The solution was stirred for 10 min with slight cooling and then a solution of 0.4 g (0.006 mole) of urea in 1 ml of H_2O and 3 drops of concentrated HCl was added. The reaction solution was immediately poured into 150 ml of cold H_2O and extracted (EtOAc). The EtOAc solution was partially dried by filtering through $CaSO_4$ and enough petroleum ether was added to force H_2O and a brown tar out of solution. The EtOAc–petroleum ether solution was decanted from H_2O and tar and clouded with more petroleum ether and allowed to crystallize at Dry Ice temperature. Properties of the product are listed in Table I.

N,N-Bis[2-(*p*-toluenesulfonoxy)ethyl]-*p*-nitrosoaniline (21). N,N-Bis[2-(*p*-toluenesulfonoxy)ethyl]aniline[9a] was nitrosated by a mixture of AcOH and 6 *N* HCl and worked up in the usual way.[9] The crude product was recrystallized from absolute EtOH to give a 75% yield of pure **21** as a green powder, mp 117–118° dec. *Anal.* ($C_{24}H_{26}N_2O_7S_2$) C, H, N.

Charge–Spatial Models. *cis*- and *trans*-3- and -4-Substituted Cyclohexyl Phosphates as Analogs of 2′-Deoxyuridine 5′-Phosphate[1]

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The isomers of 3- and 4-substituted cyclohexyl phosphates were synthesized for examination of the binding sites of thymidylate synthetase. *cis*-3-Hydroxycyclohexanecarboxylic acid treated with diphenylphosphorochloridate gave *cis*-3-diphenylphosphorylcyclohexanecarboxylic acid (**5a**). Subsequent treatment with $SOCl_2$ followed by NH_4OH gave *cis*-3-carbamoylcyclohexyl diphenyl phosphate (**5b**). Acetylation of **5b** gave *cis*-3-N-acetylcarbamoylcyclohexyl diphenyl phosphate (**5c**). The synthesis of *cis*-3-allophanoylcyclohexyl diphenyl phosphate (**5d**) was accomplished by treating the acid chloride of **5a** with urea. Pt-catalyzed hydrogenolysis of the diphenyl phosphates **5b–d** yielded the respective phosphates **1b–d**. The corresponding *trans*-3 (**2b–d**), *cis*-4 (**3b–d**), and mixed *cis-trans*-4 (**4b–d**) phosphates were prepared by the same sequence. The phosphates were found to be weak inhibitors of thymidylate synthetase; highest activity resided in *cis*-4-N-acetylcarbamoylcyclohexyl phosphate (**3c**) and *cis*-4-allophanoylcyclohexyl phosphate (**3d**).

The commonest approach to the design of enzymatic inhibitors is based on analogs of the natural metabolites of the particular enzyme. While there is no question of the productivity of this approach, the metabolite variations that can be designed as potential inhibitors are limited and in many cases the synthesis becomes extremely laborious.

Another approach to the design of enzyme inhibitors that is unlimited in scope can be built on the premise that the most important features in specific enzymatic binding reside in a maximum of two or three portions of a molecule, and the remainder of the molecule is a template structure holding the correct charge sites in the proper spatial arrangement. Binding to the enzyme through these charge sites of the molecule relies on attractive forces such as exist between unlike charges, either dipoles or ion–ion pairs, hydrophobic bonding, or other physicochemical cohesive forces.[2] An essential to binding is that these sites of the molecule are held in the correct steric–spatial relationship without steric interference of approach to the enzyme.

In an attempt to examine these postulates initial studies were directed to the inhibition of thymidylate synthetase, the enzyme catalyzing the conversion of 2′-deoxyuridine 5′-phosphate to thymidine 5′-phosphate.[3] Previous studies by many investigators have demonstrated that the phosphate group is essential for binding. In addition, studies reported on azapyrimidine nucleotides and 5-fluoro- or 5-trifluoro-2′-deoxyuridine 5′-phosphates suggest a requirement for an acidic function (pK_a = 9.5 to ~7.0) at the N_3H–C_4O portion of the pyrimidine ring.[3c,4] The correct spatial relationship of these two moieties for maximum binding has not been studied. Assuming the *syn* or *anti* configuration for the pyrimidine ring and the fact that the 5′-phosphate is theoretically freely rotating, several possible spatial arrangements can be formulated. Using the substrate 2′-deoxyuridine 5′-phosphate as the model, with a fully extended phosphate the *syn* and *anti* pyrimidine ring configurations show a range of 6.4–7.6 Å between the center of the P atom and the center of N-3.

