1-Phenylpiperazines: Potential Antagonists of Lysergic Acid Diethylamide

K. BREWSTER, D. B. COULT and R. M. PINDER.
Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, England.

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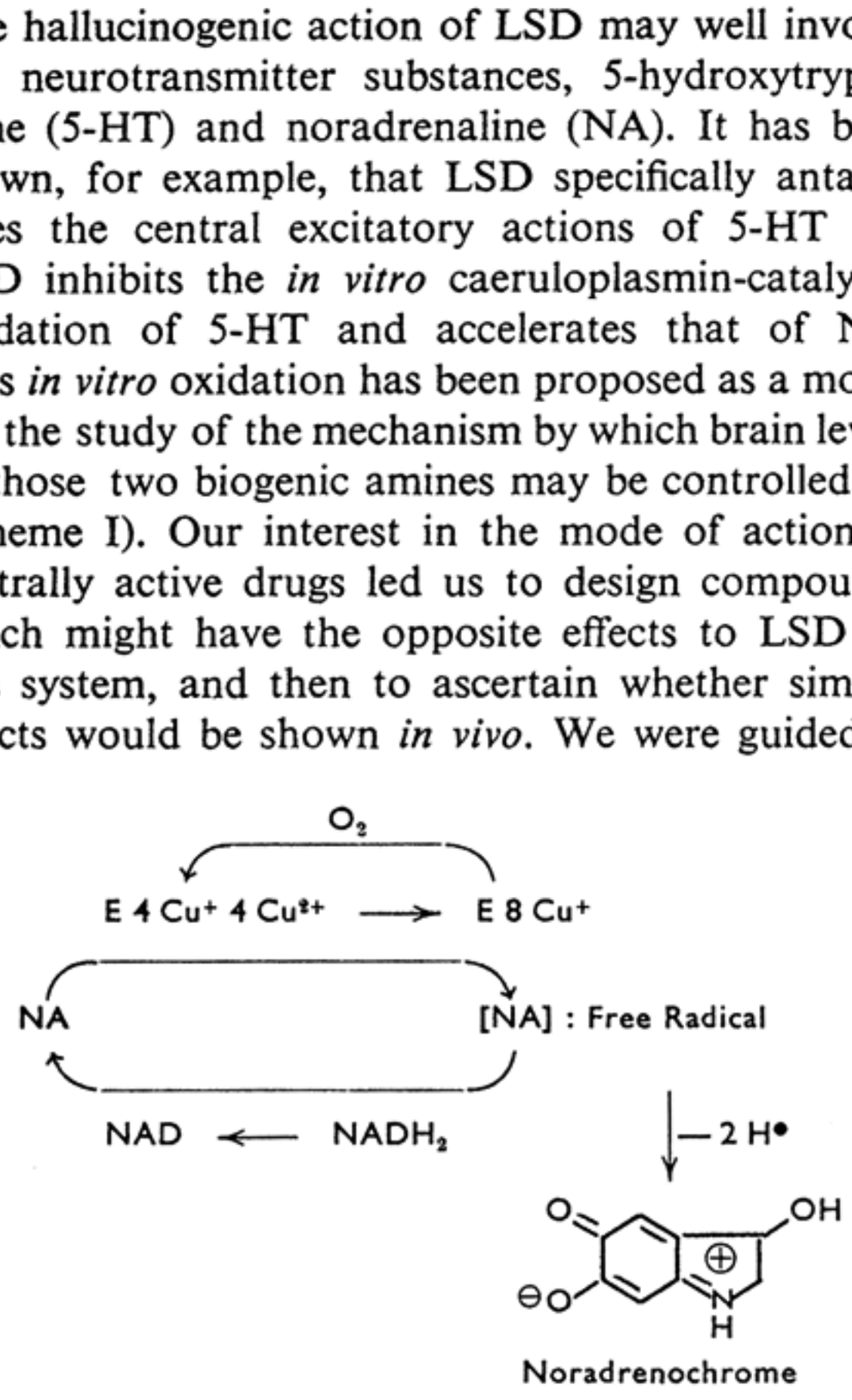
Une série de phényl-1 pipérazines est préparée par action d'arylamines primaires sur la dichloro-2,2′ diéthylamine ou son dérivé N-méthylé. Contrairement aux phényléthylènediamines, analogues à chaîne droite, les phényl-1 pipérazines ne présentent pas d'effets opposés à ceux dus au diéthylamide de l'acide lysergique (LSD) sur les oxydations de l'hydroxy-5 tryptamine et de la noradrénaline catalysées par la céruléoplasmine. De plus, ils n'inversent pas l'hyperthermie engendrée par le LSD chez le lapin et manifestent une légère action intrinsèque sur le comportement. Le rôle de l'emploi de la céruléoplasmine dans l'action du LSD sur le système nerveux central est discutée.

