The Carcinogenic Activity of Some New Derivatives of Aromatic Hydrocarbons

II. Compounds Related to 1,2-Benzanthracene*†

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INTRODUCTION

In 1939 a program of investigation was undertaken in collaboration with Professor Louis F. Fieser and his associates at Harvard University, directed toward a further study of the chemistry of artificial carcinogenesis. The main task was a biological testing for carcinogenicity of a number of new hydrocarbon derivatives synthesized by Dr. Fieser's group. Although the chief purpose of this paper is to present data on the carcinogenic tests of compounds related to 1,2-benzanthracene, some discussion of the problem as a whole is necessary in order to relate this report to those that will be published subsequently.

In spite of the enormous amount of work that has been done on synthetic carcinogenic agents, and the testing of well over 700 compounds, it is still impossible to formulate any generalization that defines the features of chemical structure necessary for carcinogenic action. It does appear that substituents in the 9-, 10-, 5-, and 6- positions of 1,2-benzanthracene are more likely to render the molecule active than if placed elsewhere in the molecule, and that hydrogenation of the aromatic rings or the substitution of large radicals reduces activity; however, none of these generalizations is definitive. Neither physical characteristics such as atomic weight, melting point, solubility, and surface tension effect nor the known chemical reactivity of the various compounds (15) has yet been successfully correlated with carcinogenic activity. Nevertheless, the striking changes in activity that follow the slightest alteration of structure imply a high degree of chemical specificity in the process of chemical carcinogenesis. This makes it appear profitable to continue the search for some common denominator among carcinogenic chemicals that will set the active ones apart from their inactive relatives, and perhaps provide a clue to the initial step in the biological process of artificial tumor induction.

In order to define the problem more clearly it seemed desirable to determine the carcinogenic activity of a considerable number of new chemical compounds, synthesized by Dr. Fieser and his group and chosen by them as those most likely to throw some light on the nature of the structural modifications that would enhance, diminish, or destroy carcinogenic activity.

Several lines of thought influenced the choice of these substances, and a number of new syntheses were evolved in order to obtain them. For example, there are 12 possible isomeric forms of monomethyl 1,2-benzanthracene, all of which have been synthesized and tested. The results have shown that carcinogenic activity is dependent to a remarkable degree on the position of the substituted methyl group in the molecule. A number of our new compounds represent members of other series of isomers, such as the dimethyl benzanthracenes and the monomethyl benzpyrenes. The results obtained with these compounds should help to define more clearly the importance of the position of substitution as it affects carcinogenic potency.

Not only the position of substitution but the nature of the substituent group has been shown to influence carcinogenic action. In general, a progressive loss of potency follows an increase in the size or complexity of the substituent. Thus 10-methyl-1,2-benzanthracene is very active, the 10-ethyl derivative is moderately so, and the 10-propyl is inactive. Substitution of functional groups usually reduces activity. We have tested a number of compounds substituted at the same molecular position but with different radicals, as, for example, the group of 5-substituted benzpyrene derivatives (13).

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† The compounds utilized in these experiments were selected and synthesized by Professor L. F. Fieser and his associates in the Department of Chemistry, Harvard University.

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Several of the new compounds have structural features common to two or more of the established carcinogens. Thus 1′,9-methylene-10-methyl-1,2-benzanthracene may be considered either as a substitution product of 10-methyl-1,2-benzanthracene or as a derivative of 10-methyl-3,4-benzpyrene, in which a 5-carbon ring has replaced one of the aromatic 6-carbon rings. Other compounds were tested that contain only a portion of an established carcinogenic nucleus; for example, the chrysene derivatives reported in a previous communication (12) lack only one benzene ring to complete the structure of benzpyrene. Active tumor production, particularly with 5-methylchrysene, shows that some simplification of the benzpyrene nucleus is possible without complete loss of carcinogenic action.

A peculiar group of carcinogenic agents is known that selectively affect the liver regardless of the route of administration. Although differing considerably in structure among themselves, one feature common to this group is the presence of nitrogen in the molecule. Many of the compounds bear no structural similarity to the carcinogenic hydrocarbons whereas others, such as 1,2,5,6-dibenzcarbazol, bear a striking resemblance to these. It was therefore decided to modify the structure of active hydrocarbons by replacing one of the ring carbons with a nitrogen atom. The resulting "aza" compounds of benzpyrene and methylcholanthrene not only failed to produce liver tumors but also lost practically all their ability to produce local tumors.

As the ultimate objective of all tests of carcinogenic agents is to throw some light on the mechanism of spontaneous tumor genesis, it would be desirable to learn not only what chemical structures are associated with the ability to induce tumors, but what reactions may take place between the carcinogenic agent and the body tissues. Preliminary studies of the metabolism of carcinogens have indicated that a certain fraction of the injected or ingested hydrocarbon is excreted unchanged, another fraction has not been traced, and a third fraction is excreted in the form of hydroxy, quinone, or other derivatives of the original compound (4, 5, 10, 19).

The chemical modification of hydrocarbons *in vivo* demonstrates that substances present in mammalian tissues can react chemically with carcinogenic hydrocarbons, but since the derivatives thus far identified are relatively inactive their formation might appear to be a process of "detoxification" rather than a part of the chemical pathway responsible for tumor production. The production of neoplasms may depend, of course, upon some direct physical action of the hydrocarbon such as a change in surface forces or tissue permeability, but it might equally well involve a chemical combination, as yet undiscovered, between the carcinogen and substances in the tissue. If the substances resulting from any such interactions were intermediates in the process of carcinogenesis they might themselves be highly carcinogenic.

