[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Sulfhydryl and Cysteine Derivatives of 1,2-Benzanthracene, 10-Methyl-1,2-benzanthracene and 3,4-Benzpyrene

BY JOHN L. WOOD[1] AND LOUIS F. FIESER

The investigation of compounds of the type mentioned in the title was prompted by a number of considerations. Information concerning the modifying influence of functional groups substituted in various positions on the biological potency of carcinogenically active hydrocarbons is interesting in itself, and derivatives having the groups OH, NH_2, Cl, Br, CN, CHO, $COCH_3$, CO_2H, SO_3H, NO_2 have already been included in the general studies. With but few exceptions (*e. g.* CN), the introduction of such substituents results in a distinct loss of biological potency, at least in the case of the rather limited number of carcinogens and positions as yet explored.[2] Since the inactive 1,2-benzanthracene is converted into carcinogens of varying degree of potency by the introduction of methyl groups at the 10-, 9-, 5- or 6-position, it is possible also that such a hydrocarbon can be endowed with carcinogenicity by suitable substitution of functional groups. Thus the 3-methoxy and hydroxy, and the 10-methoxy and amino derivatives of 1,2-benzanthracene have been found to be weakly carcinogenic.[3] Consequently, in taking up a study of sulfhydryl derivatives, we decided to attempt to introduce this group into two carcinogenic hydrocarbons and into one related non-carcinogenic substance.

Sulfhydryl derivatives of representative carcinogenic hydrocarbons seem of interest also because of the importance of sulfur compounds in tissue metabolism and because of the physiological activity so often associated with the mercapto group. Thus there are significant indications that cysteine and certain other mercaptans are capable of increasing the rate of cell proliferation.[4] Cysteine derivatives of carcinogens, like the sulfhydryl compounds, seem worthy of investigation for their possible biological actions and they are also of interest as possible end-products of metabolism (in the acetylated form). Thus bromobenzene,[5,6] naphthalene,[6] and anthracene,[7] when administered to animals are excreted in part as the mercapturic acid (acetylcysteine) derivatives. Of particular significance is the work of White and White,[8] indicating that administered methylcholanthrene, benzpyrene and pyrene enter into combination with sulfur-containing amino acids of the body and that dietary supplements of cystine and methionine counteract the growth-inhibitory effect of these hydrocarbons.

In the present state of knowledge one can only point to various possible features of interest in a series of compounds which remain to be explored, and it now seems possible that sulfur derivatives of carcinogens may be intermediates in either the detoxification reaction, the process of carcinogenesis, or both. That some form of chemical transformation of the carcinogen is involved in the series of changes resulting in tumor production is strongly suggested by the observation that the most potent carcinogens are endowed with chemical reactivity of a remarkable and unique nature,[9,10] and also by the fact that in the benzanthracene and cholanthrene series the center of this special reactivity is located at just that point in the molecule which has been recognized as a structural feature of prime importance to the development of carcinogenicity.[2] If an interaction of the carcinogen with a sulfur, oxygen or nitrogen containing constituent of the cell represents the initiating reaction leading to malignancy, as has been suggested,[2] one would expect the point of biological attack to be at the center of chemical reactivity. From this consideration, as well as for practical reasons, it seemed wise in this initial study to attempt the introduction of sulfur at the positions found to be the centers of attack in the diazo coupling[9] and lead tetraacetate oxidation[10] of the carcinogens selected.

(1) Fellow of the Finney-Howell Research Foundation.

(2) For a summary of most of the pertinent data, see Fieser, *Am. J. Cancer*, **34**, 37 (1938).

(3) Shear, *ibid.*, **36**, 211 (1939); see also Ref. 2.

(4) Brunsting and Simonsen, *J. Am. Med. Assoc.*, **101**, 1937 (1933); Hammett, *Protoplasma*, **22**, 173 (1934); Voegtlin, Johnson and Thompson, *Pub. Health Repts.*, **51**, 1689 (1936); Reimann and Toennies, *Arch. Path.*, **29**, 175 (1940).

(5) White and Jackson, *J. Biol. Chem.*, **111**, 507 (1935).

(6) Stekol, *ibid.*, **122**, 333 (1937–1938).

(7) Boyland and Levi, *Biochem. J.*, **29**, 2679 (1935); **30**, 728, 1225 (1936).

(8) White and White, *J. Biol. Chem.*, **131**, 149 (1939).

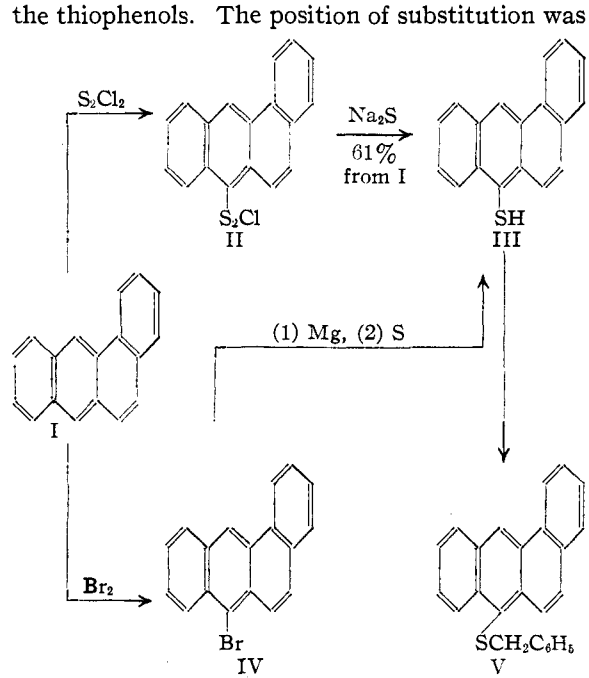
(9) Fieser and Campbell, THIS JOURNAL, **60**, 1142 (1938).

