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Anticoccidial Derivatives of 6-Azauracil. 2. High Potency and Long Plasma Life of N1-Phenyl Structures[1a]

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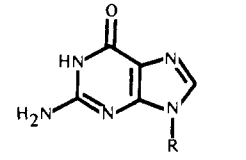
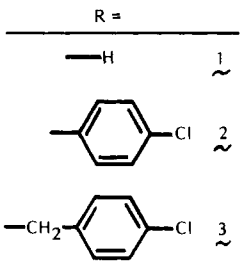
Attachment of substituted phenyl side chains at N1 of 6-azauracil caused striking increases in plasma life and anticoccidial potency. The increases were related in part to the acidity of the imide hydrogen. Maximum effects were shown by phenyl rings substituted in both meta positions by compact, electron-withdrawing, lipophilic substituents, as in 1-(3′,5′-dichlorophenyl)-6-azauracil, which had a plasma half-life of 160 h and a potency 250-fold greater than that of 6-azauracil.

The discovery that 1-benzyl side chains increased the anticoccidial activity of 6-azauracil[2] stimulated speculation on structural variations that might intensify the effect. Of the various possibilities considered, replacement of benzyl by phenyl seemed particularly attractive. Whereas the benzyl substituents probably had been limited largely to localized influences, the electronic character of a substituent on a phenyl ring would be transmitted to the heterocycle, permitting variation of the acidity of the imide proton. This variable acidity should, in turn, affect the pharmacokinetics and the binding properties of the drug. For instance, increasing the acidity of substituted uracils has been shown to improve manyfold their ability to inhibit the enzyme thymidine phosphorylase in vitro.[3] In addition, the polarizability of 1-phenyl-6-azauracils should extend throughout the entire molecule and that should facilitate adaptation to the active site.

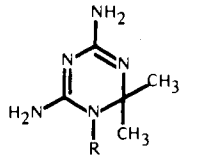
Precedent existed for the superiority of phenyl over benzyl groups in conjunction with heterocycles as enzyme inhibitors and as antiprotozoal agents. For example, 9-*p*-chlorophenylguanine (**2**) is an excellent inhibitor of rabbit

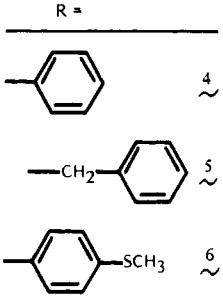


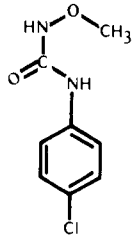


liver guanine deaminase in vitro, binding the enzyme four times better than the normal substrate, guanine (**1**), and about a 100-fold better than 9-*p*-chlorobenzylguanine (**3**).[4]

In the extensively studied dihydrofolate reductase inhibitors, the phenyl derivative **4** was about 33 times more effective as an inhibitor of the enzyme from chicken liver than the benzyl derivative **5**. The related substance **6** was







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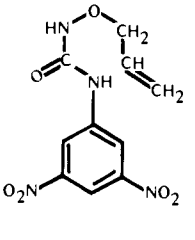

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a component of a former anticoccidial agent, trithiadol.[5] We have shown marked coccidiostat activity in **7** and **8** but not in related structures with benzyl rather than phenyl groups.[6] The mode of action of these compounds is unknown, but it seemed possible that they might be acting as fragmentary 6-azauracils, perhaps binding the orotidylate decarboxylase in a similar but less effective and specific manner.

