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# Structure and Activity in Chemical Carcinogenesis. Comparison of the Reactions of 7-Bromomethylbenz[*a*]anthracene and 7-Bromomethyl-12-methylbenz[*a*]anthracene with Deoxyribonucleic Acid *in Vitro*†

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ABSTRACT: 7-Bromomethyl-12-methylbenz[*a*]anthracene is a more effective carcinogen than 7-bromomethylbenz[*a*]-anthracene. It is shown that the more carcinogenic bromo compound reacts less extensively with DNA *in vitro* than does 7-bromomethylbenz[*a*]anthracene. Comparison of the products of reaction of each bromo compound with DNA indicates that similar products are produced in each case, through reaction of these compounds on the amino groups of the DNA bases, and that the products of reaction of the 12-methyl derivative with DNA are more light sensitive than those derived from the less carcinogenic compound. No correlation between the amounts of any hydrocarbon–DNA product formed and carcinogenic potency was found.

The precise nature of the initiating event in chemical carcinogenesis has not yet been defined for any chemical carcinogen. In order to approach this problem, detailed comparative studies of similar chemical agents which exhibit different carcinogenic potencies have been undertaken.

For example, it has been shown that 7-bromomethyl-12-methylbenz[*a*]anthracene has a shorter half-life and reacts less extensively with the nucleophile 4-(*p*-nitrobenzyl)pyridine than 7-bromomethylbenz[*a*]anthracene, and that the former compound is far more carcinogenic than the latter either after subcutaneous injection in the rat (Dipple and Slade, 1970), or topical application to mouse skin (Dipple and Slade, 1971). Since initiation may involve the interaction of a carcinogen with DNA (Brookes and Lawley, 1964), the major products of reaction of 7-bromomethylbenz[*a*]anthracene with DNA were identified (Dipple *et al.*, 1971). In the present studies the reactions of the above two bromo compounds with DNA *in vitro* have been carefully compared in order to define any differences which might possibly relate to their different carcinogenic activities.

## Experimental Section

*Radioactive 7-Bromomethylbenz[a]anthracene and 7-Bromomethyl-12-methylbenz[a]anthracene.* [$^3$H]7-Bromomethylbenz[*a*]anthracene (specific activity 165 Ci/mol) was prepared as previously described (Dipple *et al.*, 1971). [$^3$H]7-Bromomethyl-12-methylbenz[*a*]anthracene (specific activity 687 Ci/mol) was prepared by bromomethylation of radioactive 12-methylbenz[*a*]anthracene using the procedure described by Dipple and Slade (1970). Tritium-labeled 12-methylbenz-[*a*]anthracene was prepared by catalytic exchange methods at the Radiochemical Centre, Amersham, and purified in our laboratory (Duncan *et al.*, 1969). In some experiments preparations of higher specific activities than those listed above were used. 7-Bromo[$^{14}$C]methyl-12-methylbenz[*a*]-anthracene (specific activity 5.6 Ci/mol) was prepared by bromomethylation of unlabeled 12-methylbenz[*a*]anthracene using [$^{14}$C]paraformaldehyde (Radiochemical Centre, Amersham), again following the procedure of Dipple and Slade (1970).

*Reaction of [$^3$H]7-Bromomethyl-12-methylbenz[a]anthracene with Synthetic Polyribonucleotides.* Solutions of the potassium salt of poly(A) (Boehringer), the sodium salt of poly(G) (Miles) and the potassium salt of poly(C) (Miles) each at 2 mg/ml in 0.01 M sodium phosphate buffer (pH 7) were separately treated in a darkened room with a tenth volume of a dry acetone solution of the [$^3$H]bromo compound (0.2 mg/ml). After 15 min the polynucleotides were precipitated and washed as previously described (Dipple *et al.*, 1971). Portions of these polynucleotides were then hydrolyzed to mononucleotides by incubation at 37° overnight in 0.33 N potassium hydroxide (0.2 ml) and acetic acid was then added to lower the pH of these solutions to pH 8–9. These nucleotide solutions were then treated with 0.05 ml of an *Escherichia coli* alkaline phosphomonoesterase solution (1 mg/ml) and incubated at 37° for 10 hr.

