Synthesis of 2-Diphenylmethylene-1-acyl-1-alkyl Hydrazines as Potential Psychotropic Agents

FRED DeWITT REED, Jr.*, ROBERT G. BROWN, JAIME N. DELGADO, and EUGENE I. ISAACSON▲

Abstract □ A series of 2-diphenylmethylene-1-acyl-1-alkyl hydrazines was synthesized as potential psychotropic agents. The investigation was prompted by evidence of MAO inhibition by hydrazine derivatives *in vivo* in which alkyl hydrazine metabolites were implicated as the active agents. The synthetic route to the compounds involved the following steps: hydrazonation of benzophenone, hydrazone oxidation to diphenyldiazomethane, alkylation of diphenyldiazomethane with Grignard reagents, and acylations of the anion formed in the course of the Grignard alkylation. A preliminary pharmacological evaluation of the compounds, based on protection against reserpine-induced hypothermia and ptosis, was conducted and the results are presented.

Keyphrases □ 2-Diphenylmethylene-1-acyl-1-alkyl hydrazines—synthesis, potential psychotropic agents □ Hydrazines, 2-diphenylmethylene-1-acyl-1-alkyl—synthesis, potential psychotropic agents □ Psychotropic agents, potential—2-diphenylmethylene-1-acyl-1-alkyl hydrazines synthesized □ Structure–activity relationships—2-diphenylmethylene-1-acyl-1-alkyl hydrazines as potential psychotropic agents

In numerous studies involving MAO inhibitory alkyl hydrazines and 1-acyl-2-alkyl hydrazines, the acyl derivatives usually have exhibited more pronounced antidepressant properties than the parent alkyl hydrazines (1). This increased activity has been attributed to the acyl group acting as a carrier, which is subject to removal *in vivo*, yielding the alkyl hydrazine near a binding site in the "target organ" (1).

Recent literature reports describing *in vivo* inhibition of liver MAO by furazolidone, 3-[(5-nitrofurfurylidene)-amino]-2-oxazolidinone, are also worthy of note (2, 3). Partial biological degradation of the molecule has been implicated as leading to bioactivation (2). Evidence for the formation of 2-hydroxyethylhydrazine, implicated as the actual inhibitory agent, has been offered (2), and a plausible bioactivation sequence involving an initial hydrolysis of the alkylidene hydrazine moiety of furazolidone followed by hydrolysis of the oxazolidinone ring has been proposed (2) (Scheme I).

The foregoing work of Stern *et al.* (2) provides evidence that furazolidone *per se* is not an inhibitor of MAO, and that during metabolism *in vivo*, furazolidone is degraded to the MAO inhibitor 2-hydroxyethylhydrazine. A review of the literature revealed antidepressants (4, 5) and MAO inhibitors (6) which could conceivably exert their pharmacological activity in similarly biodegraded forms.

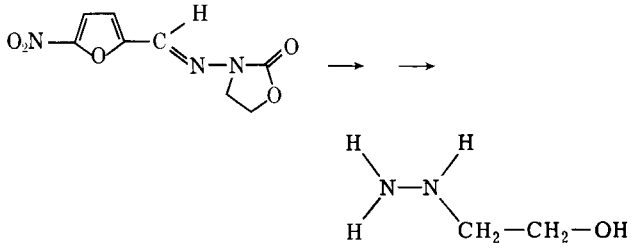
Thus, it would appear that the acyl group and the alkylidene link can serve as latentiating functions. Hence, incorporation of these two latentiating moieties into alkyl hydrazines introduces for investigation and evaluation two bioactivity parameters in the area of hydrazine-derived MAO inhibitory antidepressants. Accordingly, a series of 2-diphenylmethylene-1-acyl-1-alkyl hydrazines was synthesized with the premise in mind that both the diphenylmethylene moiety and the acyl moiety would function as carrier groups. The compounds, therefore, were anticipated to have central stimulatory properties. The compounds synthesized were subjected to a preliminary pharmacological evaluation of central activity based upon their effects upon reserpine-induced hypothermia and ptosis in mice.

DISCUSSION

Benzophenone was chosen as the ketone from which to derive the hydrazones, since it was felt that the resulting diphenylmethylene moiety would confer upon the compounds a high degree of lipophilicity and, in any event, should favor distribution to the CNS. In this connection, it is worth noting that many compounds possessing the diphenylmethyl group, *e.g.*, methadone, pipradrol, diphenhydramine, benztropine, azacyclonol, and diphenylhydantoin, have central activities which ought to be dependent, at least in part, upon the capability of this system to impart to these compounds an ability to reach the brain. The compounds reported on in this paper have the structurally closely related diphenylmethylene group. An analogy can also be drawn between the compounds described here and centrally active compounds that can be construed to include this grouping, *e.g.*, certain benzodiazepines and dibenzosuberone-derived antidepressants.

