modification of the method of Hoard and Ott[11] was used to prepare the triphosphate. The tripentylammonium salt of **5** (0.1 mmol) was dissolved in 3 ml of dry DMF and to this solution was added 160 mg (1.0 mmol) of 1,1'-carbonyldiimidazole. The mixture was allowed to stand overnight and then treated simultaneously with MeOH (73 μl, 1.8 mmol) and tripentylammonium pyrophosphate (1.0 mmol). This mixture was stirred vigorously at room temperature for 24 hr. The precipitated imidazolium pyrophosphate was removed by filtration and washed with several portions of DMF. An equal volume of MeOH was added to the filtrate, and after 30 min the solution was evaporated to dryness at reduced pressure. The residue was dissolved in 0.5 ml of H_2O and the solution streaked onto two sheets (46 × 57 cm) of Whatman No. 40 paper. Ascending chromatography was carried out with *n*-BuOH–AcOH–H_2O, 6:2:2. The major band at R_f 0.33 was cut out and the material eluted from the paper with H_2O. By uv, the yield of 6 was 20%, $\lambda_{max}^{H_2O}$ 262 nm. *Anal.* Calcd base–phosphorus ratio, 1:3; found, 1:2.9.

The product was homogeneous by polyethyleneimine–cellulose tlc (R_f 0.3, 0.8 *M* LiCl) and paper chromatography (R_f 0.35, *n*-BuOH–AcOH–H_2O, 5:2:3) and had an R_f of 0.0 on DEAE-cellulose paper (4 *M* formic acid, 0.1 *M* ammonium formate) which agrees with a previously published value.[15] Hydrolysis in 1 *N* HCl for 7 min regenerated F_3dThd-5'-P.

[6-^{3}H]-5-Trifluoromethyl-2'-deoxyuridine 5'-Phosphate and 5'-Triphosphate. The tritiation of F_3dThd was carried out for this laboratory by the Amersham-Searle Co. F_3dThd (300 mg, 1.02 mmol) was dissolved in 70% aqueous AcOH containing [^{3}H]H_2O and heated for 5 hr at 80° in the presence of prereduced PtO_2. This procedure resulted in the incorporation of 60 mCi/mmol of nonexchangeable tritium into F_3dThd. Synthetic procedures identical with those described afforded [6-^{3}H]F_3dThd-5'-P and [6-^{3}H]F_3dThd-5'-PPP.

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Synthesis and Carcinogenicity of Compounds Related to 6-Hydroxymethylbenzo[*a*]pyrene†

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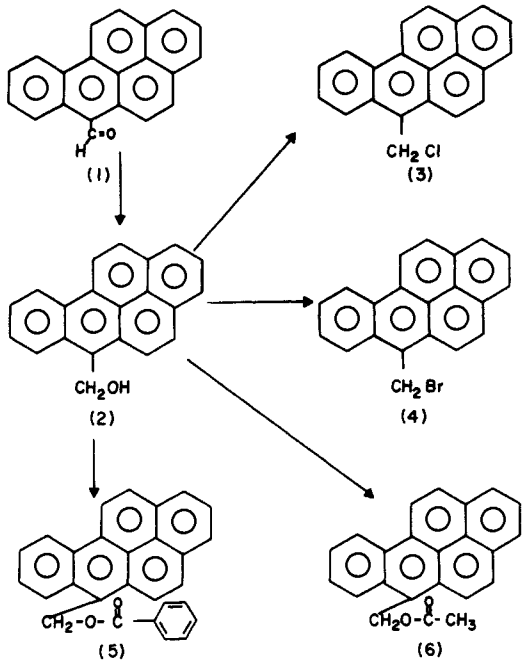
In earlier studies from this laboratory, it was postulated that 7-hydroxymethyl-12-methylbenz[*a*]anthracene, a metabolite of 7,12-dimethylbenz[*a*]anthracene, functions as a proximate carcinogen.[1,2] Evidence in support of this hypothesis was provided by observations of the carcinogenicity of 7-iodomethyl, 7-bromomethyl, 7-chloromethyl, 7-benzoyloxymethyl, 7-acetoxymethyl, 7-methoxymethyl, and 7-formyl derivatives of 12-methylbenz[*a*]anthracene. In accord with this hypothesis, each of the compounds which would be expected to be converted to 7-hydroxymethyl-12-methylbenz[*a*]anthracene was, in fact, shown to be converted, in part, to this compound. Thus, according to this hypothesis, the first step in carcinogenesis by 7,12-dimethylbenz[*a*]anthracene is hydroxylation of the 7-methyl group to form 7-hydroxymethyl-12-methylbenz[*a*]-anthracene. The second is the formation of a reactive ester bearing a good leaving group which would generate a highly reactive carbonium ion. The carbonium ion would be expected to react with critical cellular nucleophiles to initiate the chain of cellular events which result in cancer.