Chemistry. A synthesis of 1-phenyl-6-azauracil starting from aniline had been reported[7] and it proved adaptable to our purpose. This synthetic sequence is outlined in Scheme I as modified and applied to the transformation

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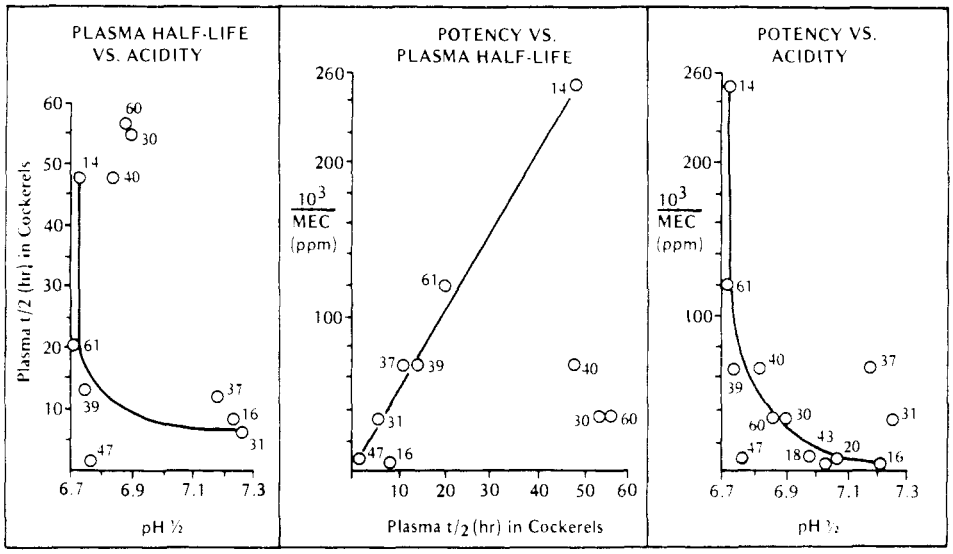
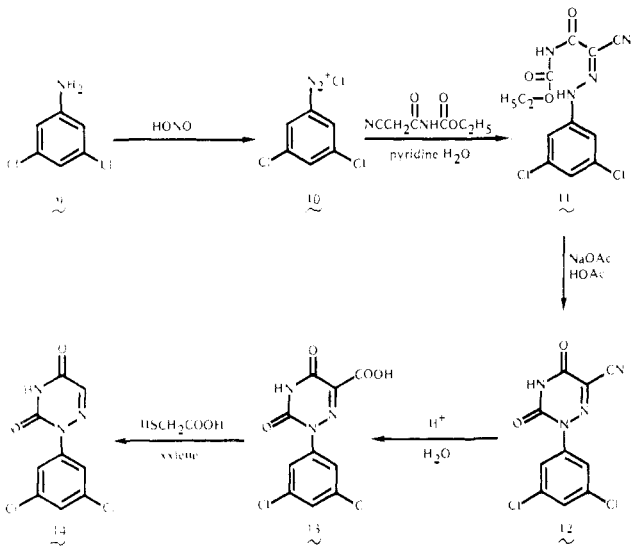


Figure 1. Relationships between acidity, plasma half-life, and potency of phenylazauracils in chickens.

Scheme I



of 3,5-dichloroaniline (**9**) to 2-(3,5-dichlorophenyl)-*as*-triazine-3,5(2*H*,4*H*)-dione (**14**), one of the most active anticoccidial agents of this series. Cyclization of **11** to **12** was improved for our use by substitution of sodium acetate in acetic acid for the aqueous base reported. Decarboxylation of neat **13** by heat as in the original procedure gave yields of **14** in the 30–50% range. These were raised in this and many other cases to 80% by catalysis of the decarboxylation by thioglycolic acid.[8] Other, less useful, catalysts for the decarboxylation of **13** were sulfur in DMF[9] and also aqueous bisulfite, the latter limited by solubility considerations. A number of the 1-phenyl-6-azauracils prepared are listed in Table I.[10] The minimum effective concentrations (MEC's) given in parts per million (ppm) by weight in feed were determined by a modification of the screening method published by Lynch[11] (as modified by Chappel et al.[12]), using *Eimeria tenella* infections in Leghorn cockerels. The values of plasma half-life were determined by the method described by Rash and Lynch.[13]

Structure–Activity Relationships. The anticipated electronic effects were indeed evident, so that anticoccidial potency was more responsive to substituent changes in the phenyl series than in the benzyl series. The acidity of the imide proton reflected the electronic influence of the substituent on the phenyl ring, and it served as a rough guide to structure design.

