[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

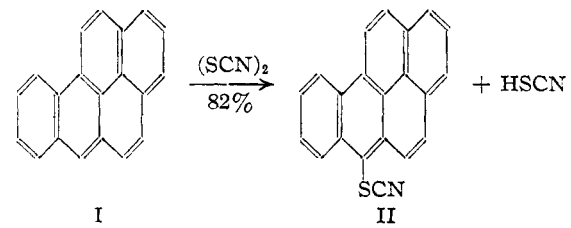
Thiocyanation of Carcinogenic Hydrocarbons

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

A previous paper reported the synthesis of certain sulfhydryl and cysteine derivatives.[2] Tests by Drs. Shields Warren and C. E. Dunlap by subcutaneous injection of the substances in tricaprylin solution into mice have given no indication of pronounced carcinogenicity for any of the six sulfhydryl and cysteine compounds described, and the derivatives of 3,4-benzpyrene and 10-methyl-1,2-benzanthracene have failed to produce tumors by the end of the latent periods observed for the parent hydrocarbons. It seems safe to conclude that these sulfur-substituted derivatives cannot function as intermediates in the process of hydrocarbon carcinogenesis but that these or isomeric substances may possibly afford one route for the metabolic detoxification of the hydrocarbons.[3] The observation that a sulfur-substituent can be introduced easily by interaction of the more potently active carcinogens with sulfur monochloride led us to suggest[2] that a tumor-initiating reaction utilizing the unique chemical reactivity of the hydrocarbons may consist in a direct combination with a proteinoid constituent of the cell by the opening of a disulfide linkage and formation of a sulfur-substituted hydrocarbon conjugate. The present paper reports a new reaction which affords a chemical model of the postulated biotransformation.

This consists in the direct nuclear or side-chain thiocyanation of the hydrocarbon: ArH + $(SCN)_2$ → ArSCN + HSCN. Heretofore thiocyanogen[4] has not been observed to react substitutively with aromatic hydrocarbons, and additions of the unstable halogenoid to the ethylenic double bond often occur only very slowly with the preformed reagent[4-9] in the dark[9,10] but can be promoted by the use of the nascent reagent[11,12,13] or by illumination.[9] Only one mole of thiocyanogen adds to acetylene[9] or to dienes[14] even under forcing conditions. That benzene is indifferent is evidenced by its use as solvent in the photocatalytic addition of thiocyanogen to alkenes,[9] and the only prior instances of aromatic substitutions are with amines and phenols.[4] These substances are thiocyanated exclusively in the para position,[7,8,9,11] if available, and even with *o*- and *m*-aminophenol only one group ordinarily is introduced (para to NH_2).[8] The limited scope of the reaction is further indicated by the fact that preformed thiocyanogen under ordinary conditions reacts with anthranilic acid[8] but not with salicylic acid,[8,13] and that the thiocyanation of the latter acid requires either special conditions of catalysis[13] or the use of nascent reagent.[11]

That certain carcinogenic hydrocarbons undergo ready thiocyanation thus provides further evidence of their special susceptibility to substitution. When treated in carbon tetrachloride with thiocyanogen prepared from lead thiocyanate and bromine,[4] 3,4-benzpyrene was converted after one hour at room temperature into a single thiocyano derivative in 82% yield; the reaction probably is promoted by the crystallization of the sparingly soluble product. The structure was established as that of 5-thiocyano-3,4-



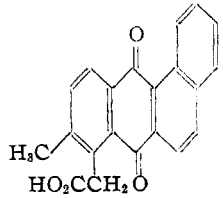
I II

benzpyrene (II) by treatment with sodium and ethanol and benzylation of the resulting crude mercaptan, which afforded a substance identical with the previously described S-benzyl-3,4-benzpyrenyl-5-mercaptan.[2] To gain some idea of the scope of the reaction as applied to hydrocarbons, we examined the behavior of anthracene. This

(1) Fellow of the Finney-Howell Research Foundation.
(2) Wood and Fieser, THIS JOURNAL, **62**, 2674 (1940).
(3) Cason and Fieser, *ibid.*, **62**, 2681 (1940).
(4) Söderbäck, *Ann.*, **419**, 217 (1919).
(5) Kaufmann and Kögler, *Ber.*, **58**, 1553 (1925).
(6) Kerstein and Hoffmann, *ibid.*, **57**, 491 (1924).
(7) Kaufmann and Küchler, *ibid.*, **67**, 944 (1934).
(8) Neu, *ibid.*, **72**, 1505 (1939).
(9) Söderbäck, *Ann.*, **443**, 142 (1925).
(10) Gardner, Pribyl and Weinberger, *Ind. Eng. Chem., Anal. Ed.*, **6**, 259 (1934).
(11) Kaufmann and Oehring, *Ber.*, **59**, 187 (1926).
(12) Kaufmann, *ibid.*, **62**, 390 (1929).
(13) Kaufmann, *Ber. deut. pharm. Ges.*, **33**, 139 (1923); Kaufmann and Liepe, *Ber.*, **56**, 2514 (1923).
(14) Bruson and Calvert, THIS JOURNAL, **50**, 1735 (1928).

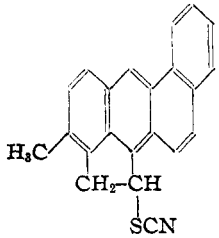
indeed can be thiocyanated and yields mixtures containing a significant amount of a disubstituted product characterized as 9,10-dithiocyanoanthracene by its oxidation to anthraquinone. Thus the reaction does not show as high a degree of specificity as diazo coupling,[15] and appears more nearly comparable with lead tetraacetate oxidation[16] and substitution by sulfur monochloride.[2] It seems rather odd that anthracene undergoes thiocyanation more readily than salicylic acid. This hydrocarbon, however, reacts much less rapidly than does 3,4-benzpyrene.

