Gafni, A., Modlin, R. L., & Brand, L. (1975) *Biophys. J. 15*, 263–280.
Gafni, A., Modlin, R. L., & Brand, L. (1976) *J. Phys. Chem. 80*, 898–904.
Grinvald, A., & Steinberg, I. Z. (1974) *Anal. Biochem. 59*, 583–598.
Grinvald, A., & Steinberg, I. Z. (1976) *Biochim. Biophys. Acta 427*, 663–678.
Inokuti, M., & Hirayama, F. (1965) *J. Chem. Phys. 43*, 1978–1989.
Konev, S. V. (1967) *Fluorescence and Phosphorescence of Proteins and Nucleic Acids*, Plenum Press, New York.
Lakowicz, J. R., & Cherek, H. (1980) *J. Biol. Chem. 255*, 831–834.
Laws, W. R., & Shore, J. (1978) *J. Biol. Chem. 253*, 8593–8597.
Laws, W. R., & Brand, L. (1979) *J. Phys. Chem. 83*, 795–802.
Lehrer, S. S. (1971) *Biochemistry 10*, 3254–3263.
McGlynn, S. P., Azumi, T., & Kinoshita (1969) *Molecular Spectroscopy of the Triplet State*, Prentice-Hall, Englewood Cliffs, NJ.
Munro, I., Pecht, I., & Stryer, L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 56–60.
Paoletti, J., & Le Pecq, J.-B. (1969) *Anal. Biochem. 31*, 33–41.
Perrin, F. (1934) *J. Phys. Radium 5*, 497–511.
Perrin, F. (1936) *J. Phys. Radium 7*, 1–11.
Privat, J.-P., Wahl, P., & Auchet, J.-C. (1980) *Biophys. Chem. 11*, 239–248.
Purkey, R. M., & Galley, W. C. (1970) *Biochemistry 9*, 3569–3575.
Rigler, R., & Ehrenberg, M. (1973) *Q. Rev. Biophys. 6*, 139–199.
Ross, J. B. A., Rousslang, K. W., & Brand, L. (1981) *Biochemistry* (preceding paper in this issue).
Rousslang, K. W., Ross, J. B. A., Deranleau, D. A., & Kwiram, A. L. (1978) *Biochemistry 17*, 1087–1092.
Rousslang, K. W., Thomasson, J. M., Ross, J. B. A., & Kwiram, A. L. (1979) *Biochemistry 18*, 2296–2300.
Schiller, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 975–979.
Small, E. W., & Isenberg, I. (1977) *Biopolymers, 16*, 1907–1928.
Subramanian, S., Ross, J. B. A., Ross, P. D., & Brand, L. (1981) *Biochemistry 20*, 4086–4093.
Sund, H., & Theorell, H. (1963) *Enzymes, 2nd Ed. 7*, 25–67.
Tao, T. (1969) *Biopolymers 8*, 609–632.
Theorell, H., & Yonetani, T. (1963) *Biochem. Z. 338*, 537–553.
Torikata, T., Forster, L. S., O'Neal, C. C., & Rupley, J. A. (1979) *Biochemistry 18*, 385–390.
Vallee, B. L., & Hoch (1957) *J. Biol. Chem. 225*, 185–189.
Wahl, P. (1969) *Biochim. Biophys. Acta* 175, 55–64.

Identification of Novel 7,12-Dimethylbenz[*a*]anthracene Adducts in Cellular Ribonucleic Acid†

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ABSTRACT: The interaction of guanosine with 7,12-dimethylbenz[*a*]anthracene (DMBA) 5,6-oxide under alkaline conditions resulted in the formation of six derivatives. These six compounds were cochromatographed with nucleosides obtained by hydrolysis of RNA isolated from rat liver cells treated with [^{3}H]DMBA. The cochromatography showed that three of these adducts were formed in cellular RNA. The three products constituted less than 5% of the total nucleoside–DMBA adducts as shown by chromatography on Sephadex LH-20 and high-pressure liquid chromatography. In one of them, the 2′-hydroxy group of the ribose moiety of guanosine was linked to the C-5, and in the second, to the C-6 position of the DMBA 5,6-oxide residue. In the third derivative, the C-8 position of guanosine was linked to the C-5 of the DMBA 5,6-oxide moiety. These results show, for the first time, modifications of the ribose moiety and of the guanine residue at the C-8 position in the cellular RNA by a metabolite of a polycyclic hydrocarbon.

