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Synthesis of Potential Hypolipidemic Agents. Reaction of Substituted Phenyl 2,3-Epoxypropyl Ethers with Adenine, Uracil, and Thymine

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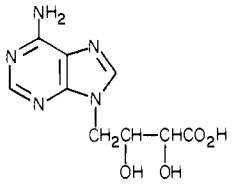
Three adenine derivatives were found to be active hypolipidemic agents at 10 mg/kg/day. The most active compound was 9-(*p*-chlorophenoxy-2-hydroxypropyl)adenine (**5**). Compound **5** significantly lowered serum triglyceride and cholesterol content in male Sprague–Dawley rats and inhibited hepatic phosphatidate phosphohydrolase activity in vitro. The synthesis of these agents involved reacting adenine, uracil, and thymine with a series of substituted phenyl 2,3-epoxypropyl ethers.

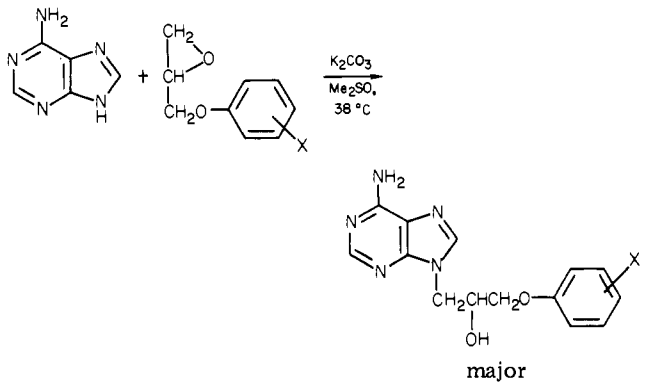
Previous work in our laboratories has shown that a series of 1,3-bis(substituted phenoxy)-2-propanones possessed excellent hypocholesterolemic activity.[1] A further extension of this work is described and is based on structure–activity studies[2] performed on eritadenine (I), a



I

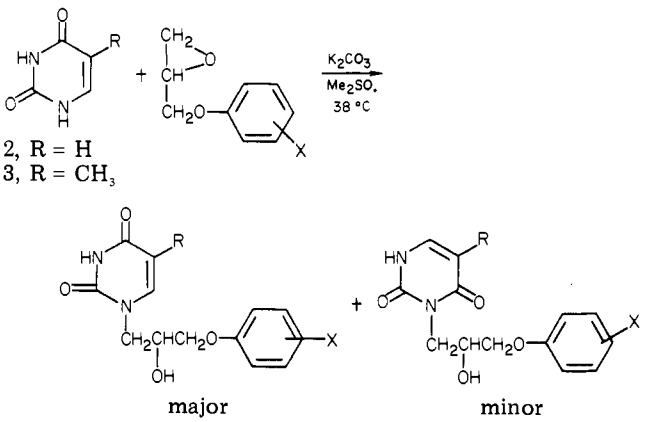
naturally occurring substance isolated from the Japenese mushroom, shiitake (*Lentinus edodes*), and other purine analogues[3] which show good hypolipidemic activity. We prepared for testing a number of 9-substituted adenine and 1-substituted pyrimidine derivatives which incorporated the phenoxy-2-propanol moiety. These derivatives were synthesized by reacting the appropriate epoxide with adenine (Scheme I), uracil, or thymine (Scheme II) in the presence of K_2CO_3 in Me_2SO. We found this method to be superior to the one using NaOH with DMF as solvent.[4] Fewer side products were observed by TLC. The epoxides were originally prepared according to standard literature procedures.[5] In an effort to improve yields we used the phase-transfer catalysis procedure (Scheme III) described by McKillop and co-workers[6] with one modification in that no organic solvent was used. We found that the reaction proceeded faster in the absence of methylene chloride and also eliminated the possibility of diaryloxymethane formation.[11]