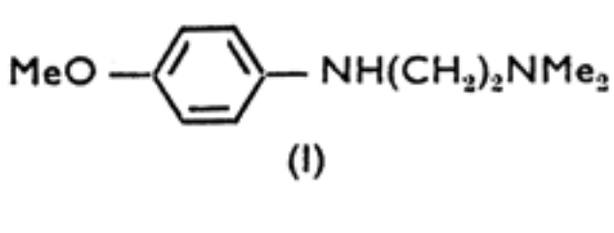
Introduction.

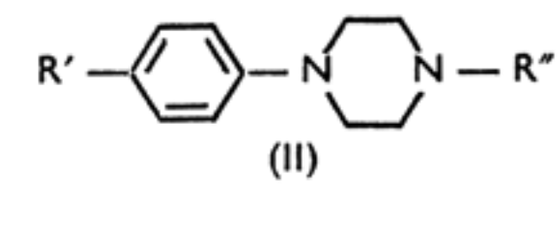
Lysergic acid diethylamide (LSD) produces a multiplicity of well defined pharmacological effects, most of which appear to be central in origin (1). The drug is best known for its psychotomimetic properties and it is the most potent hallucinogenic substance known to man. Considerable interest and speculation have centred around its possible mode of action in the central nervous system and means of counteracting that action. The usual treatment for LSD-intoxication has involved the administration of tranquillisers like chlorpromazine, or massive doses of niacin the « megavitamin therapy »; both methods are envisaged (2, 3) as restoring nicotinamide adenine dinucleotide (NAD) levels to normal.

The hallucinogenic action of LSD may well involve the neurotransmitter substances, 5-hydroxytryptamine (5-HT) and noradrenaline (NA). It has been shown, for example, that LSD specifically antagonises the central excitatory actions of 5-HT (4). LSD inhibits the *in vitro* caeruloplasmin-catalysed oxidation of 5-HT and accelerates that of NA. This *in vitro* oxidation has been proposed as a model for the study of the mechanism by which brain levels of those two biogenic amines may be controlled (5) (scheme I). Our interest in the mode of action of centrally active drugs led us to design compounds which might have the opposite effects to LSD on this system, and then to ascertain whether similar effects would be shown *in vivo*. We were guided in the design by the known substrate specificity for caeruloplasmin of aromatic diamines, aminophenols, and substituted anilines (6, 7). Preliminary work indicated that compounds like N,N-dimethyl-N′-(4-methoxyphenyl)ethylenediamine (I) were inhibitors of caeruloplasmin-catalysed oxidation of NA and accelerated oxidation of 5-HT, but they were very unstable in solution and produced dermatitic reactions. In an attempt to eliminate the undesirable biological effects and to increase stability, we have synthesised a series of 1-phenylpiperazines (II), cyclic analogues of the phenylethylenediamines, as potential antagonists of LSD.



