Amanita muscaria (Fr.) Hook, Eugster isolated an extremely unstable 4-substituted indole derivative having UV spectrum practically identical with that of psilocybin. Stein *et al.* also isolated an unknown compound from *Panaeolus venenosus* Murr. This compound also had an almost identical UV spectrum as psilocybin, with absorption maxima at 220, 265, and 288 mμ. Its high melting point (>250° dec.) suggests its identity with baeocystin.

The occurrence of baeocystin and norbaeocystin in *P. baeocystis* indicates that this fungus can serve as an appropriate organism for studying the biosynthesis and the metabolism of psilocybin. Earlier studies by Agurell *et al.* (8, 9) indicated that *Psilocybe cubensis* uses the following pathway in the biosynthesis of psilocybin: tryptophan → tryptamine → *N*-methyltryptamine → *N,N*-dimethyltryptamine → psilocin → psilocybin. Recent studies by Agurell and Nilsson (10) suggest that this fungus can also use an alternative route wherein phosphorylation precedes methylation. The high concentration of 4-hydroxytryptamine incorporated into psilocybin and the detection of psilocybin-like compounds were cited as evidence for this alternative pathway. The presence of psilocybin analogs in *P. baeocystis* suggests that the alternative pathway is probably the major route utilized by this fungus. Hence, it should be an ideal organism for further study of the alternative route.

The isolation of baeocystin and norbaeocystin offers a possibility of testing the serotonin hypothesis of mental illness (11). Norbaeocystin and baeocystin are the closest known serotonin analogs which have one of their enzyme-susceptible groups protected. Whether or not this may have any biochemical or pharmacological significance remains to be studied.

SUMMARY

The detection of unknown tryptamine derivatives in *P. baeocystis* has led to the isolation of two new compounds from submerged cultures of this fungus. The isolation procedure involved column chromatography of a methanol extract of the fungal tissue on powdered cellulose and on activated silicic acid. The structures of these compounds have been determined to be the monomethyl and demethyl analogs of psilocybin by TLC characteristics, color reactions, UV, IR, and mass spectral analyses. These compounds have been named baeocystin (monomethyl) and norbaeocystin (demethyl).

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Keyphrases

Psilocybin analogs—*Psilocybe baeocystis*
Baeocystin—isolation, identification
Norbaeocystin—isolation, identification
TLC—separation, identity
UV spectrophotometry—identity
IR spectrophotometry—identity
Mass spectroscopy—structure

N-Acyl Analogs of *N*-(2-Cyanoethyl)cyclohexylamine with CNS Activity

By W. D. ROLL

A series of substituted aryl amides of *N*-(2-cyanoethyl)cyclohexylamine was synthesized and screened for their effect on the central nervous system. Members of this series exhibit a pronounced effect on the spontaneous motor activity of mice, usually accompanied by varying degrees of activity on blood pressure in rats.

SUBSTITUTED AMIDE analogs of *N*-(2-cyanoethyl)cyclohexylamine (Abbott Laboratories) appear to act on the central nervous system either by stimulation or depression in small animals depending on the dosage. The compounds reported herein exert a depressant action on the spontaneous motor activity of mice at a dosage of 4

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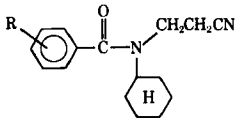
TABLE I—*N*-ACYL ANALOGS OF *N*-(2-CYANOETHYL)CYCLOHEXYLAMINE