The graphic relationships of Figure 1 show that, in general, both plasma half-life and potency increased with acidity. The least deviation from the curve occurs in the plot of potency vs. acidity. Compounds **31** and **37** were more active than indicated by their $pH_{1/2}$ and illustrate the consistent advantage of the 3,5-substitution pattern. On the other side, **47** was less active and persistent than predicted from its $pH_{1/2}$, possibly because the methyl ether group is vulnerable to metabolic cleavage, a transformation for which there is much precedent. The equipotency of **4** and **27** probably reflects metabolic N-demethylation of the latter.

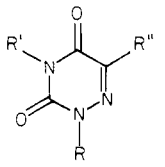
In the plot of plasma half-lives vs. acidity, the very long lives of **14**, **40**, and **60** might be ascribed to their lipophilic, metabolism-blocking substituents, although why they differ markedly in this respect from **61** is not apparent. The long plasma half-lives for **30**, **40**, and **60** were not reflected in a correspondingly high potency.

The importance of the steric factor is shown by comparison of 6-azauracils bearing different dichlorophenyl side chains. Thus, **25** with 3,4 orientation retained only 6% of the potency of **14** with 3,5 substitution. The 2,5 isomer (not included in Table I) was even less active, although its plasma life exceeded that of **14** and its $pH_{1/2}$ (6.75) was nearly the same. The 2,3-isomer **24** had less than 1% of the activity of **14**. In a related series, **37** (3-Cl, 5-Me) had an MEC of 15, **35** (4-Cl, 3-Me) had an MEC of 60 and **36** (3-Cl, 4-Me) had an MEC >250. The effect of the 3,5 substituents could not be attributed solely to blockage of metabolic attack on the phenyl ring, since **29** (4-Br, 2,3,5,6-F_4) retained only 6% of the potency of **14**.

The importance of factors other than substitution pattern is equally apparent. For example, **43** [3,5-$(OMe)_2$], isosteric with **14**, was less than 1% as active. It was somewhat less acidic than **14**, and probably the methyl ether groups underwent some metabolic cleavage. While **26** (3,4,5-Cl_3) was as active as **14**, **46** [3,4,5-$(OMe)_3$] was even less active than **43**. Beyond metabolic lability, the electron-donating influence of the 4-OMe group, as reflected by the $pH_{1/2}$ which was higher for **46** than for **43**, could be invoked as explanation.

In the benzyl side-chain series,[2] no marked difference in the therapeutic index (TI) was seen between the compound with the unsubstituted side chain, a substance rather well tolerated by the chicken, and the more potent compound with the 3-cyanobenzyl side chain. In contrast,

Table I. 2-Phenyl-*as*-triazine-3,5(2*H*,4*H*)-diones (1-Phenyl-6-azauracils), Related Structures, and Their Anticoccidial Activities[a]