Methylcholanthrene also reacts readily but the product is more soluble and the conversion is not favored by its separation. With 1.1 equivalents of thiocyanogen the yield was only 41%, but with 2.2 equivalents a yield of 89% was realized. On oxidation the sulfur substituent is eliminated and the substance is converted into 6-methyl-1,2-benzanthraquinone-5-acetic acid[17] (III). The

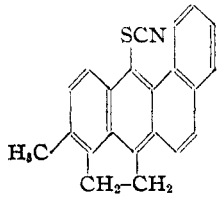


III

thiocyano group, therefore, must either have entered the *ace*-ring at the 15-position (IV) or substituted at the 11-meso position (V). Whereas



IV



V

a nuclear substitution product such as V would be expected to resemble 5-thiocyano-3,4-benzpyrene (II) in properties, the thiocyano derivative of methylcholanthrene differs considerably from this substance. In contrast to II, which is resistant to prolonged boiling with 48% hydrobromic acid and which melts sharply without decomposition at a high temperature (240°), the thiocyanomethylcholanthrene decomposes extensively at about 132°, and when it is refluxed in toluene solution the thiocyano group is eliminated and there results a hydrocarbon, m. p. 289–291°, of the composition $(C_{21}H_{14-15})_x$. In terms of formula IV for the *ace*-derivative, a likely explanation is that thiocyanic acid is eliminated and the resulting 15,16-dehydromethylcholanthrene ($C_{21}H_{14}$) polymerizes. The decomposition to a hydrocarbon seems definitely inconsistent with the alternate formulation V. Another distinctive property of the thiocyano derivative of methylcholanthrene is that it gives an immediate cherry red coloration when warmed with ferric chloride in dioxane–methanol solution. In contrast, the nuclear-substituted benzpyrenyl derivative II gives no coloration after a period of several hours.

10-Thiocyanomethyl-1,2-benzanthracene (VII), a compound of unambiguous structure which gives a similar and prompt ferric chloride color test, was prepared in good yield by the interaction of sodium thiocyanate with 10-chloromethyl-1,2-benzanthracene in boiling acetone. When heated in a capillary tube the compound melts at 138° and then solidifies and remelts sharply at 171°. A substance having the same composition and melting at the higher temperature was obtained either by conducting the preparation from the chloride VI at 100° or by heating the preformed thiocyano derivative (VII) in acetone at 100°. That the low-melting and high-melting substances are not polymorphs but contain the thiocyano and isothiocyano groups, respectively, as in formulas VII and IX, was established by their behavior toward benzylmagnesium chloride. Corresponding with the behavior of known thiocyano compounds,[18] the first substance yielded, after benzylation of the product, the known S-benzyl derivative VIII and the disulfide derivative of 1,2-benzanthryl-10-methylmercaptan.[2] The isothiocyano derivative IX, as in comparable cases,[19] afforded the addition product X. The rearrangement product IX was further characterized by conversion to the thiourethan by interaction with ethanol and sodium ethoxide,[20] and by treatment with aniline to give the phenylacetothiourea derivative. The isothiocyano compound IX gives no color when warmed with ferric chloride solution, and the test can be used to follow the course of the re-

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

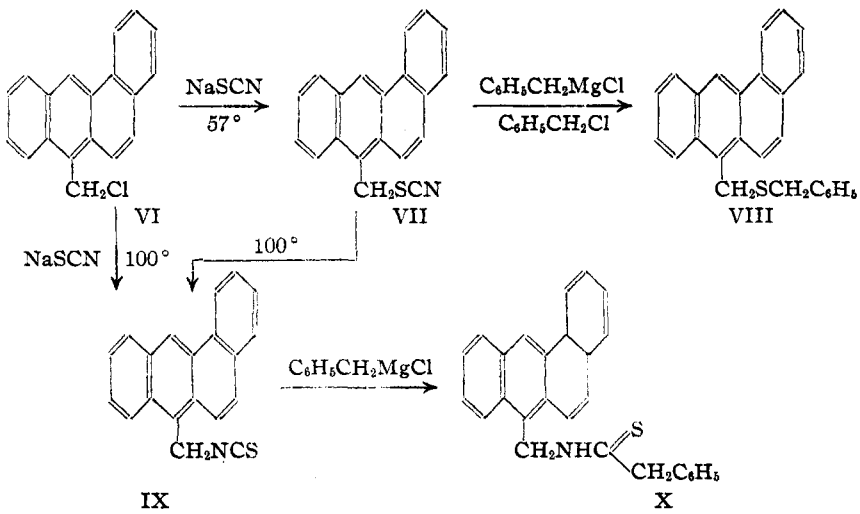
(16) Fieser and Hershberg, *ibid.*, **60**, 1893, 2542 (1938); **61**, 1565 (1939).

(17) Cook and Haslewood, *J. Chem. Soc.*, 428 (1934).

(18) R. Adams, Bramlet and Tendick, THIS JOURNAL, **42**, 2369 (1920).

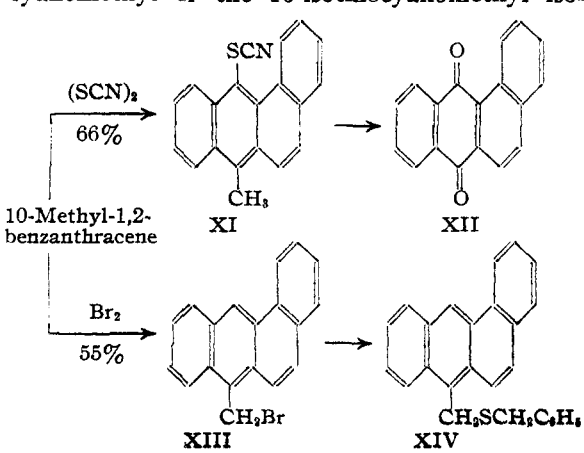
(19) Sachs and Loevy, *Ber.*, **37**, 874 (1904).

(20) Roshdestwenski, *J. Russ. Phys.-Chem. Soc.*, **41**, 1438 (1909); [*Chem. Zentr.*, **81**, I, 910 (1910)].



arrangement of 10-thiocyanomethyl-1,2-benzanthracene.