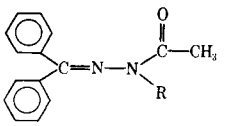
The acyl groups are acetyl, benzoyl, and phenylacetyl. These groups were chosen so that there would be a relationship between the three series that might permit preliminary observations on the effect of phenyl substitution (independent of location relative to the carbonyl) in the acyl group on bioactivity in the synthesized compounds. Additionally, it was felt that the choice of these acyl groups might permit reasonable conjecture as to the influence of the factors associated with the phenyl, *e.g.*, electronic factors contributing to stability to hydrolysis and factors affecting lipophilicity which are also important in determining bioactivity changes. It was felt that structure–activity relationship comparisons between the three series might indirectly provide an indication of the relative importance of these two factors.

To illustrate, the progression from acetyl to benzoyl may be considered. Two factors are altered. First, the phenyl of the benzoyl contributes more hydrophobic character than the methyl of the acetyl. Second, electronic factors determining stability toward hydrolysis are altered. Since the phenyl is in conjugation with the carbonyl, the electron-donating effect of the phenyl *via* resonance is introduced. The electron-withdrawing inductive effect of the phenyl is also operative but is perhaps overshadowed by the resonance effect. Hence, the benzoyl series can be expected to differ with respect to susceptibility to hydrolysis relative to the acetyl series to the extent that the cited electronic factors are operative. Since two



Scheme I

Table I—2-Diphenylmethylene-1-acetyl-1-alkyl Hydrazines

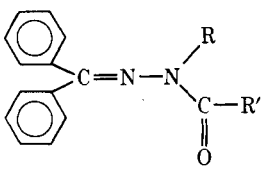


R	Diphenyldiazomethane, g., mole	Grignard Reagent, mole	Acetyl Chloride, ml., mole	Yield, g., %	Melting Point	Empirical Formula	Analysis, % Calc.	Found
a: CH_3	6.8, 0.035	0.04	2.3, 0.035	5.5, 63	81–82.5°	$C_{16}H_{16}N_2O$	C 76.16	76.06
							H 6.39	6.66
							N 11.11	11.33
b: CH_2CH_3	12.0, 0.061	0.063	4.28, 0.060	11.0, 69	78–79.5°	$C_{17}H_{18}N_2O$	C 76.67	76.84
							H 6.81	6.70
							N 10.52	10.28
c: $CH_2CH_2CH_3$	12.0, 0.061	0.063	4.28, 0.060	9.5, 57	60–62°	$C_{18}H_{20}N_2O$	C 77.09	76.92
							H 7.19	7.30
							N 9.99	9.87
d: $CH(CH_3)_2$	12.0, 0.061	0.063	4.28, 0.060	9.4, 56	88–89.5°	$C_{18}H_{20}N_2O$	C 77.09	76.91
							H 7.19	7.14
							N 9.99	9.87
e: $CH_2(CH_2)_2CH_3$	11.0, 0.0556	0.06	3.75, 0.05	6, 41	47–48.5°	$C_{19}H_{22}N_2O$	C 77.52	77.36
							H 7.54	7.47
							N 9.51	9.76

parameters are changed, lipophilicity and susceptibility to hydrolysis due to electronic differences, interpretations as to which parameter change was principally associated with bioactivity change would be difficult.

For this reason, the phenylacetyl series was included. The phenylacetyl group has the $-CH_2-C(-)=O$ group in common with the acetyl and the lipophilic phenyl in common with the benzoyl. In the phenylacetyl series, the electron-withdrawing inductive effect of the phenyl might come into play but not the electron-donating resonance effect as is the case in the benzoyl series. Since the electron-withdrawing effect is important in determining stability toward hydrolysis, members of the phenylacetyl series should display an increased sensitivity toward hydrolysis. Considering lipophilicity, the phenyl in both series will operate in the same direction; in both series there will be increased lipophilicity associated with the phenyl. Since the phenyl operates in one direction in the benzoyl series and in another direction in the phenylacetyl series with regard to stabilizing toward hydrolysis and operates in the same direction in both series with respect to lipophilicity changes, if bioactivity changes do appear when the phenyl is incorporated in the series, the bioactivity changes may be tentatively assigned either to electronic changes in the carbonyl influencing metabolic stability or to changes in the lipophilicity influencing distribution. Hence, the acyl groups may permit some judgments as to how the phenyl in the acyl group is influencing bioactivity.

