

buffer) was incubated at 37° for 15 hr and was added to 0.2 ml of 55% cold trichloroacetic acid. After centrifugation at 3000 rpm for 10 min, the supernatant (1 ml) was submitted to the inorganic phosphate determination. Under the conditions, AMP and ADP liberated 0.95 and 1.96 mol of inorganic phosphate, respectively, while adenosine 2',3'-monophosphate liberated less than 0.04 mol of inorganic phosphate.

2-*n*-Amylthioadenosine 5'-Monophosphate (2a). 2-*n*-Amylthioadenosine (1a),¹ 92.5 mg (0.25 mmol), was suspended in 0.25 ml of acetonitrile and 0.10 ml (1.2 mmol) of pyridine, and the mixture was cooled to 0°. To the suspension was added 0.10 ml (1.1 mmol) of POCl₃ and 0.010 ml (0.5 mmol) of H₂O, and the homogeneous mixture was stirred at 0° for 2 hr. It was poured into 5 ml of ice-water and was adjusted to pH 1 with 2 *N* HCl to afford a white precipitate. The precipitate was separated by centrifugation at 2000 rpm for 10 min and was redissolved in 0.5 ml of concentrated NH₄OH and submitted to preparative paper chromatography using solvent 1. Three bands (*R_f* 0.51, 0.42, and 0.29) were detected on the chromatogram by uv ray. The fastest moving major band (*R_f* 0.51) was cut out and extracted with 0.05 *N* NH₄OH and the extract was evaporated to a small volume. In order to eliminate minor contaminants and impurities it was purified again by the same preparative chromatography. The single band was extracted by 0.05 *N* NH₄OH and was evaporated to dryness and redissolved in about 2.0 ml of H₂O, which was subsequently filtered through a millipore filter (0.22 μ). To the filtrate was added 35 mg (0.08 mmol) of BaI₂ dissolved in 4 ml of EtOH. The white precipitate was separated by centrifugation at 3000 rpm for 10 min and washed with H₂O-EtOH (1:2) twice and finally with EtOH. It was dried *in vacuo* at 50° for 24 hr over P₂O₅. 2-*n*-Amylthio-AMP (2a) barium salt was obtained in a yield of 13.5% (21.0 mg) based on 1a. *Anal.* (C₁₅H₂₂O₇N₅SPBa·2H₂O) C, H, N, P. The content of inorganic phosphate was less than 0.01 mol. Labile phosphate by 5'-nucleotidase was 0.995 mol (theoretical, 1.00 mol). The sodium metaperiodate-benzidine test confirmed the presence of the 2',3'-*cis*-glycol function.

2-Benzylthioadenosine 5'-Monophosphate (2b). 2-Benzylthioadenosine (1b),¹ 105.5 mg (0.25 mmol), was suspended in 0.25 ml of acetonitrile and 0.10 ml of pyridine. The mixture was cooled to 0° and to this was added 0.10 ml of POCl₃ and 0.010 ml of H₂O. After the mixture was stirred at 0° for 2 hr, it was mixed with 5 ml of ice-water and subsequently with 0.5 ml of 2 *N* HCl. The white precipitate which separated was dissolved in concentrated NH₄OH and submitted to preparative paper chromatography as described above. Three bands (*R_f* 0.49, 0.40, and 0.26) were detected on the chromatogram. The major band (*R_f* 0.49) was extracted with 0.05 *N* NH₄OH and purified again by the chromatography. The barium salt of 2-benzylthio-AMP (2b) was obtained in a yield of 12.5% (20.0 mg) based on 1b. *Anal.* (C₁₇H₁₈O₇N₅SPBa·2H₂O) C, H, N, P. The content of inorganic phosphate was less than 0.01 mol. Labile phosphate by 5'-nucleotidase was 1.048 mol (theoretical, 1.00 mol). The sodium metaperiodate-benzidine test confirmed the presence of the 2',3'-*cis*-glycol function.