The 10-thiocyanomethyl derivative VII was prepared for comparison with the sole product isolated on conducting the direct thiocyanation of 10-methyl-1,2-benzanthracene. The reaction proceeded less readily than with the two more potently carcinogenic hydrocarbons listed above, and with only one equivalent of reagent nearly all of the starting material was recovered. With four equivalents of thiocyanogen, however, a pure reaction product was isolated in 56% yield. This substance is an isomer of 10-thiocyanomethyl-1,2-benzanthracene and has significantly different properties. It melts at 141.5–141.9° without any sign of decomposition or rearrangement, and it is not altered by prolonged heating with acetone at 100°. The product of thiocyanation afforded 1,2-benzanthraquinone on oxidation and since it is different from either the 10-thiocyanomethyl or the 10-isothiocyanomethyl isomer, it must have the structure of 9-thiocyano-10-methyl-1,2-benzanthracene (XI). This substance gives no coloration with ferric chloride and stands in contrast to the side-chain substituted isomer VII in this respect as well as in its stability to heat. The two isomers constitute models of the alternate possible structures for the product of the thiocyanation of methylcholanthrene, and the correspondence of the latter substance with VII and not with XI constitutes substantial evidence for its formulation as 15-thiocyano-20-methylcholanthrene (IV). This conclusion is supported by the results of a spectrographic study reported by Dr. R. N. Jones in an accompanying note.[21] It appears that, at least in the series of *meso*-substituted 1,2-benzanthracenes, the ferric chloride test is of diagnostic value in distinguishing between compounds of the types $ArCH_2SCN$ and ArSCN.

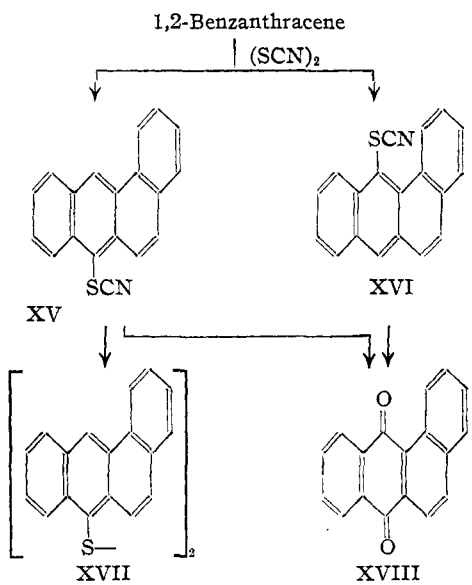


The course of the thiocyanation of 10-methyl-1,2-benzanthracene is surprising, for this is the first reported instance of the direct introduction of a functional group into the 9-position of a 1,2-benzanthracene, or the corresponding position of a cholanthrene. The methyl group of 10-methyl-1,2-benzanthracene is the only established point of attack in the oxidation of the hydrocarbon with lead tetraacetate[16] and in the condensation with sulfur monochloride,[2] although the yields in these reactions are low. According to Kamp,[22] however, the bromination of the hydrocarbon affords the 10-bromomethyl compound XIII in good yield, and on repeating his experiment we likewise encountered no other reaction product. The structure assigned by Kamp was confirmed by conversion to the known benzyl sulfide derivative[2] XIV. Although there are many analogies between thiocyanation and bromination, it is evident that in the present case the reactions take a distinctly different course. The results suggest that the former reaction is less subject to steric hindrance than the latter.

(21) R. N. Jones, THIS JOURNAL, 63, 2528 (1941).
(22) B. Kamp, Dissertation, Frankfort, 1936.

The isomeric 9-methyl-1,2-benzanthracene was found to react smoothly with one equivalent of thiocyanogen to give a monosubstitution product in 43% yield. The product yields 1,2-benzanthraquinone on oxidation and gives no coloration with ferric chloride, from which it may be inferred to have the structure of 10-thiocyano-9-methyl-1,2-benzanthracene.

1,2-Benzanthracene undergoes thiocyanation less readily than either of its meso-monomethyl derivatives. In experiments utilizing 2–4 equivalents of reagent it was possible to isolate one thiocyano derivative melting at 187° in as high as 57% yield and to separate an isomer melting at 175° in 5% yield. Both isomers afford 1,2-benzanthraquinone on oxidation and therefore are meso-substituted derivatives. The higher-melting, more abundant isomer was found to be convertible into the known bis-(1,2-benzanthryl-10)-disulfide[2] (XVII), and hence it can be assigned the structure of 10-thiocyano-1,2-benzanthracene (XV). The second isomer can hardly be an isothiocyano compound, because it was



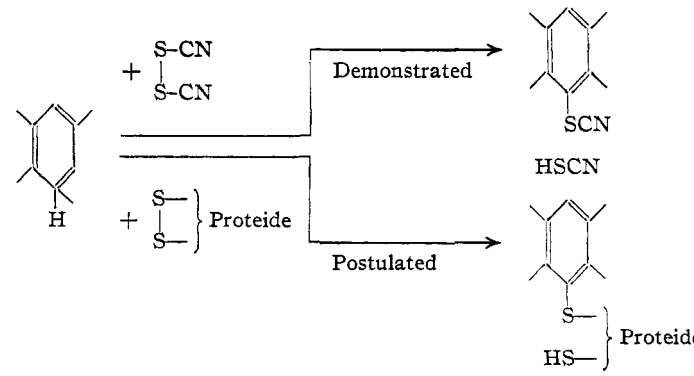
produced in a reaction conducted at room temperature and because XV shows no tendency to rearrange even at the melting point. The substance therefore must be the 9-thiocyano derivative XVI, a conclusion which is supported by spectrographic evidence.[21]

The conversion of the 10-isomer XV into the disulfide was accomplished by a novel method discovered in the course of an attempt to separate the components of the reaction mixture by chromatographic adsorption. When a benzene solution of the mixture was put through a tower of alumina, the 9-thiocyano derivative passed into the filtrate and was easily isolated in a pure form; the 10-isomer was completely retained on the column, and the material recovered on elution proved to be the disulfide XVII. The pure 10-isomer can be converted almost quantitatively into the disulfide by allowing a solution in benzene to stand in contact with activated alumina for two days. The transformation recalls the ready formation of the peroxide of 9,10-dimethyl-1,2-benzanthracene in contact with alumina.[23] The stability of the 9-isomer under the same conditions possibly is attributable to its hindered character.