Since it was felt that the most tenable structure-activity relationship for this series could be drawn among very closely related compounds, it was decided to vary the *N*-alkyl group systematically. Thus the *N*-alkyl group was varied through the homologous series: methyl, ethyl, *n*-propyl, and *n*-butyl for each of the three hydrazones, in this way permitting an evaluation of the effect of chain length of this group upon bioactivity. Isopropyl as the alkyl substituent was included to examine the effect of alkyl branching upon bioactivity.



general structure

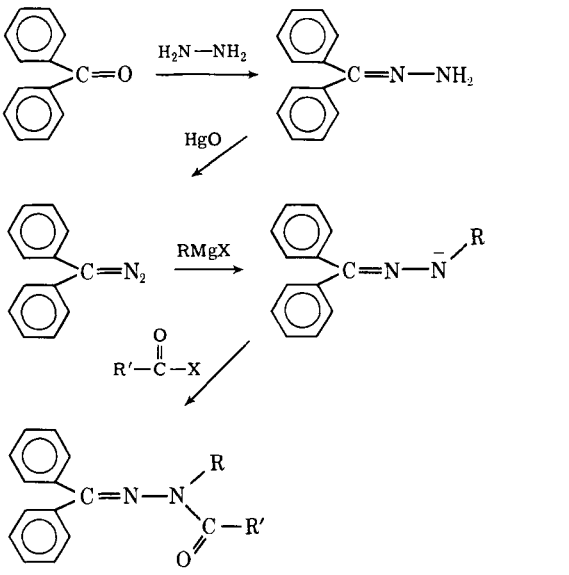
R′=	R=
I: CH_3	*a*: CH_3
II: C_6H_5	*b*: CH_2CH_3
III: $CH_2C_6H_5$	*c*: $CH_2CH_2CH_3$
	d: $CH(CH_3)_2$
	e: $CH_2(CH_2)_2CH_3$

To summarize the preceding discussion, the compounds synthesized have a general structure, where R′ identifies the three acyl series I, II, and III, each of which was varied from R = *a* through R = *e*.

Scheme II depicts the principal steps in the preparative procedures by which the compounds were synthesized.

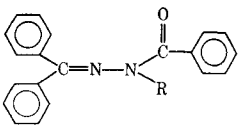
The first step in the synthesis of the compounds described was the condensation of benzophenone and hydrazine in a sealed tube to yield benzophenone hydrazone according to the method of Barton *et al.* (7). Two base-catalyzed (potassium hydroxide and triethylamine) procedures by Barton *et al.* (7) were also employed. However, the sealed-tube procedure yielded a product that could be more easily purified so it was used routinely. Next, benzophenone hydrazone was oxidized to diphenyldiazomethane using a modified method of Miller (8) which employed yellow mercuric oxide under basic (ethanolic potassium hydroxide) catalysis. The original method of Miller employs anhydrous ether as solvent. Since this solvent consistently yielded inferior products, anhydrous petroleum ether was substituted and was found to give satisfactory products.

A procedure of Smith (9), using manganese dioxide as the oxidizing agent, was also employed. The initial rate of oxidation was high but diminished rapidly, resulting in incomplete oxidation. Hence, use of this method was discontinued in favor of the modified method of Miller. The appropriate Grignard reagent was slowly added with



Scheme II

Table II—2-Diphenylmethylene-1-benzoyl-1-alkyl Hydrazines



R	Diphenyldiazomethane, g., mole	Grignard Reagent, mole	Benzoyl Chloride, ml., mole	Yield, g., %	Melting Point	Empirical Formula	Analysis, % Calc.	Found
a: CH_3	3.0, 0.0155	0.017	1.61, 0.014	3.4, 77	77.5–78.5°	$C_{21}H_{18}N_2O$	C 80.04	80.08
							H 5.77	5.73
							N 8.91	8.72
b: CH_2CH_3	12.0, 0.061	0.065	6.85, 0.059	9, 47	92.5–94°	$C_{22}H_{20}N_2O$	C 80.46	80.32
							H 6.14	6.20
							N 8.53	8.49
c: $CH_2CH_2CH_3$	11.0, 0.0556	0.06	10.0, 0.08	11, 58	75.5–77°	$C_{23}H_{22}N_2O$	C 80.71	80.70
							H 6.48	6.61
							N 8.19	8.06
d: $CH(CH_3)_2$	11.0, 0.0556	0.06	10.0, 0.08	7, 37	169.5–170.5°	$C_{23}H_{22}N_2O$	C 80.71	80.68
							H 6.48	6.73
							N 8.19	8.02
e: $CH_2(CH_2)_2CH_3$	10.5, 0.054	0.06	10.0, 0.08	16, 83.5	80–82°	$C_{24}H_{24}N_2O$	C 80.87	80.76
							H 6.79	6.72
							N 7.86	7.77

stirring under an atmosphere of nitrogen to diphenyldiazomethane in anhydrous ether cooled to −4 to −15° according to a method of Coleman *et al.* (10). The reaction mixture was allowed to stir for an additional 0.5 hr., and the respective acid chloride or (for IIc, IId, and IIe) the methyl ester of the acid was then slowly added. The reaction mixture was allowed to stand at room temperature for 0.5 hr. for the acid chloride or for 1 hr. when the methyl ester was the acylating agent; it was then cooled again to −4° and a saturated solution of ammonium chloride was added. The ether layer was collected and diluted with two volumes of anhydrous petroleum ether, and the volume was reduced under reduced pressure until precipitation appeared to be complete. Column chromatography on silica was required for the separation and purification of IIIc, IIId, and IIIe.