Scheme I



major

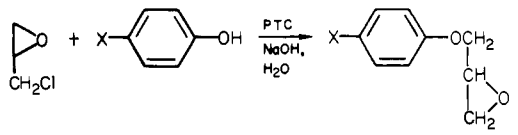
Scheme II



2, R = H
3, R = CH_3

major minor

Scheme III



20, X = Cl
21, X = F

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The NMR spectra were taken in Me_2SO-d_6 with tetramethylsilane as an internal standard on a Jeolco C60-HL spectrometer. The IR absorptions were obtained with a Perkin-Elmer 257 spectrophotometer and the UV spectra were determined in H_2O with a Cary Model 15 spectrophotometer. The spectral data were as expected and therefore only representative data are reported. TLC refers to microslides coated with Merck silica gel GF-254 and visualized by ultraviolet light. Elemental analyses agreed with the theoretical values within ±0.4% and were obtained from Atlantic Microlab,

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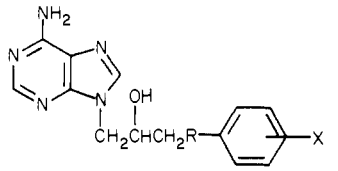
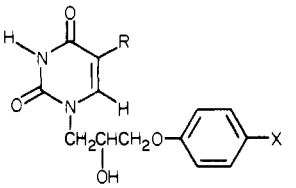
Table I. 9-(Substituted phenoxy-2-hydroxypropyl)adenine Derivatives



no.	R	X	mp, °C	formula	recrystn solvent	% yield	analyses
4	O	*p*-F	175-177	$C_{14}H_{14}N_5O_2F$	EtOH	65	C, H, N
5	O	*p*-Cl	200-201	$C_{14}H_{14}N_5O_2Cl$	EtOH	46	C, H, N, Cl
6	O	*p*-Br	207-210	$C_{14}H_{14}N_5O_2Br$	EtOH	50	C, H, N, Br
7	O	*p*-OCH_3	182-184	$C_{15}H_{17}N_5O_3$	EtOH	25	C, H, N
8	O	*p*-*t*-C_4H_9	180-183	$C_{18}H_{23}N_5O_2$	EtOH	30	C, H, N
9	O	*p*-$COCH_3$	172-175	$C_{16}H_{17}N_5O_3$	EtOH	50	C, H, N
10	O	*m*-Cl	172-174	$C_{14}H_{14}N_5O_2Cl$	EtOH-MeOH	27	C, H, N, Cl
11	O	*o*-Cl	198-200	$C_{14}H_{14}N_5O_2Cl$	MeOH-EtOH	54	C, H, N, Cl
12	S	*p*-Cl	177-178	$C_{14}H_{14}N_5SOCl$	EtOH	45	C, H, N, S, Cl
13	O	*p*-$C_6H_4COC_6H_5$	194-198	$C_{21}H_{19}N_5O$	MeOH-EtOH	20	C, H, N

Table II. Substituted Pyrimidines



no.	R	X	mp, °C	formula	recrystn solvent	% yield	analyses
14	H	Cl	160-161	$C_{13}H_{12}N_2O_4Cl$	2-propanol	32	C, H, N, Cl
15	H	OCH_3	194-196	$C_{14}H_{15}N_2O_5$	2-propanol-acetone	42	C, H, N
16	H	CH_3	178-179	$C_{14}H_{15}N_2O_4$	acetone	35	C, H, N
17	CH_3	CH_3	161-162	$C_{14}H_{17}N_2O_4$	acetone	20[a]	C, H, N
18	CH_3	Cl	181-183	$C_{13}H_{14}N_2O_4Cl$	acetone	25[a]	C, H, Cl

[a] Reaction temperature of 55-60 °C.

Atlanta, Ga. All starting materials were used as received from suppliers.