7,12-Dimethylbenz[*a*]anthracene (DMBA),[1] one of the most potent carcinogenic polycyclic aromatic hydrocarbons (Brookes & Lawley, 1964; Slaga et al., 1974), is metabolized to K-region and non-K-region dihydro diols and oxides before it reacts with nucleic acids (Sims, 1967; Keysell et al., 1973; Dipple & Nebzydoski, 1978; Tierney et al., 1978; Chou & Yang, 1978; McNicoll et al., 1980). Studies using mouse skin and embryo cells (Moschel et al., 1977; Bigger et al., 1978), hamster embryo cells (Ivanovic et al., 1978), or mouse fibroblasts (Marquardt et al., 1976) indicate that generation of the bay region diol epoxide (the C-1 to C-4 positions of DMBA) is required for formation of the majority of adducts with DNA. However, Cooper et al. (1980) have shown that in addition to the bay region diol epoxides there are other DMBA intermediates which bind to the DNA of mouse skin. Similarly, recent experiments with fluorinated derivatives of DMBA

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[1] Abbreviations used: DMBA, 7,12-dimethylbenz[*a*]anthracene; DMBA oxide, DMBA K-region oxide, 7,12-dimethylbenz[*a*]anthracene 5,6-oxide; $NaDodSO_4$, sodium dodecyl sulfate; HPLC, high-pressure liquid chromatography.

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suggested that both they bay and K regions are involved in the activation of DMBA to mutagenic and carcinogenic metabolites (Huberman & Slaga, 1979). Moreover, the K-region oxide of DMBA was shown to have the capacity to transform mouse fibroblasts in vitro (Marquardt et al., 1974) and to initiate tumors in mice (Chouroulinkov et al., 1979).

In this study, we wanted to determine whether derivatives of DMBA K-region oxide are formed in cellular RNA. We report the synthesis of six adducts formed by the interaction of guanosine with DMBA 5,6-oxide under alkaline conditions and the identification of three of them in RNA isolated from rat liver cells which were exposed to DMBA in culture.

Experimental Procedures

Materials

Chemicals. (±)-DMBA 5,6-oxide was obtained from Dr. P. Harvey, Ben May Laboratories, University of Chicago, and [^{3}H]DMBA (specific activity 5 Ci/mmol) from Amersham/Searle Co. Other chemicals and materials were obtained from the following sources: all enzymes were from Sigma, Sephadex LH-20 was from Pharmacia Fine Chemicals, cellulose TLC plates were from Eastman Kodak, silanized silica gel reverse-phase TLC plates (RP-2F) were from EM Laboratories (both types of plates contained a fluorescent indicator), phenol and 8-hydroxyquinoline were from Fisher Scientific Co., F-12K medium was purchased from Grand Island Biological Co., fetal bovine serum was from Microbiological Associates (M.A. Bio Products), and sodium dodecyl sulfate was from BDH Gallard-Schlesinger.

Methods

Synthesis and Purification of Guanosine–DMBA 5,6-Oxide Adducts. Guanosine (0.18 mmol) dissolved in equal volumes of 0.03 M NaOH (8 mL) and acetone (8 mL) was treated with the same volume of DMBA 5,6-oxide (0.32 mmol) dissolved in acetone (8 mL). The reaction mixture was incubated at 37 °C for 4 days in the dark with constant shaking. Acetone was evaporated under N_2, and the DMBA 5,6-oxide decomposition products were removed by extensive ether extraction. The ether extract was concentrated and reextracted once with 0.01 M NaOH.

The alkaline wash was combined with the main water extract and after concentration applied to a Sephadex LH-20 column (90 × 1.5 cm). The column was at first eluted with 600 mL of 0.02 M NH_4HCO_3 buffer, then with 350 mL of 40% methanol in buffer, and finally with a linear gradient of 40–90% methanol in buffer. Fractions of 3.1 mL were collected in LKB Ultrarack 7000 fraction collector and monitored at 254 nm. Absorbance and UV spectra were determined on the Gilford 240 spectrophotometer equipped with a 6040A recorder. Fractions containing DMBA moieties were combined, concentrated, and separated by HPLC (Du Pont 830) on the reverse-phase Zorbax ODS preparative column (0.25 m × 6.2 mm) which was operating at 45 °C, 2500 psi, and 30% methanol as an elution solvent.

