Biochemical and Biophysical Research Communications **290,** 275–279 (2002)
doi:10.1006/bbrc.2001.6175, available online at http://www.idealibrary.com on IDEAL®

Comparative Carcinogenicity of Picene and Dibenz[*a,h*]anthracene in the Rat

James W. Flesher,*,†,[1] Jamie Horn,*,† and Andreas F. Lehner‡
**Experimental Cancer Research Laboratory, †Department of Molecular and Biomedical Pharmacology, and ‡Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40536*

Received November 12, 2001

Early carcinogenicity tests found no evidence of activity for picene but found considerable initiating and carcinogenic activity for dibenz[*a,h*]anthracene (DBA). More recent investigation suggested that both pentacyclics were complete carcinogens when administered as single sc injections in NMRI mice, despite findings that picene acted as neither an initiating nor promoting agent. To investigate this contradiction, the complete carcinogenicities of both isomers were compared by sc injection in female Sprague–Dawley rats. The results demonstrate that 1 μmol of DBA, administered three times weekly for 20 doses, induces sarcomas in all test animals by 33 weeks (100%). Similar treatment with picene did not induce sarcoma in any test animals by 37 weeks (0%). The present results agree with the earlier studies. It follows from these results that the predictions of the unified theory for the appearance of carcinogenic properties following administration of picene and dibenz[*a,h*]anthracene to test animals have been confirmed.

Key Words: **polycyclic aromatic hydrocarbons; complete carcinogenicity; structure–activity relationships; picene; dibenz[*a,h*]anthracene.**

In 1930, dibenz[*a,h*]anthracene (DBA) was the first carcinogenic polycyclic aromatic hydrocarbon (PAH) to be identified, whereas the isomer picene was found to be carcinogenically inert (Fig. 1; 1, 2). This important discovery led to the synthesis and testing for carcinogenic properties of many new compounds and to the first attempt to formulate a quantum mechanical theory of carcinogenicity based on the fundamental proposition that the carcinogenic properties of PAH depend on the occurrence of a definite chemical reaction involving the carcinogen, *in vivo.* According to the K, L, M-region theory, the appearance of carcinogenic properties in administered unsubstituted aromatic hydrocarbons depends only on the occurrence of some addition reaction involving the carcinogen at the K-region, but is independent of the occurrence of some metabolic substitution reaction at the highly reactive meso-region or L-region positions (3).

However this theory has been disproved by the observations that unsubstituted PAH preferentially undergo metabolic methyl-substitution and electrophilic substitution reactions precisely at the highly reactive meso-region or L-region positions (5–11). Furthermore, structure–activity relationships demonstrate that methyl substitution at the reactive meso-region positions of the molecular structure can dramatically enhance carcinogenic potency. For example, methyl substitution at the L-region of dibenz[*a,h*]anthracene gives rise to a more potent carcinogen (19).

These observations lend strong support for the unified theory, which predicts that the appearance of carcinogenic properties in picene and dibenz[*a,h*]anthracene depends, in the first metabolic step, upon the occurrence of methyl-substitution at the reactive L-region positions of DBA, whereas picene cannot undergo this critical substitution reaction, since it lacks a reactive L-region (Fig. 2). Thus the unified theory predicts only DBA to possess the features of structure and reactivity necessary for the attainment of strong carcinogenic properties, whereas other theoretical approaches predict both isomers must demonstrate strong carcinogenic properties owing to the existence in the molecules of strikingly similar "bay-regions" (13).

According to the bay-region theory, the appearance of carcinogenic properties in PAH is independent of the K-region or the L-region, but is dependent only upon the occurrence of metabolic epoxidation of the 3–4 bond (or M-region) of picene and DBA followed by the formation of the M-region *trans*-3,4-dihydrodiol by the addition of water to the epoxide. The M-region dihydrodiol is a proximate carcinogen and is converted to the ultimate carcinogenic metabolite by metabolic epoxidation of the 1–2 bond (Fig. 3). Therefore, according

[1] To whom correspondence and reprint requests should be addressed. Fax: (859) 323-1981. E-mail: jwflesh@uky.edu.