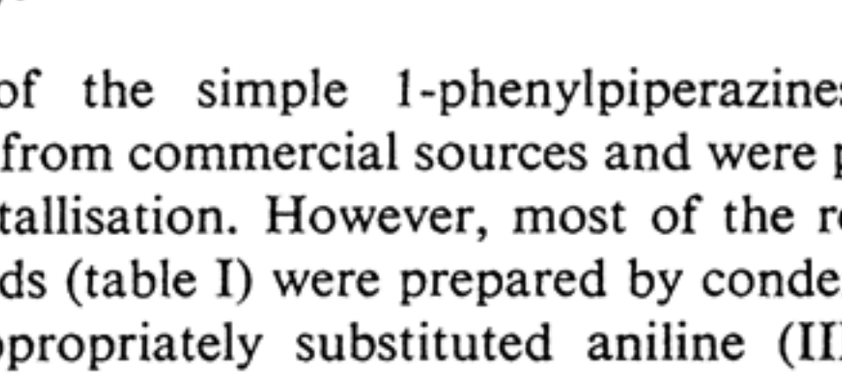
Scheme I. — Caeruloplasmin-catalysed oxidation of noradrenaline (NA); a similar scheme can be drawn for 5-hydroxytryptamine. NAD is the oxidised form of nicotinamide adenine dinucleotide.





Chemistry.

Several of the simple 1-phenylpiperazines were available from commercial sources and were purified by recrystallisation. However, most of the required compounds (table I) were prepared by condensation of an appropriately substituted aniline (III) with 2,2′-dichlorodiethylamine or its N-methyl derivative (IV) (scheme II). The originally recommended solvent, butan-1-ol (8, 9), was replaced by the higher boiling 2-butoxyethanol, which consistently gave higher yields, shorter reaction times, and improved products. Compounds containing hydroxyl substituents (II, R′ = OH) were obtained from the corresponding methyl ethers by demethylation with hydrobromic acid.

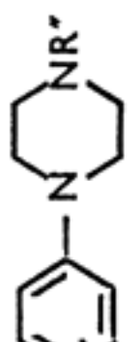


Scheme II. — Synthetic route to 1-phenylpiperazines.

Enzymology.

Caeruloplasmin from human plasma was obtained from A. G. KABI Ltd. (Sweden) as a 5 % aqueous solution. The drugs used were either obtained from

TABLE I. —



R'	R''	Yield %	Salt	B.p. (free base) °C/mm	M.p. °C (*a*)	Analysis Calc. % C	H	N	Found % C	H	N	Formula Analysis (*f*)
4-OH	H	69	2 HBr	—	291-293 (*b*)	35.52	4.74	8.24	35.25	4.67	8.39	$C_{10}H_{14}N_2O.2HBr$
4-OH	Me	63	2 HBr	—	258-260	37.31	5.12	7.91	36.36	5.21	7.59	$C_{11}H_{16}N_2O.2HBr$
4-OMe	Me	72	2 HCl	115-120/0.5 (*c*)	208-210	51.62	7.22	10.03	51.84	7.16	10.25	$C_{12}H_{18}N_2O.2HCl$
3,4-$(OH)_2$	H	43	2 HBr	—	267-268	33.73	4.53	7.87	33.38	4.37	8.01	$C_{10}H_{14}N_2O_2.2HBr$
3,4-$(OMe)_2$	H	68	HCl	140-142/2 (*d*)	235-236 (*e*)	55.70	7.40	10.83	55.75	7.49	10.88	$C_{12}H_{18}N_2O_2.HCl$
3,4,5-$(OMe)_3$	H	81	2 HCl	163-168/0.3	203-204	48.01	6.82	8.61	47.81	6.63	9.06	$C_{13}H_{20}N_2O_3.2HCl$
3,4,5-$(OMe)_3$	Me	74	2 HCl	159-161/0.6	205-206	49.56	7.13	8.26	49.85	6.89	8.47	$C_{14}H_{22}N_2O_3.2HCl$
2,4,5-$(OMe)_3$	H	52	2 HCl	147-155/0.4	231-232	48.01	6.82	8.61	47.65	6.77	8.55	$C_{13}H_{20}N_2O_3.2HCl$
2,4,5-$(OMe)_3$	Me	55	HCl	160-170/2	227-229	55.53	7.66	9.25	55.27	7.30	9.32	$C_{14}H_{22}N_2O_3.HCl$
2,5-$(OMe)_2$-4-Me	H	48	2 HCl	142-150/0.2	242-243	50.49	7.17	9.06	50.18	6.89	8.94	$C_{13}H_{20}N_2O_2.2HCl$
2,5-$(OMe)_2$-4-Me	Me	56	2 HCl	145-147/1.5	231-233	52.02	7.48	8.67	52.19	7.28	9.01	$C_{14}H_{22}N_2O_2.2HCl$