9-(*p*-Chlorophenoxy-2-hydroxypropyl)adenine (5). Adenine (10.0 g, 0.078 mol) was dissolved in 200 mL of dry Me_2SO by heating to 90 °C. After cooling to 38 °C the K_2CO_3 (5.0 g, 0.039 mol) was added. The *p*-chlorophenyl 2,3-epoxypropyl ether (21.6 g, 0.117 mol) in 50 mL of Me_2SO was added dropwise and the reaction was stirred for 18 h. The reaction mixture was filtered and to the filtrate was added 200 mL of H_2O. After allowing to stand overnight the precipitate was removed by filtration and fractional crystallization from ethanol gave 10.5 g (42%) of **5**: mp 200–201 °C; UV max 261 nm; NMR (Me_2SO-d_6) δ 8.2 and 8.1 (2 s, 2, adenine CH), 7.2 (s, 2, NH_2), 7.15 (q, 4, aromatic H), 5.8 (br, 1, OH), 4.3 (m, 2, propyl CH), 3.9–4.1 (m, 3, propyl CH). Anal. ($C_{14}H_{14}N_5O_2Cl$) C, H, N, Cl. This procedure was used for compounds in Table I.

1-(*p*-Methylphenoxy-2-hydroxypropyl)uracil (17). Uracil (3.0 g, 0.027 mol) was dissolved in 75 mL of Me_2SO. The K_2CO_3 (0.4 g, 0.003 mol) was added followed by the dropwise addition of the *p*-methyl 2,3-epoxypropyl ether (4.4 g, 0.027 mol). The reaction was stirred for 72 h. Me_2SO (50 mL) was removed in vacuo and the residue was quenched with 75 mL of hot water. The precipitate was filtered and recrystallized from acetone to give 2.5 g (34%) of **17**: mp 178–179 °C; NMR (Me_2SO-d_6) δ 7.6 and 5.55 (2 d, 2, uracil CH), 7.0 (q, 4, aromatic H), 2.9–4.2 (br, 7, propyl CH, NH, OH), 2.3 (s, 3, CH_3). Anal. ($C_{14}H_{15}N_2O_4$) C, H, N. This procedure was used for the compounds listed in Table II.

***p*-Chlorophenyl 2,3-Epoxypropyl Ether (20).** To a solution of *p*-chlorophenol (10 g, 78 mmol), NaOH (3.8 g, 95 mmol), and benzyltri-*n*-butylammonium chloride (0.5 g, 1.6 mmol) in 75 mL of H_2O was added the epichlorohydrin (10.8 g, 117 mmol) all at once. The reaction was stirred vigorously for 1 h. The organic layer was separated and the aqueous layer was washed with three 25-mL portions of ether. The organic layers were combined and washed with 10% NaOH to remove unreacted phenol and finally with brine. The organic layer was dried through magnesium sulfate and evaporated under reduced pressure. Short-path distillation (132 °C, 3 mm) gave 10.0 g (71%). This material was identical with a commercially purchased sample.

***p*-Fluorophenyl 2,3-Epoxypropyl Ether (21).** **21** was prepared in the manner described for **20**. Short-path distillation (76 °C, 0.15 mm) gave after a small forerun the desired epoxide in 74% yield.

Animals and Diet. Male Sprague–Dawley rats (Zivic Miller, Allison Park, Pa.) were fed Purina rodent lab chow with water ad libitum for the duration of the experiment. Drugs were suspended in 1% CMC (carboxymethylcellulose)–H_2O and homogenized. Doses were calculated on weekly animal weights.

Serum Hypocholesterolemic Activity. All drugs (10 mg/kg/day) were administered to male rats by oral intubation needle (0.2 cm^3) daily at 11:00 a.m. After dosing (22–24 h) blood was collected by tail vein bleeding. The blood was collected in alkali-free nonheparinized microcapillary tubes and centrifuged for 3 min to obtain the serum.[7] Duplicate 30-μl samples of nonhemolyzed serum were used to determine the mg % of serum cholesterol by a modification of the Liebermann–Burchard reaction.[8]

Serum and Triglyceride Determination. Lipids were extracted from three separate 1-g portions of liver and 0.5-mL aliquots of serum as described by Bligh and Dyer.[12] Chloroform–lipid extracts were applied to small silicic acid columns and the neutral lipid and phospholipid fractions were separated by eluting first with 100% chloroform and then 1:1 chloroform–methanol containing 10% 1 N HCl.[10] Triglyceride content of the neutral lipid fraction was determined by a slight modification of the procedure described by Haux and Natelson.[13]