For the preliminary studies this range of 6.4–7.6 Å between the acidic function and a phosphate was selected. The models used were cyclohexyl phosphates substituted *cis* or *trans* at positions 3 or 4: compounds **1b–d**, **2b–d**, **3b–d**, and **4b–d**. The substituents used at these positions were the amide which is neutral and should be inactive, the N-acetylamide, and the acylcarbamate group, both of which are weakly acidic. Although the 1,3-*trans* and the 1,4-*cis* systems are flexible, the distances between the ionizable NH and the P atom in these models, assuming extended phosphate and amide groups, are estimated to be 6.0 Å (1,3-*trans*), 6.4 Å (1,3-*cis*), 6.8 Å (1,4-*cis*), and 7.2 Å (1,4-*trans*).

Catalytic hydrogenation of *m*-hydroxybenzoic acid[5] has afforded mainly *cis*-3-hydroxycyclohexanecarboxylic acid. On the basis of more recent favorable results

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(2) For further consideration of attractive forces between molecules particularly as applied to enzymatic binding see: (a) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967; (b) J. Leyden Webb, "Enzyme and Metabolic Inhibitors," Vol. I, Academic Press, New York, N. Y., 1963.

(3) (a) M. Friedkin, *Ann. Rev. Biochem.*, **32**, 185 (1963); (b) R. L. Blakely, B. V. Ramasastri, and B. M. McDougall, *J. Biol. Chem.*, **238**, 3075 (1963); (c) P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965); (d) M. J. S. Lomax and G. R. Greenberg, *J. Biol. Chem.*, **242**, 1302 (1967); (e) E. J. Pastore and M. Friedkin, *ibid.*, **237**, 3802 (1962).

(4) C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965).

(5) (a) A. Einhorn, *Ann.*, **291**, 301 (1896); (b) W. H. Perkin and G. Tattersall, *J. Chem. Soc.*, **91**, 486 (1907); (c) E. J. Boorman and R. P. Linstead, *ibid.*, 258 (1935); (d) D. S. Noyce and D. B. Denney, *J. Am. Chem. Soc.*, **74**, 4912 (1952); (e) M. Kilpatrick and J. G. Morse, *ibid.*, **75**, 1846 (1953); (f) D. S. Noyce and H. J. Weingarten, *ibid.*, **79**, 3098 (1957); (g) H. O. House, H. Babad, R. B. Toothill, and A. W. Noltes, *J. Org. Chem.*, **27**, 4141 (1962).

using Rh catalysts,[6] the hydrogenation of *m*-hydroxybenzoic acid was conducted with Rh on alumina in absolute EtOH. The mixture was found to contain cyclohexanecarboxylic acid in addition to the expected *trans* and *cis* isomers. The *cis* isomer was obtained in 20% yields on repeated recrystallization. The hydrogenolysis side reaction to produce cyclohexanecarboxylic acid was also found when Noyce and Denney[5d] employed Pt in the same reduction.

The *trans* isomer was obtained from the solid residue of the reduction mixture by treatment with *p*-toluenesulfonic acid. The *cis* isomer formed the lactone; the *trans* isomer was separated from this mixture by extraction with $NaHCO_3$ and repeated recrystallization. The crystalline *cis*-3-hydroxycyclohexanecarboxylic acid was treated with diphenylphosphorochloridate and pyridine to give a syrup which on column chromatography yielded 46% of the desired diphenylphosphoryl ester **5a** and 38% of the lactone. The reaction products were easily identified after separation since the acid C=O of **5a** appeared at 1710 cm^{-1} while the lactone C=O appeared at 1800 cm^{-1}. Support for maintenance of the *cis* stereochemistry in the phosphate ester **5a** was derived from the nmr spectrum where the axial methine proton at the 3 position of the ring appeared as a broad peak at 4.60 ppm ($W_{1/2}$ = 20 Hz). The corresponding peak in the nmr spectrum of the *trans* isomer was seen at 5.10 ($W_{1/2}$ = 12 Hz) which represents an average of the two chair forms of the *trans* system.

