chloromethane was refluxed for 1 h under nitrogen with 0.6 g (0.00215 mol) of triphenylmethyl chloride. The solution was concentrated and applied to a 20 cm × 20 cm × 2 mm silica gel plate. After development with chloroform–ethyl acetate (1:1), the uv-active band of highest R_f was scraped off and eluted with chloroform–methanol. Recrystallization of the resulting solid from methanol gave 270 mg (26%), mp 209–211°, identical (ir, NMR, TLC) with a sample, mp 213–214°, prepared via the mesylate.[8] Anal. ($C_{26}H_{36}ClFO_5$) C, H, Cl, F.

Hydrolysis of 9d. A slurry of 150 mg of **9d** in 15 ml of methanol and 0.8 ml of 10% aqueous potassium carbonate solution was stirred at 0° for 90 min, neutralized with acetic acid, and diluted with cold water. The resulting solid was filtered and then dissolved in chloroform–methanol. The solution was dried and concentrated in vacuo to give 100 mg (91%) of 21-chloro-9-fluoro-11β,16α,17-trihydroxypregna-1,4-diene-3,20-dione, mp 265° dec, identical with an authentic sample prepared by hydrolysis of the corresponding acetonide.[12]

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Antimalarial Activity of Some Novel Derivatives of 2,4-Diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine (Pyrimethamine)

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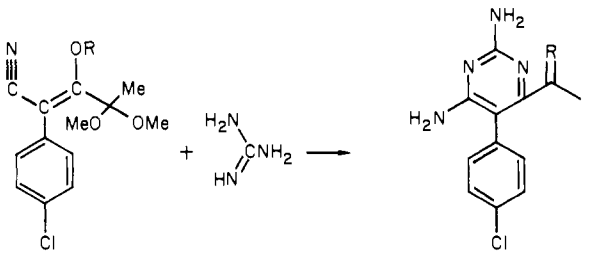
Thirteen new analogs of 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine (Daraprim, pyrimethamine) in which the α position of the 6-ethyl substituent was modified were prepared. The respective oxygen analogs (ketals, ketone, alcohol), the dimethyl hydrazone, and the nitrone displayed activities in the range of $^1/_4$ to $^1/_{16}$ that of pyrimethamine toward *Plasmodium berghei* in mice. The therapeutic ratios of some of these compounds may be slightly better than that of pyrimethamine.

Pellicularia f. sp. filamentosa sasakii 1F0 6675 hydroxylates and oxidizes the α position of the 6-ethyl substituent of pyrimethamine (3). The two products, 2,4-diamino-5-(*p*-chlorophenyl)-6-pyrimidinyl methyl ketone (6) and (+)-2,4-diamino-5-(*p*-chlorophenyl)-α-methyl-6-pyrimidinemethanol (dextro rotamer of 7), were isolated and identified by Greenspan et al.[1] For confirmation of the structure and additional biological testing, we deemed it necessary to synthesize the ketone 6 and the racemic alcohol 7 by chemical means. Furthermore, these products provided ready access to the little explored derivatives of pyrimethamine in which the α position of the 6-ethyl side chain is functionalized.

Synthesis. The most attractive approach toward the synthesis of the 6-methyl ketone 6 was essentially the one originally described by Russell and Hitchings[2]; this method was also employed by Baker and Jordaan[3] for the preparation of the 6-formyl compound. *p*-Chlorophenylacetonitrile was allowed to react with ethyl 2,2-dimethoxypropanoate in basic medium to give the enol 1, which, on treatment with diazomethane, furnished the enol ether 2. Guanidine reacted readily with the enol ether 2 in ethanolic sodium ethoxide to give 2,4-diamino-5-(*p*-chlorophenyl)-6-pyrimidinyl methyl ketone dimethyl acetal (4) in high yield. The ketal-protecting group was hydrolyzed in acidic aqueous methanol giving the methyl ketone 6, which was identical in all respects with material isolated by Greenspan et al.[1] Subsequent transformations of this ketone to the alcohol 7, the oxime 8 (for the structure of 8 and subsequent numbers, see Table I), the hydrazones 11, 12, and 13, and the nitrone 14 were conducted by standard methods and were unexceptional.