Binding of [^{3}H]DMBA to Nucleic Acids of Rat Liver Cells in Culture. K-22 epithelial rat liver cells (passage 11-14) were grown in the F-12K medium supplemented with fetal bovine serum (10% v/v) in large T-flasks at 37 °C under 5% CO_2 in the air. Confluent cells were incubated at 37 °C for 48 h in 20 mL of fresh media containing 8 μg of [^{3}H]DMBA, and 0.5% EtOH (v/v). All procedures involving exposure of cells to DMBA were performed in the absence of or in subdued light. For determination of the extent of metabolism of [^{3}H]DMBA by K-22 cells, samples of media (1 mL) were withdrawn after 24 and 48 h of incubation and extensively extracted with ethyl acetate. Radioactivity was measured in Aquasol (New England Nuclear) with a Mark II Nuclear Chicago scintillation counter.

After the 48-h incubation, the medium was removed from the T-flasks, the cells were washed twice with phosphate-buffered saline and lysed with 1% $NaDodSO_4$–citrate saline buffer (0.015 M NaCl and 0.0015 M sodium citrate, pH 6.9). Lysate was extracted 5 times with water-saturated redistilled phenol–chloroform (1:1) containing 0.1% 8-hydroxyquinoline. The water phase was extracted 5 times with equal volumes of ether. Traces of ether were removed by bubbling N_2 through the water layer. The nucleic acids were precipitated by addition of sodium acetate (pH 5) to a 0.2 M final concentration and two volumes of cold ethanol. After 17 h at −20 °C, the precipitate was pelleted, resuspended in water, and reprecipitated.

Specific activities of DNA and RNA were determined by cesium sulfate gradient centrifugation. The nucleic acids (4 A_{260} units) were dissolved in 5 mL of H_2O, mixed with 5 mL of saturated cesium sulfate containing 0.001 M EDTA, and centrifuged in cellulose nitrate tubes in the Ti50 rotor in a Beckman L5-50 centrifuge (48 K, 25 °C, 60 h). The samples were fractionated (15 drops/fraction) with an Auto-Densi-Flow (Buchler Instruments). The refractive index was determined in a Carl Zeiss refractometer using distilled water as a standard. UV absorbance at 260 nm, radioactivity, and volume were also measured.

Characterization of RNA–DMBA Adducts. DNA and RNA isolated from the cells which were treated with [^{3}H]-DMBA were dissolved in 0.02 M Tris-HCl, 0.015 M $MgCl_2$ buffer, pH 7.5, digested with RNase A (2 μg/A_{260}) at 37 °C for 3 h, followed by digestion with a mixture of snake venom and spleen phosphodiesterases and alkaline phosphatase (1 unit/A_{260} each) at 37 °C for 21 h. The synthesized UV markers were added to the enzymatic hydrolyzate and chromatographed on an LH-20 column. All fractions were scanned for radioactivity and for absorbance at 260 and 310 nm. Radioactive peaks coinciding with peaks of absorbance at both the 260 and 210 nm were concentrated and analyzed by HPLC as described above.

Results

Preparation and Characterization of Guanosine–DMBA 5,6-Oxide Markers. Modification of guanosine with DMBA 5,6-oxide under alkaline conditions resulted in the formation of guanosine–DMBA oxide derivatives. Chromatography on a Sephadex LH-20 (Figure 1) using ammonium bicarbonate as the eluent showed separation of unreacted guanosine from modified guanosine derivatives. The adducts eluting in fractions 100–170 were partially separated into three peaks. Additional minor adducts were eluted with 40% methanol. Overall, about 15% of the guanosine reacted with DMBA 5,6-oxide under these conditions.

When fractions 100–170 were combined and chromatographed on HPLC, five major G–DMBA oxide adducts were isolated (Figure 2A). Since component **1** did not show any circular dichroism (CD), suggesting that it was a mixture of two isomers, we rechromatographed it on HPLC, lowering the temperature from 45 °C to ambient. This time, component **1** separated into two compounds, **1a** and **1b** (Figure 2B). Six purified guanosine–DMBA oxide adducts were designated as **1a**, **1b**, **2**, **3**, **4**, and **5** following the initial order of elution from the HPLC.

