The Synthesis of 4,5-Dimethyl-1,2-benzanthracene and 4,5,10-Trimethyl-1,2-benzanthracene[1]

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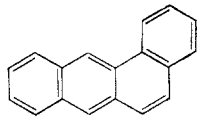
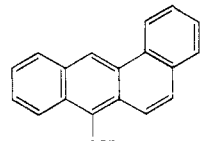
Received January 14, 1964

A new hypothesis concerning the effect of structure on cancer-producing activity of hydrocarbons in the 1,2-benzanthracene series is presented. The syntheses of the named hydrocarbons was effected by the steps outlined in the chart.

1,2-Benzanthracene (I) is not active as a carcinogenic hydrocarbon whereas 10-methyl-1,2-benzanthracene (II) is quite active.[2] In seeking an explanation for



I



II

these facts it seemed possible that I is inactive because some reaction takes place at the 10-position[3] (7-position using benz[*a*]anthracene nomenclature) which renders I inactive. In II reaction at the 10-position is blocked by the methyl group and hence the compound cannot be deactivated by reaction at the 10-position. Hence II remains and becomes involved in the sequence of reactions leading to the production of cancer.[4] However, another means of blocking reaction at the 10-position is conceivable, namely, substitution of methyl groups at the 4- and 5-positions. These methyl groups could block reaction at the 10-position by sterically hindering the deactivating process alluded to above.

That blocking of the 10-position by groups on the adjacent *peri* positions (4- and 5-) may be important is supported by the facts that 5,9-dimethyl-1,2-benzanthracene is more active than 9-methyl-1,2-benzanthracene[2] and 1,2,5,6-dibenzanthracene is more active than 1,2-benzanthracene.[2,5] Furthermore, 4- and 5-methyl-1,2-benzanthracene are quite a bit more active than I and 9-methyl-1,2-benzanthracene.[6] This is shown in Table I.

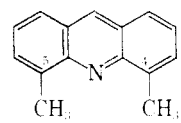
TABLE I

COMPARATIVE CARCINOGENIC ACTIVITY OF BENZ[*a*]ANTHRACENES[a]

Compound	Number of rats	% with tumors	Number of tumors	Mean latent period ± probable error in days
10-Methyl	60	93	97	201 ± 2.7
4-Methyl	58	71	58	235 ± 4.1
5-Methyl	59	61	41	265 ± 8.7
9-Methyl	61	52	39	292 ± 10.7
7-Methyl	57	5	3	396
6-Methyl	59	5	3	413

[a] This data was taken from Table I of ref. 6a.

An example of this type of steric effect is illustrated by certain properties of 4,5-dimethylacridine.[5b] This compound readily forms a salt with hydrogen chloride but does not interact with boron trifluoride[5b] which often



has stronger acidic properties than hydrogen chloride. These facts show that the size of the reagent in question is of great importance. Furthermore, 4,5-dimethylacridine *caused no irritation of the eyes, nose, or skin, whereas acridine is a very irritating compound.* These facts show that certain physiological effects can be blocked by the type of steric hindrance described above.

In order to obtain more data about this type of steric hindrance in the cancer field we decided to prepare the unknown 4,5-dimethyl-1,2-benzanthracene (VII) (6,8-dimethylbenz[*a*]anthracene) so that its carcinogenic activity could be tested.[7] In a recent letter, Huggins has reported the results of testing 4,5-dimethyl-1,2-benzanthracene (VII), 3′,6-dimethyl-1,2-benzanthracene[8] (3,9-dimethylbenz[*a*]anthracene), and 1′,9-dimethyl-1,2-benzanthracene[8] (1,12-dimethylbenz[*a*]anthracene) by a rapid testing method which will be described independently by Dr. Huggins. The results were as follows.[9] Seven rats

(1) This work was supported by a grant from the National Institutes of Health, U. S. Public Health Service.

(2) J. L. Hartwell, "Survey of Compounds Which Have Been Tested for Carcinogenic Activity," U. S. Public Health Service Publication No. 149 (1951).

(3) The 10-position in 1,2-benzanthracene is attacked preferentially by a variety of reagents. See L. F. Fieser and J. L. Hartwell, *J. Am. Chem. Soc.*, **60**, 2555 (1938), and references therein.

(4) A cooperative program to try to find out more about the factors leading to carcinogenic activity of II is under way. This program involves the synthesis of the eleven monofluoro-10-methyl-1,2-benzanthracenes in this laboratory and biological testing by Dr. James A. Miller at The University of Wisconsin. The reasons for this program are described by M. S. Newman, D. MacDowell, and S. Swaminathan, *J. Org. Chem.*, **24**, 509 (1959), and by E. C. Miller and J. A. Miller, *Cancer Res.*, **20**, 133 (1960).

(5) That the steric effect of a fused aromatic ring is similar in magnitude to that of a methyl group has been pointed out previously in other connections. See: (a) M. S. Newman and C. D. McCleary, *J. Am. Chem. Soc.*, **63**, 1537 (1941); (b) M. S. Newman and W. A. Powell, *J. Org. Chem.*, **26**, 812 (1961); and (c) J. Packer, J. Vaughan, and E. Wong, *J. Am. Chem. Soc.*, **80**, 905 (1958).