In Vivo and in Vitro Hepatic Glycerolipid Biosynthesis. In vivo glycerolipid biosynthesis was estimated by measuring the incorporation of intraperitoneally administered [1,3-^{14}C]glycerol

Table III. Percent Control of Serum Cholesterol after Administration of Test Compound at 10 mg/kg/day to Male Rats[d]

compd	$\bar{X}$ ± SD on day 4	10	16
control (1% CMC)	100 ± 7	100 ± 8	100 ± 8
4	88 ± 7	86 ± 7	87 ± 11[a]
5	100 ± 4	93 ± 7	52 ± 12[b]
6	95 ± 7	96 ± 7	80 ± 10[c]
7	91 ± 15	93 ± 7	100 ± 9.6
8	106 ± 20	110 ± 11	81 ± 10
9	93 ± 11	99 ± 8	92 ± 4
10	100 ± 11.6	97 ± 8	95 ± 6
11	105 ± 23	93 ± 9.4	95 ± 7
12	96 ± 9	79 ± 9	89 ± 16
13	96 ± 12	118 ± 13	92 ± 9
14	106 ± 13	102 ± 8	110 ± 9.3
15	91 ± 17	93 ± 7	100 ± 9.6
16	74 ± 8	89 ± 11	100 ± 6.4
17	93 ± 10	99 ± 12	95 ± 8
18	95 ± 22	96 ± 5	98 ± 9.1

[a] $p < 0.05$. [b] $p < 0.001$. [c] $p < 0.025$. [d] $N = 8$.

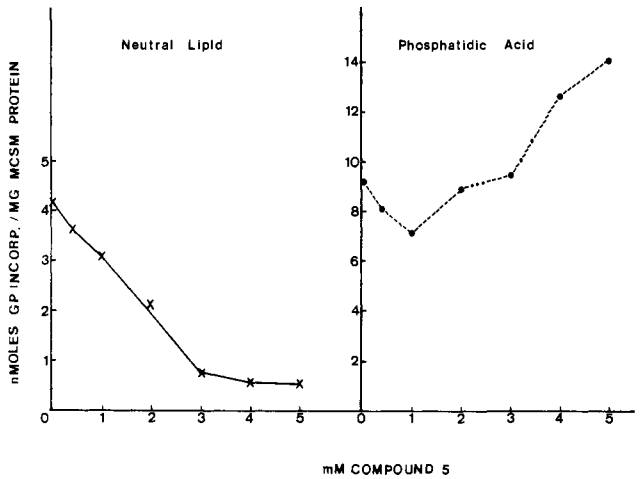


Figure 1. The effect of compound **5** in vitro on the incorporation of [1,3-^{14}C]glycerol 3-phosphate into neutral lipid (diglyceride + triglyceride) and phosphatidic acid by rat liver microsomes.

into hepatic glycerolipids.[9] The in vitro formation of glycerolipids from *sn*-[1,3-^{14}C]glycerol 3-phosphate by rat liver microsomes was determined by techniques described previously.[10]

Results and Discussion

None of the pyrimidines tested (Table II) had any effect on serum cholesterol. Of the adenine derivatives (Table I) that were synthesized, compound **5** was the most active. The ortho- (**11**) and meta-substituted (**10**) chloro derivatives were inactive. Substitution of sulfur (**12**) for oxygen in the ether linkage resulted in a compound with no activity. Replacement of the chlorine by fluorine (**4**) or bromine (**6**) in the para position gave compounds which were less active. All of the other derivatives were inactive (Table III).

Preliminary observations have suggested that in vitro hepatic phosphatidate phosphohydrolase inhibitors reduce hepatic triglyceride biosynthesis in vivo and lower serum triglyceride levels.[10] However, the agents tested in this earlier study also inhibited *sn*-glycerol-3-phosphate acyltransferase activity and it was not possible to determine which effect was primarily responsible for the reduction in hepatic triglyceride production and serum triglyceride levels.