The UV spectra (not shown) of the six adducts indicated that there were at least three types of derivatives among them, and the CD confirmed that **1a** differed from **1b** as well as from

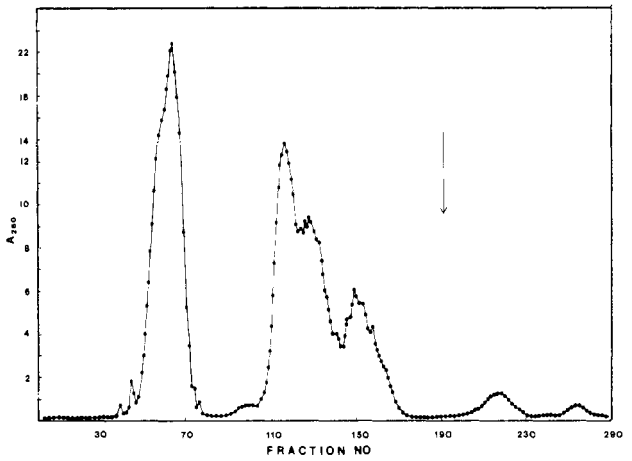

FIGURE 1: Sephadex LH-20 chromatography of the products of the reaction between guanosine and DMBA 5,6-oxide. A column (90 × 1.5 cm) was eluted with 0.02 M NH_4HCO_3 at the rate of 3.1 mL (3 min)$^{-1}$ fraction^{-1}. The arrow shows the start of elution with 40% methanol in 0.02 M NH_4HCO_3 followed by a linear gradient of 40–90% methanol in 0.02 M NH_4HCO_3. Fractions were monitored at 260 nm.

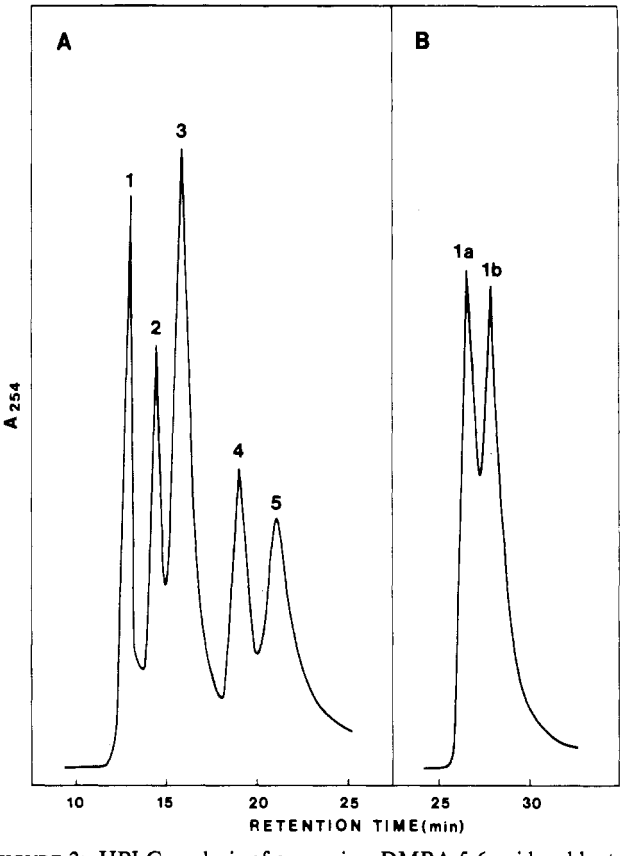

FIGURE 2: HPLC analysis of guanosine–DMBA 5,6-oxide adducts. (A) Separation of adducts was performed on a Zorbax ODS preparative column at 45 °C, 2500 psi, with 30% methanol. (B) Separation of component **1** into **1a** and **1b** at room temperature, 2500 psi, 30% methanol. Fractions were monitored at 254 nm.