*Reaction of Unlabeled 7-Bromomethyl-12-methylbenz[a]-*

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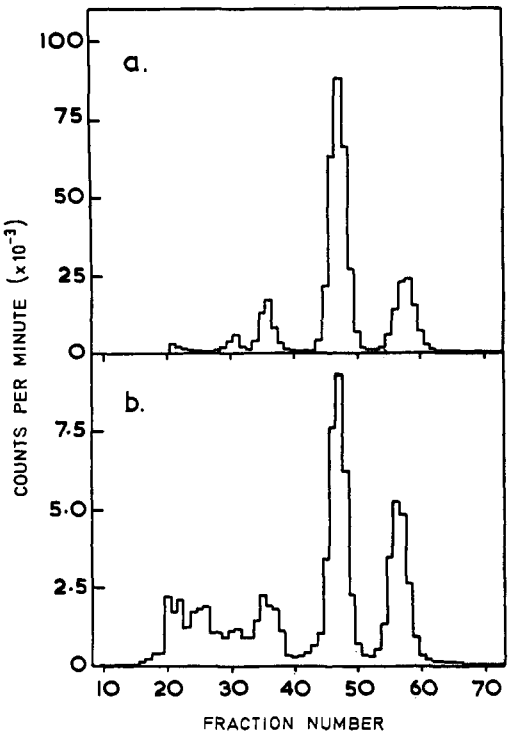


FIGURE 1: Column chromatograms of nucleoside products from DNA on Sephadex LH-20 eluted with methanol. Native salmon sperm DNA in sodium phosphate buffer (pH 7) reacted with (a) [$^3$H]7-bromomethylbenz[*a*]anthracene and (b) [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene. Reactions performed as described in the Experimental Section but no precautions were taken to protect solutions from exposure to light.

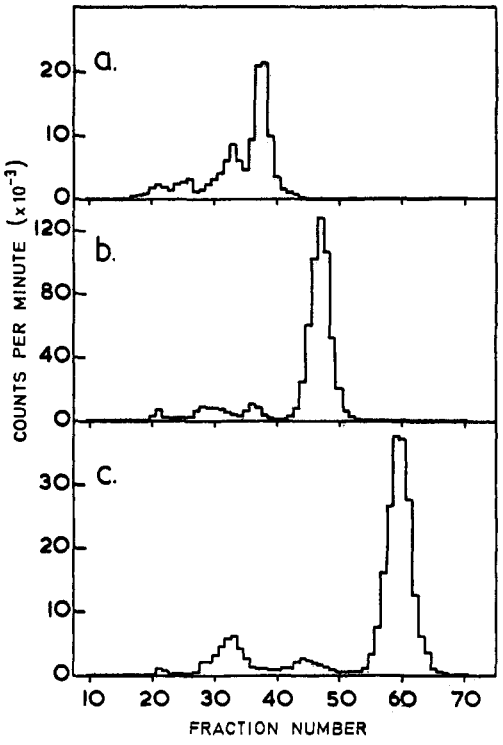


FIGURE 2: Column chromatograms on Sephadex LH-20 of nucleoside products from the reaction of [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene with (a) poly(C), (b) poly(G), and (c) poly(A). Reactions and hydrolysis to nucleosides were as described in the Experimental Section.

*anthracene with Deoxyguanosine 5'-Phosphate and Deoxyadenosine 5'-Phosphate.* Each nucleotide (50 mg) in 0.01 M sodium acetate buffer (pH 5.5, 5 ml) was treated five times with 0.5 ml of an acetone solution of 7-bromomethyl-12-methylbenz[*a*]anthracene (1 mg/ml). Between successive treatments the aqueous solution was washed with benzene (2 ml), then with ether (2 ml). After the final treatment with bromo compound the solution was washed three times with benzene and three times with ether, and any residual ether was removed by passing a stream of nitrogen through the solution. The solution was adjusted to pH 9, bacterial alkaline phosphatase (0.5 mg) was added, and the solution was incubated overnight at 37°. The resulting nucleoside digests were then separately subjected to chromatography on Sephadex LH-20 by elution with methanol as previously described (Dipple *et al.*, 1971). Unchanged deoxyguanosine and deoxyadenosine were eluted in fractions 21–30 and 22–32, respectively, and the hydrocarbon–nucleoside products were eluted in fractions 46–52 and 52–60 respectively. The fractions comprising the latter two peaks were pooled.

*Reaction of* [$^3$*H*]*7-Bromomethylbenz[a]anthracene and* [$^3$*H*]*7-Bromomethyl-12-methylbenz[a]anthracene with Native or Denatured DNA at pH 7.* Four 2-ml aliquots of a solution of Sigma salmon sperm DNA (2 mg/ml) in 0.01 M sodium phosphate buffer (pH 7) were prepared. Two were denatured by heating in a boiling-water bath for 5 min followed by cooling in ice. All four solutions were then left to warm to room temperature, and pairs of solutions (one native and one denatured) were separately treated with 0.2 ml of a dry acetone solution of either [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene (0.2 mg/ml) or [$^3$H]7-bromomethylbenz[*a*]anthracene (0.2 mg/ml). After 15 min the DNA was precipitated by the addition of one drop of 3 M sodium acetate followed by two volumes of ethanol. The precipitates were then washed twice in ethanol, twice in acetone, twice in ether, and dried.