(10) Fieser and Hershberg, *ibid.*, **60**, 1893, 2542 (1938); **61**, 1565 (1939).

The only known method of direct substitution into a hydrocarbon nucleus appeared to be the condensation with sulfur monochloride, introduced by Friedländer and Simon,[11] and applied to the single case of anthracene. This hydrocarbon was shown to be converted in nearly quantitative yield into anthryl-9-dithiochloride which, in turn, could be reduced with sodium sulfide to 9-anthrylmercaptan. No further observations concerning the reaction appear to have been reported, and the fact that benzene has been employed successfully as the solvent in other reactions of sulfur monochloride conducted at the boiling point[12] suggests that the condensation of the reagent with aromatic hydrocarbons probably is applicable only to the more reactive members of the series. It was with this very type of compound that we were concerned, and we soon found that several of the carcinogenic hydrocarbons do indeed condense with sulfur monochloride with liberation of hydrogen chloride. The reaction exhibits certain peculiarities, however, and considerable experimentation was required before suitable conditions were found. Frequently there is an induction period of considerable duration before the reaction starts, but when once initiated the reaction progresses autocatalytically and may attain such vigor as to be destructive. Catalysts evidently play an important role, as indicated by the results of parallel experiments conducted without solvent using crude anthracene and a sample of highly purified hydrocarbon. In the first case a vigorous reaction ensued at once, while with pure anthracene a slow reaction started only after the lapse of several minutes. Solvent hexane added at any stage has a marked retarding influence, and advantage was taken of this effect to control the reactions and prevent tar formation. 1,2-Benzanthracene reacts more readily than pure anthracene and tends to give a tar in the absence of solvent, while if hexane is used initially the reaction often fails to start. By allowing the components to interact without solvent for a time and then adding hexane to moderate the reaction, a smooth condensation was realized. 3,4-Benzpyrene was found to react with such readiness that it was necessary to add the diluent at the very beginning. 10-Methyl-1,2-benzanthracene reacted quite readily and invariably gave a tarry product even in the presence of hexane. Methylcholanthrene behaved similarly and seemed to condense with more vigor than even benzpyrene. The formation of tars rather than solid dithiochlorides in these two cases may be an indication of a difference in the nature of the linkage established in the reaction.

In contrast to the above hydrocarbons, 1,2,5,6-dibenzanthracene was recovered unchanged after being heated with sulfur monochloride at 65°. The approximate order of increasing reactivity to this reagent is thus: dibenzanthracene, anthracene, benzanthracene, 10-methyl-1,2-benzanthracene, benzpyrene and methylcholanthrene, and this is essentially the same as found for the reactions with *p*-nitrobenzenediazonium chloride and lead tetraacetate.

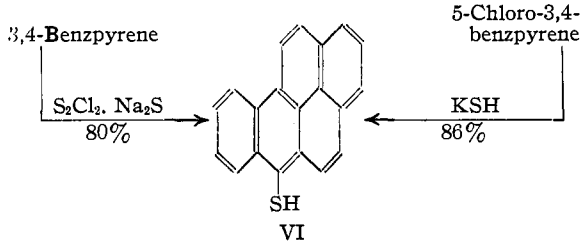
The dithiochloride from 1,2-benzanthracene was obtained as a solid but was converted without purification into the mercaptan by heating with sodium sulfide at 130°. The product was nicely crystalline, soluble in Claisen's alkali in the presence of hydrosulfite, highly sensitive to air oxidation, and gave other tests characteristic of the thiophenols. The position of substitution was



established by the preparation of the same mercaptan from the known 10-bromo compound (IV) by the interaction of the Grignard reagent[13] with sulfur, following the procedure of Taboury.[14] Comparison was made of the mercaptan samples and of samples of the stable benzyl derivative V.

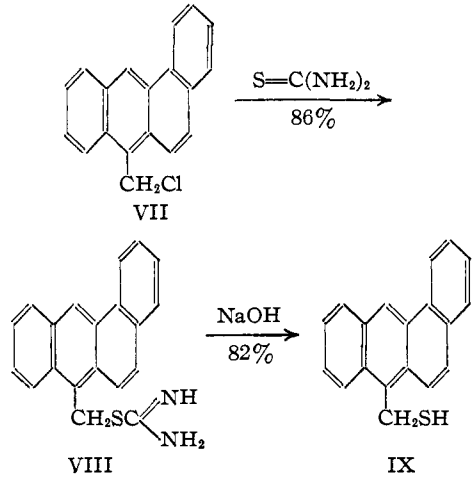
(11) Friedländer and Simon, *Ber.*, **55**, 3969 (1922).
(12) Naik and Patel, *J. Indian Chem. Soc.*, **1**, 27 (1924).
(13) Badger and Cook, *J. Chem. Soc.*, 409 (1940).
(14) Taboury, *Bull. soc. chim.*, [3] **29**, 761 (1903).

3,4-Benzpyrene was converted similarly by condensation with sulfur monochloride and reduction into a substance identified as 3,4-benzpyrenyl-5-mercaptan (VI) by its preparation from the known 5-chloro compound[15] by interaction with potassium hydrosulfide in ethanol at 150°. It is of interest to note that under similar conditions 10-bromo-1,2-benzanthracene was converted by this reagent into the hydrocarbon.



