Charge-Spatial Models. 2. *cis*- and *trans*-3- and -4-Carboxycyclohexyl Phosphates and the Carboxylate Esters as Analogs of 2′-Deoxyuridine 5′-Phosphate[1]

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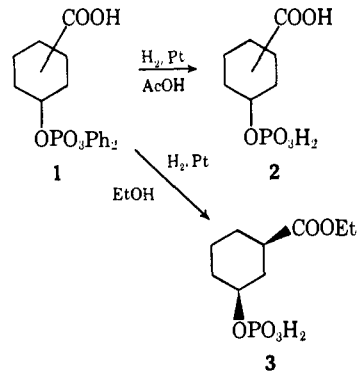
The design of enzyme inhibitors based on molecular modification of the natural substrate for a given enzymatic reaction has yielded many useful drugs. It has been generally accepted that two or three sites of a substrate model are responsible for the majority of the enzyme–substrate binding energy. If it is assumed that the remainder of the model contributes little binding energy but is simply a template holding the correct charge sites in the proper spatial arrangement then the design of new structures for specific biological effects has unlimited possibilities.

Based on this concept, a number of *cis*- and *trans*-3- and 4-substituted cyclohexyl phosphates have been synthesized[2] to simulate charge–spatial features of the substrate molecule of the enzyme thymidylate synthetase,[3] which catalyzes conversion of 2′-deoxyuridine 5′-phosphate into thymidine 5′-phosphate. As an extension of this study,[2] *cis*- and *trans*-3- and -4-carboxycyclohexyl phosphates and the corresponding carboxylate esters have now been synthesized.

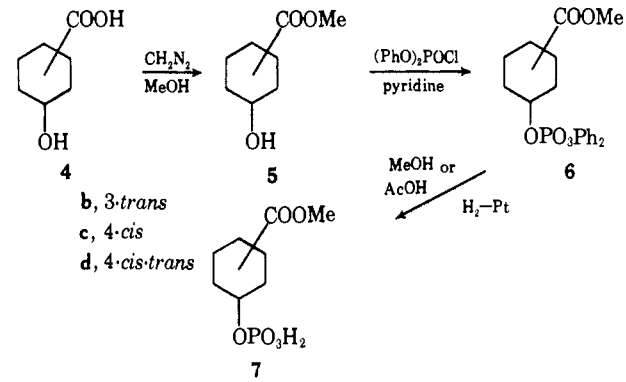
Catalytic reduction in HOAc of *cis*- and *trans*-3- and -4-diphenylphosphoryloxycyclohexanecarboxylic acids[2] (**1a–d**) gave the corresponding dihydrogen phosphates (**2a–d**). However, it was found that when abs



a, 3-*cis*
b, 3-*trans*
c, 4-*cis*
d, 4-*cis*-*trans*

EtOH was substituted as the solvent during reduction of **1a**, the resulting compound was identified as the corresponding Et ester **3** by nmr (C_2H_5 group), ir (C=O at 1735 cm^{-1}), and elemental analysis. Compounds **2a**, **2c**, and **2d** were isolated in crystalline forms, while **2b** was isolated as the corresponding diammonium salt. Compound **2d** is presumed to be a mixture of 1:1 (± 10%) cis:trans, since it was not possible to isolate *trans*-4-hydroxycyclohexanecarboxylic acid in a pure form.[2]

Hydroxycyclohexanecarboxylic acids (**4b–d**) were esterified with CH_2N_2 in MeOH and the resulting oily Me esters (**5b–d**) were treated with $(PhO)_2POCl$ to yield **6b–d**. These methyl diphenylphosphoryloxycyclohexanecarboxylates (**6b–d**) were hydrogenated in MeOH or AcOH to remove the C_6H_5 protecting groups. The oily or semisolid dihydrogenphosphates (**7b** and **7d**) were isolated as diammonium salts; **7c** was a solid. As noted previously, **7d** is a 1:1 (+10%) cis:trans mixture. The physical constants for these compounds are found in Table I.



Previously[2] it was found that weak inhibition of thymidylate synthetase resided in 4-acetylcarbamoylcyclohexyl phosphates and 4-allophanoylcyclohexyl phosphates. The purpose of this study was twofold: to determine if an acidic function was essential for activity and, secondly, to further establish the optimum distances for binding between the phosphate and the acidic function. The acids **2a–d** and the esters **3** and **7b–d** were examined *in vitro* for inhibitory activity against thymidylate synthetase. No inhibition was noted for **3**, **7b**, and **7d** at a ratio of inhibitor: substrate of 100 while only weak inhibition was found for **7c**: 15% at a ratio of 80. A fair degree of activity was found in the 1,4-cis–trans acid **2d**. At an inhibitor:substrate ratio of 2, 30% inhibition of the enzyme was found. The remaining acids (**2a–c**) were inactive at [I]/[S] of 100.