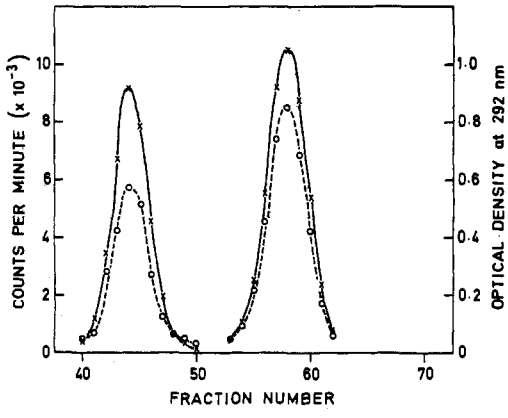


FIGURE 3: Proof that the two major products of reaction of [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene with DNA are deoxyguanosine and deoxyadenosine products. The data presented are from two separate experiments. Deoxyguanylic acid was reacted with unlabeled 7-bromomethyl-12-methylbenz[*a*]anthracene, the mixture was dephosphorylated, and the product was purified as described under Experimental Section. To this product the pooled fractions of the major radioactive peak from a chromatogram of [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene reacted DNA, as illustrated by Figure 1b, were added. The solution was concentrated and chromatographed on Sephadex LH-20 by elution with methanol, when the profiles, above left, were obtained. The profiles on the right above were the outcome of similar experiments involving deoxyadenylic acid and the second largest peak of Figure 1b: (×) counts per minute; (○) optical density at 292 nm.

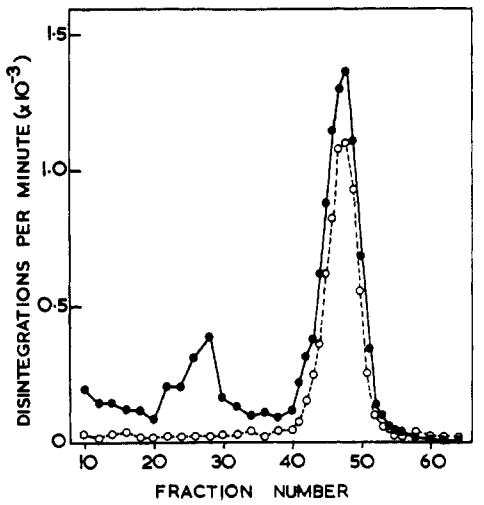


FIGURE 4: Elution profile from Sephadex LH-20 eluted with methanol of product of reaction of [8-$^3$H]deoxyguanosine 5′-phosphate with 7-bromo[$^{14}$C]methyl-12-methylbenz[*a*]anthracene after enzymatic dephosphorylation: (●) tritium disintegrations per minute; (○) carbon-14 disintegrations per minute.

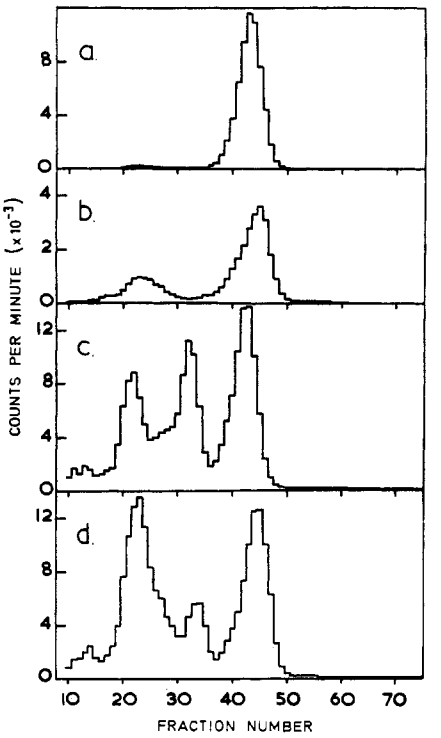


FIGURE 5: Elution profiles from columns of Sephadex LH-20 eluted with methanol of the major radioactive deoxynucleoside product of DNA reacted with [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene (the presumed $N^2$-(12-methylbenz[*a*]anthracenyl-7-methyl)deoxyguanosine) exposed to the following conditions: (a) kept in the dark under nitrogen; (b) kept under nitrogen and irradiated at a distance of 25 cm from a Hanovia fluorescence lamp, maximum emission at 366 nm, for 4.75 hr; (c) kept under oxygen and irradiated as for step b above; (d) kept under oxygen and irradiated at an average distance of 20 cm from a series of eight 20-W cool white fluorescent tubes for 4.75 hr.