IR and NMR spectrometry were utilized in the characterization of all final products. All spectra (IR and NMR) were in agreement with the assigned structures (Tables I–III).

EXPERIMENTAL[1]

Benzophenone Hydrazone—*Method I*—A method described by Barton *et al.* (7) was tested using benzophenone (3.53 g., 0.019 mole) and hydrazine 95% (6.5 ml., 0.2 mole) in 10% alcoholic potassium hydroxide solution (10 ml.). The reaction mixture was heated on a steam bath with constant stirring for 2.5 hr. Water was added until permanent cloudiness occurred; then the solution was chilled. The precipitate (yield: 2.7 g., 70%) melted after crystallization from aqueous alcohol at 97–98°.

Method II—Another method according to Barton *et al.* (7) was used in which benzophenone (3.53 g., 0.019 mole), hydrazine 95% (6.5 ml., 0.2 mole), and triethylamine (25 ml.) were heated on a water bath while being stirred for 1.5 hr. The triethylamine was then removed by distillation under reduced pressure. The product (yield: 3.38 g., 91%), when crystallized from aqueous alcohol, melted at 97–98°. The melting point of a 50% mixture with the product from Method I was undepressed.

Method III—A sealed-tube reaction was carried out according to a modified method of Barton *et al.* (7). Benzophenone (20 g., 0.12 mole) and hydrazine 64% (12 ml., 0.24 mole) were placed in a hydrogenation vessel, which was then sealed and heated in a silicone bath at 150° for 4 hr. The reaction mixture was allowed to cool to room temperature, and the contents were dissolved in hot alcohol. The solution was filtered and chilled, and the precipitate was collected (yield: 20.9 g., 89%). Crystallization from alcohol afforded brownish-white crystals which melted at 97–98°. The product was found to be identical with those of Methods I and II through mixed melting-point determinations and IR spectra.

Diphenyldiazomethane—*Method I*—According to the method of Miller (8), benzophenone hydrazone (13 g., 0.066 mole), anhydrous sodium sulfate (15 g.), anhydrous diethyl ether (200 ml.), ethanol saturated with potassium hydroxide (5 ml.), and yellow mercuric oxide (35 g., 0.16 mole) were mechanically shaken in a stoppered hydrogenation bottle wrapped with a wet towel for 1.25 hr. The purple solution was filtered, and the ether was evaporated under reduced pressure at ambient temperature in a rotary evaporator[2]. The purple residue was then washed with anhydrous petroleum ether, leaving benzophenone hydrazone (6 g.) as a precipitate. TLC of the purple petroleum ether extract revealed the presence of four different aromatic products. Further investigation of the mixture was not pursued.

Method II—The method of Miller (8) was modified using benzophenone hydrazone (13 g., 0.066 mole), anhydrous sodium sulfate (15 g.), anhydrous petroleum ether (200 ml.), ethanol saturated with potassium hydroxide (5 ml.), and yellow mercuric oxide (35 g., 0.16 mole), which were mechanically shaken in a stoppered hydrogenation bottle wrapped in a wet towel for 2.5 hr. The purple solution was filtered, and the volume was decreased under reduced pressure at ambient temperature. The oily residue was chilled to −20° and crystallized. The product (yield: 11.8 g., 90%) had a melting range of 28–31° [lit. (8) m.p. 29–32°].

Method III—Another modified method (8) used benzophenone hydrazone (13 g., 0.066 mole), anhydrous sodium sulfate (15 g.), anhydrous petroleum ether (200 ml.), ethanol saturated with potassium hydroxide (5 ml.), and red mercuric oxide (35 g., 0.16 mole), which were placed in a hydrogenation bottle. The bottle was then stoppered, wrapped with a wet towel, and mechanically shaken for 2.5 hr. The solution was filtered, and the solvent was evaporated under reduced pressure at ambient temperature. The oily residue crystallized at −20° (yield: 11.6 g., 90%) and melted at 29–31°.