Figure 1 shows that the in vitro addition of **5** (0.5–5 mM) inhibited the incorporation of [1,3-^{14}C]glycerol 3-phosphate into neutral lipid (diglyceride + triglyceride) by rat liver microsomes in the presence of ATP, palmitate, CoA, and Mg^{2+}. Phosphatidic acid accumulated under these conditions (Figure 1), suggesting that compound **5** reduced phosphatidate phosphohydrolase activity. More direct measures of the effect of compound **5** on hepatic phosphatidate phosphohydrolase activity are shown in Figure 2 and they indicate that 2.0 mmol of compound **5** produced a 50% reduction of enzyme activity. The administration of compound **5** to rats once a day for 7 days at doses of 10, 50, and 100 mg/kg of body weight significantly reduced the incorporation of intraperitoneally administered [1,3-^{14}C]glycerol into hepatic triglyceride (TG) (Table IV) and lowered serum triglyceride content (Table V).

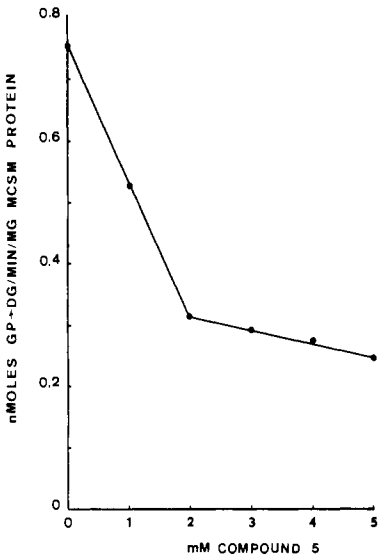


Figure 2. The in vitro effect of compound **5** on microsomal phosphatidate phosphohydrolase activity.

Table IV. Effect of Administering **5** (10, 50, and 100 mg/kg/day) for 1 Week on Hepatic Glycerolipid Formation from Intraperitoneally Administered [1,3-^{14}C]Glycerol[a]

	% control ± SEM[b] TG	PL
control	100.0 ± 8.4	100.0 ± 8.3
5 (10 mg/kg)	36.7 ± 5.6[c]	74.9 ± 12.9
5 (50 mg/kg)	29.0 ± 1.3[c]	33.5 ± 0.78[c]
5 (100 mg/kg)	31.8 ± 1.4[c]	36.7 ± 0.87[c]

[a] Expressed as nmol of [1,3-^{14}C]glycerol incorporated g^{-1} min^{-1}. [b] Control values for liver triglyceride (TG) and phospholipid (PL) were 68.9 ± 8.4 and 49.9 ± 6.1 nmol g^{-1} min^{-1}, respectively. [c] $p \leq 0.001$ level of significance from control.

Table V. Effect of Administering **5** (10, 50, and 100 mg/kg/day) for 1 Week on Serum and Hepatic Triglyceride Levels[a]

	% control ± SEM serum	liver
control	100.0 ± 5.3[b]	100.0 ± 8.4[c]
5 (10 mg/kg)	73.0 ± 3.3[d]	57.6 ± 7.5[e]
5 (50 mg/kg)	38.5 ± 2.2[e]	251.8 ± 7.7[e]
5 (100 mg/kg)	40.3 ± 3.7[e]	345.1 ± 11.0[e]

[a] Control values for serum and liver triglycerides were 100 ± 5.3 mg/100 mL and 5.9 ± 0.5 mg/g, respectively. [b] Expressed as mg/100 mL. [c] Expressed as mg/g of liver. [d] $p \leq 0.01$ level of significance from control. [e] $p \leq 0.001$ level of significance from control.

Compound **5** produced a variable effect on hepatic triglyceride content since low doses (10 mg/kg) reduced but higher doses (50 and 100 mg/kg) elevated liver triglyceride levels (Table V). The high doses (50 and 100 mg/kg) of compound **5** did not lower hepatic triglyceride biosynthesis measured in vivo more than a 10 mg/kg dose (Table IV). Therefore, the significantly lower serum triglyceride concentration associated with the 50 mg/kg dose of compound **5** compared to the 10 mg/kg dose (Table V) is probably the result of a decrease in hepatic triglyceride (very low density lipoprotein, VLDL) release since liver triglyceride content increased significantly. None of the doses (10–100 mg/kg) of compoumd **5** used in these studies killed any animals or produced abnormal alterations in body weight gain and food intake. Therefore, inhibitors of hepatic phosphatidate phosphohydrolase activity may effectively reduce hepatic triglyceride biosynthesis in vivo and lower serum triglyceride (VLDL) levels.