This apparently is the first instance in which 1,2-benzanthracene has been found to undergo direct substitution at the 9-position, in addition to predominate substitution at the less hindered meso position 10. The 10-derivatives alone have been isolated as products of the nitration of the hydrocarbon[24] (25% yield[16]), oxidation of the substance with lead tetraacetate[16] (67% yield), chloromethylation,[25] bromination,[26] and condensation with methylformanilide,[27] oxalyl chloride,[28] ethyl chloroglyoxylate[26] or sulfur monochloride.[2] The Friedel and Crafts acetylation of the hydrocarbon,[29] however, has been shown to yield, in addition to a meso derivative (9 or 10) as the chief product, the 6- and 7-aceto compounds and two other isomers, and the yields of the 10-derivatives in the other reactions listed are not such as to preclude the formation of significant amounts of other products. The extensive 9-thiocyanation observed with 10-methyl-1,2-benzanthracene, however, suggests that this reagent is more prone than others to attack the 9-position. The availability and reactivity of the corresponding nuclear position in methylcholanthrene must be comparable to that of 10-methyl-1,2-benzanthracene, and the fact that the former hydrocarbon is substituted exclusively in the *ace*-ring rather than at this nuclear site is an indication that the meso-substituted methylene group of methylcholanthrene is more reactive than the

(23) Sandin and Fieser, THIS JOURNAL, **62**, 3098 (1940).
(24) Barnett and Matthews, *Chem. News*, **130**, 339 (1925).
(25) Badger and Cook, *J. Chem. Soc.*, 802 (1939).
(26) Badger and Cook, *ibid.*, 409 (1940).
(27) Fieser and Hartwell, THIS JOURNAL, **60**, 2555 (1938).
(28) Dansi, *Gazz. chim. ital.*, **67**, 85 (1937); Dansi and Ferri, *ibid.*, **69**, 195 (1939).
(29) Cook and Hewett, *J. Chem. Soc.*, 1408 (1933).

10-methyl group of the simpler hydrocarbon. The present orienting observations indicate that the order of reactivity of the 9- and 10-methyl derivatives of 1,2-benzanthracene in the thiocyanation reaction is the reverse of the order of their rapidity of tumor induction; with this exception, a rough parallelism between the chemical reactivity and biological potency of the carcinogens studied is apparent in this as in other reactions.[30]



Thus far 1,2,5,6-dibenzanthracene, which produces tumors in mice slowly and only at a relatively high dosage level[31] but which nevertheless is an effective carcinogen in the sense that it is capable of giving tumors in a high percentage of the animals treated, has given little sign of the type of chemical reactivity displayed so spectacularly by methylcholanthrene and 3,4-benzpyrene. The hydrocarbon is attacked in the meso position or positions on chromic acid oxidation and on nitration,[32] but it is resistant to the special substitutions listed above, and even to bromination in an inert solvent.[32] The observed tendency of thiocyanogen to attack 1,2-benzanthracene and its 10-methyl derivative in the 9-position, which corresponds to the two identical meso positions in 1,2,5,6-dibenzanthracene, encouraged the attempt to thiocyanate the latter hydrocarbon, but the results were invariably negative. The trials included the use of both the preformed reagent in large excess and nascent thiocyanogen, produced by two different methods.[7,11] The sparing solubility of the hydrocarbon constitutes one limiting factor, and the tendency of the reagent to polymerize obviates much forcing of the reaction.[9,18] The ready formation of 9-thiocyano derivatives of 1,2-benzanthracenes suggests that a slightly more powerful reagent would attack the higher benzolog and affords some basis for applying to this hydrocarbon the general hypothesis[30,2] that the initiation of carcinogenesis by a hydrocarbon is associated with a substitution into either a meso position or an alkyl group located at such a position. The specific postulate[2] that this tumor-initiating substitution involves interaction with a cell constituent containing a disulfide group gains plausibility from the analogy to the observed thiocyanations. The opening of a disulfide linkage of a proteinoid substance might occur by the utilization of either an active nuclear hydrogen atom, as shown in the formulas, or the hydrogen of a meso methyl or methylene group.

The physiological significance of the thiocyanate ion and its formation as a product of the detoxification of the cyanide ion lends interest to the testing of the new thiocyano derivatives for possible carcinogenicity. Furthermore, the only functional derivatives of carcinogenic hydrocarbons which do not exhibit a marked diminution in carcinogenic potency as the result of the introduction of the functional group are certain cyano[33] and isocyano[34] derivatives.

Acknowledgments.—The authors are indebted to the Finney–Howell Research Foundation for the award of a fellowship, to the National Cancer Institute for a grant in support of the general program of cancer research in this Laboratory, and to the Jane Coffin Childs Memorial Fund for Medical Research for a grant supporting the correlated biological experimentation.

Experimental Part[35]

Solutions of free thiocyanogen were prepared according to Söderbäck[4] by adding successive portions of the calculated amount of a 10% solution of bromine in purified carbon tetrachloride to a suspension of lead thiocyanate (10% excess) in carbon tetrachloride (5–10 cc. per gram). The mixture was shaken vigorously after each addition until the color was discharged and kept slightly below room temperature by occasional ice cooling. More lead thiocyanate was added when there was any delay in the consumption of all the bromine, and when this point was reached the suspended solid was allowed to settle and the thiocyanogen solution was decanted directly into a solution or suspension of 1–2 g. of a given hydrocarbon in 15–25 cc. of carbon tetrachloride, and the lead bromide was washed by decantation with small portions of this solvent. Filtra-

(30) Fieser, *Am. J. Cancer*, **34**, 37 (1938).
(31) Shinkin and Andervont, *J. Nat. Cancer Inst.*, **1**, 57 (1940).
(32) Cook, *J. Chem. Soc.*, 3273 (1931).
(33) Newman, THIS JOURNAL, **60**, 1368 (1938).
(34) Creech, *ibid.*, **63**, 576 (1941).
(35) Microanalyses by Lyon Southworth, Eleanor Werble, and the Arlington Laboratories, Chagrin Falls, Ohio (*). All melting points are corrected.

tion of the solution is of little advantage and is not easily accomplished without the appearance of a pink coloration indicative of the presence of moisture. The reactions were conducted at room temperature with occasional shaking, usually for a period of a few hours. A reaction is evident from the yellow color of the thiocyano derivative formed, and in some cases the product crystallizes. When the material all remained in solution, except for the occasional separation of thiocyanogen polymer, this was evaporated to dryness at reduced pressure and the residue was extracted with hot benzene or acetone and the solution filtered from undissolved polymer. Since the reaction between bromine and lead thiocyanate is quantitative,[4] the amount of thiocyanogen used in a given experiment can be taken as that equivalent to the bromine used. With just one equivalent of the reagent, the reaction mixture can be tested from time to time for the presence of unchanged thiocyanogen with iron powder and ether[4,36] (red color).