On this hypothesis a number of compounds were synthesized that contained a hydrocarbon nucleus joined to various radicals theoretically available in tissues. Among the compounds thus tested were hydroxy derivatives and combinations of hydrocarbons with proteins, cysteine, amines and other radicals containing oxygen, nitrogen, sulfur, and halides. None showed sufficient activity to suggest that it might be an intermediate in the process of tumor induction.

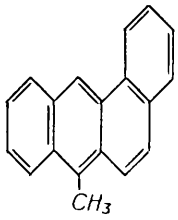
These few remarks serve to indicate some of the lines of thought that influenced the choice of substances to be tested. The detailed reasons for selecting many of them, as well as the chemical implications that may be drawn from the results of the tests, lie for the most part beyond the scope of this paper; our chief purpose is to present factual data on the carcinogenic activity of a number of new hydrocarbon derivatives.

MATERIALS AND METHODS

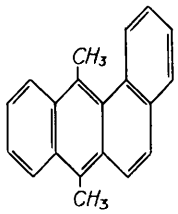
Young male mice of the C3H and Swiss strains were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were kept in groups of 5 in metal cages, and fed on Purina dog chow and water with supplements of crude cod liver oil.

These two strains were used since they have a somewhat different incidence of spontaneous tumors. The C3H strain, developed by Strong (23), has been widely used in experimental tumor work. A high percentage of the females develop spontaneous carcinoma of the breast, but tumors of this type practically never occur in the males. In old age both males and females develop occasional pulmonary adenomas, or adenomas of the liver, but spontaneous subcutaneous sarcomas in either sex are rare.

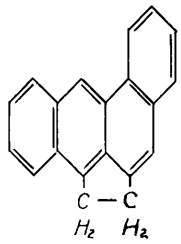
The Swiss strain has not been so widely used as the C3H. Spontaneous adenomas of the lung are frequent in old animals of both sexes, and we have observed occasional adenocarcinomas of the breast in old breeding females. The males fight among themselves, but in spite of frequent and repeated skin wounds in various parts of the body, which often became infected, subcutaneous sarcomas were rarely observed except at the site of injection of a carcinogenic agent. The exceptions were 3 sarcomas in the region of the scrotum found in 1,530 autopsies. Swiss males occasionally develop spontaneous scrotal hernias, which may become secondarily infected. The 3 scrotal



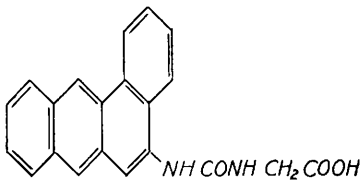
10-Methyl-1,2-benzanthracene



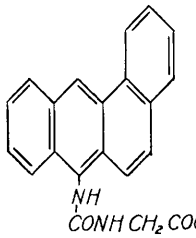
9,10-Dimethyl-1,2-benz-anthracene



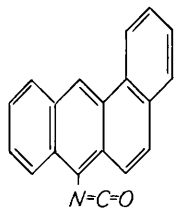
4,10-Ace-1,2-benzanthracene



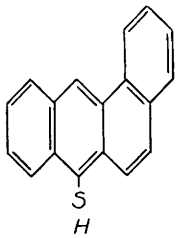
1,2-Benzanthryl-3-carbamido-acetic acid



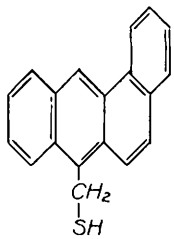
1,2-Benzanthryl-10-carb-amido acetic acid



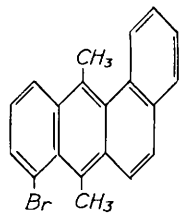
1,2-Benzanthryl-10-iso-cyanate



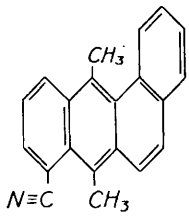
1,2-Benzanthryl-10-mercaptan



1,2-Benzanthryl-10-methyl-mercaptan



5-Bromo-9,10-dimethyl-1,2-benzanthracene



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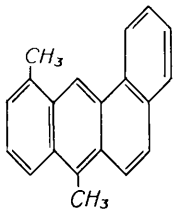
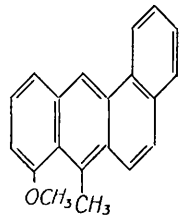
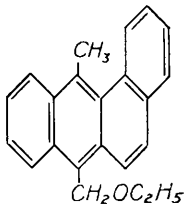


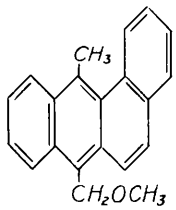
8,10-Dimethyl-1,2-benz-anthracene



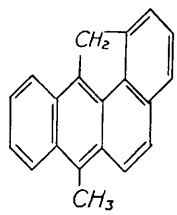
5-Methoxy-10-methyl-1,2-benzanthracene



9-Methyl-10-ethoxymethyl-1,2-benzanthracene



9-Methyl-10-Methoxymethyl-1,2-benzanthracene



10-Methyl-1',9-methylene-1-2-benzanthracene

PLATE I.—Active compounds.

sarcomas were found in association with periorchitis, but a frank hernia was demonstrated in only 1 case.

When the mice were 3 to 5 months old the hydrocarbon to be tested was injected under the skin of the rump. Tricaprylin, kindly prepared by Dr. E. B. Hershberg, was used as a solvent (17) with a few exceptions as noted in the tables. This is a synthetic triglyceride of caprylic acid, and in addition to its excellent solvent properties it has the advantage of being a pure substance of known structure, unlike sesame oil and other liquid vehicles in common use. Some of the compounds that failed to dissolve completely were injected as suspensions in tricaprylin, and a few were suspended in glycerine. The great majority of the compounds were tested at a constant dosage of 2 mgm., per animal, administered in a single injection.

