determined utilizing a Perkin-Elmer 257 spectrophotometer. Optical rotations were taken on a Perkin-Elmer Model 241 digital polarimeter. Elemental analyses were performed by Clark Microanalytical Labs, Urbana, Ill.
(7) E. A. Swinyard, W. C. Brown, and L. S. Goodman, J. Pharmacol. Exp. Ther., 106, 319 (1952).
(8) J. T. Litchfield and F. Wilcoxon, J. Pharmacol. Exp. Ther., 96, 99 (1949).
(9) M. C. Gerald and W. H. Riffee, Eur. J. Pharmacol., 21, 323 (1973).

Carcinogenicity of Derivatives of Polynuclear Compounds

Richard M. Peck,* Tie Keng Tan, and Evelyn B. Peck

The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received April 26, 1976*

In contrast to the enhanced carcinogenic activity of alkylating derivatives of polynuclear hydrocarbons and aminoacridines, a number of other derivatives, including conjugates with amino acids and peptides, showed little significant activity at comparable doses.

There is substantial evidence, reviewed by Miller and Miller,[1] that ultimate carcinogens are electrophiles. Accordingly, a "carcinogen" such as a polynuclear aromatic hydrocarbon which has no such chemical property must undergo metabolism to a reactive form. Candidate reactive forms include (1) K region epoxides[2] and (2) the carbonium ion derived from mesomethyl hydrocarbons.[3] Flesher and Sydnor's evidence is consistent with the latter possibility,[4] as is our recent finding that halomethyl derivatives of aromatic hydrocarbons are more carcinogenic than the parent hydrocarbons.[5] Alkylating derivatives of acridines, benzacridines, and their analogues are also potent carcinogens.[5]

The nucleophilic target of these alkylating carcinogens is of primary interest. The broad correlation of their carcinogenic potency with mutagenic potency, as shown by a number of investigators, most recently by McCann, Choi, Yamasaki, and Ames,[6] implicates DNA as the ultimate locus. In order to investigate whether or not the reaction could be through an intermediate conjugate with normal nucleophilic body constituents, we prepared a number of products that might be produced endogenously, as well as other conjugates designed to enhance hydrophilic character, and tested their carcinogenic activity by the same convenient mouse pulmonary adenoma assay used previously for the corresponding alkylating agents.[5]

Biological Assay. Carcinogens of essentially every known type are capable of eliciting pulmonary tumors in Strain A mice, in a convenient and relatively rapid assay.[7] Using a single low (15 μmol/kg or less) iv dose we found high activity in a number of alkylating agents that are also active both as antitumor agents[8,9] and as mutagens,[6] including agents that had been previously classified as noncarcinogenic on the basis of ip administration in the same system.[10] Small numbers of mice proved ample for detection of carcinogenicity with these potent compounds.[5]

Table I shows ten compounds bearing polynuclear groups which, when associated with an electrophilic function, are highly carcinogenic.[5] The induction period was extended somewhat over the usual 20 weeks to detect any minimal activity.

Results and Discussion

Only compound 2, the *S*-arylmethylhomocysteine derivative, showed any significant carcinogenic activity. This structure, it should be noted, is an analogue of methionine, a biological methylating agent. In separate tests, compounds 1–3, 8, and 10 gave negative results in Ames' mutagenesis test.[11]

Negative data do *not* establish absence of carcinogenic potential, only that this potential is not comparable with that of the highly electrophilic parent compounds. Therefore, the actual formation of a covalent bond by the reaction of these compounds or their related carbonium ion seems necessary to explain the high level of activity of these compounds as carcinogens,[5] mutagens,[6] and antitumor compounds.[8,9] That is, two moieties are necessary for these three related activities—a highly structure-specific polynuclear group and an electrophilic function.

Experimental Section

Melting points were taken in open capillary tubes in a Hershberg apparatus using total immersion thermometers and are reported as uncorrected values. Elemental analyses were carried out by Atlantic Microlab, Inc., of Atlanta, Ga., and unless otherwise noted were within ±0.4% of theory.

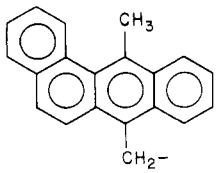
***S*-(12-Methyl-7-benz[*a*]anthrylmethyl)cysteine (1).** To a stirred solution of 2.5 g of L-cysteine hydrochloride in 90 ml of EtOH were added (1) 150 ml of C_6H_6; (2) 4.8 g of 7-iodomethyl-12-methylbenz[*a*]anthracene;[12] and about 0.5 min later, 28 ml of 1 N NaOH–MeOH. After 25 min of stirring, the bright orange color had changed to light yellow, and the voluminous precipitate was filtered, washed with EtOH and hexane, and dried. The crude product (5 g) was dissolved in 250 ml of EtOH and 16 ml of 1 N NaOH, filtered from undissolved residue, and precipitated with 16 ml of 1 N HCl. The precipitation from alcoholic alkali was repeated to give 3.25 g (69%) of product, mp 219–221 °C dec. Anal. ($C_{23}H_{21}NO_2S$) C, H, N, S.