0006-291X/02 $35.00

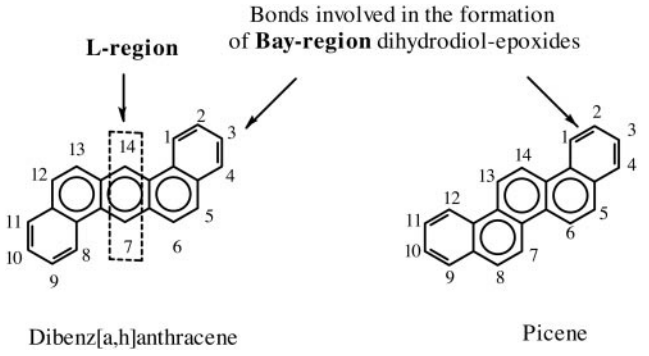

FIG. 1. Molecular structures and numbering systems for the anthracene derivative dibenz[*a,h*]anthracene and the phenanthrene derivative picene. Bonds involved in the formation of bay-region dihydrodiol-epoxides and reactive centers for electrophilic substitution contained within an L-region are shown.

to this mechanistic pathway, PAH must contain two bonds in an end ring of a phenanthrene-like structure, which forms a bay-region, capable of undergoing a series of three metabolic addition reactions for the development of pronounced carcinogenic activity (4, 12).

To investigate the contradiction between the predictions of the bay-region theory and early experimental results of Kennaway and his associates, Platt *et al.* compared the carcinogenic activity of picene to that of DBA by sc injection in the NMRI mouse, and found that the incidence of fibrosarcomas was comparable over a large dose range and that the two isomers were indistinguishable at the highest dose with an tumor incidence of 63.3% in treated animals, after 65 weeks, regardless of the isomer used (1, 2, 13). However these investigators found no evidence for initiating or promoting activity associated with picene, though considerable initiating activity was found for DBA. Therefore, it is difficult to see how picene could possibly be a strong complete carcinogen since it fails to act as either an initiating or promoting agent in the two-stage mouse skin test. Thus, further investigation of the comparative carcinogenic activity of picene and dibenz[*a,h*]anthracene is required in order to answer the important question of whether or not picene is, in fact, as potent a complete carcinogen as dibenz[*a,h*]-anthracene in the rat.

Testing for complete carcinogenic properties of these two pentacyclic isomers, picene and dibenz[*a,h*]anthracene, is of interest because it offers a means of determining the importance of the bay- and L-regions in mechanisms of activation and deactivation of these hydrocarbons.

MATERIALS AND METHODS

Animals. Twenty-three-day-old female Sprague–Dawley rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Rats, housed in polyethylene cages with wood chip bedding, were fed Purina Rat Chow and tap water ad libitum. Animals were quarantined and acclimatized to a temperature-controlled and light cycle (12 h) environment for 7 days prior to experimental use.

Test compounds. Picene was purchased from K & K Laboratories (Plainview, NY) and recrystallized from ethyl acetate, mp 366°C. It gave a single peak by reverse-phase HPLC (R_t = 5.2 min) and GC/MS. Dibenz[*a,h*]anthracene was from Eastman Organic Chemicals (Rochester, NY). When recrystallized from glacial acetic acid it had mp 267°C and gave a single peak by reverse-phase HPLC (R_t = 7.6 min) and GC/MS.

Instrumentation. Reverse-phase HPLC was conducted on a Waters system with UV and fluorescence detection (254 nm; 361 ex, 418 em). One-microgram samples were eluted from a Microsorb MV ODS column with 9:1 methanol:water at 1 ml/min. GC/MS was conducted on a Hewlett–Packard 6890 GC equipped with a HP-5MS column and a 1 ml/min He carrier gas flow. The injector port was set to 250°C with an oven temperature program of 70°C to 250°C at 8°C/min and the eluant carried through a 280°C transfer line to an interfaced 5972 mass selective detector. Electron impact fragmentation is induced at −70 eV, and generated mass spectra in the 50–500 *m/z* mass range are collected by Windows-based HP Chemstation software.

Determination of complete carcinogenicity. Complete carcinogenicity was determined as previously described (14). The test compounds in 9:1 sesame oil:DMSO were individually administered in 1