Method IV—In a hydrogenator bottle were placed benzophenone (13 g., 0.066 mole), anhydrous sodium sulfate (15 g.), anhydrous petroleum ether (200 ml.), ethanol saturated with potassium hydroxide (5 ml.), and activated manganese dioxide (9) (13.9 g., 0.16 mole). The bottle was then stoppered, wrapped in a wet towel, and mechanically shaken for 2.5 hr. Oxidation proceeded rapidly at first but slowed appreciably after 30 min. After 2.5 hr., the reaction mixture was filtered, and the petroleum ether was evaporated, leaving a residue largely composed of benzophenone hydrazone. The mixture was not investigated further.

2-Diphenylmethylene-1-acyl-1-alkyl Hydrazines—A 250-ml. three-necked flask was gassed for 15 min. with nitrogen which had

[1] The melting points are uncorrected. A Thomas-Hoover Uni-Melt apparatus was used for melting-point determinations. Elemental analyses were conducted by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. IR spectra of all final products were measured on a Beckman IR-8 spectrophotometer. NMR spectra for these compounds were obtained with a Varian A-60 spectrometer (tetramethylsilane as internal standard). In the text, alcohol refers to alcohol USP (C_2H_5OH 95%) and petroleum ether to petroleum ether of the boiling range 30–60°.

[2] Rinco.

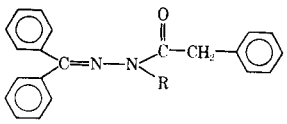


Table III—2-Diphenylmethylene-1-phenylacetyl-1-alkyl Hydrazines

R	Diphenyldiazomethane, g., mole	Grignard Reagent, mole	Phenylacetyl Chloride, ml., mole	Yield, g., %	Melting Point	Empirical Formula	Analysis, % Calc.	Found
a: CH_3	12.0, 0.061	0.065	7.9, 0.060	14.4, 73	81–82.5°	$C_{22}H_{20}N_2O$	C 80.46	80.28
							H 6.14	6.14
							N 8.53	8.76
b: CH_2CH_3	12.0, 0.061	0.065	7.9, 0.060	8.0, 39	59.5–61°	$C_{23}H_{22}N_2O$	C 80.71	80.64
							H 6.48	6.75
							N 8.19	8.28
c: $CH_2CH_2CH_3$	12.0, 0.061	0.065	7.9, 0.060	3.2, 15	Oil	$C_{24}H_{24}N_2O$	C 80.87	80.70
							H 6.79	6.99
							N 7.86	7.58
d: $CH(CH_3)_2$	12.0, 0.061	0.065	7.9, 0.060	3.5, 18	Oil	$C_{24}H_{24}N_2O$	C 80.87	80.59
							H 6.79	6.54
							N 7.86	7.67
e: $CH_2(CH_2)_2CH_3$	12.0, 0.061	0.065	7.9, 0.060	3.6, 18	Oil	$C_{25}H_{26}N_2O$	C 81.04	80.97
							H 7.07	7.34
							N 7.56	7.30

been passed through two gas washing bottles, the first containing alkaline pyrogallol (5%) solution and the second containing concentrated sulfuric acid. Then, into this flask, equipped with a mercury-sealed mechanical stirrer, a dropping funnel with a pressure equilibration tube, and a gas inlet joint, was poured diphenyldiazomethane dissolved in 50 ml. of sodium-dried anhydrous ether. The flask was chilled to −4 to −15°, and the appropriate amount of Grignard reagent was placed in the dropping funnel. The system was sealed, and the nitrogen flow was adjusted to maintain a positive pressure in the system; the pressure was monitored by observing the level of mercury in a glass U-tube, one side of which was connected to the system and the other exposed to the atmosphere.

After pressure stabilization, the Grignard reagent was slowly dropped into the ethereal solution of diphenyldiazomethane, with stirring, at a rate that did not alter the pressure in the system. The dropping interval was varied from 50 to 15 min. inversely with the temperature of the bath. The reaction mixture was stirred for 30 min. after the addition of the Grignard reagent. The appropriate acid chloride or methyl ester of the acid was added to the dropping funnel and then added slowly to the reaction mixture at a rate that did not alter the pressure in the sealed system. The acid chloride addition reaction mixture was allowed to stand 0.5 hr., the methyl ester, 1.0 hr. A saturated solution of ammonium chloride (38 g./100 ml. of water) (9) was added through a dropping funnel, the rate determined by pressure generated in the system, until all magnesium salts were precipitated. The ether layer was collected in a separator and was diluted with two volumes of anhydrous petroleum ether. The volume was decreased under reduced pressure until either crystals or a gummy residue was formed.