For conversion to nucleosides, DNA (0.2–0.6 mg) was dissolved in water (0.5 ml), 0.05 ml of 0.01 M Tris buffer (pH 7), 0.01 M in magnesium chloride, plus 0.05 ml of deoxyribonuclease solution (1 mg/ml) were added, and the solution was incubated at 37° for 1 hr. This solution was then diluted with 0.5 ml of 0.1 M Tris buffer (pH 9), 0.002 M in sodium chloride, 0.002 M in magnesium chloride, and incubated at 37° for 40–48 hr with 0.08 unit of snake venom phosphodiesterase. Finally, 0.05 ml of a solution of bacterial alkaline phosphatase (1 mg/ml) was added and the solution was incubated for a further 24 hr at 37°.

During all the above procedures solutions were protected from light in the experiments summarized in Figure 8 and Table I. For the experiments summarized in Figure 1 no special precautions against exposure to light were taken. Extents of reaction with DNA were measured by counting aliquots and measuring uv absorption after digestion with deoxyribonuclease. The extinction coefficient for a molar concentration of phosphorus in a deoxyribonuclease digest of DNA was taken to be 8750 (Brookes and Lawley, 1964).

*Effect of Light on 7-Bromomethyl-12-methylbenz[a]anthracene and 7-Bromomethylbenz[a]anthracene Deoxynucleoside Products.* After reaction of the [$^3$H]bromo compounds with DNA, enzymatic degradation of the DNA to deoxynucleosides, and chromatographic separation of the radioactive deoxynucleoside products on Sephadex LH-20 eluted with methanol, the fractions comprising the deoxyguanosine and deoxyadenosine product peaks were separately pooled from reactions with either bromo compound. These methanolic solutions were evaporated to dryness and the residues were redissolved in water which had previously been boiled to remove dissolved gases. Aliquots of these solutions were then exposed to various conditions of illumination, etc., as indicated in the legends to Figures 5, 6, and 7. The solutions (usually 1 ml) were then diluted with methanol to a final volume of 5 ml and subjected to chromatography on Sephadex LH-20.

*Reaction of 7-Bromo[$^{14}$C]methyl-12-methylbenz[a]anthracene with [8-$^3$H]Deoxyguanosine 5′-Phosphate.* A solution of [8-$^3$H]deoxyguanosine 5′-phosphate (Radiochemical Centre, Amersham) at 1.95 Ci/mmol was added to unlabeled deoxyguanosine 5-phosphate (6.13 mg) such that the resulting specific activity of this nucleotide was 9.0 Ci/mol, and the solution was then made up to a total volume of 1 ml by the addition of 0.01 M sodium phosphate buffer (pH 7). This solution was then treated with 0.2 ml of a dry acetone solution of 7-bromo[$^{14}$C]methyl-12-methylbenz[*a*]anthracene, specific activity 5.6 Ci/mol (0.4 mg/ml). After 15 min the solution was extracted twice with benzene and twice with ether, and any residual ether was removed by bubbling air through the solution. To this solution were added 1 M Tris buffer (pH 9), 0.02 M in sodium chloride and 0.02 M in magnesium chloride (0.1 ml), and 0.1 ml of a solution of bacterial alkaline phosphatase (1 mg/ml). This solution was incubated at 37° overnight, diluted to 5 ml with methanol, and examined by chromatography on Sephadex LH-20. Radioactivity from the tritiated deoxyguanosine present and unextracted hydrolyzed bromo compound was eluted in fractions 20–40 and a doubly labeled radioactive peak in the region of the chromatogram where deoxyguanosine–bromo compound products usually elute (fractions 44–54) was also present. The fractions comprising this latter peak were pooled and subjected again to chromatography on Sephadex LH-20, Figure 4.

*Chromatography on Sephadex LH-20.* Nucleoside solutions were diluted with methanol and applied to a column of Sephadex LH-20 (75 × 1.5 cm diameter) in methanol, and the column was then eluted with methanol. Fractions of 200 drops were collected, all as prevously described (Dipple *et al.*, 1971).