(*a*) All recrystallised from ethanol-diethyl ether. (*b*) Ref. (9) m.p. 275°. (*c*) M.p. 40-41° (Cyclohexane). (*d*) M.p. 98-99° (éthanol). Ref. (8) m.p. 235-236°. (*f*). Salt

commercial sources or were synthesized at the Chemical Defence Establishment following published procedures.

a) *General.*

The rate of oxygen uptake during the reactions was measured polarographically using a modified Clark electrode built at CDE. The rate of formation of aminochromes from noradrenaline and dopamine was followed by measuring, spectrophotometrically using a Perkin-Elmer 137 spectrophotometer, the change in absorption at 490 nm. The oxidation of 5-hydroxytryptamine was measured by adding reduced nicotineamide adenine dinucleotide ($NADH_2$) to the reaction solution and measuring the rate of change in absorption at 340 nm, due to disappearance of $NADH_2$, using the Perkin-Elmer 137 Spectrophotometer. The rate of formation of oxidized nicotineamide adenine dinucleotide (NAD) from $NADH_2$ in the presence of either noradrenaline, dopamine, or 5-hydroxytryptamine was measured polarographically on a Southern Analytical Differential Cathode Ray Polarograph Type A 1660.

b) *Procedures.*

Effects of compounds on the oxidation of noradrenaline and dopamine by caeruloplasmin.

To a 2×10^{-3} M solution (5 ml) of L-noradrenaline or dopamine in 0.05 M acetate buffer at pH 5.9 was added 0.01 ml of a 5 % aqueous caeruloplasmin solution and the mixed solution was placed in a 10 mm path length spectrophotometer cell in the sample compartment of a Perkin-Elmer 137 Spectrophotometer. A similar cell containing only pH 5.9 acetate buffer was placed in the reference compartment. The cell compartments were maintained at 25° and the rate of increase in optical density at 490 nm was monitored using the read-out facility of the spectrophotometer attached to a Beckmann flat-bed recorder. This experiment was repeated using known concentrations of the test compound in 5 ml of 2×10^{-3} M noradrenaline or dopamine in pH 5.9 acetate buffer.

Effects of compounds on the oxidation of reduced nicotineamide adenine dinucleotide by caeruloplasmin in the presence of substrate. (*i*) *Spectrophotometrically.*

The procedure was essentially as described above using a reaction volume of 5 ml containing 10^{-3} M 5-hydroxytryptamine (or noradrenaline or dopamine) and 2×10^{-4} M reduced nicotineamide dinucleotide in pH 5.9 acetate buffer to which was added 0.01 ml of a 5 % aqueous caeruloplasmin solution. The rate of decrease in optical density at 340 nm was monitored as described above.

(ii) *Polarographically.*

A solution (5 ml) of 10^{-3} M 5-hydroxytryptamine (or noradrenaline or dopamine) containing reduced nicotineamide adenine dinucleotide (5×10^{-4} M) in pH 5.9 acetate buffer was placed in a polarographic cell, maintained at 25°, with a quiet mercury pool anode and a dropping mercury cathode. A 5 % aqueous solution (0.01 ml) of caeruloplasmin was then added and the solution mixed by passing a stream of air through it for 5 sec. The cell was then connected to the Cell 1 input of the A1660 Cathode Ray Polarograph and the rate of increase in peak height at — 0.9 V, due to the formation of oxidised nicotineamide adenine dinucleotide (NAD) was recorded; this was expressed as μM NAD produced per minute by reference to a previously constructed calibration curve. This procedure was then repeated with the addition of known concentrations of each of the compounds under study.

Effects of compounds on the rate of oxygen removal. Noradrenaline, dopamine, or 5-hydroxytryptamine (5 ml, 2×10^{-3} M) in pH 5.9 acetate buffer was placed in a thermostatted cell maintained at 25° and a Clark-type oxygen electrode was inserted. The solution was stirred magnetically whilst a potential of + 0.6 V was applied to the electrode from a Radiometer PO4 Polarograph. The resulting current was recorded as a function of time using the recorder on the polarograph. After 5 min caeruloplasmin (0.025 ml of a 5 % aqueous solution) was added and the current was recorded for a further 20 min. This procedure was repeated with known concentrations of each of the test compounds added to the reaction solution.