The compounds thus obtained were recrystallized from petroleum ether–ether (2:1) except for I*b*, I*e*, and II*d*, where the solvents of recrystallization were petroleum ether–benzene (6:1), petroleum ether–ether (1:1), and benzene, respectively. Residues of the three higher molecular weight members of the phenylacetyl series (III*c*, III*d*, and III*e*) did not crystallize when allowed to stand in a minimum amount of anhydrous solvent, ether–petroleum ether, at −13° for 1 week. In the case of each of these residues, preliminary examination *via* TLC with ether–petroleum ether (2:1) on silica gel HF_{254} according to Stahl led to resolution into four components. The residues were then chromatographed on silica using a 30:1 ratio of adsorbent to crude product and ether–petroleum ether (1:1), benzene–methanol (99:1), and benzene–methanol (49:1) as the eluting solvents, affording the analytically pure products III*c*, III*d*, and III*e*, respectively. These pure compounds resisted all attempts at crystallization, including thermal gradient sublimation and a variety of solvent systems. However, correct elemental analyses, NMR and IR spectra, and movement as a single spot in TLC indicated purity.

Pertinent preparative data and physical constants for each of the compounds synthesized are given in Tables I–III.

PHARMACOLOGICAL EVALUATION

All of the 2-diphenylmethylene-1-acyl-1-alkyl hydrazines were subjected to a preliminary pharmacological evaluation which was based on protection against reserpine-induced hypothermia and ptosis.

Method—The method used in the investigation of protection against reserpine hypothermia was essentially that of Garattini *et al.* (11), and the parallel study of ptosis protection afforded by the test compounds was based on a report by Fujita and Tedeschi (12).

Swiss male mice, 20–29 g., were injected intraperitoneally with isomolar doses of the alkyl homologs in each acyl series of the substituted hydrazone test compounds. The test doses for each of these series, derived from preliminary toxicity data, were: acetyl, 8.82×10^{-4} mole/kg.; benzoyl, 1.21×10^{-3} mole/kg.; and phenylacetyl, 2.20×10^{-3} mole/kg. Food and water were provided *ad libitum*. Members of the acetyl and benzoyl series were tested in 2.0% acacia suspensions, whereas the phenylacetyl series was tested in polysorbate 80 USP[3] suspensions because of insufficient dispersability in the acacia system. Control experiments on the suspending agents indicated that they did not interfere with the pharmacological evaluations. Eight hours after injection of the test compound, colonic temperatures were recorded by insertion of an electric thermometer[4] probe 1.5 cm. into the rectum. Reserpine (2 mg./kg.) was then injected. Body temperatures were monitored, and ptosis observations were made at 4-, 6-, and 24-hr. intervals. Ptosis determinations were performed according to the method of Fujita and Tedeschi (12), ptosis being defined as 15 sec. or more of uninterrupted closure (70% or more) of the palpebral fissure during a 90-sec. observation period while the animal is isolated on a cage top.

Results—The experimental results are listed in Table IV.

Some preliminary relationships are apparent. Only the *n*-butyl homolog showed protection against both hypothermia and ptosis in all three series, being more statistically significant at the 24-hr. interval. This, therefore, seems to parallel an *in vitro* study in which the *n*-butyl homolog of some isonicotinyl alkyl hydrazines demonstrated optimal MAO inhibition (13).

The compound that exhibited the greatest deviation from other members of the same acyl series with respect to both high antireserpine activity and toxicity was 2-diphenylmethylene-1-phenylacetyl-1-isopropylhydrazine. This result is different from a study on the effect of α-methylation of isonicotinyl alkyl hydrazines (13) in which the ethyl homolog was more inhibitory than the isopropyl.

[3] Tween 80.
[4] Tri-R model TML, Tri-R Instruments, Jamaica, N. Y.

Table IV—Effects of Synthesized Compounds upon Reserpine-Induced Hypothermia and Ptosis in Mice (10 Mice per Group)