5-Thiocyano-3,4-benzpyrene (II).—A solution of 1 g. of 3,4-benzpyrene in 20 cc. of carbon tetrachloride was cooled to room temperature and treated with 10 cc. of carbon tetrachloride containing 1.1 equivalents of thiocyanogen (prepared from 1 cc. of a solution of 2.3 cc. of bromine in 10 cc. of carbon tetrachloride, as described above). The hydrocarbon which had crystallized soon dissolved, the solution turned deep yellow, and after one-half hour fine needles of the thiocyano compound began to separate. After one hour the product was collected and washed with fresh solvent, giving 1.0 g. (82%) of nearly pure material, m. p. 230°. The substance is very sparingly soluble in carbon tetrachloride and moderately soluble in toluene. Five crystallizations from toluene gave material of the constant melting point 240–240.8°. It crystallizes in light greenish-yellow needles, and dilute solutions show a marked fluorescence in ultraviolet light. On application of the ferric chloride test described below for the characterization of 15-thiocyano-20-methylcholanthrene, the benzpyrenyl derivative gave no coloration in the course of some four to five hours; with some samples the test remained negative after several days, while with others a weak reddish color appeared after a day or two.

Anal. Calcd. for $C_{21}H_{11}SN$: C, 81.52; H, 3.58; N, 4.53. Found: C, 81.78; H, 3.58; N, 4.51.

In an attempt to obtain the substance by heating 5-chloro-3,4-benzpyrene (10 mg.) with sodium thiocyanate (20 mg.) in acetone (2 cc.) at 100° for fifteen hours, only starting material was recovered. Under similar conditions the chloride reacts smoothly with potassium hydrosulfide.[2]

Proof of Structure.—A suspension of 100 mg. of 5-thiocyano-3,4-benzpyrene in 20 cc. of absolute ethanol was treated with portions of sodium under an atmosphere of nitrogen until most of the material had dissolved. The solution was diluted with water, partially neutralized with glacial acetic acid, and concentrated hydrochloric acid was added until precipitation ceased (odor of hydrogen cyanide). The red precipitate was washed by centrifugation and crystallized from toluene, and the purified product (25 mg.) melted at 271–272°, dec., and thus corresponds to **bis-(3,4-benzpyrenyl-5)-disulfide.**[2]

A 50-mg. sample of the 5-thiocyano compound was reduced as above and the reaction mixture containing the mercaptan was treated with three drops of benzyl chloride. A solid separated and was collected after one-half hour (45 mg.). On crystallization from acetic acid, 10 mg. of the disulfide was collected as an undissolved residue and the main product melted at 170–172° (20 mg.) and was identified as **S-benzyl-3,4-benzpyrene-5-mercaptan**[2] by mixed melting point determination.

An attempted acid cleavage of 5-thiocyano-3,4-benzpyrene was unsuccessful. A mixture of 150 mg. of the substance in 10 cc. of toluene, 10 cc. of water, and 10 cc. of concentrated hydrochloric acid was refluxed for six hours with the occasional addition of zinc. The dilute acid was replaced with concentrated acid and then with 48% hydrobromic acid, but after refluxing for a total of ten hours more the unchanged thiocyano derivative was recovered quantitatively from the toluene layer.

15-Thiocyano-20-methylcholanthrene (IV).—On treating a suspension of 1 g. of methylcholanthrene in 15 cc. of carbon tetrachloride with 1.1 equivalents of thiocyanogen in 15 cc. of this solvent, the hydrocarbon dissolved within about one-half hour and yellow needles of the product began to separate. The crystalline material collected after two hours amounted to 500 mg. (41%), m. p. 126°, dec. Purification was accomplished satisfactorily by slow crystallization from chloroform–petroleum ether. The substance decomposes at the melting point to a red liquid and with evolution of gas. A sample introduced to the melting point bath at 128° began to soften at 130°; when introduced at 132°, the compound melted instantly. When observed under a polarizing microscope, a single crystal lost its refractivity in the range 124–128°. The substance is much more soluble in organic solvents than 5-thiocyano-3,4-benzpyrene.

Anal. Calcd. for $C_{22}H_{15}SN$: C, 81.17; H, 4.67. Found: C, 81.17; H, 4.67.

The material recovered by evaporation of the mother liquors was clarified with Darco in benzene solution and the filtrate was treated with a saturated solution of picric acid in alcohol. This precipitated 300 mg. of deep purple methylcholanthrene picrate, m. p. 177–178°, corresponding to a recovery of 162 mg. (16%) of unreacted hydrocarbon. With 1 g. of methylcholanthrene in 5 cc. of carbon tetrachloride and 2.2 equivalents of thiocyanogen in 20 cc. of solvent the crystalline 15-thiocyano derivative (m. p. 120°, dec.) collected after one hour amounted to 1.08 g. (89%).

A characteristic color test is obtained by dissolving the pure thiocyanomethylcholanthrene in dioxane by heating, adding about one-half volume of methanol, followed by a drop of 1% aqueous ferric chloride solution. A permanent cherry-red coloration appears at once when the solution is warmed.

When a solution of 200 mg. of the thiocyano compound in 3 cc. of toluene was heated at the boiling point, decomposition gradually occurred, and after refluxing for thirty-five hours the ferric chloride color test was negative. The solution on cooling deposited 95 mg. of red-orange crystals, m. p. 158–165°, dec. Four recrystallizations of the **transformation product** from toluene–hexane gave material of the constant m. p. 289–291°, dec. Tests for nitrogen and sulfur were negative. The substance decomposed on attempted vacuum sublimation, and it proved to be so

(36) Lecher and Wittwer, *Ber.*, **55**, 1481 (1922).

slightly soluble in camphor that the molecular weight could not be determined by the Rast method (micro).

Anal. Calcd. for $(C_{21}H_{14})_x$: C, 94.78; H, 5.30. Calcd. for $(C_{21}H_{15})_x$: C, 94.35; H, 5.66. Found: C, 94.97, 94.65; H, 5.83, 5.56.