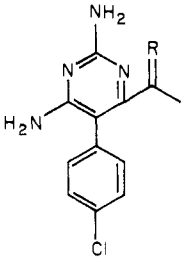
1, R = H
2, R = Me

3, R = H_2 (pyrimethamine)
4, R = $(OMe)_2$
5, R = $(OEt)_2$
6, R = O
7, R = H, OH

Raney nickel reduction of the oxime furnished the amine 9, which on acylation gave the amide 10. The guanidino compound 15 was prepared in the usual fashion. The physical constants of these compounds are listed in Table I. The racemic alcohol 7 had chromatographic mobilities and solution spectra identical with those of the resolved alcohol prepared by Greenspan.[1]

Antimalarial Activity. All compounds were tested for antimalarial activity against *Plasmodium berghei* (strain KBG 13) in mice, and the more potent ones were also tested against *Plasmodium gallinaceum* in a limited fashion by procedures described earlier.[4] Table II lists the activities of the various derivatives against *P. berghei*. The most potent compounds producing cures at relatively high levels were the acetals 4 and 5, the methyl nitrone 14, and the alcohol 7. The ketone 6 and the dimethyl hydrazone 12 produced cures at 320 mg/kg but were found to be toxic

Table I. Derivatives of 2,4-Diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine



Compd	R	Mp, °C	Recrystn solvent	Yield, %[a]	Formula	Analyses
4	$(OMe)_2$	250-255	$CHCl_3$-MeOH	22	$C_{14}H_{17}ClN_4O_2$	C, H, N, Cl
5	$(OEt)_2$	210-212	$CHCl_3$-MeOH	15	$C_{16}H_{21}ClN_4O_2$	H, N, Cl; C[b]
6	O	206-208	$CHCl_3$-MeOH	21	$C_{12}H_{11}ClN_4O_2$	C, H, N, Cl
7	HOH[c]	159, 187-188	MeOH	18	$C_{12}H_{13}ClN_4O$	C, H, N
8	NOH[d]	256-259	MeOH	13	$C_{12}H_{12}ClN_5O$	H, N, Cl; C[e]
9	HNH_2[c]	214-217	C_6H_6-EtOH	8	$C_{12}H_{14}ClN_5$	C, H, N, Cl
9a	$HNH_2 \cdot 2HCl$	280-284	EtOH	7	$C_{12}H_{16}Cl_3N_5$	C, H, N, Cl
10	HNC(=O)Me[c]	300-305 dec	DMF	8	$C_{14}H_{16}ClN_5O$	C, H, N, Cl
11	NNH_2	209-213	EtOH	16	$C_{12}H_{13}ClN_6$	C, H, N, Cl
12	$NN(Me)_2$	232-236	C_6H_6-EtOH	20	$C_{14}H_{17}ClN_6$	C, H, N, Cl
13	NNHMe[f]	248-252	$CHCl_3$-EtOH	19	$C_{13}H_{15}ClN_6$	C, H, N, Cl
14	N(→O)Me[f]	306-309	$CHCl_3$-EtOH	17	$C_{13}H_{14}ClN_5O$	C, H, N, Cl
15	$NNHC(NH_2)=NH \cdot 2HNO_3$	254 dec	MeOH-H_2O	10	$C_{13}H_{17}ClN_{10}O_6$	C, H, N, Cl
16	$HNCH(Me)(CH_2)_3N(Et)_2$[g]	138-141	EtOH	6	$C_{21}H_{33}ClN_6$	C, H, N, Cl

[a] Calculated on the basis of *p*-chlorophenylacetonitrile. [b] C: calcd, 57.1; found, 56.4. [c] Racemate. [d] Assumed to be a mixture of the syn and anti forms. [e] C: calcd, 51.9; found, 51.2. [f] Compounds 13 and 14 were cis-trans mixtures of 5:1 and 1:1, respectively, as shown by NMR spectroscopy. [g] Prepared by catalytic reduction of the respective imine, which was prepared by refluxing the ketone 6 and excess *N,N'*-diethyl-1,4-pentanediamine in EtOH.

Table II. Activity of Compounds of Table I against *P. berghei* in Mice

Compd	Increase in mean survival time at 640 mg/kg, days (cured/ treated)	Min dose giving cures, mg/kg (cured/ treated)	Min dose showing act., mg/kg	Increase in mean survival time at min dose, days
4	(2/5)	320 (1/5)	40	4.5
5	(5/5)	160 (1/5)	20	3.5
6[a]		320 (2/5)	80	5.9
7	(1/5)	None	80	4.7
8	5.4	None	160	3.4
9a	Inactive	Inactive	Inactive	Inactive
10	Inactive	Inactive	Inactive	Inactive
11	14.8	None	160	8.4
12[a]		320 (2/5)	40	3.8
13[a]		Inactive	Inactive	Inactive
14	(2/5)	640 (2/5)	160	3.1
15	Inactive	Inactive	Inactive	Inactive