2-Allylthioadenosine 5'-Monophosphate (2c). 2-Allylthioadenosine (1c),¹ 374 mg (1.0 mmol), was suspended in 1.0 ml of acetonitrile and 0.39 ml (4.8 mmol) of pyridine. The mixture was cooled to 0° and to this was added 0.40 ml (4.4 mmol) of POCl₃ and 0.040 ml (2.0 mmol) of H₂O. After the mixture was stirred at 0° for 4 hr, it was subsequently added to 5 ml of cold water. A small amount of precipitate was removed by centrifugation, and the supernatant was acidified at pH 1 with 2 *N* HCl. It was then absorbed on a column of 20 g of active carbon. The column, washed well with H₂O, was eluted with 300 ml of 10% NH₄OH-EtOH (1:1). The effluent was evaporated *in vacuo* to dryness and submitted to preparative paper chromatography as described above. Three bands (*R_f* 0.46, 0.35, and 0.18) were detected on the chromatogram, and the relative amounts of the products corresponding to these three bands estimated by optical density (273 nm, pH 1) were 75, 8.5, and 16.5%, respectively. The major band (*R_f* 0.46) was cut out, extracted with 0.05 *N* NH₄OH, evaporated to dryness, and redissolved in 6.0 ml of H₂O. It was subsequently filtered through a millipore filter and the filtrate was added to 105 mg (0.25 mmol) of barium iodide dissolved in 12 ml of EtOH. The white precipitate which separated was collected by centrifugation. It was redissolved in 6.0 ml of H₂O, filtered, and precipitated by the addition of 12 ml of EtOH. Finally it was washed with EtOH twice and dried to afford 90.0 mg (yield, 15.5%) of 2-allylthio-AMP (2c) barium salt. *Anal.* (C₁₃H₁₆O₇N₅SPBa·1.5H₂O) C, H, N, P. The content of inorganic phosphate was less than 0.01 mol. Labile phosphate by 5'-nucleotidase was 0.997

mol (theoretical, 1.00 mol). The sodium metaperiodate-benzidine test confirmed the presence of the 2',3'-*cis*-glycol function. The products corresponding to the bands, *R_f* 0.35 and 0.18, must be the 5'-diphosphate analog and 2',3'-phosphorylated analog(s) examined by paper chromatography, paper electrophoresis, and the sodium metaperiodate-benzidine test.

Platelet Aggregation Test. Platelet aggregation studies were performed on buffered platelet-rich plasma from rabbit citrated blood and platelet-rich plasma from human citrated blood, using the optical density method reported before.¹ Platelet-rich plasma from a male rabbit was immediately buffered with an equal volume of isotonic barbital buffer (pH 7.3), and the mixture was used as buffered platelet-rich plasma.

As the barium salts of 2a-c were insoluble in saline, ammonium salts were used in platelet aggregation studies. The ammonium salt of each nucleotide to be tested was prepared from the filtrate through the millipore filter of the NH₄OH extract of the spot corresponded to the nucleotide. Concentration of each nucleotide in saline was determined by optical density. The extract of filter paper by dilute NH₄OH did not contain any substances that affect platelet aggregation, and the inhibitory effect of the NH₄OH extract of 2a-c on paper chromatograms could be attributed to the ammonium salts of 2a-c.

Acknowledgments. The authors wish to express their sincere thanks to Mrs. R. Toyoshima for her technical assistance of the platelet aggregation studies and to the laboratory staffs for collection of their blood.

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Synthesis of

1,4-Bis(6-methoxy-8-quinolylaminoalkyl)piperazines as Potential Prophylactic Antimalarial Agents

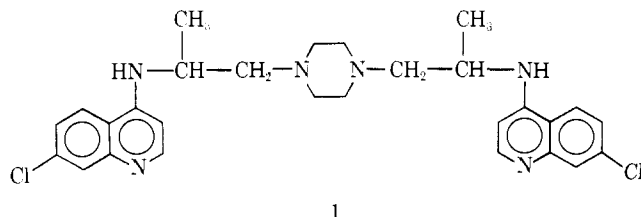
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Received June 25, 1973

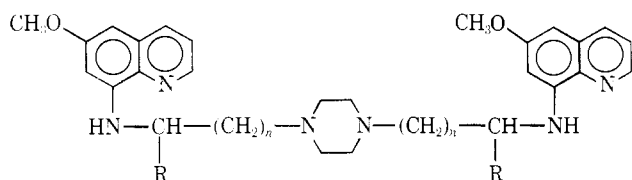
Malaria continues to be a problem, despite the years of research and screening of thousands of compounds. Today, drugs are available that interrupt the infection at its various stages, but clinically useful prophylactic anti-

malarials with repository properties are limited.¹ The known causal prophylactic drugs (proguanil, pyrimethamine, pamaquine, and primaquine) are rapidly eliminated from the body, and, except for the 8-aminoquinoline derivatives, the emergence of plasmodial resistance is a further shortcoming.