Substrate action of test compounds. This was investigated by substituting known concentrations of the test compounds for the standard substrates in the above procedures.

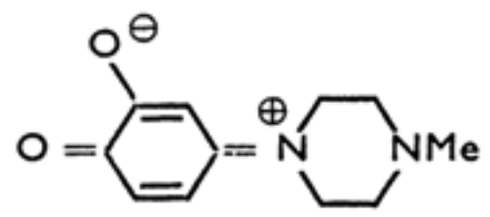
Pharmacology.

LSD normally produces a rise in rabbit rectal temperature of 1 °C for every 3 $\mu g.kg^{-1}$, i.v. (11). Since hyperthermia has been correlated with hallucinogenic potency (1, 10), the effect of the phenylpiperazines on LSD-induced hyperthermia in rabbits was measured using copper-constantin thermocouples (10).

Any intrinsic behavioural effects of the compounds were studied in Hall's open field test (12, 13), in which a novel environmental situation is used to evoke a stereotyped pattern of behaviour in rats.

Results and discussion.

The weak substrate activity for caeruloplasmin of the substituted 1-phenylpiperazines in the presence of reduced NAD ($NADH_2$) (table II) can be accounted for by the formation of a free radical. This process, involving removal of the hydrogen on the piperazine nitrogen, followed by abstraction of hydrogen from $NADH_2$ by the radical so formed, is analogous to the formation of the stable free radicals from N-alkylated aniline derivatives by caeruloplasmin (7, 14, 15). In the cases of 1-(3,4-dihydroxyphenyl)piperazine and 1-(4-hydroxyphenyl)-4-methylpiperazine, the reactive substrate action can be rationalised on the basis of the formation of highly coloured quinimines (e.g. V) by removal of hydrogen from the hydroxyl group. This process is directly analogous to the formation of aminochromes from dihydroxyphenylethylamines in the presence of caeruloplasmin (6, 15).

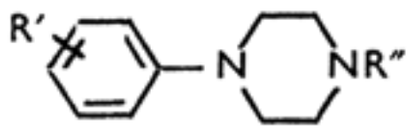


(V)

Only two compounds, 1-(4-hydroxyphenyl)-4-methylpiperazine and 1-methyl-4-(2,4,5-trimethoxyphenyl)-piperazine, inhibited NA oxidation and thereby had the opposite effect to LSD itself, but the latter

TABLE II

Results of substituted phenylpiperazines as substrates for caeruloplasmin and as modifiers of the caeruloplasmin-catalysed oxidation of noradrenaline (NA) and 5-hydroxytryptamine (5-HT).



R′	R″	Substrate Action (*a*)		Action as modifier (*b*)	
		$NADH_2 \rightarrow NAD$	Colour formation	on NA	on 5-HT
4-Cl	H	Weak	Nil	Acceleration	Inhibition
H	H	Nil	Nil	Nil	Nil
3,4-$(OH)_2$	H	+	+ (*d*) λ_{max} = 500 nm	Action masked (*c*)	
3,4,5-$(OMe)_3$	H	+	Nil	Acceleration	Masked (*c*)
3,4,5-$(OMe)_3$	Me	Weak	Nil	Nil	Nil
4-OMe	H	Weak	Nil	Acceleration	Slight inhibition
4-OMe	Me	Nil	Nil	Nil	Nil
3-OMe	H	Weak	Nil	Acceleration	Inhibition
2-OMe	H	Very weak	Nil	Slight acceleration	Slight inhibition
2,5-$(OMe)_2$-4-Me	H	+	+ λ_{max} = 575 nm	Acceleration	Inhibition
2,5-$(OMe)_2$-4-Me	Me	Nil	Nil	Nil	Nil
2,4,5-$(OMe)_3$	Me	Weak	Nil	Slight inhibition	Slight inhibition
4-OH	Me	+	+ λ_{max} = 520 nm	Slight inhibition	Masked (*c*)