VI

The sulfur monochloride method proceeded so poorly as applied to 10-methyl-1,2-benzanthracene that another method was sought for the preparation of 1,2-benzanthryl-10-methylmercaptan (IX). The interaction of the 10-chloromethyl derivative VII[16] with potassium hydrosulfide was not tried because of the known tendency of the more reactive compounds of the benzyl halide type to form sulfides and disulfides.[17] The method of Bernthsen and Klinger[18] proved applicable and the desired mercaptan was obtained by condensing

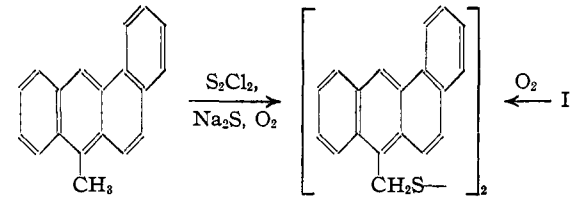


VII

VIII IX

the chloride with thiourea and hydrolyzing the resulting isothiourea derivative VIII with alkali. The benzyl derivative was obtained both by benzylation of IX and from the chloride VII and benzyl mercaptan.

Although the reaction between 10-methyl-1,2-benzanthracene and sulfur monochloride did not proceed smoothly and seemed of little preparative value, the tarry reaction product was investigated with a view to establishing the chief point of attack by the reagent. After reduction with sodium sulfide and air oxidation of the very crude mercaptan, a crystalline product was isolated in small amount having the composition of a disulfide. Since the most likely point of attachment seemed to be in the side chain, corresponding to formula XII, the disulfide in question was pre-



XI XII

pared by the air oxidation of 1,2-benzanthryl-10-methylmercaptan (IX) in aqueous dioxane containing a trace of alkali. The two disulfide preparations after somewhat laborious purification melted with decomposition at the same temperature and showed no depression when mixed. Since the melting point is high (245°) and attended with decomposition, Dr. R. N. Jones kindly made a spectrographic comparison of the samples in chloroform. Each sample gave a spectrum corresponding closely to that of 1,2-benzanthracene or its 10-methyl derivative[19] and no significant differences were discernible between the preparations. It is evident that the sulfur monochloride reaction closely parallels the lead tetraacetate oxidation and other substitutions of the hydrocarbons, both with respect to the approximate relative reactivities displayed and with regard to the position of substitution, whether this be in the nucleus or the side chain. There may be some special significance in the demonstration that sulfur can be introduced directly at a position in the molecule which seems likely to be involved in an initiating change leading to carcinogenesis. The earlier suggestion[2] that the first step consists in the introduction of a simple group such as OH, SH or NH_2 is now largely discounted, for it seems unlikely that a benzpyrene derivative of the aromatic type (ArX) could function in the subse-

(15) Windaus and Raichle, *Ann.*, **537**, 157 (1939).

(16) E. Kamp, Dissertation, Frankfort, 1936; Badger and Cook, *J. Chem. Soc.*, 802 (1939).

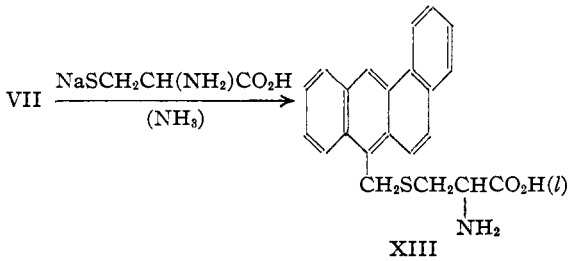
(17) Barkenbus, Friedmann and Flege, THIS JOURNAL, **49**, 2549 (1927).

(18) Bernthsen and Klinger, *Ber.*, **12**, 574 (1879).

(19) Jones, THIS JOURNAL, **62**, 148 (1940).

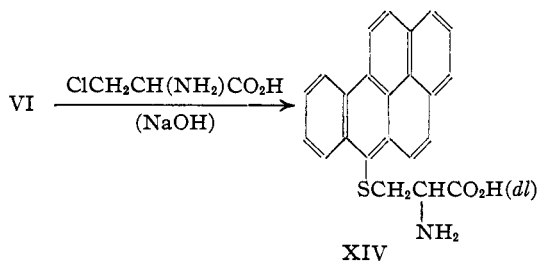
quent changes in the same manner as the structurally different type ($ArCH_2X$) derived from 10-methyl-1,2-benzanthracene and methylcholanthrene. Furthermore, such simple derivatives as have been investigated do not show enhanced carcinogenic activity and are indeed less active than the hydrocarbons. This suggests that either the very fact of reaction of the hydrocarbon is the thing of importance or that the hydrocarbon combines directly with an intact constituent of the cell protoplasm. A possible mechanism for the latter process would consist in the opening of a proteinoid S·S linkage with attachment of the carcinogen to one sulfur and of hydrogen to the other.