2 through **5**. Excitation spectra resembled those of the UV spectra. Emission (fluorescence) spectra (not shown) were all similar, with only small differences with regard to the relative intensities of the peaks, thus suggesting the same chromophore distribution in the DMBA oxide moiety. The complete structural determination of these six compounds has been published (Kasai et al., 1977; Nakanishi et al., 1980). It was accomplished by utilization of UV, CD, NMR, and mass spectra as well as Fourier-transform IR spectra and determination of pK's by monitoring changes in the CD spectra

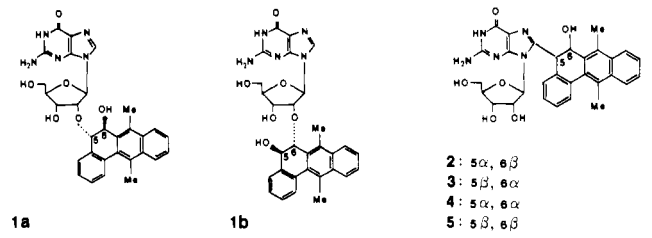

FIGURE 3: Structures of guanosine–DMBA 5,6-oxide adducts.

under acidic and alkaline conditions (Kasai et al., 1978).

As can be seen in Figure 3, DMBA oxide is attached to the 2′-hydroxy group of the ribose moiety in adducts **1a** and **1b**. The absolute configurations of **1a** and **1b** were determined to be, respectively, (5*S*)-guanosyl-(6*R*)-hydroxy and (6*R*)-guanosyl-(5*S*)-hydroxy adducts. They resulted from an attack of the 2′-OH of the ribose at the C-5 in **1a** and at the C-6 position of the DMBA oxide in **1b**, with concomitant trans opening of the oxide (Kasai et al., 1977). Although (±)-DMBA 5,6-oxide was used for modification of guanosine, diastereoisomers of **1a** and **1b** were not detected. The two major (**2** and **3**) and the two minor (**4** and **5**) adducts (Figure 3) constitute two pairs of diastereoisomers. They were formed through the trans and cis opening of DMBA oxide, respectively, by an attack of the C-8 of guanosine at the C-5 of the DMBA 5,6-oxide residue (Nakanishi et al., 1980). The absolute configurations of these compounds are shown in Figure 3.

The formation of the adducts through the guanosyl 2′-OH or the C-8 binding was promoted by the acidity of the 2′-OH (Martin et al., 1968) and by the fact that at pH 9.5 (pK_2 of guanosine is 9.5), the N-1 is 50% anionic.

Binding of [^{3}H]DMBA to RNA of Rat Liver Cells. To establish whether these six G-DMBA 5,6-oxide adducts are also formed in cells grown in the presence of DMBA, we used K-22 epithelial rat liver cells since we found they bind DMBA relatively well. The cells were incubated with tritiated DMBA (0.4 μg/mL medium) for 48 h in the dark. This concentration of DMBA at 48-h incubation time was optimal for maximum binding of this hydrocarbon to the nucleic acids. There was an almost linear increase in the rate of metabolism of DMBA by the cells. There were 16% of water-soluble metabolites formed during the first 24 h and 34% during the 48-h incubation. This concentration of DMBA was relatively nontoxic, and only about 10% dead, floating cells were present after 48 h. The yield of nucleic acids isolated after 48 h from DMBA-treated cells was only about 10% lower than that obtained from control cultures. Cells were lysed with 1% $NaDodSO_4$, and the total nucleic acids were isolated as described under Methods. Separation of DNA and RNA was achieved by cesium sulfate density gradients. Figure 4 shows that DNA and RNA were present in almost equal amounts (1.3 RNA:1 DNA), but the specific activity of the DNA was about five times higher than that of the RNA. There was one DMBA moiety bound per 7×10^4 nucleotides in DNA and one DMBA per 3.5×10^5 nucleotides in RNA. Similar results were obtained when DNA and RNA were separated prior to phenol–chloroform extraction by using NP_{40} for separation of the cytoplasm from the nuclei.

