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 $CHCl_3$. After drying ($MgSO_4$), the $CHCl_3$ 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.

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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 compounds which show some activity in this regard. Various simple trimethoxybenzamides exhibit mild sedative properties[1] 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 OCH_3 at R_1 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 R_1-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 Dehn[7] in 92% crude yield and used without further purification.

Purpurogallin Trimethyl Ether (2). Purpurogallin trimethyl ether was made[8] from purpurogallin (1) in 30% yield after recrystallization from methanol as long orange needles, mp = 179-180° (lit. mp 174°).

4-Hydroxy-5-oxo-2,3,6-trimethoxybenzocycloheptane (3). Purpurogallin trimethyl ether (2) was converted to 3 by the method described by Walker[9] in 58% yield of colorless plates melting at 78-80° (lit. mp 86-88°).

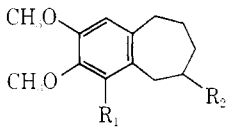
4,5-Dihydroxy-2,3,6-trimethoxybenzocycloheptane (4). This material was made from 3 by the method described by Barltrop and Nicholson[10] and recrystallized from ethanol in 90% yield to give a product melting at 145-147° (lit. mp 148°).

2,3-Dimethoxy-4-hydroxy-6-oxobenzocycloheptane (5). This material was made from 4 by methods described in the literature[8] in 72% yield and melted at 140-145° (lit. mp 140-141°).

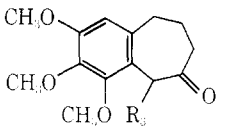
2,3-Dimethoxy-4-hydroxy-6-semicarbazonobenzocycloheptane (6). To 809.9 mg (3.4 mmol) of the hydroxy ketone **5** dissolved in hot ethanol was added about 2 ml of water, 1.0 g (9 mmol) of semicarbazide hydrochloride, and 1.5 g (11 mmol) of sodium acetate. The mixture was heated on a steam bath and almost 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-thiosemicarbazonobenzocycloheptane (7). The hydroxy ketone **5** (1.0 g, 4.2 mmol) was dissolved in hot ethanol and water added until just cloudy.

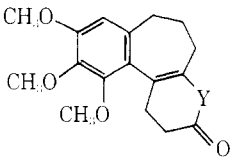
Table I. Pharmacological Results



5-18



19, 20



21, 22

Compd	R_1	R_2	LD_{50}, mg/kg	MED_{50}, mg/kg	Effects[a]	Formula	Analyses
5	–OH	=O	>100	31.6	g, efjih	$C_{13}H_{16}O_4$	
6	–OH	$=NNHCONH_2$	>100	0.237	g, bhi	$C_{14}H_{19}N_3O_4$	C, H, N
7	–OH	$=NNHCSNH_2$	>100	5.62	c	$C_{14}H_{19}N_3O_3S$	C, H, N, S
8	–OH	=NOH	>100	<1.0	g, cfej	$C_{13}H_{17}NO_4$	C, H, N
9	–OH	$-NH_2$	70.8	<1.0	g, aclkef	$C_{13}H_{19}NO_3$	C, H, N
10	–OH	$-NHCO_2CH_3$	44.7	1.78	f, ac	$C_{15}H_{21}NO_5$	C, H, N
11	–OH	$-NHCH_3$	70.8	31.6	f, a	$C_{14}H_{21}NO_3$	C, H, N
13	$-OCH_3$	$=NNHCONH_2$	>100	31.6	f, aj	$C_{15}H_{21}N_3O_4$	C, H, N
14	$-OCH_3$	$=NNHCSNH_2$	>100	5.62	c, af	$C_{15}H_{21}N_3O_3S$	C, H, N, S
15	$-OCH_3$	=NOH	31.6	1.78	a, g	$C_{14}H_{19}NO_4$	
16	$-OCH_3$	$-NH_2$	79.4	0.464	a, f	$C_{14}H_{22}ClNO_3$	C, H
17	$-OCH_3$	$-NHCO_2CH_3$	79.4	10.0	e, ac	$C_{16}H_{23}NO_5$	C, H, N
18	$-OCH_3$	$-NHCH_3$	63.1	5.62	f, ejg	$C_{32}H_{48}N_2O_{10}$	C, H, N
		R_3					
19		$-CH_2CH_2CN$	>100	0.056	h, bd	$C_{17}H_{21}NO_4$	
20		$-CH_2CH_2CO_2H$	>100	31.6	e, acf	$C_{17}H_{22}O_6$	
		Y					
21		>O	>100	3.16	e, bdmhf	$C_{17}H_{20}O_5$	
22		$>NCH_3$	>100	5.62	c, afg	$C_{18}H_{23}NO_4$	C, H
l-α-Methylphenethylamine[b]			>100	0.29	h, bdef		