The cysteine derivatives corresponding to the above three mercaptans were all prepared in a satisfactory condition, if with some difficulty. The cysteine conjugate XIII was obtained, using the benzylation technique of du Vigneaud, Audrieth and Loring,[20] by the interaction of 10-chloromethyl-1,2-benzanthracene with a solution of sodium cysteinate prepared by reducing *l*-cys-



XIII

tine in liquid ammonia with sodium. The conjugate tended to form gels and decomposed when heated, but it was obtained in an analytically pure and optically active condition. 3,4-Benzpyrenyl-5-S-cysteine (XIV) was prepared by con-



XIV

densing the 5-mercaptan in a weakly alkaline dioxane–water solution under nitrogen with α - amino - β - chloropropionic acid.[21] The same method was applied successfully to the synthesis of 1,2-benzanthryl-10-S-cysteine. Some disulfide was formed in each case but could be removed by extraction with chloroform. Both conjugates have unfavorable properties but eventually were obtained crystalline. They dissolve in the cold in acidified alcohol or dioxane giving solutions which are fluorescent in ultraviolet light. The substances seem to be more unstable than the cysteine conjugates with a single aromatic nucleus examined by Baumann.[22] While Baumann's aryl cysteines were found to decompose in alkaline solution, our substances gradually break down when warmed in a neutral aqueous solution and yield the respective disulfides. This decomposition occurs so easily that the conjugates may not persist long as such on administration to animals in the tests for carcinogenic activity now being conducted by Drs. Shields Warren and C. E. Dunlap. The corresponding mercaptans are so sensitive to oxidation in an alkaline medium that they too may undergo transformation to the disulfides in the organism. Since metabolic studies have shown, for example, that both benzylcysteine and benzyl chloride are excreted as N-acetyl-S-benzylcysteine,[23] it is also possible that the mercaptans and cysteine derivatives of the present series may undergo eventual conversion to the mercapturic acids, possibly even through the intermediary of the disulfides.

Experimental Part[24]

1,2-Benzanthracene Series

1,2-Benzanthryl-10-mercaptan, III. (a) **With Sulfur Monochloride.**—Two grams of pure, colorless 1,2-benzanthracene was covered with 4 cc. of sulfur monochloride (Dow Chemical Co.). After a short delay the mixture began to evolve hydrogen chloride and the hydrocarbon then rapidly dissolved. The reaction was moderated by adding 1 cc. of hexane, used to wash the material from the walls of the flask, followed by 15–20 cc. of petroleum ether. An oil separated and soon set to a crystalline cake; this was broken up and after cooling in an ice-bath the product was collected and washed free of sulfur monochloride (odor) with petroleum ether. The crude product, m. p. 113–114°, was added in portions with stirring to a melt of 10 g. of sodium sulfide monohydrate (gas evolution). After heating at 130° under reflux for six hours the melt was cooled, treated with saturated sodium chloride containing sodium hydrosulfite and the resulting suspension of the crystalline mercaptan sodium salt was cooled to 0° and centrifuged. After decanting the supernatant liquor

(20) Du Vigneaud, Audrieth and Loring, THIS JOURNAL, **52**, 4500 (1930).

(21) E. Fischer and Raske, *Ber.*, **40**, 3717 (1907).

(22) Baumann and Preusse, *Z. physiol. Chem.*, **5**, 309 (1881); Baumann, *Ber.*, **15**, 1731 (1882).

(23) Stekol, *J. Biol. Chem.*, **124**, 129 (1938).

(24) Microanalyses by Lyon Southworth; all melting points are corrected.

the salt was suspended in oxygen-free water in an atmosphere of nitrogen and treated with acetic acid. The liberated mercaptan was separated by centrifugation and washed with very dilute hydrochloric acid, followed by water. The wet solid was dissolved in toluene, a layer of water was separated, and the solution was boiled until anhydrous, filtered, and concentrated. After adding hexane to the saturation point and allowing the solution to cool, the mercaptan separated in the form of yellow crystals, m. p. 138–138.5°; yield 1.4 g. (61%).

The compound exhibits characteristic polymorphism. When a sample was introduced into a bath at 115° it melted at once to a clear liquid, resolidified, and remelted at 138°. It usually crystallized in fine needles, but on slow undisturbed cooling it is occasionally deposited in short, stubby prisms. The lead salt is produced as a red precipitate on adding aqueous lead acetate to a methanol solution of the mercaptan. Solutions in toluene are weakly fluorescent. A sample for analysis and biological tests was sublimed at 130° and 1 mm. and crystallized four times from toluene–hexane; m. p. 139.9–140.7°.

Anal. Calcd. for $C_{18}H_{12}S$: C, 83.06; H, 4.65. Found: C, 83.17, 83.11; H, 5.02, 5.12.

The **S-benzyl derivative** was prepared by adding 0.78 cc. of 1.5 *N* sodium methoxide in methanol to a mixture of 100 mg. of the mercaptan and 0.13 cc. of benzyl chloride under protection of an atmosphere of nitrogen. After five minutes 2 cc. of methanol was added and the mixture was warmed gently to complete the reaction. After standing for two hours at room temperature the crystalline product which had separated was collected and washed with methanol and water, giving 115 mg. of material, m. p. 126–127.5°. Further crystallization from acetic acid and from hexane gave yellow needles, m. p. 128.2–129.4°.

Anal. Calcd. for $C_{25}H_{18}S$: C, 85.67; H, 5.17. Found: C, 85.39; H, 5.10.

For **oxidation**, 52 mg. of the mercaptan was warmed with 240 mg. of sodium dichromate dihydrate in 3 cc. of acetic acid to 60° and after one hour the product was precipitated with water. This material (44 mg.) was sulfur-free (m. p. 158°) and on crystallization from acetic acid and toluene gave **1,2-benzanthraquinone,** m. p. 168–169°, identified by mixed m. p. determination.