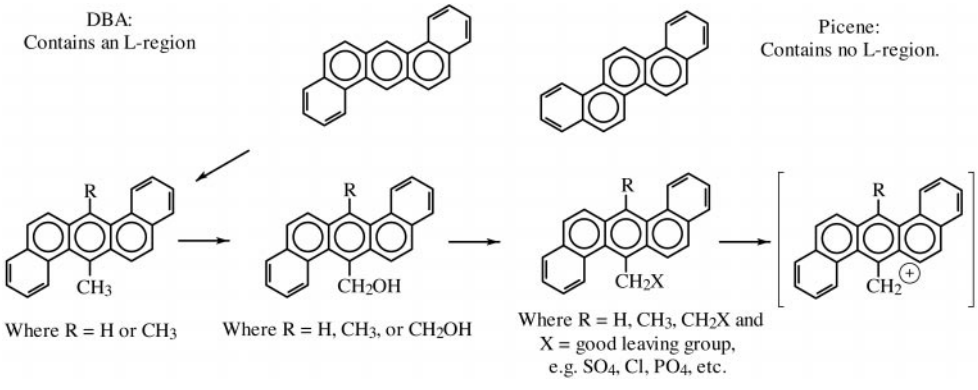

FIG. 2. The unified theory states that the first step in carcinogenesis by dibenz[*a,h*]anthracene (DBA) is methyl substitution at a reactive meso-anthracenic center with subsequent hydroxylation and conjugation with a good leaving group resulting in a benzylic type aralkylating agent as an ultimate metabolite (5, 8).

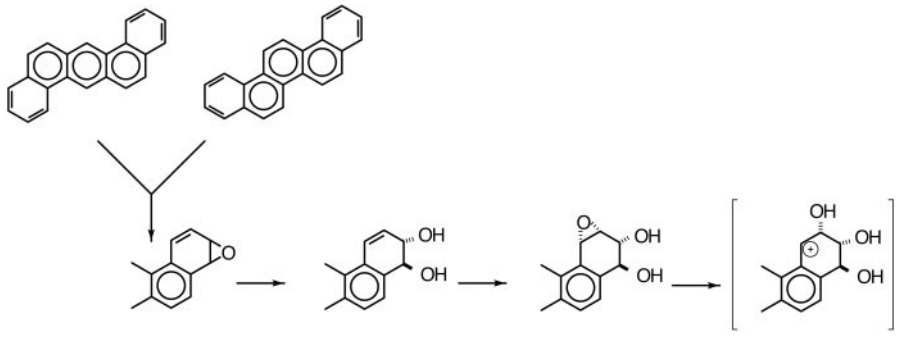

FIG. 3. The bay-region theory stipulates that PAH possessing "bay-regions," and hence terminal angular benzo-rings, will be carcinogenic owing to their metabolism to vicinal dihydrodiols and dihydrodiol-epoxides as proximate and ultimate metabolites, respectively (4).

μmol (0.3 mg)/0.1 ml doses by subcutaneous injection (*dorsal subcutis*) three times weekly for 20 doses (total dose 20 μmol, 6 mg) to a group of 12 test animals. The initial dose was administered when rats were 30 days of age. One control group of 12 female rats was administered vehicle, while another control group remained untreated. All animals were weighed once each week and examined for the presence of tumors. Ten to 30 days after the appearance of a palpable tumor, the animal was sacrificed, and all grossly pathological tissue was removed, fixed in 10% neutral formalin, and prepared for histological examination of tumor type. Complete carcinogenic potency was calculated as the percentage of animals within the treatment group that developed sarcoma at the site of injection within the 37-week experimental period.

RESULTS AND DISCUSSION

The carcinogenic activity of the two isomers, DBA and picene were compared by repeated subcutaneous injection in female Sprague–Dawley rats. The results, shown in Fig. 4, demonstrate that a 1 μmol dose of dibenz[*a,h*]anthracene administered sc three times per week for 20 doses (total dose 20 μmol), induced sarcomas at the site of injection in all 12 rats (100%) by 33 weeks, whereas the same dose of picene did not induce sarcoma in any of 12 rats (0%) by 37 weeks. There were no tumors in rats given vehicle alone or in untreated control groups.

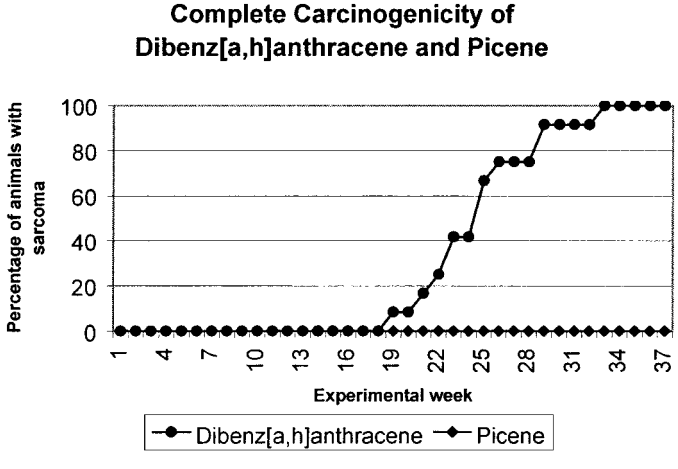

FIG. 4. By 37 weeks a total dose of 20 μmol (6 mg) DBA induced sarcomas at the site of injection in 12 of 12 test animals (100%). A total dose of 20 μmol (6 mg) picene did not induce sarcoma at the site of injection in any test animals by 37 weeks. Neither the vehicle-treated nor the untreated control group developed sarcomas within the 37-week experimental time period (controls not shown on graph).