[a] Compounds 6, 12, and 13 caused five out of five toxic deaths at 640 mg/kg.

at higher levels. The amino substituent on the 6-ethyl group of pyrimethamine (compound **9**) eliminated all activity against *P.* berghei. The amino derivative **16** had no activity against *P. gallinaceum* and was not further tested against *P. berghei*. The compounds which produced cures (the dimethyl acetal **4**, the diethyl acetal **5**, the ketone **6**, the alcohol **7**, the dimethyl hydrazone **12**, and the nitrone **14**) were tested against *P. gallinaceum* in chicks at doses of 100–160 mg/kg. All were found to be active, having a T/C of from 5.4 to 6.4 days at the respective levels, with the exception of the nitrone **14**, which showed no activity at 120 mg/kg.

In summary, it can be said that substituting the α position of the 6-ethyl substituent in pyrimethamine reduces or eliminates the antimalarial activity. The most potent compounds (**4–7**, **12**, and **14**) produce cures at minimum doses of 160–640 mg/kg in mice against *P. berghei*, thus having roughly only about $^1/_4$ to $^1/_{16}$ the activity of the standard, pyrimethamine, which produces cures at a minimum of 40 mg/kg.[5] However, pyrimethamine causes toxic death at slightly above twice the minimum curative dose, while no toxic deaths occurred (with **5**, for example) at four times the minimum curative dose. The ketone **6** seems to have the same therapeutic ratio as the parent compound, although it is less potent. The lower homologue of pyrimethamine, namely, 2,4-diamino-5-(*p*-chlorophenyl)-6-methylpyrimidine, is less potent than pyrimethamine.[6] Subsequently, it was shown that the activity is further reduced in substituting the 6-methyl group (e.g., 6-carboxyl, 6-carbethoxy, or 6-trifluoromethyl) by the diminished inhibitory effect of these compounds toward dihydrofolic reductase.[7] Thus, in both cases (6-methyl and 6-ethyl) further substitution in the alkyl group is accompanied by a decrease in potency.

Experimental Section

All melting points were taken on a Kofler block under microscopic magnification. NMR spectra were routinely recorded on a Jeolco 60 A spectrometer using 10–15% solutions in Me_2SO-d_6. The ir spectra of the crystalline products were determined in KBr disks, and those of oils were recorded neat. All spectra supported the suggested structures.

α-(*p*-Chlorophenyl)-β-methoxy-β-(1,1-dimethoxyethyl)-acrylonitrile (2). To a solution of 2.3 g (0.1 mol) of sodium metal in 60 ml of EtOH, 15.1 g (0.1 mol) of *p*-chlorophenylacetonitrile was added, followed by 16.2 g (0.1 mol) of ethyl dimethoxypyruvate, and the whole mixture was refluxed for 16 hr. After being cooled, the reaction mixture was poured into ice and extracted with Et_2O. The aqueous layer was cooled to 0°, carefully acidified with 6 N HCl, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O (three times), dried, and evaporated, leaving 8.5 g (31.5%) of α-(*p*-chlorophenyl)-α-(2,2-dimethoxypropanoyl)acetonitrile (1) as a brown oil: ir 3340 (OH), 2990, 2960, 2860, (CH), 2220 (C≡N), 1745 (CO), 1630, 1592, 1493 cm^{-1} (aromatic). This oil was dissolved in 30 ml of $(Et)_2O$, an excess of CH_2N_2 in $(Et)_2O$ (prepared from 25 g of *N*-nitrosomethylurea)

was added, and the mixture was left at 5° overnight. Work-up resulted in a brown oil: 8.0 g (87%); ir 2990, 2950, 2850 (CH), 2220 (C≡N), 1595, 1550, 1490 cm^{-1} (aromatic). An estimated 5% of the enol 1 could be detected in the ir spectrum.

2,4-Diamino-5-(*p*-chlorophenyl)-6-pyrimidinyl Methyl Ketone Dimethyl Acetal (4). To a solution of 6.9 g (0.3 mol) of sodium metal in 100 ml of EtOH there was added 8.0 g (0.028 mol) of crude **2** in 50 ml of EtOH, followed by 27.0 g of guanidine·$2HNO_3$, and the resulting mixture was stirred under reflux for 2 h. EtOH was removed in vacuo and H_2O was added until there appeared a crystalline solid: 8.0 g; mp 235–245°. Recrystallization from $CHCl_3$–MeOH gave the analytical sample (6.0 g, 70%).