[a] a = CNS depression (subjective; startle response, righting reflex, etc.); b = CNS stimulation (subjective; startle response, righting reflex, etc.); c = decreased activity (compartmental observation); d = increased activity (compartmental observation); e = ataxia; f = motor deficit (grasp reflex, traction, complex coordination on horizontal rigid wire, rotating rod); g = decreased muscle tone (trunk and limb); h = increased sensitivity in touch and sound (reactivity); i = piloerection; j = low posture; k = color change to red; l = decreased sensitivity to touch and sound (reactivity); m = increased muscle tone (trunk and limbs). [b] Aldrich Chemical Co.

Thiosemicarbazide hydrochloride (1.0 g, 7.9 mmol) was added along with 1 drop of acetic acid and heating on the steam bath continued for 20 min. The mixture was placed in an ice bath and 1.78 g of crude crystals removed by filtration. Several recrystallizations from methanol–water gave product melting at 192–194°.

2,3-Dimethoxy-4-hydroxy-6-oximidobenzocycloheptane (8). The hydroxy ketone **5** (2.48 g, 10.5 mmol), 25 ml of 10% NaOH (62.5 mmol), and 6.3 g (90 mmol) of hydroxylamine hydrochloride were mixed with enough warm ethanol to make a clear solution. The mixture was allowed to stir at steam bath temperatures for 1 hr. Cooling the solution with ice and scratching with a glass rod caused it to deposit colorless crystals, 1.93 g (73%), mp 169.5–176°.

6-Amino-2,3-dimethoxy-4-hydroxybenzocycloheptane (9). The oxime **8** (457 mg, 1.8 mmol) in 25 ml of methanol with 0.5 g of Raney nickel was hydrogenated at room temperature and atmospheric pressure. After the uptake of the theoretical amount of hydrogen (overnight) the solvent was removed *in vacuo* and the product crystallized from ethanol–ether to give 259 mg (60% yield) of white crystals (mp 153–154°).

6-*N*-Carbomethoxyamino-2,3-dimethoxy-4-hydroxybenzocycloheptane (10). To 1.27 g (5.4 mmol) of amine **9** was added 10 ml of water and 4.3 ml (10 mmol) of a sodium hydroxide solution (10%). To the resulting clear yellow solution was added dropwise 15 ml of chloroform containing 0.4 ml (5.4 mmol) of methyl chloroformate and the mixture allowed to stir overnight at room temperature. The reaction mixture was cooled to 0° in an ice bath and slowly acidified (pH 2) with dilute hydrochloric acid. After stirring for 1 hr, the layers were separated and the aqueous phase was extracted with chloroform. The combined chloroform layers were washed with water, dried with sodium sulfate, and evaporated *in vacuo*. The yellowish solid was recrystallized from benzene–ether–light petroleum yielding 0.545 g (35%) of cream-colored crystals (mp 133–135°).

2,3-Dimethoxy-4-hydroxy-6-methylaminobenzocycloheptane (11). To 1.36 g (36 mmol) of $LiAlH_4$ in 50 ml of dry tetrahydrofuran cooled to 0° was added dropwise 1.0 g (3.4 mmol) of the carbamate **10** dissolved in 15 ml of dry tetrahydrofuran. The mixture was stirred for 0.5 hr and then overnight at room temperature. The mixture was refluxed for 3 hr. The reaction was terminated with 1 ml of water in 15 ml of tetrahydrofuran and made filterable by adding 5 ml of 12% sodium hydroxide, followed by 1 ml of water, stirred 1 hr, and filtered. After washing and drying, the extract was evaporated to yield a viscous yellow oil which crystallized to give 134.9 mg (16%) of white crystals (mp 171–174°).