The observation that 7-hydroxymethyl-12-methylbenz[*a*]-anthracene and related compounds are carcinogenic suggested that a similar series of derivatives of benzo[*a*]pyrene might also be carcinogenic. The synthesis of 6-hydroxymethylbenzo[*a*]pyrene and related compounds was therefore undertaken to determine whether this is, in fact, the case.

The synthesis of 6-hydroxymethylbenzo[*a*]pyrene (**2**) was accomplished as illustrated in Scheme I by reduction

Scheme I



of 6-formylbenzo[*a*]pyrene (**1**) with $NaBH_4$. The aldehyde required for this synthesis was prepared by treatment of benzo[*a*]pyrene with *N*-methylformanilide in the presence of phosphorus oxychloride as described by Fieser and Hershberg.[3] 6-Hydroxymethylbenzo[*a*]pyrene was then converted to the chloromethyl (**3**), bromomethyl (**4**), benzoyloxymethyl (**5**), and acetoxymethyl (**6**) compounds as described in the Experimental Section.

The carcinogenicity of the compounds tested is presented in Table I. Each of the compounds tested is clearly a potent carcinogen as indicated by the high tumor incidence and short latent period. Benzo[*a*]pyrene and its formyl derivative are well-established carcinogens.[4] The carcinogenicity of 6-hydroxymethylbenzo[*a*]pyrene was recently reported from this laboratory but this is the first report of the other compounds in this series.[5]

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Table I. Induction of Sarcomata by Repeated Subcutaneous Administration of Benzo[a]pyrene and Related Compounds[a]

Benzo[a]pyrene derivative	Animals with tumors number of animals	Range, days	Mean tumor induction time, days
Benzo[a]pyrene	5/5	68–80	75
6-Hydroxymethyl	10/10	64–78	70
6-Formyl	3/5	71–78	75
6-Acetoxymethyl	3/4	71–78	73
6-Benzoyloxymethyl	5/5	78–94	87
6-Bromomethyl	3/4	86–95	92
6-Chloromethyl	5/5	74–91	81
Sesame oil	0/5		

[a]The hydrocarbon (1 mg) in sesame oil (0.1 ml) was administered by subcutaneous injection to female Sprague–Dawley rats, age 50 days, on alternate days for 20 doses. The animals were examined for appearance of palpable tumors twice weekly. Tumor negative animals were observed for 200 days. They were fed a commercial ration (Purina rat chow) *ad libitum* and given tap water to drink. Tumors were fixed in 10% neutral formalin, sectioned at a thickness of 5 μ and stained with hematoxylin and eosin. Each of the compounds tested induced fibrosarcoma. The chloromethyl compound induced a histiocytic variety of fibrosarcoma. We thank Dr. Daniel Weiss, Department of Pathology, University of Kentucky, for the interpretation of histological material.

Each of the substituted benzo[a]pyrenes described in this report would be expected to be transformed to 6-hydroxymethylbenzo[a]pyrene *in vivo*. The esters and halides would be expected to form 6-hydroxymethylbenzo-[a]pyrene by hydrolysis, whereas reduction of 6-formylbenzo[a]pyrene would form 6-hydroxymethylbenzo[a]-pyrene. Hydroxylation of 6-methylbenzo[a]pyrene to form 6-hydroxymethylbenzo[a]pyrene was recently shown to occur in rat-liver homogenates.[6]

The fact that 6-hydroxymethylbenzo[a]pyrene is a potent carcinogen supports the hypothesis that it functions as a proximate carcinogen. Thus, compounds which would be expected to be converted to 6-hydroxymethylbenzo[a]-pyrene are also carcinogenic. We therefore conclude that the relation between structure and carcinogenic activity is similar for the series of compounds related to 7-hydroxymethyl-12-methylbenz[a]anthracene and the series of compounds related to 6-hydroxymethylbenzo[a]pyrene.

Since the 6 position of benzo[a]pyrene corresponds to the 7 position of benz[a]anthracene, it would seem that compounds capable of conversion to the hydroxymethyl derivatives in the meso positions constitute proximate carcinogens for the two series.

Experimental Section

Elemental analyses were determined by Galbraith Laboratories, Knoxville, Tenn. Analytical results for the indicated elements were within ±0.4% of the theoretical values.