(6) (a) W. E. Dunning and M. R. Curtis, *J. National Cancer Inst.*, **25**, 387 (1960). (b) E. VonHaam, Ohio State University Pathology Department, private communication. This work is to be published in the near future.

(7) Dr. C. V. Huggins, Ben May Laboratory for Cancer Research, University of Chicago, was the first to test VII. Tests are now being carried out by Dr. James A. Miller, McArdle Memorial Laboratory, University of Wisconsin, Dr. W. F. Dunning, Cancer Research Laboratory, University of Miami, and Dr. E. VonHaam, The Ohio State University Department of Pathology.

(8) *The reasons for synthesis and testing of these hydrocarbons are described.* See (a) M. S. Newman, W. C. Sagar, and M. V. George, *J. Am. Chem. Soc.*, **82**, 2376 (1960); (b) M. S. Newman and M. V. George, *J. Org. Chem.*, **26**, 4306 (1961). Note ref. 7 in 8a.

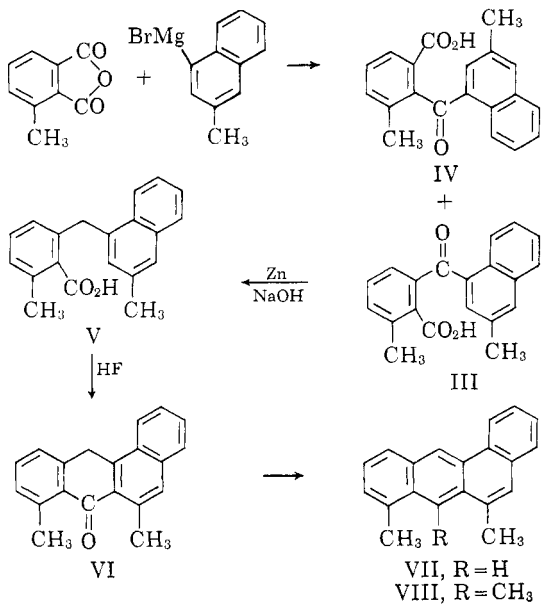
(9) Private communication from Dr. C. V. Huggins.

were injected intramuscularly, right and left legs, with the compound dissolved in sesame oil, 5 mg. in 0.5 ml. of oil in each leg. After 142 days, sarcoma had been detected in 5 of 7 rats injected with VII at 81, 92, 99, 110, and 117 days. No sarcoma was detected in any of the rats (all alive after 142 days) injected with the other two hydrocarbons.

Although comparable testing data with other methyl-substituted 1,2-benzanthracenes are not yet available, the activity of VII is apparent and lends support to the steric hindrance hypothesis of cancer-producing activity outlined above. It is hoped that further testing will be initiated.

The application of this concept to other areas of physiological activity is to be desired. This point is illustrated as follows. Let us suppose that a drug has two sites of activity, X and Y. Assume that in the body reaction at center X is desirable and that reaction at position Y is harmful, either because reaction at Y leads to removal of the drug or to a detrimental sequence of reactions. By using the steric hindrance concept described above, it may be possible to decrease the harmful reaction at Y without materially affecting the beneficial reaction at X.[10]

In this paper the synthesis of VII and of 4,5,10-trimethyl-1,2-benzanthracene (VIII) by the route outlined below is described.



The condensation of 3-methylphthalic anhydride with 3-methyl-1-naphthylmagnesium bromide afforded the acids III and IV in 53 and 14% yield.[11] The mixture of acids III and IV was separated by converting it into the corresponding normal methyl ester mixture by treating with diazomethane. Dissolution of these esters in concentrated sulfuric acid followed by dilution with water afforded III as acid and IV as methyl ester. A clean separation was thus effected.[12] The remaining steps proceeded without difficulty and details are described in the Experimental part. The fact that the anthrone (VI) reacted with methyllithium to yield as much as 38% of VIII was surprising in view of the marked hindrance in VI. This reaction did not proceed appreciably unless a large excess of methyllithium was allowed to react for a relatively long time (10 hr.).

Experimental[13]

1-Bromo-3-methylnaphthalene.—1-Nitro-2-methylnaphthalene[14] was reduced to 1-amino-2-methylnaphthalene, b.p. 141–143° (5 mm.), with iron filings in aqueous ferrous sulfate,[15] in 90% yield. Bromination of this amine was carried out as described.[16] Crude 1-amino-4-bromo-2-methylnapthalene, m.p. 71–75° (30.0 g., 52% yield), was obtained by a Soxhlet extraction using 500 ml. of petroleum ether, b.p. 35–40°, Skellysolve F. This amine was deaminated by a procedure involving hypophosphorous acid[17] to yield 1-bromo-3-methylnaphthalene, b.p. 141–145° (5 mm.), in 58% yield.