The mechanism(s) by which compound **5** might lower serum cholesterol have not been studied directly. However, the observation that compound **5** reduces serum triglyceride content (7 days) before altering serum cholesterol levels (16 days) might indicate that these responses are interrelated. This seems reasonable since it is well established that serum VLDL (triglyceride rich) is the precursor of serum LDL (low density lipoprotein, cholesterol rich).[14] Therefore, reductions in the production and secretion of hepatic triglyceride (VLDL) should lower serum cholesterol (LDL), provided reductions in the clearance of these lipoproteins do not occur.

References and Notes

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Synthesis and Carcinogenic Activity of 5-Fluoro-7-(oxygenated methyl)-12-methylbenz[*a*]anthracenes

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Treatment of 7,12-benz[*a*]anthraquinone (**2**) with methylmagnesium iodide or methyllithium yields mixtures of *cis*- and *trans*-7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracenes (**3a,b**), in which the ratio of cis to trans lies in the 3–4:1 region. Each isomer afforded high yields of 7-chloromethyl-12-methylbenz[*a*]anthracene (**5**) on treatment with hydrogen chloride in ethyl acetate. Similarly, 5-fluoro-7,12-benz[*a*]anthraquinone (**8**) afforded a mixture of *cis*- and *trans*-5-fluoro-7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracenes (**9**) which yielded 7-chloromethyl-5-fluoro-12-methylbenz[*a*]anthracene (**10**) on treatment with HCl. The chloromethyl compounds, **5** and **10**, yielded 7-acetoxymethyl-12-methylbenz[*a*]anthracene (**6**) and 7-acetoxymethyl-5-fluoro-12-methylbenz[*a*]anthracene (**11**) on treatment with acetate ion. Hydrolysis of **6** and **11** yielded 7-hydroxymethyl-12-methylbenz[*a*]anthracene (**7**) and 5-fluoro-7-hydroxymethyl-12-methylbenz[*a*]anthracene (**12**), respectively. Since neither **11** nor **12** is appreciably carcinogenic, the carcinogenic metabolism of 7,12-dimethylbenz[*a*]anthracene (DMBA) probably does not involve attack at the 7-methyl group.

One of the chief methods for preparing 7,12-dimethylbenz[*a*]anthracene (DMBA, **1**), a compound of widespread interest in cancer research, involves the reaction of 7,12-benz[*a*]anthraquinone (**2**) with methylmagnesium iodide to form 7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracene (**3**) which is converted into 7-iodomethyl-12-methylbenz[*a*]anthracene (**4**) by treatment with methanolic HI.[2] Reduction of **4** yields **1**.

Because of instability **4** is not a good intermediate if oxygenated substitution products on the 7-methyl group are desired. As we were interested in preparing such oxygenated derivatives not only of **1** but also of 5-fluoro-7,12-dimethylbenz[*a*]anthracene[3] (**7**), we have reinvestigated the above synthetic route. The oxygenated derivatives of **7** were desired for testing as to carcinogenic activity because of the hypotheses that the metabolic pathway important in the carcinogenic metabolism of all 7-methylbenz[*a*]anthracenes may involve oxygenation or other reactions at the 7-methyl groups.[4]

The reactions of **2** with methylmagnesium bromide and iodide or methyllithium are difficult to reproduce. In all cases varying amounts of unreacted quinone are present regardless of the excess organometallic reagent used or the time of reaction. We believe these results can be explained by the formation of a complex between quinone and the salt of the diol produced. This complex evidently resists further reaction with excess organometallic reagent but we do not understand why the amount of this complex varies so widely in different experiments.

Only one isomer, mp 181.5–182.5 °C, has been reported from the reaction of **2** with methylmagnesium iodide.[5] This was shown to be the cis isomer by comparison[6] with

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