(*a*) + indicates positive action; λ indicates maximum wavelength of coloured product.
(*b*) LSD strongly accelerates NA, and inhibits 5-HT, oxidations, while N,N-dimethyl-N′-(4-methoxyphenyl)ethylenediamine has the reverse effects.
(*c*) masking by colour of oxidation product, or by the formation of NAD from $NADH_2$ in the presence of the compound.
(*d*) $K_m = 3 \times 10^{-4}$ M.

compound also resembled LSD in inhibiting the oxidation of 5-HT (table II). Indeed, most of the compounds showed effects that were directly analogous to LSD, in that they accelerated NA oxidation and inhibited 5-HT oxidation. Despite this, however, they had little behavioural activity *per se* in the open field test, where 1-phenylpiperazine and its three different monomethoxylated derivatives showed effects at doses below 5 mg/kg (i.p., water) of a type associated with central depressant drugs. The effects were not well characterised and generally consisted of decreases in preening, rearing, and ambulation; LSD produces an excitatory syndrome in this test, consisting of increases in ambulation, and preening, and decreases in defaecation. None of the title compounds affected LSD-induced hyperthermia in rabbits, although two, 1-phenylpiperazine and 1-(4-methoxyphenyl)piperazine, increased rectal temperatures *per se* by 1 °C after 50 mn in doses of 10 mg/kg (i.v., water). However, 1-arylpiperazines have a wide pharmacological spectrum (8), and these effects may well be ascribable to unsuspected sympathomimetic (16) or dopaminergic (17) actions since non-hallucinogens can produce hyperthermia.

The lack of antagonistic activity towards LSD of the 1-phenylpiperazines is disappointing in view of the results with N,N-dimethyl-N'-(4-methoxyphenyl)-ethylenediamine, and the test still has to be made *in vivo* of a compound which has contrary effects to LSD in the caeruloplasmin assay. We feel that the use of caeruloplasmin as a model for the action of LSD on the central nervous system provides a valid approach to the searh for LSD antagonists. Nevertheless, it is only a model, and it is not clear whether effects upon caeruloplasmin-catalysed oxidations of NA and 5-HT have any relevance to the actual pharmacological properties of a compound *in vivo*. Thus, 2-bromolysergic acid diethylamide (BOL) has much the same effects as LSD upon these oxidations (5), yet BOL seems to be a true antagonist of LSD (2, 11). BOL is more potent as a peripheral antagonist of 5-HT than is LSD (18), but it does not have LSD's specific antagonism to the central excitatory actions of 5-HT (4). However, BOL is itself psychotomimetic at doses higher than those required for LSD-antagonism (19), and this demonstrated dose-dependence may well be manifest in relation to caeruloplasmin, since only relatively high doses were used in the initial studies (5).

Experimental section.

Melting points were determined in an Electrothermal capillary melting point apparatus and are uncorrected. Unless otherwise stated, all spectroscopic data were in accordance with the proposed structure.

1-Phenylpiperazines (table I).

(*a*) 1-(4-chlorophenyl)piperazine dihydrochloride, 1-(2-methoxyphenyl) piperazine dihydrochloride monohydrate, 1-(3-methoxyphenyl)piperazine dihydrochloride, 1-(4-methoxyphenyl)piperazine dihydrochloride, and 1-phenylpiperazine dihydrochloride, were purchased from R.N. EMANUEL. Limited and were purified by recrystallisation from ethanol-diethyl ether.

(*b*) In a typical example, 18.3 g (0.1 mole) of 3,4,5-trimethoxyaniline was refluxed for 30 hours with 2,2'-dichlorodiethylamine hydrochloride (17.8 g, 0.1 mole), anhydrous potassium carbonate (13.8 g, 0.1 mole), and 2-butoxyethanol (60 ml). The mixture was poured into water, extracted with ethyl acetate, washed with Nl NaOH and water, and dried ($MgSO_4$). Distillation gave 20.2 g (81 %) of a colourless oil, b.p. 163-168°/0.3 mm.

(*c*) Typically, 2.7 g (10 mmoles) of 1-(4-methoxyphenyl)-piperazine dihydrochloride was refluxed for 4 hours with 48 % HBr (20 ml). Excess HBr was removed under vacuum, and the brown solid residue was recrystallised from ethanol as white needles, m.p. 291-293°, yield 2.4 g (69 %).