(b) **Through the Grignard Derivative.**—A suspension of 230 mg. of dry sulfur in 5 cc. of benzene was added to the Grignard reagent prepared from 2 g. of 10-bromo-1,2-benzanthracene according to the directions of Badger and Cook.[18] The pasty reaction mixture which resulted was heated gently for one hour, cooled and decomposed with dilute hydrochloric acid. Toluene (20 cc.) was added and, after warming, the water layer was removed and the organic layer was washed with water and concentrated to a small volume. On adding hexane the product soon separated in clusters of bright yellow crystals. This material (800 mg.) seemed slightly contaminated and consequently a 700-mg. sample was extracted under nitrogen with 20 cc. of Claisen's alkali (potassium hydroxide–methanol) in three portions. The extract was freed from sediment by centrifugation and treated with ice and hydrochloric acid. The precipitated mercaptan was centrifuged and washed several times with water, then crystallized from toluene–hexane. The first crop (550 mg.) melted at 135° and on recrystallization the m. p. was 136.5–137.5° and the mixed m. p. with the above sample showed no depression. The benzyl derivative was prepared and found identical with that in (a).

Bis-(1,2-benzanthryl-10)-disulfide.—Air was bubbled through a solution of 100 mg. of the mercaptan in aqueous dioxane made alkaline with sodium hydroxide and containing a trace of ferric chloride. After several hours a yellow precipitate had formed. The mixture was acidified and diluted and the product collected by centrifugation, washed and dried (90 mg.). Repeated crystallization from toluene–hexane gave clumps of yellow needles, m. p. (evacuated capillary) 208.2–209.7°, dec.

Anal. Calcd. for $C_{36}H_{22}S_2$: C, 83.36; H, 4.28. Found: C, 83.65; H, 4.71.

Action of KSH on 10-Bromo-1,2-benzanthracene.—In an early attempt to prepare the 10-mercaptan 1 g. of the bromide was mixed with a solution prepared from 5 g. of potassium hydroxide and 20 cc. of 95% ethanol and saturated with hydrogen sulfide. The mixture was heated in a sealed tube at 180° for thirty-six hours, and on cooling the tube was filled with a crystalline deposit (680 mg.) which melted at 160.4–161° and was shown to be 1,2-benzanthracene by mixed m. p. determination.

1,2-Benzanthryl-10-S-cysteine.—Under protection of a stream of nitrogen, 1 g. of 1,2-benzanthryl-10-mercaptan was dissolved in the minimum amount of cold dioxane and the solution was made alkaline to phenolphthalein with 1.7 *N* sodium hydroxide, warmed slightly, and then treated with 670 mg. of *dl*-α-amino-β-chloropropionic acid hydrochloride,[21] added in small portions. After each addition sufficient alkali was added to restore the original alkalinity. A yellow precipitate separated and some hydrogen sulfide was evolved. After standing for one hour the mixture was acidified with hydrochloric acid and the precipitated solid collected by centrifugation (460 mg.). The decanted liquor on dilution gave 70 mg. more of the yellow crude product. The total material was extracted with chloroform until no more disulfide was removed and the residue was light yellow. This was dissolved in alcohol containing a little hydrochloric acid and the solution was treated with Darco and neutralized with ammonia. The cysteine conjugate separated as a cream-colored precipitate which became crystalline on standing overnight; yield 350 mg. (25%), m. p. 190°, dec.

On attempted crystallization from glacial acetic acid the solution rapidly turned yellow, indicating the formation of the disulfide, and the product was consequently recovered, by adding water and distilling to dryness in vacuum, and the disulfide removed by extraction with chloroform. Purification of the conjugate was then accomplished by twice repeated slow precipitation from alcoholic hydrochloric acid with ammonia. The crystals were washed with alcohol, water, and again with alcohol, and dried in vacuum over phosphorus pentoxide. When heated in an evacuated capillary the substance turned yellow at 187° and decomposed at 192–194°.

Anal. Calcd. for $C_{21}H_{17}O_2NS$: N, 4.03. Found: N, 4.14.

The chloroform extracts were evaporated to dryness and the residue crystallized twice from toluene–hexane. The yellow product melted at 209–210°, dec., and was identified

as bis-(1,2-benzanthryl-10)-disulfide by mixed melting point determination.

The benzanthrylcysteine is insoluble in water and in neutral solvents but dissolves in the cold in acidified alcohol or dioxane; dilute solutions fluoresce in ultraviolet light. In a test of the course of the decomposition of the substance a suspension of 50 mg. of the conjugate in 2.5 cc. of dioxane was boiled under reflux. In ten minutes a yellow color was perceptible in the liquid, and after four hours most of the material had gone into solution. The filtered solution was diluted and the precipitated oily product separated by centrifugation. A solution of the product in toluene was treated with Darco, concentrated to a small volume, and diluted extensively with hexane. Rosettes of fine yellow crystals slowly separated; the product (2 mg.) melted at 206–208°, dec., and did not depress the m. p. of bis-(1,2-benzanthryl-10)-disulfide.