The corresponding acid chloride of **5a** was obtained by treatment of *cis*-3-diphenylphosphorylcyclohexanecarboxylic acid with $SOCl_2$. The reaction progress was followed by ir spectra where the acid C=O at 1710 cm^{-1} was shifted to 1795 cm^{-1}.

Amide formation was effected by adding the acid chloride directly to NH_4OH which afforded an over-all yield from the acid to the amide **5b** of 28%; the *cis* stereochemistry of **5b** was confirmed by nmr.

Tran formation of the amide **5b** to the mixed imide **5c** in 60% yield was accomplished by the method of Hurd and Prapas,[7] using Ac_2O and H_2SO_4 at high temperatures. As in the case of **5b**, *cis* stereochemistry was confirmed by the phosphoryl ester CH of **5c** appearing at 4.60 ppm ($W_{1/2}$ = 20 Hz). Reaction of the acid chloride with urea without solvent gave the acylurea **5d**. The nmr spectrum of the 3-CH of **5d** was at 4.60 ppm ($W_{1/2}$ = 20 Hz) again confirming the *cis* stereochemistry.

Removal of the phenyl protecting groups to give the amide (**1b**), acylcarbamoyl (**1c**), and acylcarbamate (**1d**) phosphates proceeded smoothly in all cases *via* hydrogenolysis catalyzed by Pt.[8] The phosphates were obtained as clear, glassy, semisolids which crystallized upon washing with Et_2O. Stable NH_4 salts were prepared by freeze drying the phosphates from dilute NH_4OH.

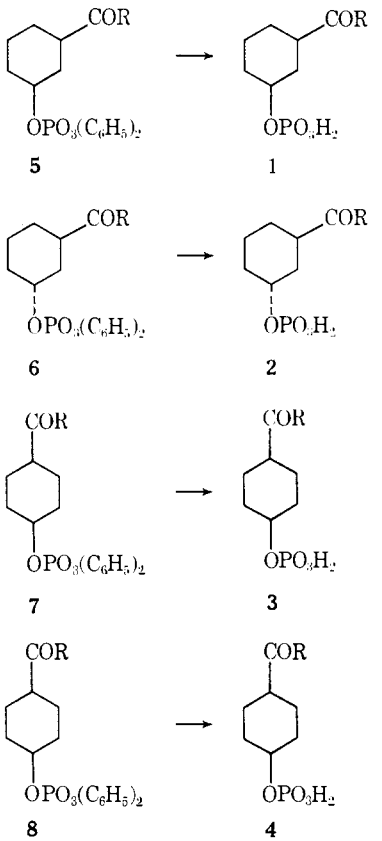
Preparation of the *trans* series (**6a–d**) (Table I) proceeded in a routine manner using the same procedures employed in the *cis* series. The *trans* stereochemistry of the diphenylphosphoryl compounds **6a–d** was supported by the 3-CH seen at 5.1 ppm ($W_{1/2}$ = 12 Hz).

(6) A. W. Burgstahler and Z. J. Bithes, *J. Am. Chem. Soc.*, **82**, 5466 (1960).

(7) C. D. Hurd and A. G. Prapas, *J. Org. Chem.*, **24**, 388 (1959).

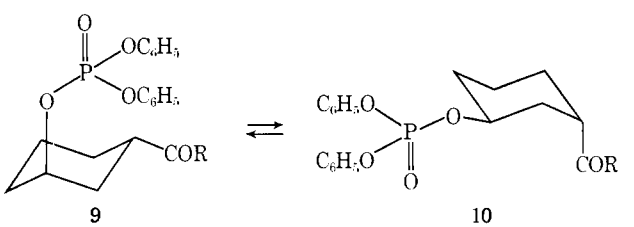
(8) J. L. Barnwell, W. A. Saunders, and R. W. Watson, *Can. J. Chem.*, **33**, 711 (1955).

Hydrogenolysis of the phenyl protecting groups was again conducted using Pt in EtOH to yield quantitatively the three desired phosphates, **2b–d** (Table II) or their NH_4 salts.



a, R = OH c, R = $NHCOCH_3$
b, R = NH_2 d, R = $NHCONH_2$

Comparison of the nmr spectra of the respective 1,3-*cis* (**5**) and -*trans* (**6**) isomers in more detail brings out the following observations. The deshielded C*H* adjacent to the phosphate was shifted further downfield in the *trans* series by about 0.4–0.5 ppm which is indicative of a predominance of conformation **9** although the peak half-width of 12 Hz shows substantial ring flip to conformation **10**. In addition the CH_2 envelope seen in the nmr of the *trans* series is much sharper than that seen in the *cis* series, which is further support for the presence of the two conformers, **9** and **10**, at room temperature.