Significant oral repository¹ and antimalarial activity² for bis(7-chloro-4-quinolyl-2-aminopropyl)-1,4-piperazine (1, 12,278 RP) and related derivatives has been reported.† There seems to be no evidence to suggest a possible metabolic degradation of 1 to two discrete 4-aminoquinoline molecules,^{1b} and as chloroquine and amodiaquine possess



no appreciable repository property, it appears that the 1,4-bis(2-aminopropyl)piperazinyl moiety contributes to the significant repository property of 1 by altering its affinity for the liver and kidney and delaying its release from liver parenchymal and Kupffer cells.^{1b} Although limited by toxicity and rapid elimination, it has been suggested³ that some 8-aminoquinoline antimalarials could act as true prophylactics against both vivax and falciparum infections, as well as achieve radical cure of relapsing malarials.¹ And, since the less toxic 2-benzyloxy analog⁴ of primaquine has demonstrated causal prophylactic activity in rats infected with *Plasmodium berghei*,‡ we were encouraged to prepare some 8-aminoquinoline analogs 2-4 of 1 for study as potential prophylactic antimalarials with repository activity. Furthermore, some aminoalkylpiperazine derivatives of 6-methoxy-8-aminoquinoline have been reported⁵ to give antiprotozoal agents of relatively low toxicity. This communication describes the synthesis and biological data for our studies.

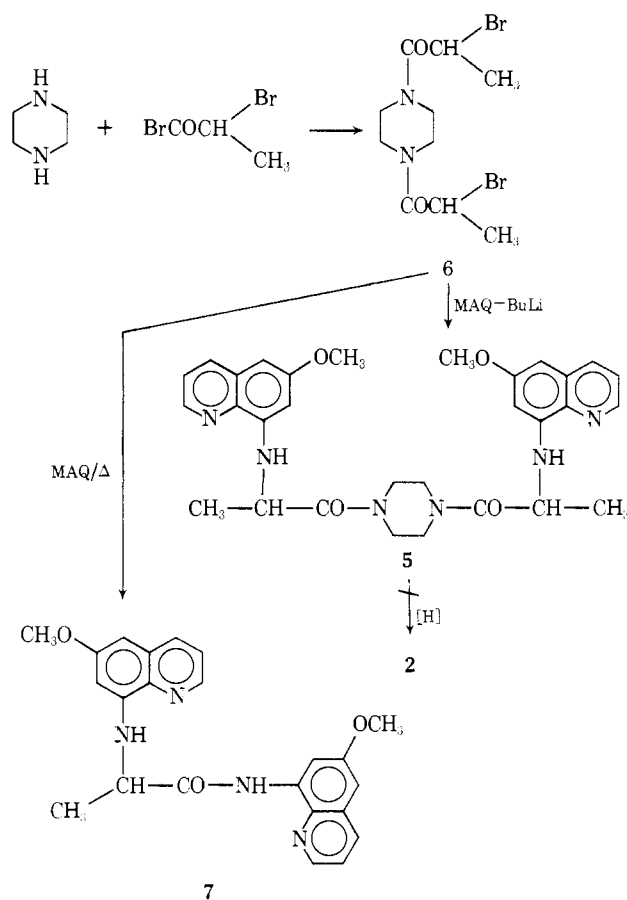


- 2, R = CH₃; n = 1
 3, R = H; n = 1
 4, R = H; n = 2

Chemistry. The initial approach was to synthesize 5 (Scheme I),⁶ which should be reducible⁷ by a variety of means to the desired target compound 2, a route extendable to the preparation of other members of the homologous series. Attempted condensation of 6 and excess 6-methoxy-8-aminoquinoline (MAQ) led to 7 instead of the desired product 5. This difficulty was circumvented by use of the lithium anion of MAQ. Attempted reduction of 5 by a variety of reagents, e.g., LiAlH₄, NaBH₄-BF₃, was unsuccessful. Use of diborane-THF apparently reduced the amide carbonyl, but the product could not be satisfactorily freed from undesirable inorganic impurities, and this approach was abandoned.