6-Oxo-2,3,4-trimethoxybenzocycloheptane (12). This material was made from the hydroxy ketone **5** by the method of Rapoport and Campion[11] in 88% yield to give a product melting at 53.5–55° (lit. mp 46–46.5°).

6-Semicarbazono-2,3,4-trimethoxybenzocycloheptane (13). The semicarbazone was made from the ketone **12** as previously described[11] in 92% yield and recrystallized several times from methanol–water to give a product melting at 192–200° (lit. mp 184–185°).

6-Thiosemicarbazono-2,3,4-trimethoxybenzocycloheptane (14). The thiosemicarbazone was made as usual from the ketone **12** and crystallized from methanol–water as colorless crystals melting at 212–214° in 54% yield.

6-Oximido-2,3,4-trimethoxybenzocycloheptane (15). This material was made as previously described[11] from the ketone **12** in 90% yield to give a product melting at 130–137° (lit. mp 130.5–131°).

6-Amino-2,3,4-trimethoxybenzocycloheptane (16). The oxime **15** (2.0 g, 7.5 mmol) dissolved in 100 ml of ethanol and 2.0 g of Raney nickel was hydrogenated at room temperature and atmospheric pressure overnight. After the theoretical amount of hydrogen was taken up, the catalyst was filtered off and the solvent evaporated *in vacuo*. A colorless oil remained. The oil was taken up in ether and isolated as the hydrochloride, 1.2 g (58%), melting at 226–230°.

6-(*N*-Carbomethoxy)amino-2,3,4-trimethoxybenzocycloheptane (17). To 2 g (7 mmol) of amine hydrochloride **16** in 30 ml of chloroform–water (1:1) mixture was added 0.53 ml (7 mmol) of methyl chloroformate. To this mixture was added 8.4 ml (21 mmol) of 10% NaOH solution and the mixture stirred for 2 hr at room temperature. Dilute hydrochloric acid was added to the ice-cold reaction mixture to pH 2. The layers were separated, the aqueous phase was extracted with chloroform, and the chloroform

was washed with water, dried, and evaporated *in vacuo*. The product, 1.89 g of pale yellow oil, was crystallized from benzene–light petroleum mixtures to give 0.994 g of colorless crystals melting at 114–116.5°.

6-(*N*-Methyl)amino-2,3,4-trimethoxybenzocycloheptane (18). The carbamate **17** (820 mg 2.7 mmol) dissolved in 15 ml of dry tetrahydrofuran was slowly added dropwise to 1.3 g (34 mmol) of $LiAlH_4$ suspended in 15 ml of dry tetrahydrofuran cooled to 0°. The mixture was stirred 0.5 hr at 0° after which time it was refluxed for 2 hr. The reaction was terminated by adding 1.3 ml of water in 10 ml of tetrahydrofuran and made filterable with 5 ml of 12% NaOH. After filtering, drying, and evaporating 643 mg (91%) of yellow oil was obtained. The compound was isolated as the oxalate, mp 195–205°.

5-β-Cyano-6-oxo-2,3,4-trimethoxybenzocycloheptane (19). This compound was made as already described[12] from the ketone **12** in 98% crude yield. Recrystallization from ethanol gave a product melting at 107–110° (lit. mp 110.5–111.2°).

β-(6-Oxo-2,3,4-trimethoxybenzocycloheptan-5-yl)propionic Acid (20). Compound **20** was made from the hydrolysis of the cyano ketone **19** by methods already described[12] in nearly quantitative yield. The ir spectrum was as expected, mp 87–89° (lit. mp 88–90°).[13]

β-[5-(6-Hydroxy-2,3,4-trimethoxybenzocyclohepten-5-yl)]-propionic Acid δ-Lactone (21). This product was made from the keto acid **20** by previously described methods[12] in 59% yield of material melting at 105–107° (lit. mp 104–105°).