6-Formylbenzo[a]pyrene (1). A mixture of benzo[a]pyrene (5 g, 20 mmol) (1), $POCl_3$ (3.3 ml, 36 mmol), *N*-methylformanilide (5.5 ml, 40 mmol), and 5 ml of *o*-dichlorobenzene was warmed on a steam bath for 2 hr. The cooled reaction mixture was poured slowly into an aqueous solution (50 ml) of 25 g of sodium acetate. The product obtained was filtered and solvent removed by steam distillation. Filtration and drying yielded 5 g of crude product which was dissolved in hot benzene (250 ml) and filtered. Evaporation of the benzene solution under reduced pressure yielded 3.3 g of dark yellow material. Chromatography on silica gel and elution with benzene gave pure product (3.0 g, 10.7 mmol) in 54% yield, mp 205–206°.

6-Hydroxymethylbenzo[a]pyrene (2). A mixture of 6-formylbenzo[a]pyrene (1 g, 3.56 mmol) and $NaBH_4$ (150 mg, 3.96 mmol) in 200 ml of ethanol was heated to reflux for 2 hr on an oil bath. The solution was treated with acetic acid and the solvent removed completely. The solid was washed with water, filtered, and recrystallized from benzene (0.9 g, 3.19 mmol) in 89% yield, mp 232–233°. *Anal.* $C_{21}H_{14}O$.

6-Chloromethylbenzo[a]pyrene (3). Thionyl chloride (0.2 ml, 2.8 mmol) was added to a suspension of 6-hydroxymethylbenzo[a]-pyrene (200 mg, 0.71 mmol) in 7 ml of dry benzene. The mixture was heated to reflux for 30 min with stirring. The solvent was removed under reduced pressure and traces of $SOCl_2$ were removed by addition of benzene and removal under vacuum. The residue was crystallized from benzene. An analytical sample was prepared by recrystallization from benzene (120 mg, 0.4 mmol) in 56% yield, mp 210–212°. *Anal.* $C_{21}H_{13}Cl$.

6-Bromomethylbenzo[a]pyrene (4). A suspension of 6-hydroxymethylbenzo[a]pyrene (500 mg, 1.77 mmol) in 30 ml of anhydrous benzene was heated under reflux with PBr_3 (0.3 ml, 3.16 mmol) for 1 hr. The solution was chilled in ice–water and excess reagent was decomposed by dropwise addition of H_2O. A crystalline material separated which was filtered, washed with benzene, and dried. Recrystallization from benzene gave an analytically pure sample (360 mg, 1.04 mmol) in 59% yield, mp 225–226°. *Anal.* $C_{21}H_{13}Br$.

6-Benzoyloxymethylbenzo[a]pyrene (5). A mixture of 6-hydroxymethylbenzo[a]pyrene (200 mg, 0.71 mmol), benzoyl chloride (1 ml, 8.6 mmol), and pyridine (4 ml) was heated on a steam bath for 2 min and then poured onto crushed ice. The oil which separated was extracted with benzene, washed with 5% $NaHCO_3$ and water, and dried. Removal of solvent gave bright yellow crystals. Recrystallization from benzene gave **5** (180 mg, 0.47 mmol) in 66% yield, mp 213–214°. *Anal.* $C_{28}H_{18}O_2$.

6-Acetoxymethylbenzo[a]pyrene (6). To 6-hydroxymethylbenzo[a]pyrene (100 mg, 0.35 mmol) was added 3 ml of dry pyridine and acetic anhydride (1 ml, 10.5 mmol) and kept at room temperature overnight to obtain a clear solution. The mixture was then poured onto crushed ice and then the product was collected by filtration. The product was found to be very soluble in cold PhH, Et_2O, and petroleum ether (bp 30–60°). It was recrystallized from small amounts of cold PhH, mp 178–179°, in high yield. *Anal.* $C_{23}H_{16}O_2$.

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Potential Antitumor Agents. 7. 4′-Diethyleneoxy Derivatives of α-(N)-Heterocyclic Carboxaldehyde Thiosemicarbazones†

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α-(N)-Heterocyclic carboxaldehyde thiosemicarbazones are potent inhibitors of the growth of a variety of transplanted rodent tumors,[1] spontaneous lymphomas of dogs,[2] and DNA viruses of the Herpes group.[3] One agent of this series, 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP), has been tested clinically and has shown weak carcinostatic potency in man.[4,5] These derivatives are strong inhibitors of the mammalian form of the enzyme ribonucleoside diphosphate reductase,[3,6,7] which catalyzes the conversion of ribonucleotides to deoxyribonucleotides. Blockade of the formation of RNA and protein also

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