Oxidation of 15-Thiocyano-20-methylcholanthrene.—A mixture of 100 mg. of the substance and 700 mg. of anhydrous sodium dichromate in 1 cc. of acetic acid was heated at the boiling point for forty minutes and diluted with water, which precipitated 50 mg. of yellow product. This was reprecipitated from soda solution and crystallized from benzene–methanol; it formed long yellow needles, m. p. 220.3–220.9, and gave a red vat test. The substance was identified as **6-methyl-1,2-benzanthraquinone-5-acetic acid** by its failure to depress the melting point of an authentic sample of the acid.[37]

9-Thiocyano-10-methyl-1,2-benzanthracene (XI).—Treatment of 1 g. of 10-methyl-1,2-benzanthracene in carbon tetrachloride solution with 1.1 equivalents of thiocyanogen for four hours resulted in the formation of only 20 mg. of the 9-thiocyano derivative. The mixture resulting from 500 mg. of the hydrocarbon and 2.2 equivalents of reagent after a five-hour reaction period yielded on fractional crystallization 270 mg. (44%) of long yellow needles of the 9-thiocyanogen compound and 60 mg. of pure 10-methyl-1,2-benzanthracene. Still better results were obtained by adding a solution of 4.4 equivalents of thiocyanogen in 20 cc. of carbon tetrachloride to a suspension of 500 mg. of the hydrocarbon in 5 cc. of the same solvent. After five hours, when the material had all dissolved, the solution was evaporated to dryness and the residue extracted with benzene. Fractionation afforded 350 mg. (56%) of pure XI, m. p. 141–141.5°, and 60 mg. (10%) of slightly less pure product, m. p. 130°; the only other substance obtained from the mother liquor was a higher melting product (15 mg.). The 9-thiocyano derivative when crystallized repeatedly from methanol, ethanol, or acetic acid formed yellow needles, m. p. 141.5–141.9° (ferric chloride test negative).

Anal. Calcd. for $C_{20}H_{13}SN$: C, 80.24; H, 4.38. Found: C, 80.46; H, 4.42.

The substance XI was recovered unchanged after being heated for fifteen hours in acetone at 100°. Treatment with sodium and ethanol resulted in the elimination of sulfur as hydrogen sulfide. Oxidation of the compound (100 mg.) with sodium dichromate as above gave 70 mg. of 1,2-benzanthraquinone, m. p. 167–169°; the material when recrystallized from toluene–hexane melted at 168.5–169.5° and gave no depression when mixed with an authentic sample.

10-Thiocyanomethyl-1,2-benzanthracene (VII).—A suspension of 500 mg. of 10-chloromethyl-1,2-benzanthracene and 500 mg. of sodium thiocyanate in 5 cc. of acetone was heated under reflux for three hours, the acetone was distilled and the product was triturated with water and collected. An initial crystallization from benzene gave 500 mg. (81%) of VII, m. p. 133–135°. After four crystallizations the substance was obtained as light yellow needles which melted at 134.2–135.8°, solidified at 137–138°, and remelted at 170.2–171°. When a test sample of the material in dioxane–methanol is treated with ferric chloride and the solution is warmed, a light cherry-red color appears in about five minutes.

*Anal.** Calcd. for $C_{20}H_{13}SN$: C, 80.24; H, 4.38. Found: C, 80.43; H, 4.22.

Reaction of VII with Benzylmagnesium Chloride.—The addition of 300 mg. of 10-thiocyanomethyl-1,2-benzanthracene in 5 cc. of warm benzene to the Grignard reagent from 0.7 cc. of benzyl chloride in benzene–ether produced an immediate yellow precipitate. After refluxing for one hour, the mixture was decomposed with dilute hydrochloric acid and extracted with benzene. Concentration of the extract afforded 120 mg. of light yellow crystals melting at 165°; this material gave a characteristic lead salt in the test with lead acetate and on treatment with benzyl chloride and sodium ethylate in ethanol it gave the known **S-benzyl derivative of 1,2-benzanthryl-10-methylmercaptan,**[2] m. p. 148–149.5°, identified by mixed melting point determination. **Bis-(1,2-benzanthryl-10-methyl)-disulfide**[2] was isolated from the residue of the benzene extraction by crystallization from chlorobenzene and identified by mixed melting point determination.

10-Isothiocyanomethyl-1,2-benzanthracene (IX).—When the 10-thiocyanomethyl derivative (50 mg.) was heated in acetone (5 cc.) at 100° for fifteen hours it was transformed quantitatively into the 10-isothiocyanomethyl compound. The latter substance was also obtained in 80–90% yield when equal quantities of 10-chloromethyl-1,2-benzanthracene and sodium thiocyanate were heated in acetone at 100° for fifteen hours. The compound crystallized from acetone–methanol or benzene–methanol in light yellow rosets; in the pure state it melted at 170.5–171.1° (ferric chloride test negative).

Anal. Calcd. for $C_{20}H_{13}SN$: C, 80.24; H, 4.38. Found: C, 80.44; H, 4.36.

Derivatives of 10-Isothiocyanomethyl-1,2-benzanthracene ($ArCH_2NCS$). (a) **N-(1,2-Benzanthryl-10-methyl)-phenylthioacetamide (X, $ArCH_2NHCSCH_2C_6H_5$).**—A solution of 150 mg. of IX in 8 cc. of benzene was added to the Grignard reagent from 0.7 cc. of benzyl chloride, and after heating for fifteen minutes the mixture was decomposed with water and 3 cc. of 6 *N* sodium hydroxide and 0.2 cc. of benzyl chloride were added and the suspension shaken vigorously for ten minutes. The mixture was acidified and extracted with benzene and on concentrating the washed and dried solution and adding hexane a yellowish crystalline product separated (225 mg., m. p. 180°). After several crystallizations the melting point was 186–187° and the sample gave positive tests for nitrogen and sulfur. Hydrogen sulfide was liberated on heating the substance with a mixture of acetic, formic, and hydrobromic acids.

*Anal.** Calcd. for $C_{27}H_{21}SN$: C, 82.83; H, 5.41; N, 3.58. Found: C, 83.58; H, 5.45; N, 3.32.