These results are in good agreement with earlier results of Cook *et al.* which did not find any evidence that picene was carcinogenic (2) and provide strong support for the unified theory for predicting PAH carcinogenicity. They do, however, conflict with the studies of Platt *et al.* which indicate that the complete carcinogenicity of picene is equal to that of DBA (13). Thus, whereas Platt *et al.* concluded that these two isomers have strikingly similar bay-regions, suggesting that they act by conversion to vicinal bay-region dihydrodiol epoxides as electrophilic mutagens and ultimate carcinogens, the results presented here and in Cook *et al.*'s original work support a conclusion that the two isomers are strikingly different in molecular structure owing to the fact that dibenz[*a,h*]anthracene possesses an exceptionally reactive L-region, whereas picene is devoid of an L-region (2, 13). This latter conclusion is also supported by the fact that picene lacks tumorigenic activity, i.e., both initiating and promoting activity (13). Nevertheless, Platt *et al.* concluded that theoretical studies predicting pronounced carcinogenic properties for picene had been confirmed and a further member had been added to the class of complete carcinogens (13). In addition, they concluded that this rare carcinogenic property of picene, which was claimed to be a strong complete carcinogen in NMRI mice, yet at most a very weak tumor initiator, might be explained in terms of its inefficient conversion to mutagenic and carcinogenic metabolites compared to the strong tumorigenic initiator dibenz[*a,h*]anthracene (13).

Microsomal metabolism studies indicate that both compounds are metabolized to M-region dihydrodiols to a comparable extent (15). However ^{32}P-postlabeling studies for polar DNA-adducts indicate that although polar-binding appears to occur through a dihydrodiol metabolite, the majority of binding by either compound

cannot be accounted for by the binding of their respective mono-*anti*- or *syn*-epoxide (16). This finding is sustained by the observations that the *trans*-3,4-dihydrodiol and *trans*-3,4-dihydrodiol-anti-1,2-epoxide derivatives of DBA express much less tumorigenic activity compared to their parent compound, DBA, on mouse skin (17). More recent data suggests that DBA activation to a *bis*-dihydrodiol-epoxide might account for more of the polar DNA-bound products of DBA than could the mono-epoxide (18).

Studies of *in vivo* bioalkylation support the theory that compounds such as DBA undergo methyl substitution at the reactive L-region in the first metabolic step (8), whereas compounds classified as phenanthrene derivatives rather than anthracene derivatives do not (20). Although picene was not included in these methylation studies, it cannot undergo methyl-substitution at an L-region, since picene does not possess this structural feature. Studies to compare the carcinogenicity of the L-region metabolites of DBA with the M-region and bay-region epoxide metabolites of DBA and picene have not been conducted. However, the unified theory is strongly supported by the fact that biological methyl substitution occurs at just that part of the molecule (the reactive L-region) that structure–activity relationships show to be particularly important for the development of very pronounced carcinogenic properties (8).

Clearly, theoretical models that predict the appearance of pronounced carcinogenic properties when picene is administered by repeated subcutaneous injection to a test animal have a definite exception in the case of the female Sprague–Dawley rat. In this regard, the unified theory could have a definite exception, if it could be shown that picene is just as potent a complete carcinogen as dibenz[*a,h*]anthracene in two animal models.

A similar argument can be advanced for the isomers benzo[*a*]pyrene and benzo[*e*]pyrene (Fig. 5). These are isomers with and without an exceptionally reactive meso-region position and are therefore predicted to be carcinogenic or non-carcinogenic isomers, respectively. This prediction of the unified theory has been confirmed by studies of complete carcinogenicity (9). Furthermore, dibenzo[*a,e*]pyrene is carcinogenic, although not as potent as benzo[*a*]pyrene owing to steric hindrance of the meso-region for electrophilic substitution, whereas dibenzo[*e,l*]pyrene, lacking an exceptionally reactive meso-region position, is carcinogenically inert (Fig. 5; 9). Also, dibenzo[*a,l*]pyrene is the most potent dibenzpyrene known having an exceptionally reactive meso-region for electrophilic substitution (9, 10). Clearly, a relationship exists between carcinogenic properties and the existence of an exceptionally reactive meso-region.