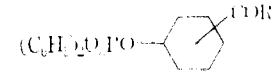


This interconversion would be expected, as the free-energy difference between the two conformers should be only about 0.5 kcal/mole based on values for conformational preference of CO_2H and OH.[9] In view of the broad CH_2 envelope in the nmr spectra of the *cis* isomers they probably exist almost exclusively in the diequatorial conformation at room temperature.

(9) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience Publishers, Inc., New York, N. Y., 1965, p 433.

TABLE I

PHYSICAL CONSTANTS OF SUBSTITUTED CYCLOHEXYL DIPHENYL PHOSPHATES

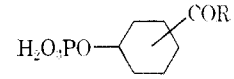


No.	Isomer	R	Method	Formula	Analyses
5a	3-*cis*	OH	A	$C_{19}H_{21}O_5P$	C, H
5b	3-*cis*	NH_2	B	$C_{19}H_{22}NO_4P$	C, H, N, P
5c	3-*cis*	$NHCOCH_3$	C	$C_{21}H_{24}NO_5P \cdot H_2O$	C, H, N
5d	3-*cis*	$NHCONH_2$	D	$C_{20}H_{23}N_2O_5P$	C, N, P; H[a]
6a	3-*trans*	OH	A	$C_{19}H_{21}O_5P$	C, H
6b	3-*trans*	NH_2	B	$C_{19}H_{22}NO_4P$	C, H, N, P
6c	3-*trans*	$NHCOCH_3$	C	$C_{21}H_{24}NO_5P \cdot 0.5H_2O$	C, H, N
6d	3-*trans*	$NHCONH_2$	E	$C_{20}H_{23}N_2O_5P$	C, H, N, P
7a	4-*cis*	OH	A	$C_{19}H_{21}O_5P$	C, H
7b	4-*cis*	NH_2	B		
7c	4-*cis*	$NHCOCH_3$	C		
7d	4-*cis*	$NHCONH_2$	D		
8a	4-*cis*-*trans*	OH	A	$C_{19}H_{21}O_5P \cdot H_2O$	C, H
8b	4-*cis*-*trans*	NH_2	B	$C_{19}H_{22}NO_4P$	C, H, N
8c	4-*cis*-*trans*	$NHCOCH_3$	C	$C_{21}H_{24}NO_5P$	N
8d	4-*cis*-*trans*	$NHCONH_2$	D	$C_{20}H_{23}N_2O_5P$	C, H, N

[a] H: calcd, 5.54; found, 5.95.

TABLE II

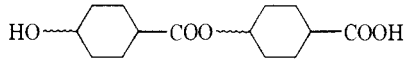
PHYSICAL CONSTANTS OF SUBSTITUTED CYCLOHEXYL PHOSPHATES[a]



No.	Isomer	R	Formula	Analyses
1b	3-*cis*	NH_2	$C_7H_{14}NO_5P \cdot XH_2O$	N/P 1.04
1c	3-*cis*	$NHCOCH_3$	$C_9H_{16}NO_6P \cdot XH_2O$	N/P 0.99
1d	3-*cis*	$NHCONH_2$	$C_8H_{15}N_2O_6P \cdot XH_2O$	N/P 2.05
2b	3-*trans*	NH_2	$C_7H_{14}NO_5P$	C, H, N
2c	3-*trans*	$NHCOCH_3$	$C_9H_{16}NO_6P \cdot NH_3 \cdot XH_2O$	N/P 2.08
2d	3-*trans*	$NHCONH_2$	$C_8H_{15}N_2O_6P \cdot XH_2O$	N/P 1.99
3b	4-*cis*	NH_2	$C_7H_{14}NO_5P \cdot 2NH_3 \cdot 2H_2O$	H, N; C[b]
3c	4-*cis*	$NHCOCH_3$	$C_9H_{16}NO_6P \cdot 2NH_3 \cdot 2H_2O$	C, N; H[c]
3d	4-*cis*	$NHCONH_2$	$C_8H_{15}N_2O_6P$	C, H, N
4b	4-*cis*-*trans*	NH_2	$C_7H_{14}NO_5P \cdot NH_3 \cdot XH_2O$	N/P 2.02
4c	4-*cis*-*trans*	$NHCOCH_3$	$C_9H_{16}NO_6P \cdot 2NH_3 \cdot XH_2O$	N/P 3.14
4d	4-*cis*-*trans*	$NHCONH_2$	$C_8H_{15}N_2O_6P \cdot C_2H_5OH$	N

[a] Method F was used for synthesis. [b] C: calcd, 30.54; found, 31.11. [c] H: calcd, 7.62; found, 8.07.