At weekly intervals after injection the mice were inspected for tumors. All were kept alive as long as possible, and at death were autopsied. All tissues suggestive in the gross of neoplasia were examined histologically. The induction time was computed as the number of days between injection and the first appearance of a readily palpable nodule at the injection site—provided that the nodule showed progressive growth and proved to be a malignant tumor on histological examination. Practically all the verified tumors were either fibrosarcomas or rhabdomyosarcomas.

Certain arbitrary standards were adopted as minimal criteria of the adequacy of the tests. No compound was considered inactive unless it had failed to produce tumors after administration to at least 20 mice, of which at least 10 survived for 6 months or more after injection. Tumors appearing elsewhere than at the site of injection are noted in the tables, but were not considered as evidence of carcinogenic activity. Compounds producing malignant tumors at the injection site in one or more of the test mice were arbitrarily classed as "active," even though it is realized that the induction of a single tumor is weak evidence of carcinogenic activity. In this connection it might be mentioned that in another series of experiments involving the subcutaneous injection of India ink, a subcutaneous sarcoma arose in one of 40 C3H mice at the site of injection of the ink. Commercial India ink has been injected into innumerable experimental animals without producing tumors, and we do not consider this one growth sufficient justification for placing it in the increasing list of carcinogenic agents.

The great majority of the compounds tested were substitution products of the four familiar hydrocarbons, 1,2-benzanthracene, 20-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene. The derivatives of each and an additional group of miscellaneous substances will be considered separately in a series of papers. Part I (12) dealt with chrysene derivatives and the present section, Part II, will be devoted to derivatives of 1,2-benzanthracene.

Carcinogenic Tests On 1,2-Benzanthracene Derivatives

Of the 6 possible hydrocarbons consisting of 4 condensed aromatic rings (3) 1,2-benzanthracene has been the most thoroughly studied from the point of view of carcinogenic derivatives. Although 1,2-benzanthracene itself is inactive (18) some of its methyl and dimethyl substitution products are among the most rapidly acting agents known. About 100 compounds that may be considered immediate derivatives of 1,2-benzanthracene have been synthesized and tested, as outlined in reviews by Fieser (15) and by Cook and his associates (7, 2) and listed in a survey by Hartwell (18), and over a third of them have produced tumors in mice or rats when applied by skin painting or injection. The present paper reports the results of tests on 38 additional derivatives.

In order to provide a basis for comparison control tests were carried out with the 4,10-ace-(or dimethylene), 10-methyl-, and 9,10-dimethyl- derivatives of 1,2-benzanthracene, three compounds that are among the most actively carcinogenic derivatives of 1,2-benzanthracene hitherto reported (20). In our hands they induced tumors in an average time of 161 days, 128 days, and 100 days respectively.

RESULTS

The results obtained with the new derivates of 1,2-benzanthracene are presented in Tables II and III, which are for the most part self-explanatory. Table I gives the details of the tests on the 3 active control substances; Table II includes the 12 compounds that produced tumors; and Table III contains the 26 inactive compounds. Several of the derivatives deserve special comment.

1,2-Benzanthryl-3-Carbamidoacetic Acid
1,2-Benzanthryl-10-Carbamidoacetic Acid

Synthesized by Dr. Hugh J. Creech, these are closely related to the compounds developed by Creech and Franks in an attempt to obtain antigenic carcinogens (8); though theirs showed antigenic properties the preservation of carcinogenic activity was not clearly demonstrated. The new compounds under discussion are both sparingly soluble in weak aqueous solutions of sodium hydroxide, and the only tumors obtained followed the injection of aqueous solutions or suspensions. But since only a single tumor was

produced in each instance, neither of these compounds is sufficiently potent to render it useful as a water-soluble carcinogen. Such a compound is probably available in ε-(1,2,5,6,-dibenzanthryl-9-carbamido)-caproic acid, as will be reported in a later paper (14).

None of the 6 other carbamido-linked substitution products of 1,2-benzanthracene (Table III) produced tumors, a particularly disappointing outcome in the case of the two conjugated proteins prepared by linking horse-serum albumin to benzanthracene. The successful synthesis of these conjugated proteins *in vitro* (9) suggests the theoretical possibility of a protein-hydrocarbon combination *in vivo,* furnishing a hypothetical point of attack by a carcinogenic agent on living tissues. An attempt was made in collaboration with Dr. R. Norman Jones to demonstrate a protein-hydrocarbon complex in extracts of the tissues of rats injected with dibenzanthracene, but serious technical difficulties were encountered and the experiment was not successful.

Seven other sulfur-containing derivatives, including 4 thiocyanates, 1 isothiocyanate and two containing cysteine, were tested with negative results (Table III). The thiocyanate groups were substituted in the benzanthracene molecule at the "active" 9- and 10- positions, and since these positions are optimal for the expression of the carcinogenic effects of other substituents it is doubtful that any monothiocyanate of 1,2-benzanthracene will prove active.

1,2-Benzanthryl-10-Isocyanate

Isocyanates of 1,2-benzanthracene have not previously been tested for carcinogenic properties. Of the 3 available in this series 1,2-benzanthryl-10-isocyanate gave a single tumor, while the 3-isocyanate and the 10-methyl-3-isocyanate were inactive. Since 10-methyl-1,2-benzanthracene is such a potent carcinogen the weak activity of a compound with isocyanate in the 10- position would suggest that the isocyanate group has limited ability to confer carcinogenic properties, a conclusion that gains support from the negative results obtained with the other 2 isocyanates. However, we have observed (14) that the substitution of isocyanate in the 9- position of dibenzanthracene results in an increase of activity over that of the parent compound, and here again the nature of the substituent group may be of less importance than the molecular position of substitution and the structure of the parent molecule.