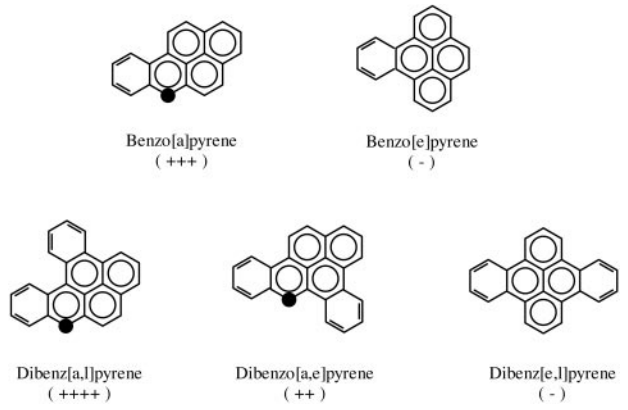

FIG. 5. Structure–activity relationships indicate that the appearance of carcinogenic properties of administered isomeric PAH is dependent upon the existence of a reactive meso-region position capable of undergoing substitution (shown as a bold dot), but is independent of the existence of a terminal benzo ring (bonds capable of undergoing addition shown) that forms part of a bay-region.

SUMMARY AND CONCLUSIONS

1. The pronounced carcinogenicity of dibenz[*a,h*]anthracene and the lack of carcinogenicity of picene has been confirmed in the rat. These results are in perfect agreement with studies of tumorigenicity.

2. The presence of bay-regions in picene is not a sufficient structural feature for the attainment of pronounced carcinogenic activity. Picene belongs in the class of phenanthrene derivatives that are inactive or only very weakly active.

3. Although the metabolic activation and carcinogenicity of dibenz[*a,h*]anthracene might depend, in part, on the formation of a bay-region diol-epoxide, the possibility that the appearance of carcinogenic properties depends, in the first metabolic step, upon methyl-substitution at the L-region, followed by meso-methyl hydroxylation and esterification to form ultimately a powerful aralkylating metabolite, bearing a good leaving group, cannot be ignored.

4. The validity of some hypothesis or theory of polycyclic aromatic hydrocarbon carcinogenesis must ultimately depend on the ability to correctly predict carcinogenic properties of specific molecular structures. Therefore, further studies, in at least two animal models, are required to convincingly demonstrate that picene is just as potent a complete carcinogen as dibenz[*a,h*]anthracene.

ACKNOWLEDGMENTS

Acknowledgment is made to the University of Kentucky and NIH/NCI Grant CA85134 for generous support.