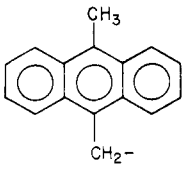
***S*-(12-Methyl-7-benz[*a*]anthrylmethyl)homocysteine (2).** To a solution, stirred in an ice bath, of 3.0 g of DL-homocysteine thiolactone hydrochloride, 65 ml of 1 N NaOH–MeOH, and 100 ml each of EtOH and C_6H_6 was added portionwise 4.9 g of 7-iodomethyl-12-methylbenz[*a*]anthracene.[12] After stirring 20 min, 40 ml of 1 N AcOH was added, and the nearly clear solution diluted to 400 ml. The aqueous–alcoholic layer was removed, and the C_6H_6 layer was extracted three times with NaOH in 70% EtOH. The extracts were acidified with AcOH to give 3.7 g (74%) of product, mp 219–222 °C dec. Three precipitations from aqueous alcoholic alkali failed to change the analysis. Anal. ($C_{24}H_{23}NO_2S$) C, H, N, S; C: calcd, 74.0; found, 72.65.

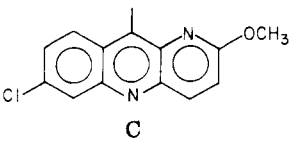
***S*-(12-Methyl-7-benz[*a*]anthrylmethyl)glutathione (3).** To a stirred, cooled solution of 1.3 g of glutathione in 20 ml of MeOH, 12 ml of 1 N NaOH–MeOH, and 8 ml of C_6H_6 was added 0.75 g of 7-iodomethyl-12-methylbenz[*a*]anthracene.[12] After 1 h of stirring, the mixture was filtered and the crude product (salt) precipitated with hexane (1.1 g). This was redissolved in 30 ml of 1:1 H_2O–MeOH, filtered, and acidified with AcOH to give a gelatinous precipitate, which was digested by overnight stirring at 30–40 °C, when filtration was possible; the yield was 0.4 g (36%). Needles were obtained by recrystallization from 1.5 ml of 50% EtOH (40% recovery): mp 207–210 °C dec. Anal. ($C_{30}H_{31}N_3SO_6$) C, H, N, S.

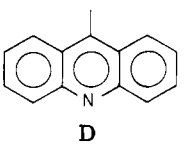
***S*-(12-Methyl-7-benz[*a*]anthrylmethyl)thioglycerol (4).** A solution of 2.4 g of thioglycerol (Evans) in 20 ml of 1 N NaOH–MeOH and 10 ml of C_6H_6 was stirred and cooled while 2.1 g of 7-iodomethyl-12-methylbenz[*a*]anthracene[12] was added. After 3 h of stirring at 0 °C, the clear mixture was diluted with

Table I. Pulmonary Adenomas from Derivatives of Polynuclear Aromatic Nuclei


A


B


C


D

No.	Nucleus	Side chain	Dose,[a] μmol/kg	Vehicle	Induction period, weeks	No. of adenomas/ no. of mice	Av no./ mouse
		Controls[b]				154/431	0.36
1	A	*S*-Cysteine[c]	15	PG	24	2/6	0.3
2	A	*S*-Homocysteine[c]	15	PG	24	5/6	0.8[d]
3	A	*S*-Glutathione[c]	15	PG	24	2/6	0.3
4	A	$-SCH_2CHOHCH_2OH$[c]	15	PG	26	2/6	0.3
5	A	$-NH(CH_2)_3N(C_2H_5)-CH_2CH_2OH\cdot 2HI$[e]	15	S	24	0/5	0.0
6	A	$[-NH(CH_2)_3N(C_2H_5)-CH_2CH_2]_{43}HSA$	15[f]	S	24	1/6	0.2
7	B	$-SCH_2CHOHCH_2OH$	15	PG	26	0/6	0.0
8	B	-Sarcosylglycylglycine	15	S	24	0/6	0.0
9	C	$-NH(CH_2)_3NHCH_2-CH_2OH\cdot 2HCl$[g]	15	S	24	1/6	0.2
10	D	-Glycylglycylglycine	15	S	24	3/6	0.5

[a] Compounds were given iv to 8–11-week-old female mice in 0.1–0.4 ml of vehicle. Depending on solubility, vehicle was either 0.9% saline (S) or 50% aqueous propylene glycol (PG). [b] This number of control mice was based on combining our controls with Shimkin's[10] statistically compatible controls.[5] [c] At least one animal in each of these groups exhibited heavy hair loss. [d] Only this group of mice developed a statistical excess number of adenomas ($p = 0.0274$). [e] As reported by Peck et al.[13] [f] Of hapten. [g] As reported in ref 9.

ice water; the product was removed by filtration and washed with aqueous EtOH and C_6H_6–hexane to give 1.6 g of crude product. Crystallization from aqueous EtOH and from C_6H_6 gave 1.3 g (57%) of product containing solvent of crystallization. Sublimation in vacuo gave a product of mp 118–121 °C. Anal. ($C_{23}H_{22}O_2S$) C, H, S.