Table I: Active Control Compounds

Compound	Date of injection	Number of mice	Strain	Dose in mgm.	Vehicle*	Route	Effectual total†	Tumors at site of injection	Induction time of earliest tumor, days	Average induction time, days	Transplants	Comments
10-Methyl-1, 2-benzanthracene	Feb. 29, 1940	10	Swiss	5.0	0.2 cc tricap.	subcut.	10	7	96	128	....	2 mice with pulmonary adenoma at 96 and 231 days respectively.
9, 10-Dimethyl-1, 2-benzanthracene	Jan. 9, 1940	20	Swiss	2.0	0.1 cc. tricap.	subcut.	16	10	67	100	....	2 mice with epidermoid carcinoma, 6 with leukemia, and 3 with adenoma of lung, the latter at 100, 112, and 114 days respectively.
	Oct. 28, 1941	15	Swiss	8.0	0.6 cc tricap.	subcut.	5	5	86	101	....	
4, 10-Ace-1, 2-benzanthracene	Oct. 28, 1941	20	C3H	2.0	0.2 cc tricap.	subcut.	18	6	99	161	....	

* tricap = tricaprylin.

† Effectual total = number of mice alive when first tumor appeared.

1,2-Benzanthryl-10-Mercaptan
1,2-Benzanthryl-10-Methylmercaptan

These sulfur-containing derivatives both proved to be of only weak carcinogenic activity, yet they have considerable theoretical interest. Wood and Fieser (24), in their article describing the syntheses, suggest among other things that sulfur derivatives of carcinogens, formed *in vivo,* may represent biological intermediates in the process of carcinogenesis, or of "detoxification," or both. It will be noted that in one instance carcinogenic activity was conferred on the inert 1,2-benzanthracene molecule by substituting a sulfhydryl group in the 10- position, whereas in the other instance the potent carcinogen, 10-methyl-1,2-benzanthracene, was largely inactivated by substituting sulfhydryl for one of the methyl hydrogens. In these compounds the presence of sulfur in the substituent group appears to have less biological importance than the position of substitution.

5-Bromo-9,10-Dimethyl-1,2-Benzanthracene

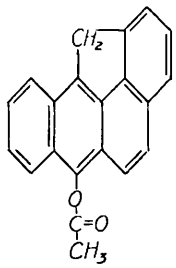
This is the product of substituting bromine in the highly carcinogenic 9,10-dimethyl-1,2-benzanthracene molecule. The tests showed very little loss of activity, and tumors appeared in all surviving mice in an average time of only 117 days. The trimethyl analogue, 5,9,10-trimethyl-1,2-benzanthracene, which Bachmann and his co-workers (1) tested by skin painting, appears to be less active.