The isomeric mixture of *cis*- and *trans*-4-hydroxycyclohexanecarboxylic acid was prepared as in the case of the 1,3 series by Rh-catalyzed reduction. After recrystallization, the *cis* isomer was separated as the lactone formed by dehydration with *p*-toluenesulfonic acid. After bicarbonate extraction, alkaline hydrolysis of the residual lactone and recrystallization afforded pure *cis*-4-hydroxycyclohexanecarboxylic acid.[5e] In contrast to the 1,3 series, the bicarbonate extracts of the dehydration mixture afforded a mixture of the desired *trans*-4-hydroxycyclohexanecarboxylic acid contaminated by appreciable amounts of mixed esters (**11**) from both *cis*- and *trans*-hydroxy acids.



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The *cis*-1,4-hydroxy acid was converted to the respective diphenyl phosphates **7a–d** by procedures used in the synthesis of **5b–d**. The mixed isomers, *cis*- and *trans*-4-diphenyl phosphates **8a–d**, were also prepared according to the sequence used in **5b–d** (Table I).

Hydrogenolysis of **7b–d** yielded the respective phosphates **3b–d** and similarly the **8b–d** series gave **4b–d** isolated as the free phosphates or the NH_4 salts[10] (Table II).

The nmr spectrum of the lactone of *cis*-4-hydroxycyclohexanecarboxylic acid showed the 4-proton at 4.89 ppm ($W_{1/2}$ = 7 Hz); hydrolysis to the acid moved the 4-proton upfield to 3.60 ppm ($W_{1/2}$ = 12 Hz). The isomer mixture, *cis*-*trans*-4-hydroxycyclohexanecarboxylic acid, showed two slightly overlapping peaks for the 4-protons. The low-field peak (3.60 ppm, $W_{1/2}$ = 12 Hz) is from the *cis*-4-proton and the upfield peak centered at 3.30 ppm ($W_{1/2} \cong 20$ Hz) is therefore assigned to the *trans*-4-proton which from the relative chemical shift and half band width is the expected 4-axial proton. The integrated areas show the mixture to be approximately 50:50 ± 10% *cis*-:*trans*-4-hydroxycyclohexanecarboxylic acid. This mixture was used as such in the synthesis of **8a–d** and therefore the phosphates **4b–d** are presumed to be composed of 40–60% *trans* isomer with the remainder *cis*.

(10) Elemental analysis of the phosphates or their NH_4 salts proved difficult due to the persistence of polyhydrates even after long drying in high vacuum. For this reason the N/P ratio derived from elemental analysis was used often as the criteria of purity.

Biological Results.—The results of inhibition of thymidylate synthetase are recorded in Table III as the inhibitor:substrate (2′-deoxyuridine 5′-phosphate) ratio necessary to achieve 50% inhibition of the rate of enzymatic formation of thymidylic acid.[3e,11] To eliminate the possibility of one-point binding as the mode of inhibition of thymidylate synthetase, models of this series of compounds containing either the phosphate or acylurea groups were examined. Both trifluoroacetylurea and cyclohexylcarboxylurea showed a very low order of inhibition with an $([I]/[S])_{0.5}$ greater than 500.[12] Cyclohexyl phosphate[13] showed an $([I]/[S])_{0.5}$ of 1250.