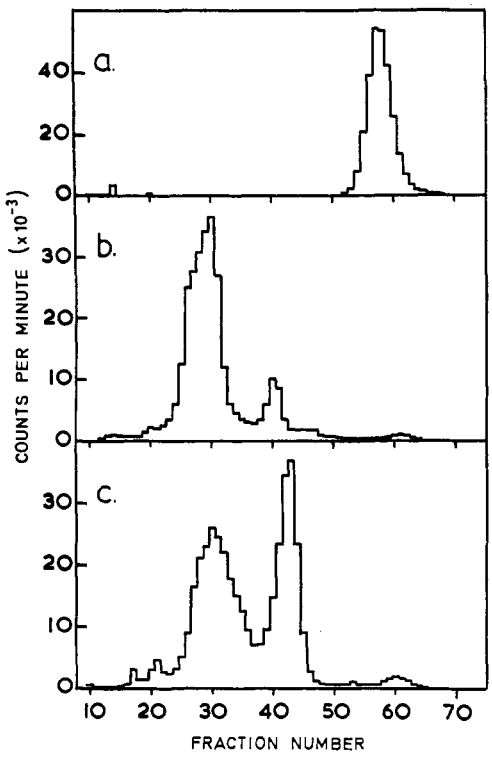


FIGURE 6: Elution profiles from columns of Sephadex LH-20 eluted with methanol of the second most abundant radioactive deoxynucleoside product from DNA reacted with [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene (*i.e.*, the presumed $N^6$-(12-methylbenz[*a*]-anthracenyl-7-methyl)deoxyadenosine) exposed to the following conditions: (a) kept in the dark under oxygen; (b) kept under nitrogen and irradiated at a distance of 25 cm from a Hanovia fluorescence lamp, maximum emission at 366 nm, for 4.75 hr; (c) kept under oxygen and irradiated as for step b above.

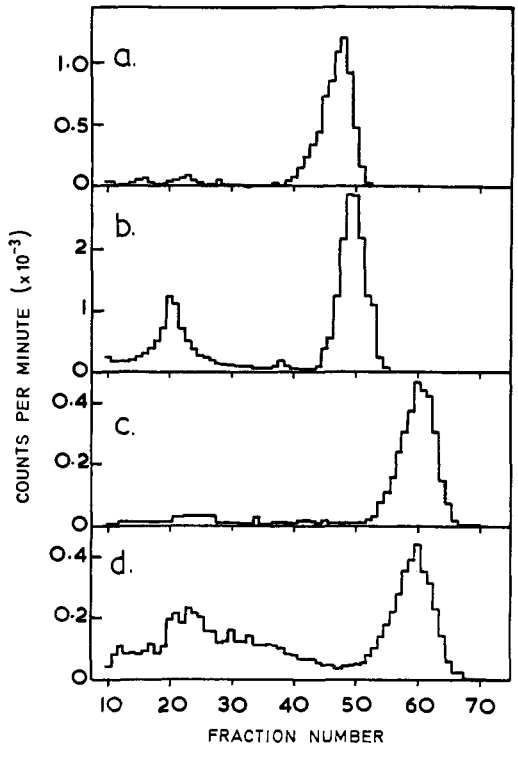


FIGURE 7: Elution profiles from columns of Sephadex LH-20 eluted with methanol of the major radioactive deoxynucleoside products from DNA reacted with [$^3$H]7-bromomethylbenz[*a*]anthracene. (a) [$^3$H]$N^2$-(benz[*a*]anthracenyl-7-methyl)deoxyguanosine kept in the dark; (b) [$^3$H]$N^2$-(benz[*a*]anthracenyl-7-methyl)deoxyguanosine irradiated under oxygen at a distance of 25 cm from a Hanovia fluorescence lamp, maximum emission at 366 nm, for 4.75 hr; (c) [$^3$H]$N^6$-(benz[*a*]anthracenyl-7-methyl)deoxyadenosine kept in the dark; (d) [$^3$H]$N^6$-(benz[*a*]anthracenyl-7-methyl)deoxyadenosine irradiated under oxygen as for step b above.

Aliquots (1 ml) of each fraction were counted in a Packard liquid scintillation counter using a phosphor containing toluene (6 l.), Triton X-100 (4 l.), 2,5-diphenyloxazole (70 g), and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (3.5 g).