TABLE II: ACTIVE COMPOUNDS

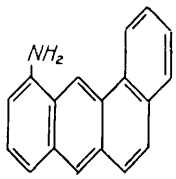
Compound	Date of injection	Number of mice	Strain	Dose in mgm.	Vehicle*	Route	Effectual total†	Tumors at site of injection	Induction time of earliest tumor, days	Average induction time, days	Transplants	Comments
1, 2-Benzanthryl-3-carbamido-acetic acid	Nov. 14, 1939	30	Swiss	3×1.0	0.5 cc H_2O	subcut.	20	1	310	...	yes	2 mice with pulmonary adenoma at 394 and 681 days respectively.
	Mar. 26, 1940	15	Swiss	1.0	0.5 cc H_2O	subcut.	9	0	...	...	...	2 mice with pulmonary adenoma at 310 and 569 days respectively.
	Aug. 12, 1941	15	C3H	2.0	0.2 cc tricap.	subcut.	13	0	...	...	...	
1, 2-Benzanthryl-10-carbamido-acetic acid	Nov. 7, 1939	10	Swiss	4×1.0	0.5 cc H_2O	subcut.	6	1	643	...	...	
	Nov. 14, 1939	15	Swiss	4×1.0	0.5 cc H_2O	subcut.	6	0	...	...	...	1 mouse with pulmonary adenoma at 538 days.
	Mar. 26, 1940	15	Swiss	1.0	0.5 cc H_2O	subcut.	8	0	...	...	...	1 mouse with pulmonary adenoma at 115 days.
	Sept. 9, 1941	10	C3H	2.0	0.2 cc tricap.	subcut.	8	0	...	...	...	
1, 2-Benzanthryl-10-isocyanate	Nov. 14, 1939	10	Swiss	2.0	0.2 cc tricap.	subcut.	6	0	...	...	...	1 mouse with pulmonary adenoma at 622 days.
	"	5	Swiss	4.0	0.3 cc tricap.	subcut.	1	0	...	...	...	1 mouse with epidermoid carcinoma of bladder at 123 days.
	Apr. 22, 1940	10	C3H	1.0	0.2 cc H_2O	subcut.	9	0	...	...	...	
	"	15	Swiss	1.0	0.2 cc H_2O	subcut.	14	1	332	...	...	3 mice with pulmonary adenoma at 451, 628, and 777 days respectively.
1, 2-Benzanthryl-10-mercaptan	Apr. 22, 1940	10	Swiss	4.0	0.2 cc tricap.	subcut.	10	0	...	...	...	1 mouse with pulmonary adenoma at 451 days.
	"	20	Swiss	2.0	0.1 cc tricap.	subcut.	19	0	...	...	...	1 mouse with pulmonary adenoma at 658 days.
	"	10	C3H	2.0	0.1 cc tricap.	subcut.	9	1	281	...	yes	
	Aug. 19, 1941	20	C3H	2.0	0.2 cc tricap.	subcut.	16	2	155	173	...	
1, 2-Benzanthryl-10-methyl-mercaptan	Feb. 29, 1940	15	Swiss	2.0	0.1 cc tricap.	subcut.	10	0	...	...	...	2 mice with pulmonary adenomas at 408 and 441 days respectively.
	Mar. 4, 1941	10	C3H	2.0	0.2 cc tricap.	subcut.	9	1	241	...	...	1 mouse with leukemia.
5 Bromo-9, 10-dimethyl-1, 2-benzanthracene	July 15, 1941	20	C3H	2.0	0.2 cc tricap.	subcut.	19	19	84	117	...	1 mouse with leukemia.
5, cyano-9, 10-dimethyl-1, 2-benzanthracene	Sept. 9, 1941	5	C3H	2.0	0.2 cc tricap.	subcut.	2	2	94	97	...	
8, 10-Dimethyl-1, 2-benzanthracene	Oct. 17, 1939	5	C3H	1.0	0.1 cc tricap.	subcut.	5	5	140	175	...	
	"	5	Swiss	1.0	0.1 cc tricap.	subcut.	3	1	147	...	...	1 mouse with pulmonary adenoma at 172 days.
	"	5	Swiss	2.0	0.2 cc tricap.	subcut.	1	0	...	...	...	1 mouse with pulmonary adenoma at 353 days.
	Aug. 19, 1941	10	C3H	2.0	0.2 cc tricap.	subcut.	9	4	113	133	...	
	"	10	Swiss	2.0	0.2 cc tricap.	subcut.	3	0	...	...	...	
5-Methoxy-10-methyl-1, 2-benzanthracene	Feb. 29, 1940	10	Swiss	2.0	0.2 cc tricap.	subcut.	6	0	...	...	...	1 mouse with pulmonary adenoma at 583 days.
	Jan. 14, 1941	20	C3H	2.0	0.2 cc tricap.	subcut.	15	7	100	198	yes	
9-Methyl-10-ethoxymethyl-1, 2-benzanthracene.	Dec. 10, 1940	20	C3H	2.0	0.2 cc tricap.	subcut.	19	6	176	244	yes	
9-Methyl-10-methoxymethyl-1, 2-benzanthracene	Nov. 12, 1940	20	C3H	2.0	0.2 cc tricap.	subcut.	12	3	238	335	...	
10-Methyl-1'9-methylene-1, 2-benzanthracene	Nov. 7, 1939	15	Swiss	1.0	0.2 cc tricap.	subcut.	2	1	441	...	...	1 mouse with pulmonary adenoma at 519 days.
	"	5	Swiss	2.0	0.3 cc tricap.	subcut.	5	2	231	258	...	1 mouse with lymphoma at 330 days.
	Feb. 25, 1941	20	C3H	2.0	0.2 cc tricap.	subcut.	19	7	149	218	...	

* tricap = tricaprylin.

† Effectual total = number of mice alive when first tumor appeared. In groups in which no tumors appeared, the number alive 6 months after injection is used.



10-Acetoxy-1',9-methylene-1,2-benzanthracene



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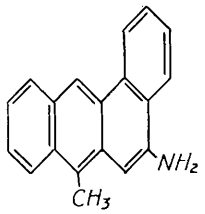

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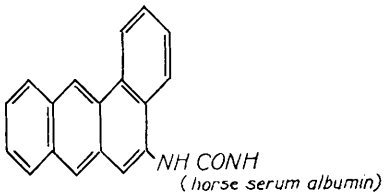
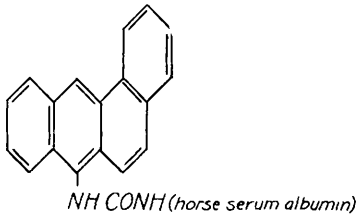
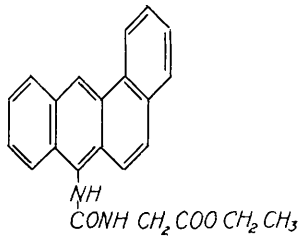


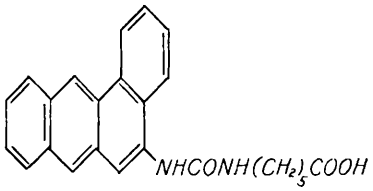
1,2-Benzanthryl-3-carbamido-horse serum albumin



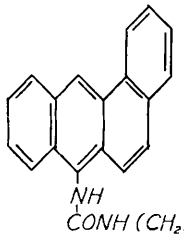
1,2-Benzanthryl-10-carbamido-horse serum albumin



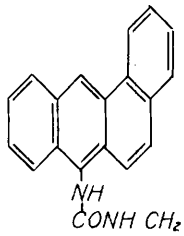
Ethyl ester of 1,2-benzanthryl-10-carbamidoacetic acid



ε-(1,2-Benzanthryl-3-carbamido)-caproic acid



ε-(1,2-Benzanthryl-10-carbamido)-caproic acid



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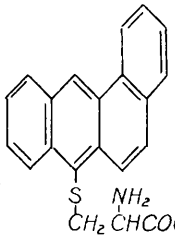
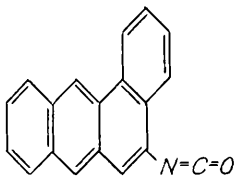
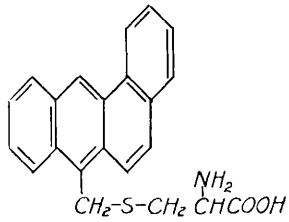