R	0–4 hr. Mean ΔT[a]	0–4 hr. p* Value[b]	0–4 hr. Ptosis[c]	0–6 hr. Mean ΔT	0–6 hr. p* Value	0–6 hr. Ptosis	0–24 hr. Mean ΔT	0–24 hr. p* Value	0–24 hr. Ptosis
2-Diphenylmethylene-1-acetyl-1-alkyl Hydrazines (Dose: 8.82×10^{-4} mole/kg.)									
Methyl	−3.3	0.001	10	−4.4	0.1		−1.5	0.01	17
Ethyl	−2.2	0.01		−2.6	0.001		−0.2	0.002	
n-Propyl	−4.3	ns[d]	20	−4.9	ns		−0.8	0.001	67
Isopropyl	−3.5	0.05		−3.5	0.001		−0.1	0.001	
n-Butyl	−3.0	0.1	50	−4.8	0.1	10	−1.4	0.01	83
2-Diphenylmethylene-1-benzoyl-1-alkyl Hydrazines (Dose: 1.21×10^{-3} mole/kg.)									
Methyl	−3.6	ns	20	−3.4	0.002	30	−0.6	0.001	17
Ethyl	−4.5	ns		−5.2	ns		−2.3	ns	
n-Propyl	−3.3	0.1	30	−4.1	0.01		−2.2	0.1	
Isopropyl	−2.9	0.05	30	−4.3	0.02	20	−1.5	0.01	33
n-Butyl	−2.4	0.02	80	−3.0	0.001	50	−1.0	0.001	83
2-Diphenylmethylene-1-phenylacetyl-1-alkyl Hydrazines (Dose: 2.20×10^{-3} mole/kg.)									
Methyl	−3.8	ns	20	−4.9	ns		−3.1	ns	20
Ethyl	−3.7	0.1		−4.8	0.1		−3.0	ns	
n-Propyl	−3.5	0.1	60	−2.7	0.001	30	−1.8	0.05	20
Isopropyl[e]	−0.5	0.001	100	−1.4	0.001	100	−0.9	0.002	100
n-Butyl	−2.2	0.05	70	−2.2	0.001	20	−1.1	0.001	80
Reserpine Controls (Dose: 2 mg./kg.)									
	−4.5			−5.9			−3.2		

[a] Mean ΔT = average temperature depression. [b] p value as determined by statistical evaluation using Student's t test. [c] Number of animals protected from reserpine-induced ptosis expressed as percent:

$$\% = 100 - \left(\frac{\text{experimental}}{\text{control}}\right) \times 100$$

Zero and negative values have been deleted. [d] ns = not significant. [e] Five of the experimental animals died within 12 hr. after administration of the test compound.

The most active acyl-substituted alkyl hydrazines are the acetyl, which may be related to the lower lipophilicity of the series. This activity relationship may be reflected by the inversion of the relative toxicities of the corresponding acid hydrazides; benzhydrazide is more toxic than acethydrazide (14).

The possible inverse relationship between activity and lipophilicity may be examined further. Among bioactive compounds, it is frequently seen that increasing lipophilicity to a point increases activity and further increases in lipophilicity decrease activity. All of the reported compounds possess the highly lipophilic diphenylmethylene group. Further substitution with lipophilic groups then can yield compounds of decreasing activity. It is pertinent at this point to consider the phenylacetyl series. A reason for incorporation of this series was to evaluate the hydrophobic role of the phenyl, separated, to the degree that considering only the major electronic factors involved in governing stability to hydrolysis permits, from its effect on hydrolysis, as was not directly possible in the case of the benzoyl. It can thus be conjectured that in the phenylacetyl series the phenyl is decreasing activity largely *via* increasing lipophilicity because the behavior toward hydrolysis would be in the opposite direction to the extent that the cited electronic factors are operative. If the phenyl decreases activity *via* its hydrophobic effect in the phenylacetyl series, it is possible that its presence in the benzoyl series is decreasing activity largely *via* a hydrophobic effect, since hydrophobic factors are additive. If hydrophobic character is the principal factor determining activity, then differences in metabolic stability that are a function of the cited electronic factors must be of less importance. The data are in accord with these interpretations, since the two series that possess the phenyl in the acyl, regardless of where that phenyl is placed relative to the carbonyl, are less active than the acetyl series. If the foregoing conjecture that the phenyl is decreasing activity *via* increasing hydrophobic character in the compounds is correct, the premise for the incorporation of the diphenylmethylene group in the compounds may be reexamined. Fundamentally, the premise for its incorporation is substantiated, because the compounds are centrally active. Importantly, however, the diphenylmethylene group may not be the optimal group to promote central activity among the compounds, since it may be too hydrophobic for optimal activity.

The argument that the phenyl in the phenylacetyl and benzoyl series is decreasing activity principally by decreasing distribution to the CNS because of too high a lipophilicity rather than by principally effecting hydrolytic bioactivation affords a possible explanation for the reason why the phenyl decreases activity. Of course, other explanations could be advanced. For example, steric hindrance to hydrolysis by the phenyl could be involved in the benzoyl and phenylacetyl series. Nonspecific perturbation of hydrolytic enzymes, resulting in a retarded rate of hydrolysis by operation of hydrophobic forces due to the phenyl, could be implicated. Also, it could be rationalized that the lipophilic phenyl in the phenylacetyl and benzoyl series renders the compounds so lowly soluble *in vivo* that hydrolysis is retarded. Conjecture aside as to why the phenyl in these compounds affects activity as it does, the data do permit the conclusion that the phenyl in the acyl group decreases activity, and this decrease is independent of where the phenyl is located relative to the carbonyl.