It can be concluded from this and the previous study[2] that the acid function is essential for binding and that in this series optimal binding is achieved when the two binding functions are 1,4-trans. Further studies are in progress to evaluate the effect of extending the distance between the two binding groups in charge–spatial models of the substrate deoxyuridine 5′-phosphate.

Experimental Section

Melting points were determined in a Thomas-Hoover apparatus and are uncorrected. Spectra were determined with a Beckman IR-10 and a Varian A-60A using $SiMe_4$ or DSS as internal standards; the results were as expected. The nmr spectra were recorded using 15–20% concentration.

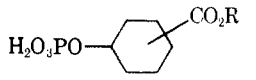
Silica gel for column chromatography refers to Brinkman (0.05–0.20 mm) product activated as 110° and deactivated with 10% H_2O. Alumina for column chromatography refers to Woelm neutral alumina. All hydrogenations were carried out at atmo-

(1) This work was supported by Research Grant CA 5639 and by a Career Development Award CA 10,739 from the National Cancer Institute, National Institutes of Health.

(2) (a) M. P. Mertes and E. A. Coats, *J. Med. Chem.*, **12**, 823 (1969). (b) E. A. Coats, Ph.D. Thesis, University of Kansas, Lawrence, Kan., 1968.

(3) (a) A. J. Wahba and M. Friedkin, *J. Biol. Chem.*, **237**, 3794 (1962). (b) R. Blakley, *ibid.*, **238**, 2113 (1963). (c) P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965).

TABLE I
PHYSICAL CONSTANTS OF SUBSTITUTED CYCLOHEXYL PHOSPHATES



No.	Isomer	R	Method	Yield, %	Mp, °C	Formula	Anal.
2a	3-cis	H	A		179–181.5[a]	$C_7H_{13}O_6P$	C, H
2b	3-trans	H	A	45		$C_7H_{13}O_6P$	C, H
2c	4-cis	H	A	78	175–177[a]	$C_7H_{13}O_6P$	C, H
2d	4-cis-trans	H	A	76	146–149	$C_7H_{13}O_6P$	C, H
3	3-cis	C_2H_5	B	74		$C_9H_{17}O_6P \cdot 2NH_3$	C, H, N
7b	3-trans	CH_3	C	90		$C_8H_{15}O_6P \cdot 2NH_3$	C, H, N
7c	4-cis	CH_3	D	86	106–110	$C_8H_{15}O_6P$	C, H
7d	4-cis-trans	CH_3	D	88		$C_8H_{15}O_6P$	C, H

[a] Recrystd from MeAc–$CHCl_3$.

spheric pressure at 25° using PtO_2 as the catalyst purchased from Engelhard Industries. Microanalyses were carried out on F and M 185 instrument at the University of Kansas, Lawrence, Kan.; elemental analyses were within ±0.4%.

trans-**3-Carboxycyclohexyl Phosphate (2b). Method A.**—A soln of *trans*-3-diphenylphosphoryloxycyclohexanecarboxylic acid[2] (**1b**) (0.45 g, 1.2 mmoles) in 10 ml of HOAc was added to prereduced PtO_2 (0.1 g) in 10 ml of HOAc and hydrogenated for 7 hr. During this time the uptake of H_2 amounted to 265 ml (235 ml theoretical). The catalyst was removed by filtration and washed well with the solvent. The filtrate and the washings were coevapd and the resulting viscous oil was washed with $CHCl_3$–cyclohexane and dried under vacuum to yield 0.122 g (45%) of the product. *Anal.* ($C_7H_{13}O_6P$) C, H.

The oily product was treated with a few milliliters of dil NH_3 soln and subjected to freeze-drying. This provided 0.104 g of the hygroscopic diammonium salt.

Compounds **2a**, **2c**, and **2d** were prepared in the same manner and isolated as crystalline free acids.

cis-**3-Carboethoxycyclohexyl Phosphate (3). Method B.**—A soln of *cis*-3-diphenylphosphoryloxycyclohexanecarboxylic acid[2] (**1a**) (1.04 g, 2.8 mmoles) in abs EtOH was added to prereduced PtO_2 (0.1 g) in the same solvent and hydrogenated for 9 hr. The uptake of H_2 during this time amounted to 545 ml (545 ml theoretical). The catalyst was filtrated and washed well with the solvent. The washings and the filtrate were combined and the solvent removed under vacuum without using heat. The resulting oil was treated with a few milliliters of dil NH_3 soln and filtered. The filtrate was washed twice with Et_2O and the Et_2O layers were discarded. The aq layer was freeze-dried to yield 0.61 g (74%) of the diammonium salt as a white powder. *Anal.* ($C_9H_{23}N_2O_6P$) C, H, N.