***N*-Ethyl-*N*-[3-(12-methyl-7-methylaminobenz[*a*]anthryl)propyl]aminoethyl Horse Serum Albumin (6).** To a stirred solution of 2 g of horse serum albumin (Pentex, Fr V) in 91 ml of water, 12 ml each of 1 N $NaHCO_3$ and 1 N Na_2CO_3, and 115 ml of ethanol in a low actinic flask (unnecessary light was avoided throughout the preparation) was added portions of a solution of 1 g of N^1-[12-methyl-7-(benzanthryl)methyl]-N^3-(ethyl-2-chloroethyl)propanediamine dihydrochloride[13] in 10 ml of H_2O, over about 1 h. The mixture was stirred in a cold room (5 °C) for 4.5 h; 2 ml of 1 N NaOH was added after addition was complete; another 2 ml was added midway during the 4.5-h period. The mixture was dialyzed exhaustively against cold water and 0.001 N AcOH and centrifuged to give 610 ml of clear solution containing 2.90 mg/ml of protein (Kjeldahl). It was taken to 0.84 M with $(NH_4)_2SO_4$ and the precipitate dialyzed exhaustively vs. 0.001 N AcOH and 0.1% NaCl and then centrifuged to give 90 ml of clear solution containing 11.6 mg/ml. Spectrophotometric analysis[14] showed the presence of 43 hapten groups per molecule albumin.

***S*-(10-Methyl-9-anthrylmethyl)thioglycerol (7)** was prepared by the same method as its benzanthryl analogue in 51% yield: mp 142–144 °C. Anal. ($C_{19}H_{20}SO_2$) C, H, S.

***N*-(10-Methyl-9-anthrylmethyl)sarcosylglycylglycine (8).** To a stirred cooled solution of 1.2 g of sarcosylglycylglycine (Nutritional Biochemicals Corp., Cleveland, Ohio) in 20 ml of MeOH and 6 ml of 1 N NaOH–MeOH was added 14 ml of C_6H_6, followed by 2 g of 9-iodomethyl-10-methylanthracene.[12] Stirring was continued at 0 °C for 3 h and overnight at 5 °C, and an additional gram of iodomethylmethylanthracene was added. Again the mixture was stirred at 0 °C for 3 h and overnight at 5 °C and then filtered. The filtrate was evaporated to dryness in vacuo, taken up in 1:4 MeOH–H_2O plus excess NaOH, and filtered from Norit; acidification with AcOH gave 0.5 g of product; crystallization from 3:1 MeOH–H_2O gave 0.45 g (18%) of product, mp 192–193 °C. Anal. ($C_{23}H_{25}N_3O_4$) C, H, N.

***N*-(9-Acridinyl)glycylglycylglycine (10).** A mixture of 2.1 g of 9-chloroacridine, 1.9 g of glycylglycylglycine (Nutritional Biochemicals Corp., Cleveland, Ohio), and 25 ml of diethanolamine was stirred at 105–110 °C (internal) for 1.5 h, poured into water, and filtered from the by-product acridone. The filtrate was acidified with AcOH, and crystallization was induced by intense local cooling and scratching. When crystallization was complete, the product was removed; it weighed 1.0 g. It was taken up in dilute NaOH and the viscous solution was filtered and acidified with cooling and seeding. The yield was 0.9 g (25%). Anal. ($C_{19}H_{18}N_4O_4$) C, H, N.

Acknowledgment. This work was supported by U.S. Public Health Service Grants CA-13986, CA-06927, and RR-05539 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.

References and Notes

(1) E. C. Miller and J. A. Miller in "Chemical Mutagens", Vol. 1, A. Hollaender, Ed., Plenum Press, New York, N.Y., 1971, p 85 ff.
(2) P. L. Grover and P. Sims, *Biochem. Pharmacol.*, **19**, 2251 (1970).
(3) A. Dipple, P. D. Lawley, and P. Brookes, *Eur. J. Cancer*, **4**, 493 (1968).
(4) J. W. Flesher and K. L. Sydnor, *Cancer Res.*, **31**, 1951 (1971).
(5) R. M. Peck, T. K. Tan, and E. B. Peck, *Cancer Res.*, **36**, 2423 (1976).
(6) J. McCann, E. Choi, E. Yamasaki, and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 5135 (1975).
(7) M. B. Shimkin and G. D. Stoner, *Adv. Cancer Res.*, **21**, 1–58 (1975).
(8) R. M. Peck and A. P. O'Connell, *J. Med Chem.*, **15**, 68 (1972).
(9) H. J. Creech, R. K. Preston, R. M. Peck, and A. P. O'Connell, *J. Med. Chem.*, **15**, 739 (1972).
(10) M. B. Shimkin, J. H. Weisburger, E. K. Weisburger, N. Gubareff, and V. Suntzeff, *J. Natl. Cancer Inst.*, **36**, 915 (1966).
(11) B. N. Ames, personal communication.
(12) R. B. Sandin and L. F. Fieser, *J. Am. Chem. Soc.*, **62**, 3098 (1940).
(13) R. M. Peck, A. P. O'Connell, and H. J. Creech, *J. Med. Chem.*, **13**, 284 (1970).
(14) H. J. Creech and R. M. Peck, *J. Am. Chem. Soc.*, **74**, 463 (1952).