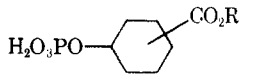
TABLE I
PHYSICAL CONSTANTS OF SUBSTITUTED CYCLOHEXYL PHOSPHATES



| 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 $4.8 \times 10^{-5}$ *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.

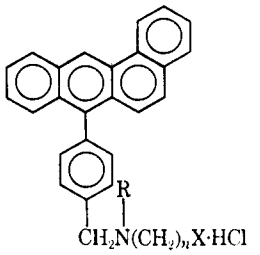
(5) See R. M. Peck, A. P. O'Connell, and H. J. Creech, *J. Med. Chem.*, **13**, 284 (1970), for the most recent ref.

anthracene and have them screened for possible antitumor activity.

Of the several possible approaches, it appeared, based on our experience with benz[a]anthracene derivatives,[6] that the best approach would be to attach the mustard after the polycyclic system had been built. Therefore 7-(4-methylphenyl)benz[a]anthracene[7] was photochemically converted into 7-(4-bromomethylphenyl)benz[a]anthracene. This was treated with an amino alcohol leading to the polycyclic amino alcohols shown in Table I which were isolated as hydrochlorides. These were converted into the corresponding mustards.

TABLE I

ALCOHOLS AND MUSTARDS DERIVED FROM 4-BENZ[a]ANTHRACEN-7-YLBENZYLAMINE




| R | X | n | Yield, % | Mp, °C | Formula[a] |
|---|---|---|---|---|---|
| $CH_3$ | OH | 2 | 80 | 202–203 | $C_{28}H_{25}NO \cdot HCl$ |
| $C_2H_5$ | OH | 2 | 80 | 227–228 | $C_{29}H_{27}NO \cdot HCl$[b] |
| $CH_2CH_2OH$ | OH | 2 | 80 | 184–185 | $C_{29}H_{27}NO_2 \cdot HCl$[c] |
| H | OH | 3 | 80 | 218–219 | $C_{28}H_{26}NO \cdot HCl$ |
| $CH_3$ | Cl | 2 | 75 | 209–210 | $C_{28}H_{24}ClN \cdot HCl$ |
| $C_2H_5$ | Cl | 2 | 75 | 220–221 | $C_{29}H_{26}ClN \cdot HCl$ |
| $CH_2CH_2Cl$ | Cl | 2 | 70 | 203–204 | $C_{29}H_{25}Cl_2N \cdot HCl$[d] |
| H | Cl | 3 | 90 | 244–245 | $C_{28}H_{24}ClN \cdot HCl$[e] |

[a] All compds were analyzed for C, H, Cl, N. [b] C: calcd, 78.8; found, 78.3. [c] C: calcd, 76.0; found, 75.5. [d] C: calcd, 70.4; found, 70.9. [e] C: calcd, 75.3; found 74.8.

### Experimental Section[8]

**7-(4-Bromomethylphenyl)benz[a]anthracene.**—To a soln of 64 g (0.20 mole) of 7-(4-methylphenyl)benz[a]anthracene[7] in 800 ml of $CCl_4$ heated under reflux, there was added 3 g of benzoyl peroxide followed by 36 g of NBS (excess), added in small portions. The soln, while being irradiated with two 200-W lamps, was refluxed for 90 min after all the NBS had been added, then cooled, and filtered. The filtrate was concd to 200 ml and filtered, and the filtrate was taken to dryness. The yellow solid was crystd from heptane and gave 64g (80%) of white rhombic crystals, mp 158–159°. *Anal.* ($C_{25}H_{17}Br$) C, H, Br.

***p*-Benz[a]anthracen-7-yl-*N*-(2-hydroxyethyl)-*N*-methylbenzylamine·HCl.**—To a soln of 10 g (0.025 mole) of 7-(4-bromomethylphenyl)benz[a]anthracene in 200 ml of $C_6H_6$ there was added 2 ml of *N*-methylethanolamine and the mixture was refluxed for 4 hr. The soln was cooled, washed ($H_2O$) 3 times, and dried ($MgSO_4$). When ethereal HCl was added, the product pptd and was recrystd from dry EtOH.

***p*-Benz[a]anthracen-7-yl-*N*-(2-chloroethyl)-*N*-methylbenzylamine·HCl.**—The above amino alcohol (10 g, 0.023 mole) was added to 500 ml of $CHCl_3$ and heated under reflux. Excess pure $SO_2Cl_2$ was then added slowly and the mixture was refluxed for 90 min. Removal of the solvent and excess $SO_2Cl_2$ left a solid which was crystd from dry EtOH.