3,4-Benzpyrene Series

3,4-Benzpyrenyl-5-mercaptan, VI. (a) **With Sulfur Monochloride.**—A suspension of 1 g. of 3,4-benzpyrene in 4 cc. of petroleum ether (20–40°) was treated with 2 cc. of sulfur monochloride, added in small portions. The reaction tended to become violent but could be controlled by suitable addition of hexane. When the evolution of hydrogen chloride had nearly ceased, petroleum ether (2–3 volumes) was added, the mixture was cooled in ice and the crystalline product was collected and washed well with petroleum ether. The crude dithiochloride (1.3 g.) was fused with hydrated sodium sulfide at 130° for ten hours and the mercaptan was isolated by the procedure described above in the benzanthracene series. The crystalline product obtained from toluene–hexane amounted to 800 mg. (80%) and three crystallizations from dioxane–water gave small, pale yellow needles which decomposed at 205–206° and corresponded to the sample described in (b) except, apparently, for a somewhat higher state of purity. The more easily purified and better characterized benzyl derivative had the same melting point and mixed melting point as that described below.

(b) **From 5-Chloro-3,4-benzpyrene.**—A solution of 6 g. of potassium hydroxide in 15 cc. of 95% alcohol was saturated with hydrogen sulfide, 900 mg. of 5-chloro-3,4-benzpyrene[15] was added, and the mixture was heated in a sealed tube at 150° for fifty-five hours. The resulting solution was concentrated to about half its volume, diluted with water, acidified with acetic acid, and the precipitate collected by centrifugation and washed with dilute hydrochloric acid and with water. The moist solid was dissolved in hot toluene and the red solution was decanted from the water layer, concentrated and diluted slowly with hexane. The crystalline red product which separated was collected and dried under nitrogen; yield 770 mg. (86%), m. p. 194–195.5°, dec. This material was suspended in toluene–hexane and shaken with oxygen-free Claisen alkali. The alkaline extract was centrifuged and acidified with acetic acid under a stream of nitrogen and the precipitated material was washed twice with water by centrifugation and taken up in toluene. After distillation of the water the solution was filtered through a mat of charcoal, concentrated, and diluted with hexane. The mercaptan separated as short, red prisms, decomposing at 197–198° Three further crystallizations from toluene–hexane and one from toluene gave material decomposing at 197–198.5°. This reacted normally with aqueous lead acetate (red precipitate from methanol–dioxane) and the toluene solution showed a strong fluorescence. On combustion the sample left a residue (0.77%) and the analysis is corrected accordingly.

Anal. Calcd. for $C_{20}H_{12}S$: C, 84.45; H, 4.25. Found: C, 84.25; H, 4.40.

The **S-benzyl derivative** was prepared as above except that the amount of benzyl chloride was reduced to 1.1 equiv.; 100 mg. of mercaptan yielded 110 mg. of derivative. Crystallized twice from acetic acid and four times from methanol–toluene–ligroin the substance formed yellow crystals which decomposed at 170.2–172.2°.

Anal. Calcd. for $C_{27}H_{18}S$: C, 86.59; H, 4.85. Found: C, 86.69; H, 5.11.

The **disulfide** was prepared by aerating a solution of 100 mg. of mercaptan in 10 cc. of dioxane containing 2 drops of 2 *N* sodium hydroxide and a trace of ferric chloride; the metal halide formed a dark complex with the mercaptan which gradually disappeared as the oxidation proceeded. The disulfide separated in quantitative yield on acidification and dilution, and on repeated crystallization from chlorobenzene–toluene it formed bright bronze-red crystals, m. p. (vacuum) 271–272°, dec. The analysis was corrected for 0.4% residue.

Anal. Calcd. for $C_{40}H_{22}S_2$: C, 84.77; H, 3.91. Found: C, 84.65; H, 4.17.

3,4-Benzpyrenyl-10-S-cysteine (XIV).—The conjugation of the mercaptan VI (700 mg. in 5 cc. of dioxane) with *dl*-α-amino-β-chloropropionic acid (510 mg.) was conducted exactly as described in the benzanthracene series. On extracting the crude solid (770 mg.) with chloroform (100 cc.) and evaporating the extract there was obtained 250 mg. of red starting material, m. p. (after crystallization) 199–201°. The chloroform-insoluble material was then extracted with warm alcohol containing hydrochloric acid; this left a residue of 250 mg. which when crystallized from chlorobenzene–toluene melted at 270–272° dec., and was identified as bis-(3,4-benzpyrenyl-5)-disulfide (mixed m. p.). The alcoholic extract was neutralized with ammonia and after standing overnight the yellow cysteine conjugate was collected (170 mg.). This was dissolved in methanol containing hydrochloric acid, a few drops of water were added, and the solution was allowed to evaporate in a vacuum desiccator over sodium hydroxide. The material slowly separated in clumps of minute pale yellow crystals, which when washed with methanol, water and methanol and dried in vacuum weighed 60 mg. and decomposed with gas evolution at 146.7–147.5°. The decomposition point is highly dependent upon the rate of heating and melting points some 10–15° above that reported have been observed on inserting the capillary in a preheated bath. Neutralization of the mother liquor gave 60 mg. of less pure conjugate.

Anal. Calcd. for $C_{28}H_{17}O_2NS$: N, 3.77. Found: N, 3.50.

10-Methyl-1,2-benzanthracene Series

1,2-Benzanthryl-10-S-isothiourea (VIII).—The 10-chloromethyl-1,2-benzanthracene was prepared according to

Badger and Cook[16] from 1,2-benzanthracene, trioxymethylene and hydrogen chloride in acetic acid and crystallized twice from benzene, m. p. 190–190.6°. A solution of 3 g. of the halide in 60 cc. of boiling benzene was treated with 0.75 g. of thiourea in 20 cc. of absolute alcohol. The solution was heated under reflux until crystals began to appear and then taken to dryness on the steam-bath. The residual solid was triturated with benzene and collected, affording 3.3 g. (86%) of crude **hydrochloride.** This is insoluble in neutral organic solvents or water but can be dissolved in pyridine or acidified alcohol. A very dilute solution in methanol containing a few cc. of 2 *N* hydrochloric acid was concentrated to a small volume, and on cooling pale yellow crystals were obtained. After repeating the process the substance melted at 213–214°, dec.