The conjecture that hydrophobic changes involved in the distribution of the compounds, rather than electronic changes involved in hydrolytic bioactivation, are the major factors in determining activity in these compounds should not be taken to rule out that hydrolysis is not occurring in the compounds. Rather, it seems likely that hydrolysis is occurring in all of the active compounds. The finding that the compounds are active appears to allow the conjecture that the compounds are being hydrolyzed *in vivo*, since in accordance with the case of furazolidone, they should not be active *per se*.

From the pharmacological data, it can be concluded that most of the compounds do exhibit significant CNS antidepressant properties in accordance with the basis for this investigation: the expectation that the synthesis of 2-diphenylmethylene-1-acyl-1-alkyl hydrazines patterned after furazolidone and other hydrazine derivatives might yield CNS stimulatory agents.

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Accepted for publication May 30, 1972.
* Present address: University of Heidelberg, Heidelberg, Germany.
▲ To whom inquiries should be directed. Present address: College of Pharmacy, Idaho State University, Pocatello, ID 83201

Application of Fluorescence Spectroscopy to Study of Intramolecular Hydrogen Bonding in Pamaquine

STEPHEN G. SCHULMAN▲ and KENNETH ABATE

Abstract □ The fluorescences of the antimalarial pamaquine and of its precursor 8-amino-6-methoxyquinoline were studied in sulfuric acid and *n*-heptane and throughout the pH range in water. Quenching of the fluorescences of both molecules in concentrated acid solutions upon addition of water was found to be the result of dissociation in the excited state from the protonated 8-amino groups. The anomalously long wavelengths of fluorescence of singly protonated pamaquine in the biological pH range in water and in *n*-heptane are shown to be due to intramolecular proton transfer, in the excited state, from the diethylamino group of the side chain to the heterocyclic nitrogen atom of the quinoline ring. This phenomenon appears to be mediated by the presence of an intramolecular hydrogen bond between the protonated diethylamino group and the ring nitrogen atom in the ground state, a result that may be of significance in the interpretation of the pharmacodynamics of pamaquine.

Keyphrases □ Pamaquine—fluorescence, intramolecular hydrogen bonding, acidity dependence □ Fluorescence, pamaquine—effect of intramolecular hydrogen bonding, acidity dependence □ Spectrophotofluorometry—pamaquine fluorescence, acidity dependence

The fluorescence of pamaquine has been a subject of some controversy. Brodie *et al.* (1) found that the naphthoate salt of pamaquine emitted a blue fluorescence in alkaline solutions. Irvin and Irvin (2) subsequently studied the free base dissolved in concentrated sulfuric acid solutions, and they observed fluorescence in solutions greater than 7 *M* in sulfuric acid. No fluorescence was observed at lower acidities, and the emission in concentrated sulfuric acid was attributed to a solvent effect and protonation of the methoxy group. Later work by Udenfriend *et al.* (3) showed the fluorescence of pamaquine naphthoate to be due to the naphthoate anion. Recently, Schulman and Sanders (4) showed that the failure of 8-aminoquinoline to fluoresce in fluid dilute acid solutions was a result of hydrogen bonding of the monocation with the solvent, water, in the lowest excited singlet state. The reason for the failure of the dication of the aminoquinoline to fluoresce in fluid concentrated sulfuric acid solutions was less certain and was attributed either to hydrogen-bonding-assisted internal conversion, as for the monocation, or to the acidity of the dication in the excited state which may have been so great that the latter species could not form even in the most concentrated sulfuric acid solutions available.

To evaluate the nature of the fluorescence of pamaquine in concentrated acid solutions in light of modern fluorescence theory, the present study of the acidity dependence of the fluorescence of pamaquine and of its simple aromatic precursor, 8-amino-6-methoxyquinoline, was undertaken.

EXPERIMENTAL

Instrumentation—Absorption spectra were obtained using a spectrophotometer[1]. Fluorescence measurements were performed on a fluorescence spectrophotometer[2] whose monochromators were calibrated against the xenon line emission spectrum and whose output was corrected for instrumental response by means of a rhodamine-B quantum counter. The pH measurements were made using a digital pH meter[3] with a silver–silver chloride–glass combination electrode[4].

Reagents—8-Amino-6-methoxyquinoline[5] and pamaquine phosphate[6] were used without further purification.

Analytical reagent grade sulfuric acid was purchased[7] and used without further purification. Solutions of varying acidity for fluoro-

[1] Beckman DG-BT.
[2] Perkin-Elmer MPF-2A.
[3] Orion model 801.
[4] Beckman.
[5] K & K Laboratories, Inc., Plainview, N. Y.
[6] Sterling-Winthrop Research Institute, Rensselaer, N. Y.
[7] Mallinckrodt Chemical Works, Inc., St. Louis, Mo.