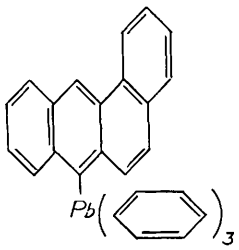
1,2-Benzanthryl-10-S-dl-cysteine



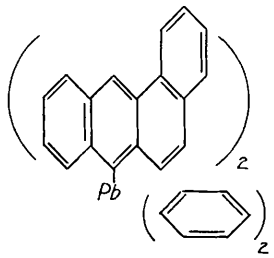
1,2-Benzanthryl-3-isocyanate



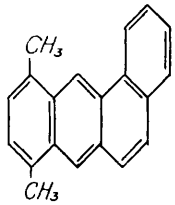
1,2-Benzanthryl-10-methyl-S-l-cysteine



10-(1,2-Benzanthryl)-triphenyl-lead

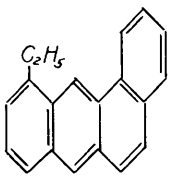


10-(Di-1,2-benzanthryl)-diphenyllead

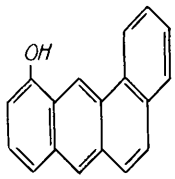


5,8-Dimethyl-1,2-benzanthracene

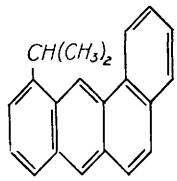
PLATE II.—Inactive compounds.



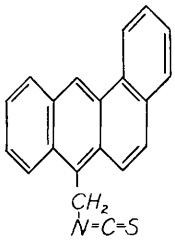
8-Ethyl-1,2-benzanthracene



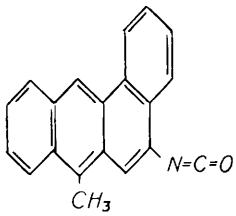
8-Hydroxy-1,2-benzanthracene



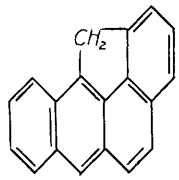
8-Isopropyl-1,2-benzanthracene



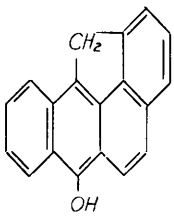
10-Isothiocyanomethyl-1,2-benzanthracene



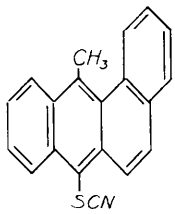
10-Methyl-1,2-benzanthryl-3-isocyanate



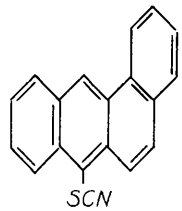
1',9-Methylene-1,2-benzanthracene



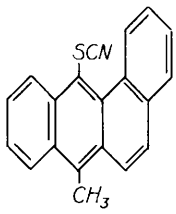
1',9-Methylene-1,2-benz-10-anthranol



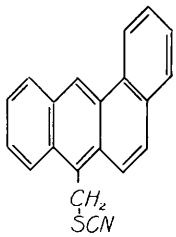
9-Methyl-10-thiocyano-1,2-benzanthracene



10-Thiocyano-1,2-benzanthracene



9-Thiocyano-10-methyl-1,2-benzanthracene



10-Thiocyanomethyl-1,2-benzanthracene

PLATE III.—Inactive compounds (continued).