Anal. Calcd. for $C_{20}H_{17}N_2SCl$: N, 7.94. Found: N, 7.75.

The **free base** was precipitated from a solution of the hydrochloride in pyridine by the addition of water. The product was collected by centrifugation and washed free of halide ion with water. It was obtained as a yellowish powder which when inserted in a bath at 160° melted and then resolidified to a waxy solid which did not become liquid at 235°.

Anal. Calcd. for $C_{20}H_{16}N_2S$: N, 8.85. Found: N, 8.62.

1,2-Benzanthryl-10-methylmercaptan (IX).—A suspension of 3 g. of the isothiourea hydrochloride in 12.5 cc. of 2 *N* sodium carbonate containing 0.1 g. of sodium hydrosulfite, 10 cc. of water and 60 cc. of methanol was refluxed for three and one-half hours. Water was added and most of the methanol was removed by distillation. Acidification with 2 *N* hydrochloric acid in an atmosphere of nitrogen precipitated a crystalline product, which was filtered and washed under nitrogen and dried over sulfuric acid in a desiccator filled with nitrogen. The crude mercaptan weighed 1.9 g. (82%), m. p. 168–173°. After three crystallizations from dioxane, one from chloroform–petroleum ether, and four from benzene–hexane the substance formed small yellow prisms, m. p. 172.5–173.9° (a sublimed and recrystallized sample melted at 172.7–174.7°).

Anal. Calcd. for $C_{19}H_{14}S$: C, 83.17; H, 5.14. Found: C, 83.21; H, 5.13.

The **benzyl derivative** was prepared (a) from 100 mg. of the mercaptan IX and 0.13 cc. of benzyl chloride and sodium methylate in methanol, giving a product which when crystallized from glacial acetic acid melted at 150.2–150.6°. Also (b), a suspension of 500 mg. of 10-chloromethyl-1,2-benzanthracene with 0.5 cc. of benzylmercaptan and 1.3 cc. of 1.5 *N* sodium methylate solution in 5 cc. of methanol and 5 cc. of benzene was warmed gently, when the material rapidly dissolved and colorless needles began to separate. The crystalline product collected after cooling weighed 550 mg. (87%), m. p. 148–149.5°. A sample repeatedly crystallized from glacial acetic acid melted at 150.1–150.5° and the mixed melting point with (a) showed no depression.

Anal. Calcd. for $C_{26}H_{20}S$: S, 8.79. Found: S, 8.43.

Bis-(1,2-benzanthryl-10-methyl)-disulfide (XII). (a) From the Mercaptan IX.—Air oxidation of 100 mg. of mercaptan by the method described above gave 70 mg. of crude disulfide, m. p. 230–232°, dec. After repeated crystallization from chlorobenzene–hexane the substance formed pale yellow microcrystals, m. p. (vacuum) 244.5–245°, dec. Decomposition occurred on attempted sublimation in high vacuum. The analysis was corrected for 0.35% ash.

Anal. Calcd. for $C_{38}H_{26}S_2$: C, 83.48; H, 4.79. Found: C, 83.24; H, 5.06.

(b) **From 10-Methyl-1,2-benzanthracene and Sulfur Monochloride.**—The required **10-methyl-1,2-benzanthracene** was prepared by a convenient new process based upon observations of Dr. R. B. Sandin in this Laboratory. A solution of 9 g. of stannous chloride crystals in 10 cc. of concentrated hydrochloric acid and 10 cc. of dioxane was added to a solution of 3 g. of 10-chloromethyl-1,2-benzanthracene in 50 cc. of dioxane. A transient yellow color was observed and the solution then became colorless. After warming on the steam-bath for one-half hour, 500 cc. of water was added, giving a nearly white precipitate. A single crystallization from alcohol gave 2.15 g. of pure hydrocarbon, m. p. 139.6–140.0°, identified as 10-methyl-1,2-benzanthracene by mixed melting point determination. A second crop of 150 mg. was obtained from the mother liquor.

On adding 1 cc. of sulfur monochloride to a suspension of 800 mg. of the hydrocarbon in 4 cc. of hexane at room temperature there was an induction period of about one-half hour before an evolution of hydrogen chloride was observed. The reaction then proceeded rapidly and resulted in the formation of a viscous gum. This was triturated with several successive portions of petroleum ether, which was decanted, and eventually obtained as a sticky solid. This was suspended in 30 cc. of methanol containing 10 g. of hydrated sodium sulfide and the mixture was boiled for a time under reflux. The methanol was then allowed to distil slowly until the temperature had risen to 130°. Saturated sodium chloride solution containing hydrosulfite was added and the precipitated salt collected by centrifugation, washed with salt solution, suspended in water and acidified with acetic acid. The precipitated crude mercaptan (pale yellow lead salt in the test with lead acetate) was oxidized with air in alkaline dioxane–methanol in the presence of ferric chloride as usual. The crude product was oily, but after suitable processing was obtained crystalline from toluene–hexane (210 mg., 222–227°; 70 mg., 185°). Repeated crystallization from chlorobenzene–hexane–methanol finally afforded 40 mg. of yellow crystalline material of the constant m. p. (vacuum) 243–244°, dec. This gave no depression when mixed with the sample prepared as in (a).