Compd. No.	R	M.p., °C.	UV[a] λ, mμ	UV[a] ε	IR C=O	IR CN	Yield, %	Formula	Anal., % Calcd.	Anal., % Found
1	*p*-CH_3O-	77–78	282	1679	1,630	2,265	76.8	$C_{17}H_{22}N_2O_2$	C, 71.30 H, 7.74	C, 71.33 H, 7.78
2	*m*-CH_3O-	Oil[b]	282	2040	1,630	2,265	75.0	$C_{17}H_{22}N_2O_2$	C, 71.30 H, 7.74	C, 71.77 H, 7.83
3	*o*-CH_3O-	43–44	282	2611	1,630	2,265	74.5	$C_{17}H_{22}N_2O_2$	C, 71.30 H, 7.74	C, 71.45 H, 7.80
4	*p*-Cl-	93–94	266	1500	1,630	2,265	79.4	$C_{16}H_{19}ClN_2O$	C, 66.08 H, 6.58	C, 66.20 H, 6.50
5	*m*-Cl-	48–49	266	757	1,630	2,265	75.0	$C_{16}H_{19}ClN_2O$	C, 66.08 H, 6.58	C, 66.12 H, 6.63
6	*o*-Cl-	56–57	266	391	1,630	2,265	78.4	$C_{16}H_{19}ClN_2O$	C, 66.08 H, 6.58	C, 66.14 H, 6.60
7	*p*-CH_3-	74–75	264.5	1220	1,630	2,265	72.5	$C_{17}H_{22}N_2O$	C, 75.52 H, 8.20	C, 75.80 H, 8.25
8	*m*-CH_3-	48–49	264.5	1785	1,630	2,265	71.5	$C_{17}H_{22}N_2O$	C, 75.52 H, 8.20	C, 75.89 H, 8.29
9	*o*-CH_3-	56–57	264.5	451.6	1,630	2,265	72.5	$C_{17}H_{22}N_2O$	C, 75.52 H, 8.20	C, 75.92 H, 8.30

[a] Measured in 95% EtOH. [b] Purified by chromatography (eluted with petroleum ether) on silica gel, n_D^{25} 1.5390. TLC showed single spot.

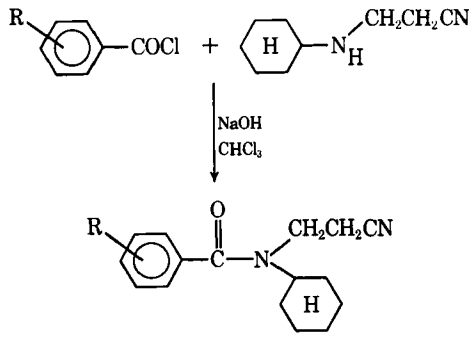
mg./kg. and, in addition, they produce a fall in blood pressure in rats. The methoxybenzyol analogs (Compounds 1, 2, 3) caused the most profound effects on both the central nervous system and the blood pressure; the chlorobenzoyl analogs (Compounds 4, 5, 6) demonstrated similar but intermediate activity; and the toluoyl analogs (Compounds 7, 8, 9) appeared to have the least effect. Within each group the *para*-substituted derivative was the most potent compound.

At a dosage of 2 mg./kg. the *para*- and *meta*-methoxybenzoyl analogs (Compounds 1 and 2) produced an increase in the spontaneous activity of mice, and the former compound antagonized the depressant action of perphenazine. The *para*-chlorobenzoyl analog (Compound 4) also increased the spontaneous motor activity of mice at this dosage.

EXPERIMENTAL

Analyses for carbon and hydrogen were obtained with a Coleman carbon-hydrogen analyzer. Melting points were determined using a Mettler FP-1 melting and boiling point apparatus. IR absorption spectra were obtained with a Perkin-Elmer model 137-B spectrophotometer and UV data were obtained with a Bausch and Lomb Spectronic 600.

Syntheses—The compounds were prepared (Scheme I) by a modification of the Schotten-Baumann (1, 2) reaction. A mixture of 15 ml. of chloroform, 0.01 mole of *N*-(2-cyanoethyl)cyclohexylamine, 60 ml. of 5% sodium hydroxide, and 0.01 mole of acyl halide was shaken in a separator at room temperature until the exothermic reaction was complete. The chloroform layer was washed with water, dried with anhydrous sodium sulfate, and evaporated *in vacuo* to give viscous yellow oils which crystallized on standing for a period of several weeks. The crude products were recrystallized from aqueous ethanol to give the pure products listed in Table I.



where R = *o*, *m*, *p*-OCH_3 (Compounds 3, 2, 1)
o, *m*, *p*-Cl (Compounds 6, 5, 4)
o, *m*, *p*-CH_3 (Compounds 9, 8, 7)

Scheme I

Pharmacology—The depressant/excitatory effects of the compounds were determined in mice with actophotometers (Metro Industries, Inc.). The experimental design and statistical treatment of data were that described by Watzman and co-workers (3). The total body movements of single animals were recorded at 30-min. intervals over a period of 2 hr. and the mean square root of the actual number of counts was plotted against time (Figs. 1–4). Twelve animals were used to study the effect of each compound at each dosage level.