TABLE III: INACTIVE COMPOUNDS

Compound	Date of injection	Number of mice	Strain	Dose in mgm.	Vehicle*	Route	Number of mice alive at 6 months	Comments
10-Acetoxy-1'9-methylene-1, 2-benzanthracene	Jan. 25, 1940	20	Swiss	1.5	0.2 cc. tricap.	subcut.	18	1 mouse with pulmonary adenoma at 530 days;
	Aug. 12, 1941	15	C3H	2.0	0.2 cc. tricap.	subcut.	11	1 with sarcoma of shoulder at 496 days, not at site of injection.
8-Amino-1, 2-benzanthracene	Oct. 17, 1939	5	C3H	1.0	1.0 cc. tricap.	subcut.	3	1 mouse with pulmonary adenoma at 345 days.
	"	5	Swiss	1.0	1.0 cc. tricap.	subcut.	5	
	"	5	Swiss	2.0	0.2 cc. tricap.	subcut.	1	
3-Amino-10-methyl-1, 2-benzanthracene	Dec. 17, 1940	20	C3H	2.0	0.2 cc. tricap.	subcut.	11	
1, 2-Benzanthryl-3-carbamido-horse serum albumin	July 10, 1940	15	C3H	2.0	1.0 cc. water	subcut.	10	
	"	15	Swiss	2.0	1.0 cc. water	subcut.	10	
1, 2-Benzanthryl-10-carbamido-horse serum albumin	July 10, 1940	15	C3H	2.0	0.7 cc. water	subcut.	9	2 mice with pulmonary adenoma at 353 and 497 days respectively.
	"	15	Swiss	2.0	0.7 cc. water	subcut.	7	1 mouse developed sarcoma of foot.
Ethyl ester of 1, 2-benzanthryl-10-carbamidoacetic acid	Nov. 14, 1939	20	Swiss	2.0	0.2 cc. tricap.	subcut.	14	
	Sept. 9, 1941	10	C3H	2.0	0.2 cc. tricap.	subcut.	6	
ε-(1, 2 Benzanthryl-3-carbamido)-caproic acid	Nov. 21, 1939	25	Swiss	3×0.5	0.5 cc. water	subcut.	5	1 mouse with pulmonary adenoma at 253 days.
	Dec. 12, 1939	10	Swiss	1.0	1.0 cc. water	intra-peritoneal	4	
	Mar. 11, 1941	10	C3H	2.0	0.2 cc. tricap.	subcut.	9	
ε-(1, 2-benzanthryl-10-carbamido)-caproic acid	Nov. 21, 1939	25	Swiss	3×0.5	0.5 cc. water	subcut.	6	1 mouse with pulmonary adenoma at 811 days.
	Dec. 12, 1939	15	Swiss	1.0	1.0 cc. water	intra-peritoneal	7	1 mouse with pulmonary adenoma at 253 days.
	Mar. 11, 1941	15	C3H	2.0	0.2 cc. tricap.	subcut.	14	
1, 2-Benzanthryl-10-carbamidoethanol	Nov. 21, 1939	25	Swiss	1.0	0.25 cc. tricap.	subcut.	9	1 mouse with pulmonary adenoma at 584 days.
	Mar. 4, 1941	10	C3H	2.0	0.2 cc. tricap.	subcut.	9	
1, 2-Benzanthryl-10-S-d1-cysteine	Dec. 10, 1940	20	C3H	2.0	0.2 cc. tricap.	subcut.	11	
1, 2-Benzanthryl-3-isocyanate	Nov. 14, 1939	10	Swiss	2.0	0.2 cc. tricap.	subcut.	7	
	Jan. 14, 1939	5	Swiss	4.0	0.4 cc. tricap.	subcut.	5	1 mouse with pulmonary adenoma at 415 days.
	Apr. 22, 1940	10	C3H	1.0	0.2 cc. water	subcut.	9	
	"	15	Swiss	1.0	0.2 cc. water	subcut.	14	1 mouse with pulmonary adenoma at 371 days.
1, 2-Benzanthryl-10-methyl-S-1-cysteine	Jan. 25, 1940	15	Swiss	2.0	0.2 cc. glycerine	subcut.	7	
	Feb. 25, 1941	10	C3H	2.0	0.2 cc. tricap.	subcut.	7	
10-(1, 2-Benzanthryl)-triphenyllead	June 10, 1941	20	C3H	2.0	0.2 cc. tricap. (suspension)	subcut.	10	
10-(Di-1, 2-benzanthryl)-diphenyllead	June 10, 1941	20	C3H	2.0	0.2 cc. tricap. (suspension)	subcut.	12	

* tricap = tricaprylin.

TABLE III: INACTIVE COMPOUNDS—*Continued*

Compound	Date of injection	Number of mice	Strain	Dose in mgm.	Vehicle*	Route	Number of mice alive at 6 months	Comments
5, 8-Dimethyl-1, 2-benzanthracene	Aug. 29, 1939	15	C3H	1.0	0.7 cc. tricap.	subcut.	14	
	"	10	Swiss	1.0	0.7 cc. tricap.	subcut.	10	2 mice with pulmonary adenoma at 481 and 530 days respectively and 1 with uterine leiomyoma at 551 days.
	Sept. 16, 1939	5	C3H	2.0	0.1 cc. tricap.	subcut.	4	
	"	10	C3H	2.0	0.25 cc. glycerine	subcut.	9	
	"	5	Swiss	2.0	0.1 cc. tricap.	subcut.	5	
	"	10	Swiss	2.0	0.25 cc. glycerine	subcut.	10	1 mouse with pulmonary adenoma at 471 days.
8-Ethyl-1, 2-benzanthracene	Oct. 10, 1939	10	C3H	2.0	0.05 cc. tricap.	subcut.	9	
	"	5	Swiss	2.0	0.05 cc. tricap.	subcut.	1	
	"	5	C3H	1.0	0.2 cc. glycerine	subcut.	4	
	"	10	C3H	1.0	0.2 cc. glycerine	subcut.	5	3 mice with pulmonary adenoma at 430, 569, and 594 days respectively.
8-Hydroxy-1, 2-benzanthracene	Oct. 10, 1939	5	C3H	2.0	0.1 cc. tricap.	subcut.	0	
	"	5	Swiss	2.0	0.1 cc. tricap.	subcut.	4	
	Jan. 7, 1941	14	C3H	2.0	0.2 cc. tricap.	subcut.	12	
8-Isopropyl-1, 2-benzanthracene	Feb. 10, 1940	20	C3H	2.0	0.2 cc. tricap.	subcut.	13	
10-Isothiocyanomethyl-1, 2-benzanthracene	May 19, 1941	20	C3H	2.0	0.2 cc. tricap.	subcut.	16	
10-Methyl-1, 2-benzanthryl-3-isocyanate	Dec. 17, 1940	20	C3H	2.0	0.2 cc. tricap.	subcut.	14	
1′, 9-Methylene-1, 2-benzanthracene	Aug. 29, 1939	15	C3H	1.0	0.5 cc. tricap.	subcut.	14	1 mouse with 3 tumors: pulmonary adenoma, hepatic adenoma, and benign hemangioma of rump at 539 days.
	"	15	Swiss	1.0	0.5 cc. tricap.	subcut.	10	
	Sept. 16, 1939	5	C3H	2.0	0.1 cc. tricap.	subcut.	5	
	"	5	Swiss	2.0	0.1 cc. tricap.	subcut.	2	
	"	10	C3H	2.0	0.25 cc. glycerine	subcut.	7	
	"	5	Swiss	2.0	0.25 cc. glycerine	subcut.	1	
1′, 9-Methylene-1, 2-benz-10-anthranol	Jan. 9, 1940	10	Swiss	1.0	0.03 cc. tricap.	subcut.	3	1 mouse with pulmonary adenoma at 608 days.
	"	5	Swiss	4.0	0.12 cc. tricap.	subcut.	5	
	June 24, 1941	15	C3H	2.0	0.2 cc. tricap.	subcut.	10	
9-Methyl-10-thiocyano-1, 2-benzanthracene	Dec. 17, 1940	20	C3H	2.0	0.2 cc. tricap.	subcut.	17	
	June 10, 1941	20	C3H	2.0	0.2 cc. tricap.	subcut.	16	
10-Thiocyano-1, 2-benzanthracene	May 6, 1941	20	C3H	2.0	0.2 cc. tricap.	subcut.	14	
9-Thiocyano-10-methyl-1, 2-benzanthracene	June 3, 1941	15	C3H	2.0	0.2 cc. tricap.	subcut.	15	
10-Thiocyanomethyl-1, 2-benzanthracene	May 13, 1941	20	C3H	2.0	0.2 cc. tricap.	subcut.	13	