no.	R	R′	R″	mol formula	mp, °C	$pH_{1/2}$[b]	plasma half-life, h	MEC, ppm[c]
14	3,5-$Cl_2C_6H_3$-	H	H	$C_9H_5Cl_2N_3O_2$	168-169	6.72 7.59[e]	48C[d] 160B	4
15	H-	H	H	$C_3H_3N_3O_2$				1000
16	C_6H_5-	H	H	$C_9H_7N_3O_2$	204-210	7.24	8C	500
17	4-F-C_6H_4-	H	H	$C_9H_6FN_3O_2$	273-274	7.80[e]		>125
18	4-Cl-C_6H_4-	H	H	$C_9H_6ClN_3O_2$	231-233	7.00		125
19	3-Cl-C_6H_4-	H	H	$C_9H_6ClN_3O_2$	212-214			125
20	4-Br-C_6H_4-	H	H	$C_9H_6BrN_3O_2$	246-248	7.08		250
21	3-Br-C_6H_4-	H	H	$C_9H_6BrN_3O_2$	206-208	7.01		125
22	2-Br-C_6H_4-	H	H	$C_9H_6BrN_3O_2$	169-170	6.88		>250
23	3-I-C_6H_4-	H	H	$C_9H_6IN_3O_2$	200-202	6.99		250
24	2,3-Cl_2-C_6H_3-	H	H	$C_9H_5Cl_2N_3O_2$	213-215	7.84[e]		500
25	3,4-Cl_2-C_6H_3-	H	H	$C_9H_5Cl_2N_3O_2$	225-226			60
26	3,4,5-Cl_3-C_6H_2-	H	H	$C_9H_4Cl_3N_3O_2$	215-220		120B	4
27	3,5-Cl_2-C_6H_3-	CH_3	H	$C_{10}H_7Cl_2N_3O_2$	129-130			4
28	3,5-I_2-C_6H_3-	H	H	$C_9H_5I_2N_3O_2$	230-230.5	7.64[e]		30
29	4-Br-2,3,5,6-F_4-C_6-	H	H	$C_9H_2BrF_4N_3O_2$	177-178			60
30	3-CN-C_6H_4-	H	H	$C_{10}H_6N_4O_2$	197-198	6.90	55C	30
31	3,5-$(CH_3)_2$-C_6H_3-	H	H	$C_{11}H_{11}N_3O_2$	168.4-169	7.26	6C 4B	30
32	3,4-$(CH_3)_2$-C_6H_3	H	H	$C_{11}H_{11}N_3O_2$	145-147	7.41		>60
33	3-F-5-CH_3-C_6H_3-	H	H	$C_{10}H_8FN_3O_2$	182-184			125
34	3-C_2H_5-C_6H_4-	H	H	$C_{11}H_{11}N_3O_2$	87-89	7.52		>250
35	4-Cl-3-CH_3-C_6H_3-	H	H	$C_{10}H_8ClN_3O_2$	210-212	7.12		60
36	3-Cl-4-CH_3-C_6H_3-	H	H	$C_{10}H_8ClN_3O_2$	229-229.3			>250
37	3-Cl-5-CH_3-C_6H_3-	H	H	$C_{10}H_8ClN_3O_2$	179.5-180.5	7.18	12C 20B	15
38	3,4-Cl_2-5-CH_3-C_6H_2-	H	H	$C_{10}H_7Cl_2N_3O_2$	206-208			25
39	4-Br-3-CF_3-C_6H_3-	H	H	$C_{10}H_5BrF_3N_3O_2$	145-146	6.74	13C	15
40	4-F-3-CF_3-C_6H_3-	H	H	$C_{10}H_5F_4N_3O_2$	141-143	6.83	48C	15
41	3,5-$(CF_3)_2$-C_6H_3-	H	H	$C_{11}H_5F_6N_3O_2$	173-175			25
42	3-CH_3-5-CF_3-C_6H_3-	H	H	$C_{11}H_8F_3N_3O_2$	185-187			45
43	3,5-$(OCH_3)_2$-C_6H_3-	H	H	$C_{11}H_{11}N_3O_4$	205-206	7.75[e]		500
44	3,4-$(OCH_3)_2$-C_6H_3-	H	H	$C_{11}H_{11}N_3O_4$	78-81	7.23		500
45	3,4-$(OCH_2O)_2$-C_6H_3-	H	H	$C_{10}H_7N_3O_4$	250-252	7.26[e]		>60
46	3,4,5-$(OCH_3)_3$-C_6H_2-	H	H	$C_{12}H_{13}N_3O_5$	210-212	7.98[e]		>500
47	3,5-Cl_2-4-CH_3O-C_6H_2-	H	H	$C_{10}H_7Cl_2N_3O_3$	180-182	7.47[e]	2C	250
48	4-NO_2-C_6H_4-	H	H	$C_9H_6N_4O_4$	182-184			250
49	3,5-$(NO_2)_2$-C_6H_3-	H	H	$C_9H_5N_5O_6$	242-250			>8
50	2,4-$(NO_2)_2$-C_6H_3-	H	H	$C_9H_5N_5O_6$	159-161	6.00		>250
51	4-Cl-3-(*i*-PrOCO)-C_6H_3-	H	H	$C_{13}H_{12}ClN_3O_4$	169-171			>125
52	3,5-$(C_3H_7OOC)_2$-C_6H_3-	H	H	$C_{17}H_{19}N_3O_6$	164.2-164.8	7.62[e]		
53	3,5-(*i*-PrOCO)$_2$-C_6H_3	H	H	$C_{17}H_{19}N_3O_6$	188-190	7.60[e]		>250
54	3-Cl-5-CH_3-C_6H_3-	H	H	$C_{10}H_8ClN_3OS$[g]	199-201			125
55	2-thiazolyl	H	H	$C_6H_3N_4O_2S$	250-250.5	6.81[f]		>250
56	2-benzthiazolyl	H	H	$C_{10}H_6N_4O_2S$	275-275.4			500
57	3,5-Cl_2-4-($MeSO_2$)-C_6H_2-	H	H	$C_{10}H_7Cl_2N_3O_4S$	235-237			60
58	C_6H_5-	H	C_6H_5-	$C_{15}H_{11}N_3O_2$	265-267			>250
59	3,5-$(CH_3)_2$-C_6H_5-	H	C_6H_5-	$C_{17}H_{15}N_3O_2$	202-203			60
60	3-CF_3-C_6H_4-	H	H	$C_{10}H_6F_3N_3O_2$	125-126	6.88	57	30
61	3-CF_3-4-Cl-C_6H_4-	H	H	$C_{10}H_5ClF_3N_3O_2$	159-161	6.71	20	8