Anal. Calcd. for $C_{38}H_{26}S_2$: C, 83.48; H, 4.79. Found (corrected for ash): C, 83.12; H, 4.97.

1,2-Benzanthryl-10-methyl-S-*l*-cysteine (XIII).—Anhydrous liquid ammonia (50 cc.) was collected in a test-tube fitted with a stopper and a soda-lime tube and a few glass beads were added to the liquid to aid in stirring, followed by a piece of sodium the size of a pea. *l*-Cystine ($[\alpha]^{20}D$ −220°) was added until the blue color was discharged, another piece of sodium was added, and the process was repeated until 1 g. of cystine had been reduced. Enough sodium was used to cause the blue color to persist for ten

minutes, and ammonium chloride was then added until a colorless solution resulted. At this point 10 cc. of dry toluene and 1 g. of finely pulverized 10-chloromethyl-1,2-benzanthracene were added and the suspension was shaken vigorously for twenty to thirty minutes with occasional immersion of the test-tube in a carbon dioxide cooling-bath. The ammonia was allowed to evaporate and the residue was warmed on the steam-bath with 10 cc. of benzene for fifteen minutes. The solid was then collected and suspended in water, and the mixture rendered acidic to congo red with hydrochloric acid. The crude conjugate was then collected, washed and dried (2 g.) and crystallized from a hot mixture of dioxane and 2 *N* hydrochloric acid, the solution, after clarification with Norit, being allowed to cool slowly. The substance at first separated as a gel but became crystalline on standing. It was collected and washed, dried over phosphorus pentoxide, recrystallized from dioxane–hydrochloric acid, and finally digested at room temperature with dilute sodium bicarbonate solution and then with water. After thorough drying at 60°, the pale yellow, microcrystalline material decomposed at 205.7–206.7° with gas evolution when inserted in a bath at 205°.

Anal. Calcd. for $C_{22}H_{19}O_2SN$: N, 3.89. Found: N, 4.09.

A solution of 50 mg. of the conjugate in 5 cc. of a 2:1 mixture of dioxane and 2 *N* hydrochloric acid showed the rotation $[\alpha]^{23}D$ −7.5°. The conjugate is insoluble in water, in aqueous acid or alkali, and neutral organic solvents. The solutions in hot acidified alcohol or dioxane, which are strongly fluorescent in ultraviolet light, deposit the substance in an initially gelatinous condition on either dilution with water or neutralization of the acid.

Summary

On reaction with sulfur monochloride, followed by the reduction of the resulting dithiochloride with sodium sulfide, 3,4-benzpyrene gives the 5-mercaptan and 1,2-benzanthracene is converted into its 10-mercapto derivative, as shown by the synthesis of the identical compounds from known halides by reaction with potassium hydrosulfide, and through the interaction of the magnesium derivative with sulfur, respectively. 1,2-Benzanthryl-10-methylmercaptan was prepared best from the 10-chloromethyl derivate and thiourea, but the corresponding disulfide was also obtained from the tarry product of the reaction of the hydrocarbon with sulfur monochloride. From these observations and certain qualitative tests it is shown that, in a series of typical carcinogenic hydrocarbons and related compounds, the relative order of reactivity and the position of substitution is the same as in the reaction with lead tetraacetate.

Cysteine conjugates were prepared in the 3,4-benzpyrene and 1,2-benzanthracene series from the mercaptans and α-amino-β-chloropropionic acid. 1,2-Benzanthryl-10-methyl-S-*l*-cysteine was obtained by the interaction of the chloride with sodium cysteinate in liquid ammonia.

Certain inferences are presented concerning the process of hydrocarbon carcinogenesis.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASSACHUSETTS RECEIVED JULY 20, 1940

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Synthesis of 4′,8′-Dihydroxy-1,2,5,6-dibenzanthracene and its Relation to Products of Metabolism of the Hydrocarbon

BY JAMES CASON[1] AND LOUIS F. FIESER

Following their very thorough and significant study of the metabolism of anthracene in rats and rabbits,[2] Boyland and Levi[3] undertook to determine the form in which the carcinogenic 1,2,5,6-dibenzanthracene is eliminated from the animal organism. From the urine of rabbits which had been fed on a diet containing 0.04% of the hydrocarbon they succeeded in isolating a phenolic substance melting at 350–360°. This was characterized by certain tests and by the preparation of several derivatives as a dihydroxydibenzanthracene with the hydroxyl groups at some positions other than 4, 8, 9 and 10. Dobriner, Rhoads and Lavin[4] have reported the isolation from the urine of rabbits injected subcutaneously or intramuscularly with the hydrocarbon of a crystalline phenolic substance (m. p. 355–358°) corresponding precisely in absorption spectrum with the metabolite of Levi and Boyland. Although the small amount of material isolated sufficed for tests in only a few animals, they found the dihydroxy derivative to be definitely less carcinogenic (no tumors in six months) than the original hydrocarbon (tumors in all controls). On this basis

(1) Research Fellow on a grant from the National Cancer Institute.

(2) Boyland and Levi, *Biochem. J.*, **29**, 2679 (1935); **30**, 728, 1225 (1936).

(3) Levi and Boyland, *Chemistry and Industry*, **15**, 446 (1937).

(4) Dobriner, Rhoads and Lavin, *Proc. Soc. Exptl. Biol. Med.*, **41**, 67 (1939).