***p*-Benz[a]anthracen-7-yl-*N*-{2-[(2-chloroethyl)thio]ethyl}-*N*-methylbenzylamine·HCl.**—To a hot soln of the above mustard (10 g, 0.022 mole) in $CHCl_3$ was added 5.0 g (0.064 mole) of 2-mercaptoethanol dissolved in NaOEt and 4 g of Na in 100 ml of EtOH. The soln was refluxed for 2 hr and then cooled and poured into $H_2O$. The product was extd with $Et_2O$, dried ($MgSO_4$), ethereal HCl was added, and the mixture was cooled overnight (refrigerator). The solid was filtered and dissolved in 200 ml of $CHCl_3$, excess $SO_2Cl_2$ was added, and the mixture was refluxed for 2 hr. The solvent and excess $SO_2Cl_2$ were distd, and the residue was recrystd 3 times from EtOH, yielding 7.2 g (65%), mp 182–183.[9] *Anal.* ($C_{30}H_{28}ClNS \cdot HCl$), C,[10] H, Cl, N, S.

***p*-Benz[a]anthracen-7-ylbenzyl 2-Chloroethyl Sulfide.**—To a soln of 10 g (0.025 mole) of 7-(4-bromomethylphenyl)benz[a]anthracene in EtOH was added 5.0 g (0.065 mole) of 2-mercaptoethanol dissolved in NaOEt. The soln was refluxed for 2 hr, cooled, and poured into $H_2O$. The product was extd with $Et_2O$, the soln dried, and the solvent distd, leaving an oil which was crystd from EtOH giving the alcohol as bright yellow plates, mp 116–117°, 8.4 g (85%). *Anal.* ($C_{27}H_{22}OS$) C, H, S. This alcohol was dissolved in $CHCl_3$ and excess $SO_2Cl_2$ was added. The soln was refluxed for 10 min and then taken to dryness under reduced pressure. The resulting oil was crystd from EtOH and gave 7.0 g (80%) of product, mp 108–109°. *Anal.* ($C_{27}H_{21}ClS$) C, H, Cl, S.[11]

**Biological Testing.**—All the mustards were tested by CCNSC against L-1210 lymphoid leukemia and none was found to be active.

(6) F. A. Vingiello, L. Ojakaar, and R. Kelsey, *J. Med. Chem.*, **8**, 144 (1955).

(7) F. A. Vingiello, A. Borkovec, and J. Shulman, *J. Amer. Chem. Soc.*, **77**, 2320 (1955).

(8) All melting points were taken on a Fisher-Johns melting apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

(9) Two additional mixed N and S mustards were prepared: *p*-benz[a]anthracen-7-yl-*N*-{2[(2-chloroethyl)thio]ethyl}-*N*-ethylbenzylamine·HCl, ($C_{31}H_{30}ClNS \cdot HCl$) C, H, Cl, N, S, mp 153–154° (70%); *p*-benz[a]anthracen-7-yl-*N*-{3-[(2-chloroethyl)thio]propyl}benzylamine·HCl, ($C_{30}H_{28}ClNS$) C: calcd, 71.1; found, 68.0. Although a satisfactory C analysis could not be obtained, this compound was included in the antitumor screen. Anal. was satisfactory for H, Cl, N, S, mp 211–212° (60%).

(10) C: calcd, 71.1; found, 65.7. Although a satisfactory C anal. could not be obtained, this compd was included in the antitumor screen.

(11) Cl: calcd, 8.6; found, 8.1. S: calcd, 7.8; found, 7.3.

## Synthesis and Anticancer Activity of Cytosine Arabinoside 3-*N*-Oxide (Ara-C 3-*N*-Oxide)[1]

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Cytosine arabinoside[3] has been shown to undergo a rapid enzymatic deamination in man to form arabinofuranosyluracil, an inactive metabolite.[4–7] The inhibition of pyrimidine nucleoside deaminase, the enzyme which causes this process, has been the subject of much study. Tetrahydrouridine (THU, $H_4$-U, 3,4,5,6-tetrahydrouridine) has been shown to be an effective inhib-

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(2) The recipient of a University of Utah Research Committee Fellowship, 1968–1970.

(3) Synonyms for cytosine arabinoside are: Ara-C, cytarabine, and 1-β-D-arabinofuranosylcytosine.

(4) G. W. Camiener and C. G. Smith, *Biochem. Pharmacol.*, **14**, 1405 (1965).

(5) R. V. Loo, M. J. Brennan, and R. W. Talley, *Proc. Amer. Ass. Cancer Res.*, **6**, 41 (1965).

(6) R. J. Papac, W. A. Creasy, P. Calabresi, and A. D. Welch, *ibid.*, **6**, 50 (1965).

(7) W. A. Creasey, R. J. Papac, M. E. Markiuo, P. Calabresi, and A. D. Welch, *Biochem. Pharmacol.*, **15**, 1417 (1966).