[a] All compounds in this table were prepared by the method outlined under the Chemistry section, except for 29 and 50. These were prepared by direct arylation with aryl fluorides as described under the Experimental Section. [b] Because of the low solubility of these compounds in water, $pH_{1/2}$ determinations were made in 1:1 DMF-H_2O solutions unless otherwise noted (see footnotes *e* and *f*). In the 2:1 DMF-H_2O solutions, the readings averaged 0.7 $pH_{1/2}$ unit higher than in the 1:1 solutions. [c] These values are the minimum amounts (parts per million by weight in feed mixtures) of drugs required to prevent formation of detectable disease lesions. [d] The letter B indicates that the plasma half-life measurements were made in broiler chickens (6-8 weeks old). The letter C indicates that the measurements were made in cockerels or 2-week-old chickens. [e] Titrated in 2:1 DMF-H_2O solution. [f] Titrated in 3:1 DMF-H_2O solution. [g] A 4-thio-6-azauracil.

1-phenyl-6-azauracil was not well tolerated at effective dose levels, but the TI increased with potency among its analogues as shown in Figure 2. Thus, the most active candidate **14** had a TI of 10, while **61** with 50% of its potency had a TI of 6, **39** and **40** both with 25% of its potency had a TI of 2–3, and **19** with 3% of its potency had a TI of 2.

Discussion and Conclusions

While medicinal chemists sometimes encounter structural congeners (at least within narrow structural ranges)

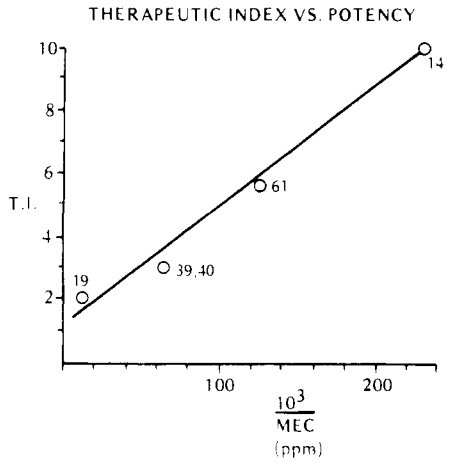


Figure 2. Relationship between therapeutic index and potency of phenylazauracils fed to chickens.