2,4-Diamino-5-(*p*-chlorophenyl)-6-pyrimidinyl Methyl Ketone (6). Compound **4** (5 g, 0.016 mol) was dissolved in 200 ml of MeOH and 20 ml of concentrated HCl and the whole mixture was refluxed for 2.0 h. Concentration of the methanolic solution to one-third volume caused precipitation of the hydrochloride salt, which was filtered (4.8 g), dissolved in warm water, and precipitated with concentrated NH_4OH, giving the desired ketone (4.0 g, 95%), mp 200–204°. Recrystallization from $CHCl_3$–MeOH gave the analytical sample.

***dl*-2,4-Diamino-5-(*p*-chlorophenyl)-α-methyl-6-pyrimidinemethanol (7).** Compound **6** was reduced in MeOH by $NaBH_4$, yielding 88% of the respective alcohol.

Acknowledgment. Tests with *P. berghei* and *P. gallinaceum* were conducted by the late Dr. Leo Rane, to whom we are indebted for these test results. Dr. Greenspan of these laboratories supplied us with comparative samples of microbial transformation products of pyrimethamine, for which we express our thanks.

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Conformationally Rigid Amphetamine Analogs as Inhibitors of Monoamine Uptake by Brain Synaptosomes

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Four 3-phenyl-2-amino-*trans*-decalin isomers were synthesized in order to obtain derivatives of phenylethylamine with a rigid conformation between the phenyl ring and the amino function. The stereoisomers were tested as inhibitors of catecholamine uptake by rat brain synaptosomes, and their potency was compared with that of amphetamine. The most potent inhibitor of catecholamine uptake was the diaxial 2(a)-amino-3(a)-phenyl-*trans*-decalin, which was one-fourth to one-third as potent as (±)-amphetamine. As a dopamine uptake inhibitor in the striatum, this compound was competitive. The results differ from those obtained earlier with similar analogs with a norepinephrine moiety incorporated into the decalin structure, since a gauche derivative [2(a)-amino-3(e)-3,4-dihydroxyphenyl-3-*trans*-decalol] was then the most potent and over 20 times as potent as the diaxial anti derivative. It remains to be seen whether this indicates that the mode of binding of phenylethylamines is different from that of catecholamines.

In recent work at these laboratories 3-catechol derivatives of 2-amino-*trans*-3-decalol were studied as inhibitors of dopamine uptake.[1] In these compounds the structure of sterically rigid norepinephrine can be found. Four racemic isomers were utilized: in one of them the relation between the catechol ring and the amino group was anti; in the three others it was gauche. One of the gauche derivatives, (±)-2(a)-amino-3(e)-3,4-dihydroxyphenyl-3-*trans*-decalol, was by far the most potent dopamine uptake inhibitor, and the potency was one-half that of (–)-norepinephrine. The result was interpreted as suggesting that a gauche conformation is preferable for uptake inhibition and that the uptake site might require the substrate in this conformation as well.

The present experiments extended the study to amphetamine-like *trans*-decalin derivatives. These compounds have been tested previously as inhibitors of 5-hydroxytryptamine and histamine uptake by rabbit blood platelets, but no clear structural correlation was found in those experiments.[2,3]

Results and Discussion

Active Uptake of Dopamine and Norepinephrine. The uptake characteristics of dopamine agreed closely with those observed previously.[1] A tissue–medium ratio of 90.0 ± 11.5 (SD) was obtained in 5 min when the substrate concentration was 10^{-7} M (the ratio was calculated for the original wet weight of striatal tissue and would thus be much higher for the synaptosomal fraction actually used). The uptake was saturable, and the kinetic constants were $K_m = 8.8 \times 10^{-8}$ M and $V_{max} = 11.2$ nmol/g/5 min.

Norepinephrine uptake in the hypothalamic homogenate was somewhat more active than in cortical homogenates in identical experimental conditions,[4] which is in agreement with the results of Snyder and Coyle.[5] A tissue–medium ratio of 6.4 ± 1.0 (SD) was obtained in 5 min at a substrate concentration of 10^{-8} M.

Inhibition of Uptake. All four decalin derivatives were less potent inhibitors of norepinephrine and dopamine uptake than (±)-amphetamine, and the most potent derivative was the diaxial anti isomer (Table I, Figure 1). These results differ clearly from those obtained with the catechol derivatives, since the anti isomer of the catechol derivatives was significantly less potent than one of the gauche isomers, 2(a)-amino-3(e)-3,4-dihydroxyphenyl-3-*trans*-decalol.[1] The difference between dopamine uptake and norepinephrine uptake was also smaller than in experiments with catechol derivatives (cf. Figure 1).