Finally, 2 was prepared successfully by heating an intimate mixture of 1,4-bis(2-chloropropyl)piperazine hydro-

Scheme I

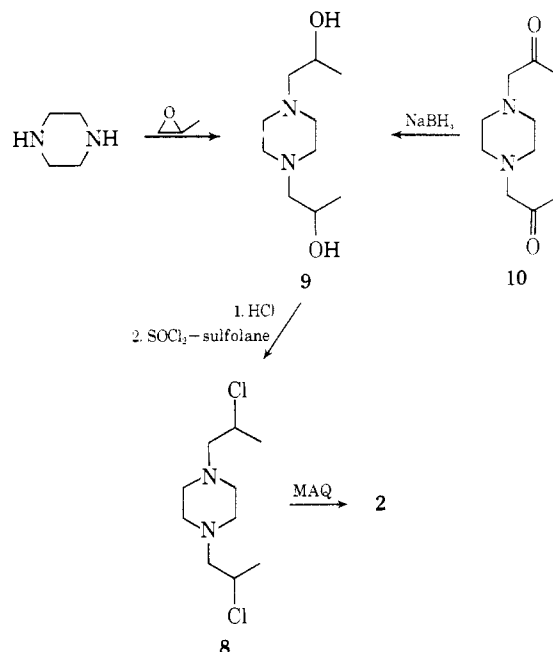


MAQ = 6-methoxy-8-aminoquinoline

chloride (8) and MAQ (Scheme II). Compound 8 was prepared from the appropriate bisalcohol 9, obtained by reaction of piperazine with propylene oxide. The bisalcohol 9 was also prepared by NaBH₄ reduction of the diketone 10, obtained from chloroacetone and piperazine. Other homologs, 3 and 4, were prepared similarly from readily available halo compounds.

Biological Data. The antimalarial test results were

Scheme II



†For reviews, see ref 1a-c and literature cited therein.

‡Personal communication, Walter Reed Army Institute of Research.

Table I. 1,4-Bis-Substituted Piperazines

$\begin{array}{c} \text{X} \\ \\ \text{RCH}-(\text{CH}_2)_n-\text{N} \begin{array}{c} \diagup \quad \diagdown \\ \diagdown \quad \diagup \\ \text{N} \end{array} -(\text{CH}_2)_n-\text{CHR} \\ \\ \text{X} \end{array}$							
No.	R	X	n	Mp, °C	Yield, %	Formula	Analyses
12	CH ₃	Cl	1	228–230 dec	58	C ₁₀ H ₂₂ N ₂ Cl ₄ ^a	C, H, N, Cl
13	H	Cl	2	285–290 dec	85	C ₁₀ H ₂₂ N ₂ Cl ₄ ^a	
14	H	Cl	1	305–310 dec ^b	89	C ₃ H ₁₃ N ₂ Cl ₄ ^a	
2	CH ₃	Q ^c	1	203–205	30	C ₃₀ H ₃₄ N ₆ O ₆	C, H, N
3	H	Q ^c	2	148–151	55	C ₃₀ H ₃₈ N ₆ O ₆	C, H, N
4	H	Q ^c	1	151–155	21	C ₂₃ H ₃₄ N ₆ O ₂	C, H, N

^aDihydrochloride, ^bLit.¹¹ mp 293–295°. ^cQ = (6-methoxy-8-quinoly)amino.

provided by the Walter Reed Army Institute of Research. The activity was assessed against *P. berghei* in mice by the method of Rane and coworkers.⁸ The compounds were dissolved or suspended in sesame or peanut oil and were administered to groups of five mice in a single subcutaneous dose 72 hr postinfection. The mean survival time (MST) of untreated mice was 6.1 days. Compounds are arbitrarily considered to be "active" when they produce at least 100% increase in the mean survival time of treated mice. Animals that survive to 60 days postinfection are considered "cured" (C). None of the compounds showed interesting antimalarial activity at 640 mg/kg, although 4, a position isomer of 2, as well as 1, did reveal moderate activity at 320 and 640 mg/kg (Δ MST, 6.5 and 7.2, respectively). For comparison, the lead compound 1 was "active" at 20 mg/kg (Δ MST, 8.5) and curative at 40 mg/kg (3/5 C). None of the compounds were toxic (T) in this system (death prior to 6 days). The 8-aminoquinoline, primaquine phosphate, was "active" at 80 mg/kg (Δ MST, 9.4) with toxic deaths at 160 mg/kg (2/5 T).