TABLE III

INHIBITION OF THYMIDYLATE SYNTHETASE[a] BY SUBSTITUTED CYCLOHEXYL PHOSPHATES

	$([I]/[S])_{0.5}$[b]			
R	1,3-*cis* (1)	1,3-*trans* (2)	1,4-*cis* (3)	Mixed isomers ~50% 1,4-*trans* (4)
NH_2 (**b**)	1270	650	610	1360
$NHCOCH_3$ (**c**)	340	320	190	190
$NHCONH_2$ (**d**)	280	180	180	260

[a] The rate of the enzymatic reaction was monitored at 340 mμ, a measure of the formation of dihydrofolic acid. Inhibitors were dissolved in H_2O. The reference used lacked only the substrate. 2′-Deoxyuridine 5′-monophosphate was present in 4.8×10^{-5} *M* concentration. The enzyme source was *E. coli* B. [b] $([I]/[S])_{0.5}$ refers to the ratio of the molar concentrations of the inhibitor and substrate necessary for 50% inhibition. The values were obtained by plotting [I] *vs.* enzymatic rate for a range of concentrations of inhibitor and where necessary extrapolating to 50% inhibition.

Examination of the results show, in general, a low order of activity. However, a comparison within this series reveals several differences. An extremely low order of activity, probably through one-point binding, is seen in the amide series **1b**, **2b**, **3b**, and **4b**. Conversion to the more acidic N-acylamides **1c**, **2c**, **3c**, and **4c** gives a two- to eightfold increase in activity. The acylureas of the 1,3 series (**1d** and **2d**) are slightly more active than the imides **1c** and **2c**. However the acylureas **3d** and **4d** show the same order of activity as the imides **3c** and **4c**.

Examination of the relative distances encountered in the acylamide **c** and acylurea series **d** shows the 1,3-*trans* to be more effective than the 1,3-*cis* while a higher order of activity resides in the 1,4 series. Although the results are somewhat inconclusive, it would appear from the relative concentrations for 50% inhibition, the 1,4 mixed isomers (**4**) have approximately the same activity as the 1,4-*cis*-**3**. It can therefore be assumed that the 1,4-*trans* system is no more effective than the 1,4-*cis*.

The low order of activity in these compounds would suggest nonspecific binding and the resultant poor inhibition. However, from the model studies on one-point binding, the predictable requirement for the acidic function and the phosphate for two-point binding found in series **c** and **d** contrasted to the probable single-point binding in the neutral amide, series **b**, is confirmed by the relative activities.

(11) Methods for the assay[3e] were the same as those reported previously: M. P. Mertes and N. R. Patel, *J. Med. Chem.*, **9**, 868 (1966). A Gilford multiple-sample absorption spectrophotometer was used with a full scale setting of 0.0–0.1 absorbance units.

(12) Solutions in DMSO were examined. Insolubility at concentrations greater than 10^3 *M* in the assay media necessitated an estimation of the $([I]/[S])_{0.5}$ value.

(13) H. A. C. Montgomery and J. H. Turnbull, *J. Chem. Soc.*, 1963 (1958).

Experimental Section

Nmr spectra were obtained on Varian A-60 and A-60 A instruments (Me_4Si as standard). Ir spectra were obtained on Beckman IR-8 and IR-10 instruments. All melting points were obtained on a calibrated Thomas–Hoover capillary melting point apparatus. Tlc were run using Eastman silica gel chromatogram sheets and column chromatography was done using Brinkman silica gel (0.05–0.20 mm). Elemental analyses were conducted by Midwest Microlabs, Indianapolis, Ind.; Huffman Laboratories, Wheatridge, Colo.; and on an F and M 185 instrument, University of Kansas. Except where indicated analyses were within ±0.4%.

cis-**3-Diphenylphosphorylcyclohexanecarboxylic Acid (5a). Method A.**—A solution of 12.5 g (0.087 mole) of *cis*-3-hydroxycyclohexanecarboxylic acid in 20 ml of C_5H_5N was cooled to 0° in an ice bath and 23.4 g (0.087 mole) of diphenylphosphorochloridate was added dropwise. A white precipitate formed and the resulting mixture was stirred overnight at 25°. The reaction mixture was poured into 200 ml of ice–H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give a yellow syrup which was chromatographed on 80 g of silica gel. Elution with C_6H_6 afforded 4.7 g (38%) of the lactone of 3-hydroxycyclohexanecarboxylic acid, mp 115° (lit.[5c] mp 120°), ir (CCl_4) 1800 cm^{-1}. Elution with Et_2O–C_6H_6 (1:1) afforded 15.0 g (46%) of the desired product **5a**, ir (liquid film) and nmr as expected. *Anal.* ($C_{19}H_{21}O_5P$) C, H.