Direct blood pressure measurements were conducted in Wistar rats under urethan anesthesia, 1.2

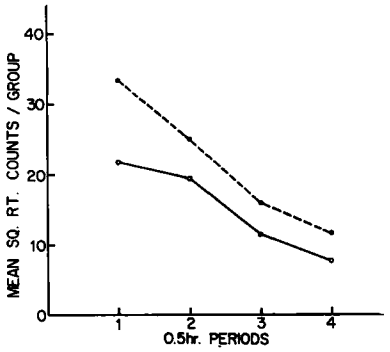


Fig. 1—The effect of N-(p-*methoxybenzoyl*)-N-(2-*cyanoethyl*)*amine at elevated dosage, 4 mg./kg. Key:* ●, *propylene glycol.*

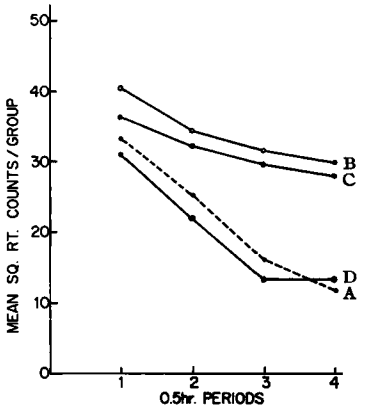


Fig. 2—The effects of N-*methoxybenzoyl analogs on the spontaneous motor activity of mice. Key:* A, *propylene glycol;* B, p-*methoxy;* C, m-*methoxy;* D, o-*methoxy.*

g./kg. i.p., with a mercury manometer connected to the carotid artery. Injections were made *via* the femoral vein. The effects of Compounds 1, 2, and 4 (Figs. 5–7) represent the mean responses of eight test animals.

Indirect blood pressure measurements were carried

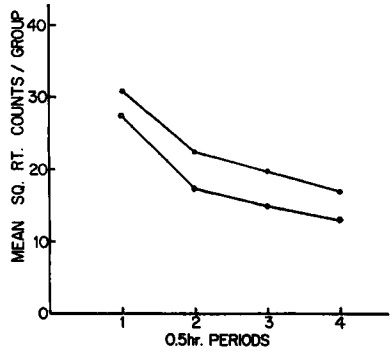


Fig. 3—Spontaneous motor activity of mice: the antagonizing effect of N-(p-*methoxybenzoyl*)-N-(2-*cyanoethyl*)*cyclohexylamine on perphenazine-treated animals. Key:* ◐, *perphenazine;* ●, *perphenazine +* p-*methoxybenzoyl analog.*

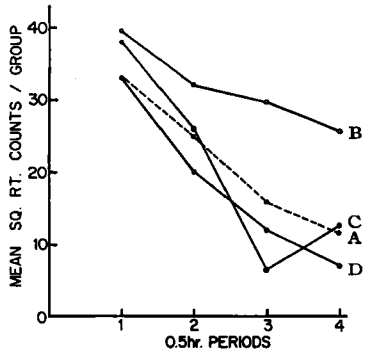


Fig. 4—The effects of N-*chlorobenzoyl analogs on the spontaneous motor activity of mice. Key:* A, *propylene glycol;* B, p-*chloro;* C, m-*chloro;* D, o-*chloro.*

out in normotensive Wistar rats using the photoelectric tensometer (Metro Industries, Inc.). The rats were trained for indirect systolic blood pressure determinations and the methodology was that of Coates *et al.* (4). Each systolic blood pressure value in Fig. 8 represents the mean response of eight animals.

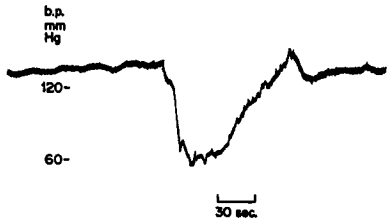


Fig. 5—The effect of Compound 1 on blood pressure in the rat.

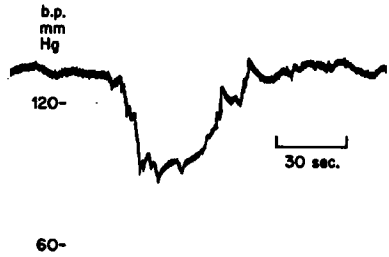


Fig. 6—The effect of Compound 2 on blood pressure in the rat.