Compounds 2–4 were evaluated for prophylactic action in chicks.^{9a} In this test, white Leghorn cockerels were parasitized by the intrajugular injection of *Plasmodium gallinaceum* sporozoites. The compounds were suspended in peanut oil and were administered subcutaneously in a single dose on the day of infection. Prophylactic value was assessed by comparing the maximum survival time of treated sporozoite-infected chicks and the survival time of untreated sporozoite-infected controls. A compound is considered to have potential prophylactic value if it produces a minimum increase of 100% in survival time of the untreated controls. None of the compounds tested in graded dose levels possessed prophylactic activity based on the above criteria.

Compounds 3 and 4 were also evaluated for potential prophylactic action in the sporozoite-mouse test system (*P. berghei yoelii* and *Aspergillus stephensi*).^{4,9b} The compounds were given subcutaneously to a group of five infected mice for 3 consecutive days: the day before, the day of, and the day after sporozoite infection. None of the compounds tested were effective at 480 mg/kg, whereas primaquine gave no evidence of parasitemia at 30 mg/kg. The activity patterns were not considered adequate to justify expanded testing or further extension of the present group.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Satisfactory ir (Perkin-Elmer 237B grating spectrophotometer, KBr) and nmr (Hitachi Perkin-Elmer R20A high-resolution nmr spectrometer and Me₄Si as internal reference) spectra were obtained for all new compounds (DMSO-*d*₆). Tlc were performed on Eastman chromatogram sheets, type 6060 (silica gel).

6-Methoxy-8-[2-(6-methoxy-8-quinoly)aminopropionamido]-

quinoline (7). 1,4-Bis(2-bromopropionyl)piperazine (6)⁶ (3.56 g, 0.01 mol) and 8-amino-6-methoxyquinoline (10 g, 0.057 mol) were dissolved in 150 ml of *i*-PrOH and heated on an oil bath at 80–90° for 0.5 hr. The temperature of the bath was then raised to 130–140° and maintained at this temperature for 1.5 hr. The *i*-PrOH was allowed to evaporate and the resulting melt cooled at room temperature. Ether (50 ml) was added to the solid mass and the residue remaining after filtration was digested with 750 ml of benzene (250-ml portions) on a water bath and filtered. Ether and benzene filtrates were combined and concentrated *in vacuo*. Addition of 100 ml of cold Me₂CO to the residue gave an off-white solid (4.5 g, 56.2%); mp 197–200°. The analytical sample was recrystallized from Me₂CO; mp 201–203°. *Anal.* (C₂₃H₂₂N₄O₃) C, H, N.

1,4-Bis[2-(6-methoxy-8-quinoly)aminopropionyl]piperazine (5). In a three-necked round-bottomed flask fitted with a mechanical stirrer and inlet for nitrogen was placed 45 ml of 15% BuLi (0.105 mol) in hexane. This solution was cooled in an ice-salt bath and slowly treated with 19 g (0.109 mol) of 8-amino-6-methoxyquinoline dissolved in 200 ml of THF, previously dried over 5A molecular sieve. To the resulting deep red solution was added 17.7 g (0.05 mol) of 1,4-bis(2-bromopropionyl)piperazine (6),⁶ dissolved in 100 ml of THF. After the addition was completed, the reaction mixture was allowed to warm to room temperature and stirred for 2 hr. Pouring the mixture into 2 l. of ice water gave a sticky solid, which was dispersed in Me₂CO and filtered. Several washings with Me₂CO gave an off-white material (14 g, 51.8%); mp 258–263°. *Anal.* (C₃₀H₃₄N₆O₄) C, H, N. Recrystallization from DMF gave mp 278–280° dec.