cis-**3-Carbamoylcyclohexyl Diphenyl Phosphate (5b). Method B.**—A solution of 12.4 g (0.03 mole) of **5a** in 20 ml of $SOCl_2$ was stirred at 25° overnight with exclusion of moisture. The resulting clear solution was subjected to aspirator vacuum with slight warming to remove excess $SOCl_2$ and the residue was added to an ice-cooled solution of 100 ml of concentrated NH_4OH. The mixture was allowed to warm to 25° over 1 hr, then diluted with H_2O to 200 ml. The H_2O mixture was then extracted with six 100-ml portions of EtOAc and the combined extracts were dried ($MgSO_4$). Filtration and evaporation of the filtrate *in vacuo* afforded 8.0 g of a syrup which solidified and was recrystallized from EtOAc to give 3.5 g (28%) of the product **5b** as a white solid, mp 119–120°, ir and nmr as expected. *Anal.* ($C_{19}H_{22}NO_5P$) C, H, N, P.

cis-**3-N-Acetylcarbamoylcyclohexyl Diphenyl Phosphate (5c). Method C.**—Following the procedure of Hurd and Prapas,[7] a solution of 3.50 g (0.009 mole) of **5b** in 2.50 g (0.024 mole) of Ac_2O was treated with 5 drops of H_2SO_4 and heated at 140° for 5 min. The resulting light brown solution was poured into 80 ml of ice–H_2O and the mixture was extracted with four 50-ml portions of EtOAc. The combined EtOAC extracts were dried ($MgSO_4$) and filtered, and the filtrate was evaporated to give 3.6 g of a yellow syrup. This material was dissolved in Et_2O, washed with five 25-ml portions of H_2O, and dried ($MgSO_4$), and the solvent was removed to give 2.4 g of **5c** (62%) as a light yellow syrup, ir and nmr as expected. *Anal.* Calcd ($C_{21}H_{24}NO_6P \cdot H_2O$) C, H, N.

cis-**3-Allophanoylcyclohexyl Diphenyl Phosphate (5d). Method D.**—A solution of 4.61 g (0.01 mole) of **5a** in 6.0 g (0.05 mole) of $SOCl_2$ was stirred at 25° overnight with exclusion of moisture. The resulting clear solution was subjected to aspirator vacuum with slight warming to remove excess $SOCl_2$ leaving 5.0 g of oil (ir, liquid film 1795 cm^{-1}). This oil was treated with 2.9 g (0.05 mole) of urea with warming at 50–60° for 2 days. The resulting brown mass was dissolved in $CHCl_3$ and the solution was washed with H_2O and dried (Na_2SO_4). Filtration and evaporation of the filtrate afforded 2.5 g (49%) of **5d** as a brown syrup. Purification was carried out by column chromatography on silica gel with elution of the product in 2% MeOH–$CHCl_3$; ir and nmr as expected. *Anal.* ($C_{20}H_{23}N_2O_6P$) C, N, P; H: calcd, 5.54; found, 5.95.

cis-**3-Carbamoylcyclohexyl Phosphate (1b). Method F.**—A solution of 0.20 g (0.0005 mole) of **5b** in 20 ml of absolute EtOH was added to a stirred suspension of 0.35 g of prereduced Pt in 100 ml of absolute EtOH and the resulting mixture was subjected to 1 atm of H_2 at 25° for 3.5 hr. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* at room temperature and washed with Et_2O to give a quantitative yield of the

product **1b** as a highly hygroscopic solid, nmr (DMSO-d_6) as expected. *Anal.* ($C_7H_{14}NO_5P \cdot XH_2O$) N/P: calcd, 1.00; found, 1.04.

The NH_4 salt of **1b** was prepared by dissolving the phosphate in several milliliters of 2% NH_4OH and freeze drying to a white powder. *Anal.* ($C_7H_{14}NO_5P \cdot 2NH_3 \cdot XH_2O$) N/P: calcd, 3.00; found, 2.98.