which yield a good parabolic curve merely by plotting potency against lipophilicity,[14] the 1-phenyl-6-azauracil system was more complex. Compact, electron-withdrawing, lipophilic groups of low polarizability in the 3′,5′ positions were preferred. The potency peak was reached at 14 with 3′,5′-dichloro substituents. It was 250 times more active than 6-azauracil (**15**), 125 times more active than 1-phenyl-6-azauracil (**16**), and 30 times more active than 1-(3′-chlorophenyl)-6-azauracil (**19**). Smaller or larger halogens were less effective.

Compound 14 was coccidiocidal and did not permit the emergence of resistant strains. At 4 ppm, it controlled all important species of *Eimeria*, except *E. acervulina* which required 8 ppm. At the latter level, it thoroughly suppressed oocyst production in all species. Even though it was efficiently absorbed orally in the chicken and excreted very slowly, it was well tolerated, with a therapeutic index of 10. However, the phenomenally long plasma half-life of 160 h, which might have been an asset in certain types of drugs where it would allow infrequent dosage, was undesirable for a drug of this type. Attempts to divorce potent activity from persistence led to the design of the more elaborate side chains that will be described in later papers of this series. Publications from other sources on these more elaborate compounds are cited.[12,13,15-17]

Experimental Section

Melting points were determined on a calibrated Kofler hotstage microscope. The $pH_{1/2}$ titrations were done on a Metrohm potentiograph Model 436 with a Swiss combination glass–calomel electrode and a special 1-mL capacity syringe delivery tube. Solvents used were analytical reagent grade, where pertinent, protected from water by storage over molecular sieves.

3,5-Dichlorobenzenediazonium Chloride (10). A mixture of 8.1 g (0.050 mol) of 3,5-dichloroaniline, 10 mL of concentrated HCl, and 50 mL of H_2O was cooled to 5 °C. Separately, a solution of 3.6 g (0.050 mol) of $NaNO_2$ in 7.2 mL of H_2O was cooled to 5 °C and then added to the aniline hydrochloride slurry with the addition tube beneath the liquid surface. The temperature was maintained at 5 °C during the addition and for 1 h thereafter.

Ethyl *N*-[[[Cyano(3,5-dichlorophenyl)hydrazinylidine]-methyl]carbonyl]carbamate (11). A mixture of 45.8 g (0.295 mol) of cyanoacetylurethane, 3280 mL of pyridine, 1080 g of ice, and 200 mL of water was held at 5 °C while a slurry prepared according to the preceding procedure but containing 52.4 g (0.250 mol) of diazotized 3,5-dichloroaniline was added during 15 min with stirring. After an additional hour of stirring the mixture at the same temperature, the orange solid which formed was removed by filtration. The crude yield was 41.3 g (50%), mp 196–198 °C.

2-(3,5-Dichlorophenyl)-6-cyano-*as*-triazine-3,5(2*H*,4*H*)-dione (12). A mixture of 31 g (0.095 mol) of **17**, 9.0 g (0.110 mol) of NaOAc, and 140 mL of HOAc was refluxed for 75 min. The resulting clear solution was concentrated at reduced pressure, and the solid that separated was removed by filtration and washed with water. Recrystallization from 95% ethanol yielded 14.5 g (53%) of **12**, mp 239–241 °C.

2-(3,5-Dichlorophenyl)-3,5(2*H*,4*H*)-dioxo-*as*-triazine-6-carboxylic Acid (13). A mixture of 14.2 g (0.050 mol) of **12**, 190 mL of 6 N HCl, and 500 mL of dioxane was refluxed for 12 h. On cooling, a solid separated and was removed by filtration. Recrystallization from MeOH–H_2O yielded 8.0 g (50%) of **13**, mp 255–256 °C (gas evolution).