1,4-Bis(2-hydroxypropyl)piperazine (9). Method A. A mixture of 8.6 g (0.1 mol) of piperazine, 13.2 g (0.23 mol) of propylene oxide, and 60 ml of DMF was heated in a pressure bottle for 9 hr at 100–110°. Reaction mixtures from 14 batches were combined and DMF was removed *in vacuo* to give 207 g. Recrystallization from EtOAc gave the desired product in 59% yield; mp 113–116°. *Anal.* (C₁₀H₂₂N₂O₂) C, H, N.

The dihydrochloride was prepared in 77% yield by treating a CHCl₃ solution with HCl gas; mp 212–217° (EtOH). *Anal.* (C₁₀H₂₄N₂O₂Cl₂) C, H, N.

Method B. Compound 9 was also prepared by NaBH₄ reduction of the ketone 10 [*Anal.* (C₁₀H₁₈N₂O₂) C, H, N], which was obtained from the reaction of chloroacetone and piperazine¹⁰ in absolute EtOH at room temperature. Anhydrous K₂CO₃ was employed as the HCl scavenger. The yield from piperazine was 21%.

1,4-Bis(3-hydroxypropyl)piperazine (11).[§] This compound can be prepared in 34–40% yield by the procedure of Gardner and Schneider.^{11a} Yields may be improved by a modification of the procedure. A mixture of piperazine (8.6 g, 0.1 mol), allyl alcohol (23.2 g, 0.4 mol), and NaOH (8g, 0.2 mol) was heated at 137–140° for 24 hr. The gelatinous material was dissolved in water, and the solution was saturated with NaCl and extracted with CHCl₃. After drying (Na₂SO₄), the CHCl₃ was removed *in vacuo* to give a white solid in 79% yield; mp 138–140°. The analytical sample was obtained by recrystallization from THF; mp 139–141° (lit.¹¹ 142–143°). *Anal.* (C₁₀H₂₂N₂O₂) C, H, N.

1,4-Bis(chloroalkyl)piperazine Dihydrochlorides (Table I, 12–14). The 1,4-bis(hydroxyalkyl)piperazine dihydrochloride was suspended in sulfolane,¹² heated in an oil bath to 120°, and slowly treated with 1 equiv of SOCl₂. The temperature was raised to 130–140° and maintained at this temperature for 15 min. The cooled reaction mixture was filtered and the filtrate treated with absolute EtOH and ether (1:11.5). The precipitate was collected

§1,4-Bis(2-hydroxyethyl)piperazine is commercially available.

and recrystallized from absolute ethanol.

1,4-Bis[(6-methoxy-8-quinolyl)aminoalkyl]piperazines (Table I, 2-4). An intimate mixture of the appropriate piperazine 12-14 (1 equiv) and 6-methoxy-8-aminoquinoline (6 equiv) was heated under nitrogen at 120-125° for 19 hr. The melt was cooled at room temperature, rendered basic with 1 *N* NaOH, and extracted with CHCl₃. After drying (MgSO₄), the CHCl₃ was removed *in vacuo* and the oily residue dissolved in ethanol and treated with excess alcoholic oxalic acid. The insoluble precipitate (oxalate salt of the desired product, *e.g.*, 2) was collected. Unreacted 6-methoxy-8-aminoquinoline remained in solution as the oxalate salt. The alcoholic insoluble product was stirred with 10% NaOH solution for 0.5 hr and filtered. The solid residue was dissolved in benzene, treated with charcoal, and concentrated *in vacuo*, and the free amine precipitated with *n*-hexane. The analytical sample was prepared by several reprecipitations from benzene by hexane.

In the preparation of 3 and 4, it was not necessary to prepare oxalate salts to separate product from unreacted 6-methoxy-8-aminoquinoline. The product solidified in a fairly pure state when the oily residue was treated with ether.

Acknowledgments. We acknowledge the U. S. Army Medical and Development Command under Contract DADA 17-71-C-1068 for support of this work. This is Contribution No. 1178 from the Army Research Program on Malaria. The authors wish to thank Drs. R. E. Strube and T. R. Sweeney of WRAIR for interest and encouragement during the course of this work.