For hydrolysis of the RNA–[^{3}H]DMBA complex into modified ribonucleosides, the mixture was enzymatically treated as described under Methods. Products from the reaction of guanosine with DMBA 5,6-oxide at alkaline pH were added to the hydrolysate and cochromatographed on a Sephadex LH-20 column. Figure 5 shows a profile of such cochromatography. The enzymes and the unhydrolyzed ma-

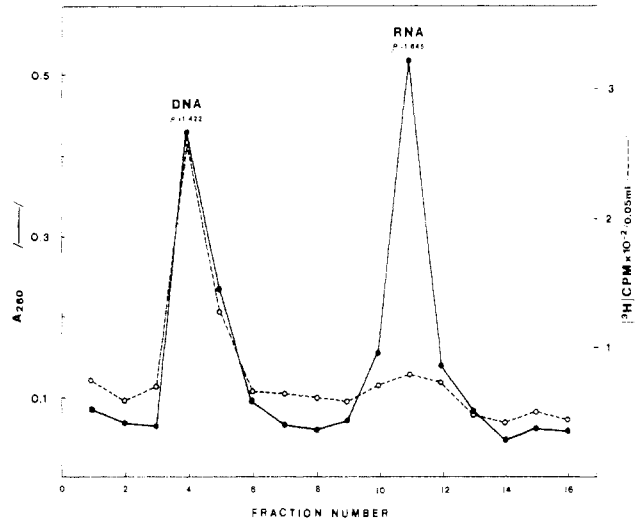

FIGURE 4: Profile of cesium sulfate gradient of DNA and RNA from rat liver cells incubated with [^{3}H]DMBA for 48 h. Fractions were scanned for radioactivity (---) and absorbance at 260 nm (—).

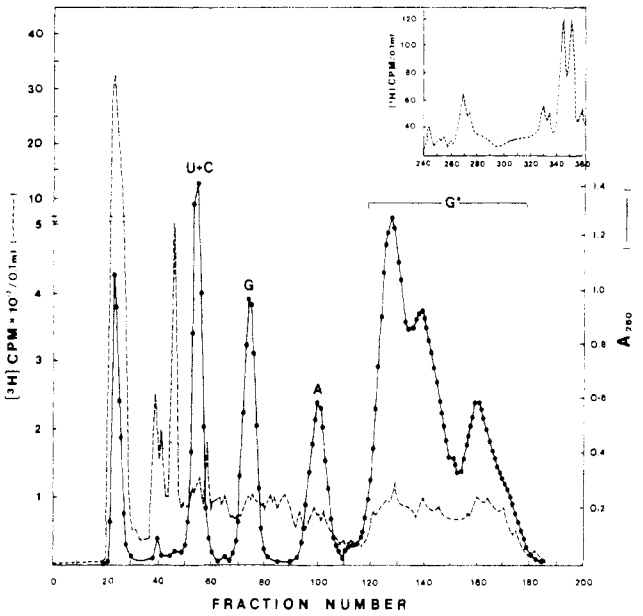

FIGURE 5: A Sephadex LH-20 cochromatography of an enzymatic digest of RNA from [^{3}H]DMBA-treated rat liver cells in culture and the G-DMBA 5,6-oxide markers **1a**, **1b**, and **2–5** (G*). The column was eluted with 0.02 M NH_4HCO_3 followed by 30% methanol in 0.02 M NH_4HCO_3 (not shown) and by a linear gradient of 30–90% methanol in 0.02 M NH_4HCO_3 (inset). Fractions were scanned for radioactivity (---) and absorbance at 260 nm (—).

terial eluted in the void volume. By using bicarbonate as the initial eluent, almost 40% of the total radioactivity was removed from the column. The modified oligonucleotides eluted first, followed by relatively well-separated nucleosides and finally by the modified nucleosides. This last region (fractions 120–180) also contained the markers **1a**, **1b**, and **2–5** and about 10% of the total radioactivity. When the eluent was changed to 30% methanol in buffer, followed by a linear gradient of 30–90% methanol in buffer, additional radioactive peaks appeared (see Figure 5, inset). Fractions between 240 and 280 contained 15% and fractions 320–360 over 25% of the radioactivity.