SUMMARY.

A series of 1-phenylpiperazines was prepared by reaction of anilines with 2,2'-dichlorodiethylamine or its N-methyl derivative. Unlike their straightchain analogues the phenylethylenediamines, the 1-phenylpiperazines did not have opposite effects to those produced by lysergic acid diethylamide (LSD) on the caeruloplasmin-catalysed oxidations of 5-hydroxytryptamine and noradrenaline. Moreover, they did not reverse LSD-induced hyperthermia in rabbits and they had little behavioural activity *per se*. The significance of the use of caeruloplasmin as a model for the action of LSD on the central nervous system in discussed.

ZUSAMMENFASSUNG.

Eine Reihe von 1-Phenylpiperazinen wurde durch Einwirkung von Anilinen auf 2,2'-Dichloräthylmin oder dessen N-Methylderivat dargestellt. Im Gegensatz zu den analogen geradkettigen Phenyläthyldiaminen besitzen die 1-Phenylpiperazine keine Effekte, die den von Lysergsäurediäthylamid auf Caeruloplasmin-katalysierte Oxydationen von 5-Hydroxytryptophan und Noradrenalin hervorgerufenen entgegengestzt sind. Ueberdies verändern sie nicht die LSD-induzierte Hyperthermie bei Kaninchen und hatten wenig Wirkung auf das Verhalten. Die Bedeutung des Caeruloplasmins als Modell für dei Wirkung des LSD auf das zentrale Nervensystem wird diskutiert.

BIBLIOGRAPHY.

(1) A. HOFMANN in *Drugs Affecting the Central Nervous System*, Ed. by A. Burger, Edward Arnold, London, 1968, p. 169.
(2) A. HOFFER and H. OSMOND, *The Hallucinogens*, Academic Press, New York and London, 1967.
(3) A. HOFFER, *Manual for Treating Schizophrenia and Other Conditions using Megavitamin Therapy*, Modern Press, Saskatoon, 1967.
(4) R. J. BOAKES, P. B. BRADLEY, I. BRIGGS and A. DRAY, *Brit. J. Pharmacol.*, 1970, **40**, 202.
(5) B. C. BARRASS and D. B. COULT, *Biochem. Pharmacol.*, 1972, **21**, 677.
(6) J. A. MCDERMOTT, C. T. HUBER, S. OSAKI and E. FRIEDEN, *Biochim. biophys. Acta*, 1968, **151**, 541.
(7) P. O. GUNNARSSON, A. LINDSTROM and G. PETTERSSON, *Acta chem. scand.*, 1971, **25**, 770.
(8) P. C. JAIN, V. KAPOOR, N. ANAND, A. AHMAD and G. R. PATNAIK, *J. med. Chem.*, 1967, **10**, 812.
(9) V. PRELOG and Z. BLAZEK, *Collect. Czechosl. chem. Communic.*, 1934, **6**, 211.
(10) R. W. BRIMBLECOMBE, *Int. J. Neuropharmacol.*, 1967, **6**, 423.

(11) A. HORITA and J. H. GOGERTY, *J. Pharmacol. Exper. Therapeut.*, 1958, **122**, 195.
(12) C. S. HALL, *J. Compt. Psychol.*, 1934, **18**, 385.
(13) R. W. BRIMBLECOMBE, *Psychopharmacologia*, 1963, **4**, 139.
(14) J. PZEISACH and W. G. LEVINE, *J. biol. Chem.*, 1965, **240**, 2284.
(15) E. WALAAS and O. WALAAS, *Arch. Biochem. Biophys.*, 1961, **95**, 151.
(16) A. HORITA and A. E. HAMILTON, *Science*, 1969, **164**, 78.
(17) H. F. HILL and A. HORITA, *J. Pharm. Pharmacol.*, 1971, **23**, 715.
(18) H. ISBELL, E. J. MINER and C. R. LOGAN, *Psychopharmacologia*, 1959, **1**, 20.
(19) J. R. BERTINO, G. D. KLEE and H. WEINTRAUB, *J. Clin. exper. Psychopath.*, 1959, **20**, 218.