## Results

In a previous study (Dipple *et al.*, 1971) the two major products of reaction of [$^3$H]7-bromomethylbenz[*a*]anthracene with DNA were characterized, after enzymic conversion of the DNA to nucleosides, as $N^2$-(benz[*a*]anthracenyl-7-methyl)-deoxyguanosine and $N^6$-(benz[*a*]anthracenyl-7-methyl)deoxy-adenosine. A third product was tentatively identified as $N^4$-(benz[*a*]anthracenyl-7-methyl)deoxycytidine. The profile of elution of these nucleoside products from Sephadex LH-20 is illustrated in Figure 1a. Two minor unidentified radioactive products were eluted first, followed by the deoxycytidine product, then $N^2$-(benz[*a*]anthracenyl-7-methyl)deoxyguanosine, and finally $N^6$-(benz[*a*]anthracenyl-7-methyl)-deoxyadenosine. Figure 1b illustrates an analogous elution profile for a nucleoside mixture from DNA reacted with [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene. The similarities in the positions of elution of the last three peaks of Figure 1a,b suggested that 7-bromomethyl-12-methylbenz-[*a*]anthracene probably attacks DNA at the same sites as does 7-bromomethylbenz[*a*]anthracene.

Studies of the reaction of 7-bromomethyl-12-methylbenz-[*a*]anthracene with homopolyribonucleotides supported this view since, after degradation to nucleosides, the major products from the reaction of this bromo compound with poly(C), poly(G), and poly(A) were eluted from the Sephadex LH-20 system (Figure 2) in similar positions to the three major DNA products of Figure 1b.

Further evidence for the structures of these 7-bromomethyl-12-methylbenz[*a*]anthracene reaction products was obtained from comparison of the two major radioactive products from DNA with deoxynucleoside products from the reactions of unlabeled bromo compound with deoxyguanosine 5′-phosphate and deoxyadenosine 5′-phosphate (Figure 3). The uv-absorbing products and the radioactive products were eluted in coincidence in both cases and after concentration of the fractions comprising these peaks, the radioactivity and uv absorption were found to be inseparable by thin-layer chromatography (Polygram sheets SIL N-HR/UV, Camlab) in the following solvents: acetone, ethyl acetate, methanol, and benzene–butan-1-ol (1:1). This established that the major radioactive peak of Figure 1b represented a deoxyguanosine product and that the second largest peak represented a deoxyadenosine product, and thereby further emphasized the similarities in the reactions of the two bromo compounds with DNA.

Since Pochon and Michelson (1971) and Pochon *et al.* (1971) have suggested that 7-bromomethylbenz[*a*]anthracene predominantly attacks the C-8 position of guanine residues in polynucleotides and DNA, the reaction of 7-bromo[$^{14}$C]-methyl-12-methylbenz[*a*]anthracene with [8-$^3$H]deoxyguanosine 5′-phosphate was examined. After reaction, enzymatic

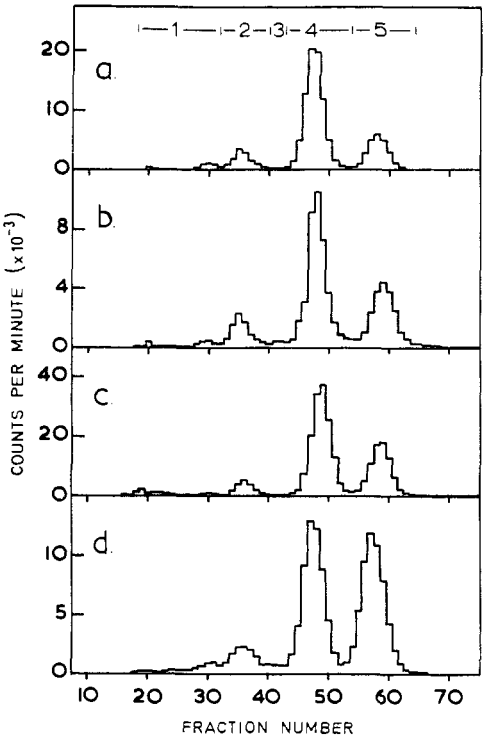


FIGURE 8: Column chromatograms on Sephadex LH-20 eluted with methanol of nucleoside products from native and heat-denatured DNA reacted with either [$^3$H]bromo compound as described under Experimental Section. (a) Native DNA reacted with [$^3$H]7-bromomethylbenz[*a*]anthracene; (b) heat-denatured DNA reacted with [$^3$H]7-bromomethylbenz[*a*]anthracene; (c) native DNA reacted with [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene; (d) heat-denatured DNA reacted with [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene. The percentage of total radioactivity found under each peak indicated in Figure 8a is listed in Table I for all DNA samples. At all stages of these experiments precautions were taken to protect solutions from exposure to light.