2-(3,5-Dichlorophenyl)-*as*-triazine-3,5(2*H*,4*H*)-dione (14).

A. A 6.0-g (0.019 mol) sample of **13** was heated at 260–270 °C under N_2 for 20 min as CO_2 was evolved. Crystallization of the residue from EtOH–H_2O yielded 1.92 g (40%) of **14**, mp 168–169 °C.

B. Another 6.0-g sample of **13** was mixed with 100 mL of xylene and 1 drop of thioglycolic acid. After refluxing for 2 h, the solvent was removed at reduced pressure. Recrystallization yielded 3.84 g (80%) of **14**, mp 168–169 °C.

2-(4-Bromo-2,3,5,6-tetrafluorophenyl)-*as*-triazine-3,5-(2*H*,4*H*)-dione (29). To a solution of 22.4 g (0.200 mol) of potassium *tert*-butoxide in 120 mL of DMF was added 11.3 g (0.100 mol) of 6-azauracil. To this stirred suspension was added dropwise during 50 min 29.6 g of bromopentafluorobenzene. The temperature of the reaction mixture was held at 30–40 °C during the addition. After the addition was complete, the temperature was raised to 130 °C. At the end of 20 h at this temperature, the mixture was cooled, 50 mL of MeOH was added, and the solid that separated was removed by filtration. The filtrate was acidified with 6 N HCl and extracted with $CHCl_3$. Concentration of the $CHCl_3$ solution gave a solid mixture, which was leached with hot benzene. Concentration of the benzene yielded 530 mg of **29**, mp 177–178 °C.

2-(2,4-Dinitrophenyl)-*as*-triazine-3,5(2*H*,4*H*)-dione (50). To 11.3 g (0.110 mol) of 6-azauracil dissolved in aqueous KOH (16.8 g or 0.300 mol of KOH in 100 mL of H_2O) was added dropwise during 2 h a solution of 22.3 g (0.120 mol) of 2,4-dinitrofluorobenzene in 20 mL of Me_2SO. After 20 h at ambient temperature the reaction mixture was extracted with ether. The aqueous raffinate was next adjusted to pH 6 with concentrated HCl and extracted with $CHCl_3$. Evaporation of the solvent left a brown gum. Fractionation of this gum on a column containing 150 g of Florisil by development with 4:1 benzene–EtOAc yielded the desired product. Recrystallization from benzene–EtOAc gave 3.95 g (14%) of colorless **50**, mp 159–161 °C.

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[3-(1,4-Cyclohexadienyl)-L-alanine,8-lysine]vasopressin: Synthesis and Some Pharmacological Properties

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[3-(1,4-Cyclohexadienyl)-L-alanine,8-lysine]vasopressin, otherwise known as [3-(2,5-dihydrophenylalanine),8-lysine]vasopressin or [DiHPhe^3]lysine-vasopressin, has been synthesized in an attempt to utilize 2,5-dihydrophenylalanine (DiHPhe) to evaluate the contribution of aromaticity in position 3 to biological activity. The analogue has the same primary structure as lysine-vasopressin, except that two additional hydrogen atoms are present on the ring moiety of the phenylalanine residue in position 3. The key intermediate was the protected nonapeptide *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyldihydrophenyl-L-alanyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-N^{ϵ}-tosyl-L-lysylglycinamide that was synthesized stepwise by the solid-phase technique. Deprotection with sodium in liquid ammonia was followed by sulfhydryl oxidation with I_2 to give the hormone analogue. [DiHPhe^3]lysine-vasopressin exhibited 125–130 units/mg of antidiuretic, 129–132 units/mg of rat pressor, and 6 units/mg of rat uterus contracting activity. To confirm the presence of DiHPhe in the analogue, an enzymatic procedure employing *Aspergillus oryzae* was developed that liberates in high yield the amino acid residue in position 3 of the posterior pituitary hormone structure. This study should be applicable to other biologically active peptides.