(b) **Thiourethan,** $ArCH_2NHCSOC_2H_5$.—When a suspension of 300 mg. of IX in 5 cc. of absolute ethanol containing a trace of sodium ethoxide was heated under reflux for six hours the thiourethan formed a crust on the walls of the flask. It was dissolved in acetone and the filtered solution was concentrated and diluted with methanol. On recrystallization from benzene–hexane the substance melted at 166–168° (140 mg.), and after three more crystallizations the melting point was 167.5–168.9° (crystal powder).

(37) Fieser and Hershberg, THIS JOURNAL, **60**, 2542 (1938).

Anal. Calcd. for $C_{22}H_{19}ONS$: N, 4.25. Found: N, 3.95.

(c) **N-(1,2-Benzanthryl-10-methyl)-N'-phenylthiourea,** $ArCH_2NHCSNHC_6H_5$.—A solution of 200 mg. of IX in 2 cc. of aniline was heated at 90° for three hours and poured into dilute hydrochloric acid. The washed and dried product melted at 130–140° (260 mg.), and after removing considerable insoluble material by extraction with benzene the material melted at 200°. After three crystallizations from toluene–hexane and three more from chloroform–hexane the derivative formed yellowish rosets, m. p. 225–227°, dec.

Anal. Calcd. for $C_{26}H_{20}N_2S$: N, 7.14. Found: N, 7.06.

Bromination of 10-Methyl-1,2-benzanthracene.—On dropwise addition of 4 cc. of 1 *N* bromine solution to 500 mg. of the hydrocarbon in 6 cc. of carbon bisulfide at room temperature, the absorption of bromine was immediate and the product soon began to separate. After standing overnight at 5° the crystalline material was collected and combined with that obtained by evaporation of the filtrate and crystallized from benzene, affording 370 mg. of 10-bromomethyl-1,2-benzanthracene, m. p. 197–198°, dec., which is in agreement with the results of Kamp.[22] The bromide reacted with alcoholic silver nitrate in dioxane solution, and on treatment with benzyl mercaptan in methanol containing sodium ethoxide it yielded the S-benzyl derivative of 1,2-benzanthryl 10-methylmercaptan.[2]

10-Thiocyano-9-methyl-1,2-benzanthracene.—A mixture of 285 mg. of 9-methyl-1,2-benzanthracene and 1.1 equivalents of thiocyanogen in a total of 15 cc. of carbon tetrachloride gave a straw colored solution after three hours. On evaporation to dryness, extraction with benzene and dilution of the filtrate with hexane, there was obtained 150 mg. (43%) of yellow thiocyano derivative, m. p. 151.2–152°. After four recrystallizations the product melted at 153–154°.

Anal. Calcd. for $C_{20}H_{13}SN$: C, 80.24; H, 4.38. Found: C, 80.49; H, 4.38.

The oxidation of a 50-mg. sample with dichromate gave 20 mg. of crystallized 1,2-benzanthraquinone, m. p. 168–170° (mixed melting point determination).

Thiocyanation of 1,2-Benzanthracene.—In exploratory experiments it was found that treatment of the hydrocarbon in carbon tetrachloride or carbon bisulfide with only 1.1 equivalents of reagent afforded only somewhat yellowish starting material. When a suspension of 1 g. of 1,2-benzanthracene in 10 cc. of carbon tetrachloride was treated with 4.4 moles of thiocyanogen in 30 cc. of carbon tetrachloride, solution was complete after five hours at room temperature. After evaporation to dryness, the product was extracted with benzene and the solution was clarified with Norit, concentrated and diluted with methanol, giving 720 mg. (58%) of a crystallizate mixture. Fractional crystallization from benzene–methanol and from acetone yielded 100 mg. (8%) of pure **10-thiocyano-1,2-benzanthracene,** m. p. 186–187°, in the form of greenish-yellow rosets (acetone), and a second crop of 65 mg. (5%) of less pure material, m. p. 181–183°. Both the solid substance and its solutions fluoresce brilliantly in ultraviolet light.

*Anal.** Calcd. for $C_{19}H_{11}SN$: C, 79.97; H, 3.89. Found: C, 80.05; H, 3.89.

The residues afforded 120 mg. of a crystallizate mixture which was dissolved in benzene and passed through a column of activated alumina. The 10-thiocyano derivative was effectively retained on the column, and on concentration of the filtrate long yellow needles of **9-thiocyano-1,2-benzanthracene** separated. Repeated crystallization from benzene–methanol yielded 60 mg. (5%) of material melting at 174.3–174.6°. The substance gives no color with ferric chloride even after several days.

*Anal.** Calcd. for $C_{19}H_{11}SN$: C, 79.97; H, 3.89. Found: C, 79.46; H, 4.00.

An attempt was made to separate the isomeric thiocyanogen derivatives produced in a duplicate experiment by adsorption of the reaction mixture on alumina from benzene solution and successive elutions with benzene, ether, acetone and methanol. The benzene eluate yielded 15 mg. of 1,2-benzanthracene and 490 mg. of **bis-(1,2-benzanthryl-10)-disulfide,**[2] which appeared in a non-fluorescent extract. The acetone eluate was fluorescent as it passed through the column, but the solution on concentration deposited only 40 mg. more of the disulfide. None of the 9-isomer was found.

In a further experiment 2 g. of 1,2-benzanthracene and 2.2 equivalents of thiocyanogen in a total of 40 cc. of carbon tetrachloride yielded, after standing overnight, 1.43 g. (57%) of 10-thiocyano-1,2-benzanthracene in a slightly impure condition, m. p. 175–185°; this was easily isolated by crystallization from benzene. The mother liquors, after passage through an adsorption tower, afforded 120 mg. of rather impure 9-isomer.

The **conversion of 10-thiocyano-1,2-benzanthracene into bis-(1,2-benzanthryl-10)-disulfide under the influence of alumina** was demonstrated when a solution of 50 mg. of the pure 10-isomer in 3 cc. of benzene was allowed to stand over 200 mg. of activated alumina. Within a short time the odor of hydrogen cyanide was noticeable, and after forty-eight hours the mixture was filtered and the alumina eluted with ethanol and benzene. The filtrate yielded 35 mg. of **bis-(1,2-benzanthryl-10)-disulfide,** m. p. 208–209°, which did not depress the melting point of an authentic sample.[2] When the experiment was repeated with the substitution for the alumina of 100 mg. of Super-Cel, which had been used as a diluent in the adsorption columns employed in the above experiments, only the unchanged 10-thiocyanogen derivative (35 mg.) was recovered.