Fractions 120–180 (Figure 5) which contained the markers were combined, concentrated, and separated by HPLC. Figure 6 shows that among the radioactive peaks there were three which eluted at the same retention time as the UV markers: **1a**, **1b**, and **2**. Similar results were obtained when the HPLC

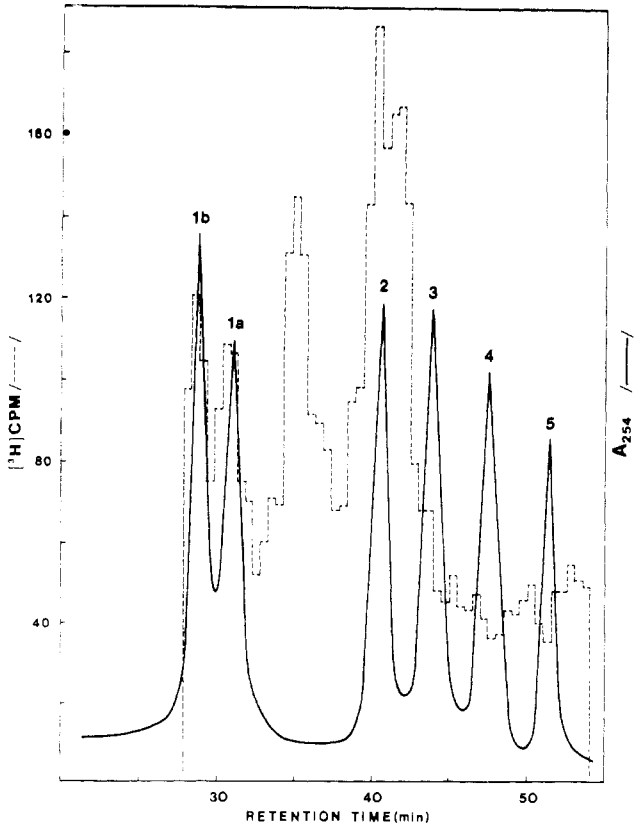

FIGURE 6: HPLC analysis of concentrated fractions 120–180 (see Figure 5) on a Zorbax ODS column. Fractions were collected in scintillation vials and radioactivity (---), and absorbance at 254 nm (—) was measured.

was performed under various temperature, pressure, and elution conditions and also by TLC on cellulose or reverse-phase (RP-2F) silanized silica gel plates (not shown).

Thus, from these results, we conclude that the three minor adducts formed in the RNA by DMBA treatment of rat liver cells in culture correspond to the three guanosine–DMBA 5,6-oxide adducts **1a**, **1b**, and **2** synthesized under alkaline conditions.

Discussion

Although some of the recent studies show that the major DMBA–DNA adducts formed in different cells treated in vivo with DMBA arise from 3,4-dihydro-3,4-dihydroxy-7,12-DMBA 1,2-oxide (Moschel et al. 1977; Ivanovic et al. 1978; Cooper et al., 1980), other minor products were also detected which have not yet been identified (Cooper et al., 1980). One of the possible metabolites involved in the interaction with nucleic acid residues could be DMBA 5,6-oxide which is formed in hepatic microsomes (Keysell et al. 1973). Jeffrey et al. (1976a,b) defined the structures of four guanosine adducts formed by the reaction of DMBA 5,6-oxide with poly(guanylic acid) at neutral conditions, but none of these adducts was detected in mouse skin or hamster embryo cells treated with DMBA in culture (Jeffrey et al., 1979).

In the present study, we prepared six additional adducts of DMBA 5,6-oxide with guanosine, and three of these were detected in RNA hydrolysate of liver cells treated with DMBA in culture. Two of these products provide the first evidence that a metabolite of a polycyclic hydrocarbon is capable of reaction with the 2′ oxygen of the ribose moiety in cellular RNA. The third derivative having an oxide moiety attached to the C-8 position of guanine is similar to that formed by an interaction of 2-(acetylamino)fluorene (Kriek et al. 1967), *N*-hydroxy-2-naphthylamine (Kadlubar et al., 1980), or *N*-

methyl-4-aminoazobenzene (Lin et al., 1975; Beland et al., 1980) and guanine residues in nucleic acids. These three products constituted less than 5% of the total nucleoside–DMBA adducts formed in the RNA of the cell culture. We did not attempt to characterize the rest of the RNA adducts, but from the chromatographic behavior on the Sephadex-LH-20, some of them could be derived from the "bay-region" DMBA metabolites.

Althogh the two ribose adducts could not be formed in DNA, the third DMBA product bound to the C-8 of guanosine could possibly arise in DNA also, similarly to modification by other aromatic carcinogens (Kriek et al., 1967; Lin et al., 1975; Kadlubar et al., 1980). Since it is difficult to assess the significance of the minor adducts in nucleic acids, further studies are required to obtain more information on the possible role of the major as well as the minor nucleic acid–carcinogen adducts in the initiation stage of carcinogenesis.