* tricap = tricaprylin.

The presence of bromine in the molecule opens the possibility of substituting radioactive bromine to serve as a tracer in studying the metabolism of the carcinogen at the site of tumor induction, and a similar suggestion has previously been made concerning the sulfur atom in 4,9-dimethyl-5,6-benzthiophanthrene (11). However, work on the latter compound at Dr. Fieser's laboratory has not yet perfected a synthesis sufficiently economical of sulfur to be practicable, and the present synthesis of the 5-bromo compound is likewise not adapted to the introduction of radioactive bromine.

5-Cyano-9,10-Dimethyl-1,2-Benzanthracene

Only a few milligrams of this material were available for testing, but the meager data suggest that it is highly active. Shear (21) found comparable activity in the closely related compound, 5-cyano-10-methyl-1,2-benzanthracene, whereas his 6-cyano-isomer was inactive.

8,10-Dimethyl-1,2-Benzanthracene

Fourteen of the 28 possible dimethyl isomers of 1,2-benzanthracene had previously been tested (18). This new member of the series, only moderately active, serves again to indicate the importance of a substituent at the 9- or 10- positions in imparting activity to benzanthracene derivatives. In our hands the 5,8-dimethyl isomer yielded no tumors, nor did the 8-amino, 8-ethyl, 8-isopropyl or 8-hydroxy derivatives, and Shear (22) found 8-methyl-1,2-benzanthracene inactive. The evidence at hand indicates that benzanthracene derivatives with substituents at the 8 position are likely to be carcinogenically weak or inert.

5-Methoxy-10-Methyl-1,2-Benzanthracene
9-Methyl-10-Ethoxymethyl-1,2-Benzanthracene
9-Methyl-10-Methoxymethyl-1,2-Benzanthracene

These 3 compounds have in common the presence of oxygen in one of the substituent groups. Similar compounds in the past have shown at best only slight or moderate activity (15). If the parent substances be considered as 9-methyl- and 10-methyl-1,2-benzanthracene the results indicate that the substitution of the oxygen-containing functional groups resulted in each case in a partial loss of activity.

10-Methyl-1',9-Methylene-1,2-Benzanthracene

This compound, as previously noted, is related structurally to both 1,2-benzanthracene and benzpyrene. The 1',9-methylene group completes a 5-carbon ring structure in the position occupied by a 6-carbon ring in benzpyrene. However, the 5-membered ring in the 1',9- position does not appear to confer any carcinogenic properties, since the compound proved weaker than its parent substance, 10-methyl-1,2-benzanthracene. It should also be noted (Table III) that 1',9-methylene-1,2-benzanthracene, 1',9-methylene-1,2-benz-10-anthranol, and 10-acetoxy-1',9-methylene-1,2-benzanthracene were completely inactive. The results obtained with these compounds do not accord with the suggestion of Bergman (6) that substituents "produce active carcinogens if they imitate rings which enhance tumor production."

Amino Compounds

8-amino- and 3-amino-10-methyl-1,2-benzanthracene failed to produce tumors. The 8- and 3- positions may both be considered unfavorable for testing the effect of amine as an activating substituent in benzanthracene, for the amino substituent is not incompatible with carcinogenic activity, as is shown by the fact that Shear (21) obtained tumors with 10-amino-1,2-benzanthracene; and we have found 9-amino-1,2,5,6-dibenzanthracene to be very active (14).

SUMMARY

1. Thirty-eight derivatives of 1,2-benzanthracene have been tested on mice for carcinogenic activity.

2. Twelve of these compounds produced one or more tumors each, whereas 26 were inactive.

3. Substitution of functional groups in the benzanthracene nucleus resulted in the formation of weak or inactive compounds, in keeping with the results of other workers. Exceptions to this rule were found in the cases of 5-bromo- and 5-cyano-9,10-dimethyl-1,2-benzanthracene, both of which proved to be very active.

4. The substitution of bromine for the methyl group in the 5- position of 5,9,10-trimethyl-1,2-benzanthracene apparently enhanced activity.

5. Four thiocyano and 2 amino substitution products were inactive.

6. The substitution of a methylene group in the 1',9- position of benzanthracene results in a ring structure with features common to benzpyrene and benzanthracene. However, compounds with this structural feature had little or no activity. In one instance the activity of a potent compound, 10-methyl-1,2-benzanthracene, was greatly diminished by introducing a 1',9-methylene substituent.

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