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Preliminary Pharmacology of Some Benzocycloheptane Derivatives Related to Amphetamine

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Recent interest in drug abuse and the quest for compounds that may act as blocking agents for drugs, such as the amphetamines, has brought forth a number of com-

pounds which show some activity in this regard. Various simple trimethoxybenzamides exhibit mild sedative properties¹ and are capable of antagonizing the action of amphetamine.² In contrast to the psychomimetic activity of 3,4-dimethoxyamphetamine, β -(3,4-dimethoxyphenyl)-2-propanol, the oxygen analog, is a CNS depressant.³ The *N,N*-diallyl derivative of amphetamine has been reported to have antagonistic activity toward amphetamine.⁴ Other more complicated *N*-substituted amphetamines and amphetamine derivatives show antagonistic activity.^{5,6}

During the course of work not related to drug abuse, a series of compounds was generated and given a preliminary screening for pharmacological activity in mice. All of these compounds are benzocycloheptane derivatives with methoxy and/or hydroxy substitution on the benzene ring and are easily obtained in good yield from purpurogallin. Their relationship to amphetamine prompts us to publish the preliminary pharmacology.

Results and Discussion

Amphetamine exhibited a spectrum of pharmacological effects (Table I) which included those most clearly referable to CNS stimulation (b, d, and h). On the basis of these effects, compounds 19 and 21 most closely approximated amphetamine while compounds 5 (h) and 6 (b, h) also appeared to be predominantly stimulant. None of the other compounds exhibited any of these properties and all except 18 showed at least one opposing effect referable to CNS depression. Compound 9 appeared to be the most clearly depressant (a, c, l).

It is of interest that substitution of OH for OCH₃ at R₁ in most compounds had little effect on activity or enhanced depressant effects (16 *vs.* 9) but reversed activity in one case (13 *vs.* 6). The slight stimulant activity of 5 was enhanced in R₁-methylated derivatives with appropriate substitution adjacent to the keto function (19) even when the latter was enolized in lactone formation (21). Interestingly, however, stimulant activity was absent in the corresponding lactam 22 and in the carboxyl analog 20 of the nitrile 19. It is possible that limitations on the stimulant actions of 19 and 21 could result *in vivo* from nitrile and lactone hydrolysis, respectively, which would yield inactive 20 in each case.

Experimental Section

Purpurogallin (1). Purpurogallin was made from pyrogallol by the method of Evans and Dehn⁷ in 92% crude yield and used without further purification.

Purpurogallin Trimethyl Ether (2). Purpurogallin trimethyl ether was made⁸ from purpurogallin (1) in 30% yield after recrystallization from methanol as long orange needles, mp = 179-180° (lit. mp 174°).

1-Hydroxy-5-oxo-2,3,6-trimethoxybenzocycloheptane (3). Purpurogallin trimethyl ether (2) was converted to 3 by the method described by Walker⁹ in 58% yield of colorless plates melting at 78-80° (lit. mp 86-88°).

4,5-Dihydroxy-2,3,6-trimethoxybenzocycloheptane (4). This material was made from 3 by the method described by Barltrop and Nicholson¹⁰ and recrystallized from ethanol in 90% yield to give a product melting at 145-147° (lit. mp 148°).

2,3-Dimethoxy-4-hydroxy-6-oxobenzocycloheptane (5). This material was made from 4 by methods described in the literature⁸ in 72% yield and melted at 140-145° (lit. mp 140-141°).

2,3-Dimethoxy-4-hydroxy-6-semicarbazobenzocycloheptane (6). To 809.9 mg (3.4 mmol) of the hydroxy ketone 5 dissolved in hot ethanol was added about 2 ml of water, 1.0 g (9 mmol) of semicarbazide hydrochloride, and 1.5 g (11 mmol) of sodium acetate. The mixture was heated on a steam bath and almost immediately white crystals formed. After heating an additional 15 min, cooling and filtering gave 882.1 mg (88%) of white crystals melting at 220-223°.

2,3-Dimethoxy-4-hydroxy-6-thiosemicarbazobenzocycloheptane (7). The hydroxy ketone 5 (1.0 g, 4.2 mmol) was dissolved in hot ethanol and water added until just cloudy.