9-Thiocyano-1,2-benzanthracene is not affected by the alumina treatment. Thus from 10 mg. which had been in contact with 20 mg. of alumina in benzene solution for three days, there was recovered 5 mg. of unchanged material, m. p. 169–172°.

On oxidation, 65 mg. of 10-thiocyano-1,2-benzanthracene yielded 30 mg. of crude product, m. p. 165°; crystallization afforded 1,2-benzanthraquinone, m. p. 168–169°. Similarly, 25 mg. of the 9-isomer yielded 10 mg. of 1,2-benzanthraquinone.

Thiocyanation of Anthracene.—A suspension of 500 mg. of anthracene in a total of 15 cc. of carbon tetrachloride containing 4.4 equivalents of thiocyanogen was allowed to stand overnight and the resulting solution was evaporated to dryness at reduced pressure. Extraction with hot benzene and dilution of the filtered solution with methanol gave a crystallizate of long, bright yellow needles of **9,10-**

dithiocyanoanthracene, m. p. 198–201°; yield 250 mg. (45%). After five crystallizations from benzene–methanol the substance melted at 206.7–207.8° (ferric chloride test negative). Oxidation yielded anthraquinone, m. p. 284.1–284.7° (mixed melting point). The disubstitution product was also isolated in an experiment utilizing only one equivalent of reagent.

Anal. Calcd. for $C_{16}H_8S_2N_2$: C, 65.72; H, 2.76. Found: C, 65.52; H, 2.79.

The mother liquors of the above preparation afforded 350 mg. of fine yellow needles of a mixture which resisted attempted fractionation from benzene–methanol, acetic acid, or methanol, and which melted consistently at about 125–145°. When a benzene solution of 150 mg. of the mixture was allowed to stand in contact with alumina, hydrogen cyanide was evolved and elution of the material adsorbed on the alumina afforded a small amount of a substance which when crystallized repeatedly melted at 211–212° (6 mg.). This probably is impure 9,9'-dianthryl disulfide (m. p. 223°)[38] arising from the 9-thiocyano derivative, for a small comparison sample prepared by the method of Cook, Heilbron and Walker[38] melted at the same temperature and gave no depression on admixture.

Trials with 1,2,5,6-Dibenzanthracene.—In typical experiments suspensions of 500-mg. portions of the hydrocarbon in either benzene or carbon tetrachloride containing 4.4 equivalents of thiocyanogen were shaken for three to four days, but the hydrocarbon was largely recovered unchanged (48–82% recovery) and no thiocyano derivative could be isolated. In another trial a solution of 150 mg. of dibenzanthracene in 100 cc. of acetic acid was cooled to 15° and treated with 0.5 g. of sodium thiocyanate in 10 cc. of acetic acid, followed by 0.16 cc. of bromine in 3 cc. of acetic acid, added by drops. After standing overnight, the material precipitated by water was processed by crystallization and chromatographic adsorption, but it afforded only starting material (130 mg., m. p. 264–265°). In trials with black copper thiocyanate[7] there was no sign of a reaction.

(38) Cook, Heilbron and Walker, *J. Chem. Soc.*, **127**, 2254 (1924).

Summary

The ready reaction of at least some of the more potently active carcinogens with thiocyanogen provides an interesting chemical analogy lending plausibility to the hypothesis that an administered carcinogen can combine with a proteinoid constituent of the cell by virtue of the opening of a disulfide linkage and fixation of the hydrocarbon to one of the sulfur atoms.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASSACHUSETTS RECEIVED MAY 27, 1941

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

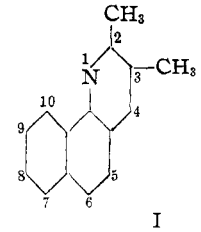
The Nitrogen Compounds in Petroleum Distillates. XXIII. Isolation of 2,3-Dimethylbenzo[h]quinoline (I) and 2,4-Dimethylbenzo[h]quinoline (II) from California Petroleum

BY LESLIE M. SCHENCK AND J. R. BAILEY

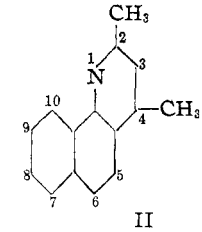
Introduction

Cumulative extraction[1] of gas oil bases in the 355–365° range from California petroleum[2] effects relatively sharp segregation of the component base hydrochlorides in the ratio 1:4 between the aqueous and solvent phases, respectively. The high refractivity of the basic admixture from the aqueous hydrochloride layer suggested possible trinuclear structure. Processing of this material yielded 2,3-dimethylbenzo[h]quinoline (I) and 2,4-dimethylbenzo[h]quinoline (II), neither of which have been reported previously from a natural source.

From selenium dioxide oxidation of (II) was obtained a $C_{13}H_7N(COOH)_2$ dicarboxylic acid; this behavior is suggestive of a structure in which methyl groups are in the positions alpha and gamma to nitrogen. Lack of material prevented



I



II

decarboxylation of this acid to the parent compound. Eliminating an acridine homolog on the basis of active methyl groups, the $C_{15}H_{13}N$ base (II) was one of the three isomeric 2,4-dimethylbenzoquinolines. Synthesis of 2,4-dimethylbenzo[h]quinoline[3] from α-naphthylamine and acetylacetone yielded a product identical with II.

Selenium dioxide oxidation of (I) yielded a

(1) Perrin and Bailey, THIS JOURNAL, **55**, 4136 (1933).

(2) This material was supplied by the Union Oil Company of California.

(3) Synthesis of this base from the same intermediates has been reported previously by Combes, *Compt. rend.*, **106**, 1537 (1888); and with acetaldehyde and acetone as the carbonyl reagents by Reed, *J. prakt. Chem.*, [2] **35**, 312 (1887). In both cases the melting point of the base from petroleum ether is reported as 44° (uncor.). In this work, both the natural and synthetic products were found to melt at 55–56° (cor.) from the same solvent.