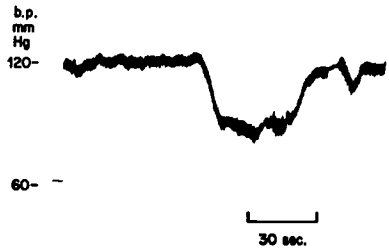


Fig. 7—The effect of Compound 4 on blood pressure in the rat.

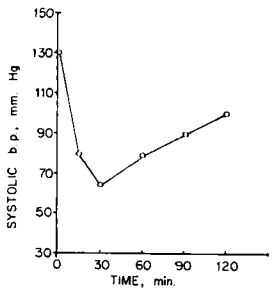


Fig. 8—The effect of Compound 1 on systolic blood pressure, 4 mg./kg. i.p.

RESULTS

The oral administration of 4 mg./kg. of Compound 1, *N*-(*p*-methoxybenzoyl)-*N*-(2-cyanoethyl)cyclohexylamine, in propylene glycol to mice resulted in a significant reduction in the spontaneous motor activity of mice (Fig. 1). A quieting or "taming" effect was seen in the animal at this dosage. Ataxia was clearly discernable in the animal at elevated dosages, *i.e.*, 10 mg./kg. The rat responded even more dramatically to elevated dosages, assuming a cataleptoid posture.

At slightly lower oral dosages, *i.e.*, 2 mg./kg. in propylene glycol, Compounds 1 and 2, *N*-(*m*-methoxybenzyl)-*N*-(2-cyanoethyl)cyclohexylamine, produced excitation in mice, while Compound 3, the *o*-methoxybenzoyl analog caused a slight reduction in total activity (Fig. 2). This dosage level, in the case of Compound 1, rather effectively antagonized the tranquilizing activity of orally, simultaneously administered 0.4 mg./kg. doses of perphenazine (Fig. 3).

Oral doses, 2 mg./kg. in propylene glycol, of Compound 4, *N*-(*p*-chlorobenzoyl)-*N*-(2-cyanoethyl)-cyclohexylamine, produced a high degree of excitation (Fig. 4), but its antagonism to 0.4-mg./kg. doses of perphenazine was insignificant.

Although depression was the predominant symptom of animals receiving 4 mg./kg. of this compound, the degree of reduction in spontaneous motor activity was much less than in the case of Compound 1.

The *m*-chlorobenzoyl analog, Compound 5, caused excitation during the first hour of measurement, but spontaneous activity rapidly decreased during the next 1.5-hr. period. Like Compound 3, the *o*-chlorobenzoyl analog (Compound 6) produced a slight reduction in total activity.

Blood pressure is markedly depressed by Compound 1, and to a somewhat lesser degree by Compounds 2 and 4 by doses of 4 mg./kg. i.v. (Figs. 5–7).

Compound 1 produced a significant hypotensive effect when administered intraperitoneally to unanesthetized normotensive rats (Fig. 8).

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Keyphrases

N-(2-Cyanoethyl)cyclohexylamine, *N*-acyl analogs—synthesis
CNS activity—*N*-(2-cyanoethyl) cyclohexylamine *N*-acyl analogs
UV spectrophotometry—structure
IR spectrophotometry—structure

Antitumor Activity of *Juglans nigra* (Black Walnut) Extractives

By UMESH C. BHARGAVA and BERTIS A. WESTFALL

Antitumor activity of compounds present in *Juglans nigra* were studied on spontaneous and/or transplanted tumors in mice. Ellagic acid, juglone, and isolated fractions (strong acids, weak acids, and alkaloids) were injected intraperitoneally for 9–12 days. The results showed that ellagic acid, juglone, and the "strong acids" fraction depressed the tumor growth rate significantly.

CAIN (1) observed that certain water-soluble polyphenolic compounds have the property to retard the growth rate of some experimental rodent tumors; however, no proof was shown experimentally. Furthermore, certain quinone derivatives (2) have been observed to increase the life span and decrease the tumor size in mice. Since some species of walnut (3–5) contain polyphenolic and/or quinone constituents, and also since *Juglans nigra* has been reported to inhibit the growth rate of some other plants (6), it was thought that active principles of walnut

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