Methyl *trans*-**3-Diphenylphosphoryloxycyclohexanecarboxylate (6b).**—Diphenyl phosphorochloridate (2.0 g, 7.5 mmoles) was added dropwise to a soln of methyl *trans*-3-hydroxycyclohexanecarboxylate (**5b**) (0.5 g, 3.16 mmoles) in 5 ml of anhydrous C_5H_5N at 0° with stirring.[2] Stirring was continued overnight at 25° and the reaction mixture was then poured over ice–water mixture (50 ml). It was allowed to stand overnight and then extd with Et_2O. The ext was successively washed with HCl (5%), $NaHCO_3$ (5%), and H_2O, and dried (Na_2SO_4) and the solvent was removed at 25° under vacuum. The resulting oil was chromatographed over a neutral alumina (30 g) column. Elution with $CHCl_3$ and the evapn of the eluant solvent provided 1.18 g (95%) of pure product as a colorless oil. *Anal.* ($C_{20}H_{23}O_6P$) C, H.

Compounds **6c** and **6d** were prepared in a similar fashion in quantitative yields. *Anal.* ($C_{20}H_{23}O_6P$) C, H.

trans-**3-Carbomethoxycyclohexyl Phosphate (7b). Method C.**—A soln of methyl *trans*-3-diphenylphosphoryloxycyclohexanecarboxylate (**6b**) (0.5 g, 1.3 mmoles) in 10 ml of abs MeOH was added to a stirred suspension of prereduced PtO_2 (0.1 g) in 100 ml of the same solvent and hydrogenated for 7 hr. The uptake of H_2 at this stage amounted to 270 ml (250 ml theoretical). The catalyst was removed by filtration and washed well with the solvent. The filtrate and washings were combined and the solvent was removed under reduced pressure at 25°. The resulting oil was washed twice with Skellysolve B and converted into the diammonium salt by addn of a few milliliters of dil NH_3 soln followed by freeze-drying to yield 0.31 g (90%) of **7b** as a white powder. *Anal.* ($C_8H_{21}N_2O_6P$) C, H, N.

Methyl *cis*-**4-Carbomethoxycyclohexyl Phosphate (7c). Method D.**—A soln of methyl *cis*-4-diphenylphosphoryloxycyclohexanecarboxylate (**6c**) (0.4 g, 1.05 mmoles) in 10 ml of HOAc was added to a stirred suspension of prereduced PtO_2 (0.1 g) in 10 ml of the same solvent and hydrogenated for 12 hr. The absorption of H_2 during this time amounted to 250 ml (205 ml theoretical). The catalyst was removed by filtration and washed well with the solvent. The filtrate and the washings were combined and the solvent removed by freeze-drying. This provided 0.21 g (86%) of the product as white crystals, mp 106–110°. *Anal.* ($C_8H_{15}O_6P$) C, H.

Compound **7d** was prepared in the same fashion.

Enzyme Testing.—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. 2'-Deoxyuridine 5'-monophosphate was present in 4.8×10^{-5} *M* concn. The enzyme source was *Escherichia coli* B.[2,4] The enzyme, inhibitor, cofactor, and buffers were incubated at 32° until there was no change in absorbances at 340 mμ; this usually required about 15–30 min. The substrate (dUMP) was added to the sample cuvette, an equal vol of H_2O was added to the reference cuvette, and the change in absorbance was monitored, using full scale equals 0.1 absorbance unit in a Gilford multiple sample absorbance spectrophotometer. Control rates were measured under identical conditions without the inhibitor. The compounds were examined at concns in the assay media that give a ratio of inhibitor: substrate of 100. Limited soly in the assay soln for **7c** required an evaulation of ratios below [I]/[S] of 100.

(4) M. P. Mertes and N. R. Patel, *J. Med. Chem.*, **9**, 868 (1966).

Mustards Derived from 7-Phenylbenz[*a*]anthracene[1]

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In view of the antitumor activity of 7-phenylbenz[*a*]-anthracene[4] and of mustards derived from polycyclic aromatic hydrocarbons,[5] it seemed desirable to prepare a series of mustards derived from 7-phenylbenz[*a*]-

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(3) Taken in part from the Ph. D. Thesis of R. G. D. presented to the Virginia Polytechnic Institute in 1967.

(4) Tests performed by Cancer Chemotherapy National Service Center.

(5) See R. M. Peck, A. P. O'Connell, and H. J. Creech, *J. Med. Chem.*, **13**, 284 (1970), for the most recent ref.