β-[5-(6-Hydroxy-2,3,4-trimethoxybenzocyclohepten-5-yl)]-propionic Acid δ-*N*-Methyllactam (22). The crude keto acid **20** (528 mg, 1.6 mmol) was added to 25 ml of ethanol which had been saturated with methylamine. The mixture was heated in a stainless steel bomb at 140° for 8 hr. The product remaining after the evaporation of the ethanol was purified by chromatography on a silica gel plate using chloroform as a solvent (R_f = 0.05–0.25). The product was removed from the plate and crystallized from light petroleum to give 230 mg (44%) of colorless microcrystals which melted at 104–106°. The ir spectrum was as expected.

Biological Evaluation. Male albino mice of the Swiss-Webster strain weighing 20–25 g were used. All injections were *via* the intravenous route (tail) in volumes not exceeding 0.01 ml/g of body weight. Testing protocol consisted of suspending or dissolving all compounds in water or 0.5% methylcellulose. Approximate 0.5 log-spaced doses were employed to characterize a "no effect" to 100% lethal response. Four animals were used at each dose level. Each animal was evaluated for significant activity at 5, 10, 15, 30, and 60 min postinjection and thereafter at 2, 4, and 24 hr. The statistical method, employed for the calculation of LD_{50} and MED_{50}, is that described by Weil.[14] The MED_{50} shown in Table I is based on the first pharmacological effect shown in the "effects" column; other effects (those shown after the comma) may or may not be observed at the MED_{50} dose. The MED_{50} is based on the pharmacological effect shown at the lowest dose which gave any clear effect.

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Puromycin Analogs.[1] Synthesis and Biological Activity of 5′-Deoxypuromycin and Its Aminonucleoside, 6-Dimethylamino-9-(3′-amino-3′,5′-dideoxy-β-D-ribofuranosyl)purine†

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The antibiotic puromycin derives its antimicrobial and antitumor activity from its ability to cause a premature release of growing polypeptide chains from ribosomes.[2] The aminonucleoside derived from puromycin is devoid of antibacterial activity but is three to four times as cytotoxic as the parent antibiotic against mammalian cells.[3] Consequently, an additional cytotoxic effect on the host may result from the release of aminonucleoside if hydrolytic or enzymatic removal of the amino acid moiety from administered puromycin occurs[4] (Figure 1). Recent studies demonstrate that the aminonucleoside is monodemethylated[5] and subsequently converted to the 5′-nucleotide.[6] It is not unreasonable to assume, therefore, that nucleotide formation is a prerequisite to cytotoxic activity. This same metabolic scheme may also account for the severe nephrotoxic manifestations, including renal lesions, resulting from administered puromycin aminonucleoside.

In an attempt to improve the selective toxicity of the antibiotic, an assessment of the requirement for the 5′-OH in both the antimicrobial activity of puromycin and in the cytotoxic activity of the aminonucleoside is desirable. Previous studies with carbocyclic analogs have been useful in defining the ribosomal binding requirements of the antibiotic.[7] However, the absence of a sugar moiety in these analogs precludes the assessment of the 5′-OH group's contribution to the cytotoxic effect. Thus, we have developed

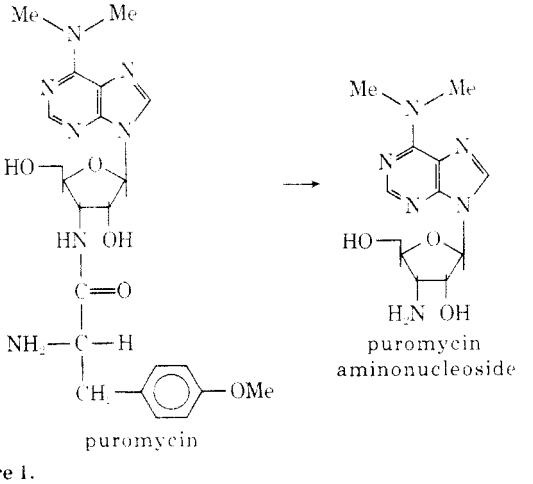

Figure 1.

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