References

Beland, F. A., Tullis, D. L., Kadlubar, F. F., Straub, K. M., & Evans, F. E. (1980) *Chem.-Biol. Interact. 31*, 1–17.

Bigger, A., Tomaszewski, J. E., & Dipple, A. (1978) *Biochem. Biophys. Res. Commun. 80*, 229–235.

Brookes, P., & Lawley, P. D. (1964) *Nature (London) 202*, 781–784.

Chou, M. W., & Yang, S. K. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5466–5470.

Chouroulinkov, I., Gentil, A., Tierney, B., Grover, P. L., & Sims, P. (1979) *Int. J. Cancer 24*, 455–460.

Cooper, C. S., Ribeiro, O., Hewer, A., Walsh, C., Grover, P. L., & Sims, P. (1980) *Chem.-Biol. Interact. 29*, 357–367.

Dipple, A., & Nebzydoski, J. A. (1978) *Chem.-Biol. Interact. 20*, 17–26.

Huberman, E., & Slaga, T. J. (1979) *Cancer Res. 39*, 411–414.

Ivanovic, V., Geacintov, N. E., Jeffrey, A. M., Fu, P. P., Harvey, R. G., & Weinstein, I. B. (1978) *Cancer Lett. 4*, 131–140.

Jeffrey, A. M., Blobstein, S. H., Weinstein, I. B., Beland, F. A., Harvey, R. G., Kasai, H., & Nakanishi, K. (1976a) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2311–2315.

Jeffrey, A. M., Blobstein, S. H., Weinstein, I. B., & Harvey, R. G. (1976b) *Anal. Biochem. 73*, 378–385.

Jeffrey, A. M., Weinstein, I. B., & Harvey, R. G. (1979) *Proc. Am. Assoc. Cancer Res. 20*, 131.

Kadlubar, F. F., Unruh, L. E., Beland, F. A., Straub, K. M., & Evans, F. E. (1980) *Carcinogenesis 1*, 139–149.

Kasai, H., Nakanishi, K., Frenkel, K., & Grunberger, D. (1977) *J. Am. Chem. Soc. 99*, 8500–8502.

Kasai, H., Nakanishi, K., & Traiman, S. (1978) *J. Chem. Soc., Chem. Commun.*, 798–799.

Keysell, G. R., Booth, J., Grover, P. L., Hewer, A., & Sims, P. (1973) *Biochem. Pharmacol. 22*, 2853–2867.

Kriek, E., Miller, J. A., Juhl, U., & Miller, E. C. (1967) *Biochemistry 6*, 177–182.

Lin, J. K., Schmall, B., Sharpe, I. D., Miura, I., Miller, J. A., & Miller, E. C. (1975) *Cancer Res. 35*, 832–843.

McNicoll, A. D., Grover, P. L., & Sims, P. (1980) *Chem.-Biol. Interact. 29*, 169–188.

Marquardt, H., Sodergren, J. E., Sims, P., & Grover, P. L. (1974) *Int. J. Cancer 13*, 304–310.

Marquardt, H., Grover, P. L., & Sims, P. (1976) *Cancer Res. 36*, 2059–2064.

Martin, D. M. G., Reese, C. B., & Stephenson, G. F. (1968) *Biochemistry 7*, 1406–1412.

Moschel, R. C., Baird, W. M., & Dipple, A. (1977) *Biochem. Biophys. Res. Commun. 76*, 1092–1098.

Nakanishi, K., Komura, H., Miura, I., Kasai, H., Frenkel, K. & Grunberger, D. (1980) *J. Chem. Soc., Chem. Commun.* 82–83.

Sims, P. (1967) *Biochem. J. 105*, 591–598.

Slaga, T. J., Bowden, G. T., Schribner, J. D., & Boutwell, R. K. (1974) *J. Natl. Cancer Inst. 53*, 1337–1340.

Tierney, B., Hewer, A., McNicoll, A. D., Gervasi, P. G., Rattle, H., Walsh, C., Grover, P. L., & Sims, P. (1978) *Chem.-Biol. Interact. 23*, 243–257.