***trans*-3-Allophanoylcyclohexyl Diphenyl Phosphate (6d). Method E.**—A solution of 1.0 g (0.0026 mole) of *trans*-3-diphenylphosphorylcyclohexanecarboxylic acid (**6a**) in 25 ml of anhydrous C_6H_6 was treated with 0.4 g (0.0032 mole) of oxalyl chloride at 25°. After the initial effervescence had subsided the reaction mixture was stirred overnight at 25°, heated for 1 hr at 60°, and evaporated *in vacuo* to give a syrup. The syrup was then treated with 0.50 g (0.008 mole) of urea at 65° for 2 days. The resulting brown mass was partitioned between EtOAc and H_2O and the EtOAc extracts were dried ($MgSO_4$). Subsequent filtration and evaporation afforded 1.0 g (90%) of the desired product (**6d**) as an impure brown oil. Purification was accomplished on silica using 2% MeOH–$CHCl_3$ as the eluent; ir (liquid film) and nmr as expected. *Anal.* Calcd ($C_{20}H_{23}N_2O_6P$) C, H, N, P.

Trifluoroacetylurea.—Urea (26 g, 0.44 mole) was dissolved in 100 ml of CF_3COOH, and 93 g (0.44 mole) of $(CF_3CO)_2O$ and 0.1 ml of H_2SO_4 were added. The solution was heated to 100° for 1 and stirred at 25° for 2 hr, and 500 ml of H_2O was added. The solid product was collected and recrystallized from EtOH; mp 184–186° (lit.[14] 189°). *Anal.* ($C_3H_3F_3N_2O_2$) C, H, N.

Cyclohexylcarboxylurea.—Cyclohexylcarboxylic acid (2 g, 16 mmoles) was stirred with 15 ml of $SOCl_2$ overnight. The solution was evaporated to a thick syrup and heated to 70° for 1 day with 2 g (33 mmoles) of urea. Upon the addition of 30 ml of H_2O a solid formed which was filtered and dried to yield 2.26 g of the product (83%) which was recrystallized from EtOH; mp 230–232°. *Anal.* ($C_8H_{14}N_2O_2$) C, H, N.

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A Physicochemical Model for the Mechanism of Action of Antihistaminics and Cortisol

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The mechanism of action of antihistaminics and cortisol has been studied at the molecular level using "coupled" ion-exchange membrane electrodes on an *in vitro* system consisting of bovine serum albumin as the receptor and various antihistaminics and cortisol as competitors. The data obtained indicate that, when the antihistaminic concentration is above a critical value (1×10^{-4} *M*), histamine does not induce those changes in bovine serum albumin which are necessary for an interaction. At the same time, structural analogs without antihistaminic activities have shown no influence on the binding of histamine, when tested in the same experimental conditions. Furthermore, the access of histamine to the biopolymer is inhibited by molar concentrations of antihistaminics which closely approximate those previously found for inorganic monovalent ions. The correlation between ionic strength and antihistaminic action is discussed in terms of a stabilizing effect of the antihistaminic on a given conformation of the biopolymer. This conformation is unable to bind histamine. When antihistaminics are replaced by cortisol, this steroid prevents the binding of histamine to the macroion at molarities at which antihistaminics show no effect. In fact, under the same experimental conditions, cortisol is more effective by a factor of 10 than antihistaminics in stabilizing that conformation of the biopolymer which does not interact with histamine.

The investigation of pharmacological problems has been greatly aided by the study of model systems using a purely physicochemical approach. This is particularly true for the mechanism of action of antihistaminics. Many of the mechanisms postulated[1] may be tested at the molecular level using a synthetic *in vitro* system. Even though the model system is merely a method for visualizing a problem in simpler molecular terms and is not an attempt to reproduce physiological conditions, it can serve the purpose of eliminating those mechanisms which violate the principles obtained from these studies.

Kier's considerations[2] on the interatomic distances in the cortisol and histamine molecules and theoretical speculations on a possible competition between the two as an explanation of the role of cortisol in controlling the inflammatory response have found experimental support in the electrochemical data presented in this paper. We have demonstrated that an electrostatic competition between cortisol and histamine does occur and no binding of histamine to the macroion takes place in the presence of a given molar concentration of cortisol.

It is commonly believed[1] that the antihistaminics function by competing with histamine for a specific receptor site on a protein. This receptor is ill defined and has not, as yet, been isolated or identified.

On the assumption[3] that protein–drug interaction produces a change in the structure of the biopolymer and consequently a variation in the mean ionic activity of the saline medium, potentiometric measurements have been carried out by means of "coupled" ion-exchange membrane electrodes previously described.[4] This new technique is useful for the study of unstable biologically important compounds and of biopolymers which undergo conformational changes.[5]

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