Interest in examining the biological peoperties of 2,5-dihydrophenylalanine [3-(1,4-cyclohexadienyl)-L-alanine, DiHPhe] in peptide form as an analogue of phenylalanine followed from the finding that DiHPhe is a highly effective antagonist of phenylalanine for the rat and a variety of bacteria.[1,2] Although the molecular basis of the antagonism of DiHPhe had not been explored at that time, the question arose whether substitution of this amino acid in a phenylalanine-containing peptide hormone would produce inhibitory properties that could be pharmacologically useful. Lysine-vasopressin (LVP), the antidiuretic hormone of the pig that has the structure Cys-Tyr-Phe-Gln-Asn--Asn-Cys-Pro-Lys-Gly-NH_2, seemed a good candidate for such a study. Substitution of DiHPhe for Phe in LVP had additional interest inasmuch as the substitution would be in residue-3, a position thought to be important in binding of neurohypophyseal hormones to their receptors.[3] In the three-dimensional structure proposed for neurohypophyseal hormones, the amino acid residue in position 3 is thought to occupy one of the corners of a β turn in a way that would allow its side chain to participate in intermolecular interactions.[4-6] Moreover, it is one of the positions (3, 4, and 8) undergoing substitution in the nine recognized naturally occurring oxytocic and antidiuretic neurohypophyseal peptides,[7] which suggested that this position confers some specificity to the biological properties.

In [DiHPhe^3]LVP the aromatic character of the ring of the phenylalanine residue that might contribute to the binding of LVP would be replaced by the π electron atmosphere of two isolated double bonds. As judged from values calculated from heats of hydrogenation for related olefins, the resonance energy of 1,4-cyclohexadiene is very low and similar to that of 1,3-cyclohexadiene: benzene, 36.0; 1,3-cyclohexadiene, 1.8; 1,3-cyclopentadiene, 2.9; 1,3-pentadiene, 4.2; 1,4-pentadiene, −0.2 kcal/mol. Heats of hydrogenation of 1,4-cyclohexadiene and 1,3-cyclohexadiene are −53.6 and −53.9 kcal/mol, respectively.[8,9] It can be accepted that 1,4-cyclohexadiene differs markedly from benzene in having little resonance energy and little diamagnetic ring current due to electron density. This is reflected, for example, in their relative effects in inducing shifts in the ^{1}H NMR spectrum of the dipole acetonitrile, which are 0.01 and 1 ppm, respectively.[10] This marked difference in ring current is expected to apply also to DiHPhe and Phe. In this respect, DiHPhe should have an advantage over β-2-thienylalanine which has recently served a similar purpose, i.e., to assess the contribution of aromaticity to biological activity by substitution for Phe in LVP.[11] The thiophene ring has about 70% of the resonance energy of the benzene ring.

Like Phe, DiHPhe is considered to have a planar ring and, differing only by 2 H atoms, it should have very similar molecular dimensions. In any comparison of peptides of DiHPhe and Phe to evaluate consequences of electronic differences, steric differences that might arise when other amino acids are substituted in this position, therefore, should be unimportant and need little consideration. In this respect, DiHPhe should have an advantage over β-cyclohexylalanine [Phe($3H_2$)], the cyclohexyl ring of which is puckered and nonplanar.[12] Past experiments designed to assess the contribution of aromaticity of Phe to biological activity by substitution of the Phe($3H_2$) residue include the synthesis of an analogue of the gastrin C-terminal tetrapeptide that had full biological activity[13,14] and [Phe($3H_2$)^3]oxytocin.[15] As compared to [Phe^3]-oxytocin, the analogue had a considerably lower potency in all oxytocin- and vasopressin-like activities observed but showed a greater intrinsic activity for the uterine receptor.

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