdot 2H_2O$, 0.06; $NaHCO_3$, 0.5; and glucose, 0.5) maintained at 37° and oxygenated with pure oxygen.

The strip was permitted to stretch in the organ bath for 1 hr., after which the minimum amount of serotonin required to give a response of 4–5 cm. was determined. This varied from 0.1–1.0 ng./ml. of bathing solution. This amount of serotonin was added to the bath at 6-min. intervals and the tissue washed twice between doses. Three equivalent responses to serotonin were followed by the addition of a known quantity of the drug, diluted in normal saline, 1 min. before the next addition of serotonin. If inhibition occurred, the tissue was washed until recovery was noted, and two equivalent responses to serotonin were recorded before the tissue was used for another test. Four different doses of each drug were selected so that the highest dose gave a complete blockade of serotonin and the lowest a 5–10% blockade. The volume of each dose was less than 0.4 ml.

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