dephosphorylation, and chromatography on Sephadex LH-20, a peak, eluting in the same region of the chromatogram as the deoxyguanosine product from DNA was found which exhibited a $^3$H:$^{14}$C ratio of 1.3. Since on this chromatogram vast numbers of tritium counts were eluted in fractions 20–40 (attributable to unreacted deoxyguanosine) the fractions comprising the product peak were pooled and rerun on Sephadex LH-20 (Figure 4). The average $^3$H:$^{14}$C ratio through the peak was again 1.3. From the design of the experiment it was estimated that this ratio should be 1.6 if attack was in fact on the amino group and zero of course if attack was on C-8. The experimental result was sufficiently close to the predicted value for attack on the amino group to establish that attack did not occur on C-8 of guanine. It is reasonable, therefore, to proceed on the assumption that the 7-bromomethyl-12-methylbenz[*a*]anthracene products arise as a result of reaction of the bromo compound with the amino groups of the DNA bases, since this has been proven to be the case for 7-bromomethylbenz[*a*]anthracene (Dipple *et al.*, 1971).

The main difference in the reaction of the two bromo compounds with DNA is that a greater proportion of radioactivity is eluted in fractions 20–35 for 7-bromomethyl-12-methylbenz[*a*]anthracene (Figure 1b) than for 7-bromomethylbenz[*a*]anthracene (Figure 1a), and it was eventually shown that the majority of the radioactive products eluted in fractions 20–35 of Figure 1b had in fact arisen from the action of light on the major radioactive products. Thus, when the major radioactive product from the reaction of [$^3$H]7-bromomethyl-12-methylbenz[*a*]anthracene with DNA (the presumed $N^2$-(12-methylbenz[*a*]anthracenyl-7-methyl)-deoxyguanosine) was recovered from a chromatogram such as that illustrated in Figure 1b and kept in the dark under nitrogen, it was stable (Figure 5a). Irradiation at 366 nm even under nitrogen caused some decomposition (Figure 5b) and in the presence of oxygen even more decomposition was apparent (Figure 5c). Figure 5d illustrates that this light-induced decomposition occurs also on exposure of this product to light from fluorescent tubes used for laboratory lighting. Figure 6 illustrates the analogous decomposition of the deoxyadenosine product (the presumed $N^6$-(12-methylbenz-[*a*]anthracenyl-7-methyl)deoxyadenosine) on irradiation at 366 nm under nitrogen (Figure 6b) or in the presence of oxygen (Figure 6c). The 7-bromomethylbenz[*a*]anthracene products were less readily affected by light (Figure 7) although some decomposition was observed when either $N^2$-(benz[*a*]anthracenyl-7-methyl)deoxyguanosine or $N^6$-(benz[*a*]anthracenyl-7-methyl)deoxyadenosine was irradiated at 366 nm in the presence of oxygen, Figure 7b,d, respectively.

It was clear, therefore, that quantitative studies of the reactions of the bromo compounds with DNA were only meaningful if maximum precautions were taken to protect reaction solutions from exposure to light. Under such conditions, native and heat denatured DNA were separately treated with each bromo compound, and each DNA sample after enzymic degradation to nucleosides was analyzed for radioactive products by chromatography on Sephadex LH-20 (Figure 8 and Table I). Comparison of Figures 8a and 1a shows that normal laboratory lighting does not substantially affect the elution profiles found for the 7-bromomethylbenz-[*a*]anthracene products, while comparison of Figures 8c and 1b shows that even this ill-defined but presumably low level of irradiation can profoundly influence the experimental findings in the case of 7-bromomethyl-12-methylbenz[*a*]-anthracene products.

## Discussion

It has previously been shown that 7-bromomethyl-12-methylbenz[*a*]anthracene is more carcinogenic than 7-bromomethylbenz[*a*]anthracene and the object of the present study was to define any differences in the chemistry of reaction of these two bromo compounds with DNA which might ultimately be related to their different biological activities. The major products of reaction of 7-bromomethylbenz[*a*]anthracene with DNA had previously been identified and sufficient similarities between the products of reaction of the two bromo compounds with DNA were established to warrant the assumption that the 12-methyl derivative attacks the same sites in DNA as does 7-bromomethylbenz[*a*]anthracene, *i.e.*, the amino groups of the DNA bases.