REFERENCES

1. Kennaway, E. L., and Hieger, I. (1930) Carcinogenic substances and their fluorescence spectra. *Br. Med. J.*, 1044–1046.
2. Cook, J. W., Hieger, I., Kennaway, E. L., and Mayneord, W. V. (1932) The production of cancer by pure hydrocarbons—Part 1. *Proc. R. Soc. London (Biol.),* **111,** 455–484.
3. Pullman, A., and Pullman, B. (1955) Electronic structure and carcinogenic activity of aromatic molecules: New developments. *In* Advances in Cancer Research (Greenstein, J. P., and Haddow, A., Eds.), Vol. 3, pp. 117–169, Academic Press, New York.
4. Jerina, D. M., Yagi, H., Lehr, R. E., Thakker, D. R., Schaefer-Ridder, M., Karle, J. M., Levin, W., Wood, A. W., Chang, R. L., and Conney, A. H. (1978) The bay-region theory of carcinogenesis by polycyclic aromatic hydrocarbons. *In* Polycyclic Hydrocarbons and Cancer: Environment, Chemistry and Metabolism (Gelboin, H. V., and Ts'O, P. O. P., Eds.), pp. 141–179, CRC Press, Boca Raton.
5. Flesher, J. W., and Sydnor, K. L. (1973) Possible role of 6-hydroxymethylbenzo[a]pyrene as a proximate carcinogen of benzo[a]pyrene and 6-methylbenzo[a]pyrene. *Int. J. Cancer* **11,** 433–437.
6. Stansbury, K. H., Flesher, J. W., and Gupta, R. C. (1994) Mechanism of aralkyl-DNA adduct formation from benzo[a]pyrene *in vivo*. *Chem. Res. Toxicol.* **7,** 254–259.
7. Surh, Y.-J., Liem, A., Miller, E. C., and Miller, J. A. (1989) Metabolic activation of the carcinogen 6-hydroxymethylbenzo-[a]pyrene: Formation of an electrophilic sulfuric acid ester and benzylic DNA adducts in rat liver *in vivo* and in reactions *in vitro*. *Carcinogenesis* **10,** 1519–1528.
8. Flesher, J. W., Myers, S. R., Bergo, C. H., and Blake, J. W. (1986) Bioalkylation of dibenz[a,h]anthracene in rat liver cytosol. *Chem.–Biol. Interact.* **57,** 223–233.
9. Flesher, J. W., and Myers, S. R. (1991) Rules of molecular geometry for predicting carcinogenic activity of unsubstituted polynuclear aromatic hydrocarbons. *Teratogen. Carcinogen. Mutagen.* **11,** 41–54.
10. Flesher, J. W., Horn, J., and Lehner, A. F. (1996) Molecular modeling of carcinogenic potential in polycyclic hydrocarbons. *J. Mol. Struct.* **362,** 29–49.
11. Flesher, J. W., Myers, S. R., and Blake, J. W. (1988) Bioalkylation of polynuclear aromatic hydrocarbons *in vivo:* A predictor of carcinogenic activity. *In* Polynuclear Aromatic Hydrocarbons: A Decade of Progress (Cooke, M., and Dennis, A. J., Eds.), Tenth International Symposium, pp. 261–276, Battelle Press, Columbus.
12. Jerina, D. M., and Lehr, R. E. (1977) The bay-region theory: A quantum mechanical approach to aromatic hydrocarbon-induced carcinogenicity. *In* Microsomes and Drug Oxidations (Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R. W., and Conney, A. M., Eds.), pp. 709–720, Pergamon Press, Oxford.
13. Platt, K. L., Pfeiffer, E., Petrovic, P., Friesel, H., Beerman, D., Hecker, E., and Oesch, F. (1990) Comparative tumorigenicity of picene and dibenz[a,h]anthracene in the mouse. *Carcinogenesis* **11,** 1721–1726.
14. Flesher, J. W., and Sydnor, K. L. (1971) Carcinogenicity of derivatives of 7,12-dimethylbenz[a]anthracene. *Cancer Res.* **31,** 1951–1954.
15. Platt, K. L., Petrovic, P., Seidel, A., Beerman, D., and Oesch, F. (1988) Microsomal metabolism of picene. *Chem.–Biol. Interact.* **66,** 157–175.
16. Lecoq, S., Pfau, W., Ni She, M., Platt, K. L., Seidel, A., Oesch, F., Phillips, D. H., and Grover, P. L. (1993) Comparison of the metabolic activation of dibenz[a,h]anthracene and picene using ^{32}P-postlabeling. *In* Polycyclic Aromatic Compounds: Synthesis, Properties, Analytical Measurements, Occurrence and Biological Effects (Garrigues, P., and Lamotte, M., Eds.), Vol. III (Suppl.), pp. 921–928, Gordon and Breach Science.
17. Slaga, T. J., Gleason, G. L., Mills, G., Ewald, L., Fu, P. P., Lee, H. M., and Harvey, R. G. (1980) Comparison of the skin tumor-initiating activities of dihydrodiols and diol-epoxides of various polycyclic aromatic hydrocarbons. *Cancer Res.* **40,** 1981–1984.
18. Carmichael, P. L., Platt, K. L., She, M. N., Lecoq, S., Oesch, F., Phillips, D. H., and Grover, P. L. (1993) Evidence for the involvement of a *bis*-diol-epoxide in the metabolic activation of dibenz[a,h]anthracene to DNA-binding species in mouse skin. *Cancer Res.* **53,** 944–948.
19. Shear, M. J., and Leiter, J. (1940) Studies in carcinogenesis XIV. 3-Substituted and 10-substituted derivatives of 1,2-benzanthracene. *J. Natl. Cancer Inst.* **1,** 303–336.
20. Flesher, J. W., and Myers, S. R. (1990) Bioalkylation of benz-[a]anthracene as a biochemical probe for carcinogenic activity. Lack of bioalkylation in a series of six non-carcinogenic aromatic hydrocarbons. *Drug Metab. Dispos.* **18,** 163–167.