One important difference between the products of reaction of the two bromo compounds with DNA was that the products of reaction of the 12-methyl derivative were quite sensitive to the effects of exposure to light. Although the 7-bromomethylbenz[*a*]anthracene products were also photosensitive the effect of light on these products was less pronounced. The higher photosensitivity of the 7-bromomethyl-12-methylbenz[*a*]anthracene–DNA products relative to that of the 7-bromomethylbenz[*a*]anthracene–DNA products can be compared to the greater ease of production of photooxides from 7,12-dimethylbenz[*a*]anthracene than from 7-methyl-

TABLE I: Reaction of [$^3$H]Bromomethylbenz[*a*]anthracenes with Native and Denatured DNA.[a]

| Compound | DNA | Extent of Reaction in mmol/mol of DNA-*p* | Percentage of Total Radioactivity Recovered as | | | | | dAdo:dGno Products | Av Recov of Radioactivity (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Peak 1 | Peak 2[b] | Peak 3 | Peak 4[c] | Peak 5[d] | | |
| 7-Bromomethylbenz[*a*]anthracene | Native | 6.1 | 6.8 ± 3.2 | 9.6 ± 1.0 | | 62.3 ± 2.5 | 20.6 ± 0.4 | 0.33 | 98.4 |
| | Denatured | 4.1 | 7.6 ± 3.0 | 10.4 ± 1.0 | 1.3 ± 0.1 | 52.8 ± 1.4 | 25.7 ± 1.3 | 0.49 | 95.5 |
| 7-Bromomethyl-12 methylbenz[*a*]anthracene | Native | 1.4 | 3.1 ± 0.7 | 6.6 ± 0.6 | | 60.0 ± 2.1 | 29.5 ± 0.6 | 0.49 | 103 |
| | Denatured | 1.0 | 7.9 ± 1.1 | 8.0 ± 1.1 | 1.8 ± 0.8 | 39.3 ± 0.9 | 41.8 ± 1.1 | 1.06 | 102 |

[a] Reactions were performed and DNA was degraded, assayed, and chromatographed as described under Experimental Section. The column headings are defined in Figure 8a. Each analysis was repeated three times and the average is presented in the table together with the standard deviation. Reaction solutions were protected from exposure to light. [b] Deoxycytidine product. [c] Deoxyguanosine product. [d] Deoxyadenosine product.

benz[*a*]anthracene (Cook and Martin, 1940). Although attempts were made to determine whether oxygen was necessary for the light-induced changes of the hydrocarbon–nucleoside products to occur, a definitive answer was not obtained (Figures 5 and 6). The changes observed differed in the presence of either nitrogen or oxygen but even the purest nitrogen available was not totally oxygen free. It is possible that the photosensitivity of 7-bromomethylbenz[*a*]anthracene is responsible for the formation of cross-links in DNA which has been treated with this compound (Pochon *et al.*, 1971; Michelson and Pochon, 1972; Venitt and Shooter, 1972).

Quantitative comparisons of the reaction products from DNA treated with either 7-bromomethyl-12-methylbenz[*a*]anthracene or 7-bromomethylbenz[*a*]anthracene revealed that the more carcinogenic 12-methyl derivative reacts less extensively with DNA than does the less carcinogenic compound. Also, both compounds react less extensively with denatured DNA than with native DNA and this decreased extent of reaction is almost wholly attributable to a decrease in the number of guanine residues attacked for both compounds. This finding shows that the native DNA structure promotes reaction with the amino groups of guanine residues which lie in the narrow groove of the DNA helix, while reactions on the amino group of adenine, for example, which lies in the wide groove, occur with equal ease in either native or denatured DNA. The ratio of adenine residues substituted: guanine residues substituted is greater for the more carcinogenic 12-methyl derivative than for 7-bromomethylbenz[*a*]anthracene but, as can be seen from Table I, the actual amount of each product per mole of DNA phosphorus is greater in every case for the less carcinogenic than for the more carcinogenic bromo compound. Thus, the only positive correlations between carcinogenic potency and reaction with DNA *in vitro* which emerge from these studies are (1) the products formed from the more carcinogenic bromo compound are more light sensitive than those from 7-bromomethylbenz[*a*]anthracene and (2) 7-bromomethyl-12-methylbenz[*a*]anthracene exhibits a higher specificity for attack on adenine residues in DNA than does the less carcinogenic 7-bromomethylbenz[*a*]anthracene. It would be premature to attempt to devise mechanisms for carcinogenesis based upon these correlations, or to discard DNA as a likely target for these chemical carcinogens on the basis of these findings. However, having increased our understanding of the reactions of these bromo compounds with DNA *in vitro*, it seems that it is now important to examine the interaction of these compounds with DNA *in vivo* in the situation where tumors arise.

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