3-Methylphthalic Anhydride.—A mixture of 140 g. of pure maleic anhydride, 135 g. of freshly distilled 2-methylfuran, and 0.2 g. of hydroquinone in 600 ml of dry ether was heated until homogeneous and a mildly exothermic reaction began. After standing for 1 hr., stirring and cooling were initiated A total of 205 g. (76%) of adduct, m.p. 83–85° after air drying, was obtained in two crops. This material was added to 1 l. of concentrated sulfuric acid held at −10 to −15° during addition and for 1 more hr. After coming to 5° during 2 more hr. the solution was poured on ice. The crude product was collected and washed with dilute sodium carbonate solution. Recrystallization from benzene–petroleum ether (b.p. 65–70°) afforded 70 g. (38%) of 3-methylphthalic anhydride,[18] m.p. 112–114°.

2-(3-Methyl-1-naphthoyl)-6-methylbenzoic Acid (III) and 2-(3-Methyl-1-naphthoyl)-3-methylbenzoic Acid (IV).—The Grignard reagent prepared from 40 g. of 1-bromo-3-methylnaphthalene in 100 ml. of ether and 20 ml of benzene was added rapidly to a hot stirred solution of 33 g. of 3-methylphthalic anhydride in 150 ml. of benzene. After 6 hr. of stirring at reflux the reaction mixture was cooled and added to cold dilute hydrochloric acid. The entire acid portion (about 43 g.), isolated in the usual way, was esterified with diazomethane and this ester mixture added to 500 g. of concentrated sulfuric acid held at room temperature. After 2 hr. this solution was poured on ice and separated into acid and neutral fractions in the usual way. The acid fraction, m.p. 181–183°, amounted to 29.1 g. (53%). A pure sample of III, m.p. 182–183°, was obtained by crystallization from toluene. The isomeric acid (IV), m.p. 215–216°, was obtained from the neutral fraction as described below.

Anal.[19] Calcd. for $C_{20}H_{16}O_3$: C, 78.9; H, 5.3. Found for III: C, 79.1; H, 5.6. Found for IV: C, 78.9; H, 5.2.

The neutral fraction obtained as described above yielded 8.0 g. (14%) of the methyl ester of IV, m.p. 132.0–133.5°, on crystallization from methanol. Alkaline hydrolysis yielded IV in almost quantitative yield.

Anal. Calcd. for $C_{21}H_{18}O_3$: C, 79.2; H, 5.7. Found: C, 79.2; H, 5.5.

(10) If any reader of this article has a specific drug in mind which has both desirable and undesirable effects, I would be happy to discuss cooperation in which I might undertake the synthesis involved if suitable testing of the resulting drug can be arranged. M. S. Newman.

(11) Compare with the results obtained with *m*-xylylmagnesium bromide [M. S. Newman and C. D. McCleary, *J. Am. Chem. Soc.*, **63**, 1542 (1941)] wherein the ratio of comparable ketoacids was about 10:1.

(12) For an explanation of the mechanism upon which this separation is based see ref. 5a.

(13) All melting points are uncorrected. Melting points below 200° are accurate to within 0.5°. The term, "worked up in the usual way," means that an ether-benzene extract of the organic products was washed with water, dilute acid or base as needed, and saturated sodium chloride solution, and dried by filtration through a bed of anhydrous magnesium sulfate. The solvent was then distilled or evaporated in a rotary evaporator.

(14) H. E. Fierz-David and E. Mannhart, *Helv. Chim. Acta*, **20**, 1024 (1937).

(15) This procedure, as applied by H. H. Hodson and D. E. Hathway, *J. Chem. Soc.*, 538 (1944), was superior to the reduction described in ref. 14.

(16) A. Fischer, W. J. Mitchell, G. S. Ogilivie, J. Packer, J. E. Packer, and J. Vaughan, *ibid.*, 1426 (1958).

(17) N. Kornblum, *Org. Reactions*, **2**, 294 (1944).

(18) We thank Dr. K. Greenlee for the directions for this preparation which is comparable to that used in the synthesis of 3,6-dimethylphthalic anhydride by M. S. Newman and B. T. Lord, *J. Am. Chem. Soc.*, **66**, 733 (1944).

(19) Analyses by Micro-Analysis Lab., Inc., Marshallton, Wilmington 8, Del.

Proof of Structure of III and IV.—The structures of III and IV were proved by decarboxylation and comparison of the resulting ketones with authentic samples.[20] Because both 3-methyl-1-naphthyl *m*-tolyl ketone and 3-methyl-1-naphthyl *o*-tolyl ketone were liquids, comparison was effected by means of the 2,4-dinitrophenylhydrazones. The 2,4-DNPH derivative of the *m*-tolyl isomer, prepared by reaction of 3-methyl-1-naphthylmagnesium bromide with *m*-tolunitrile, melted (alone and mixed with the 2,4-DNPH derivative of the ketone formed by decarboxylation of III) at 234–236° dec., after recrystallization from benzene–isopropyl alcohol. The infrared spectra of the two samples were also identical. Similarly the 2,4-DNPH derivative of the *o*-tolyl isomer, prepared by reaction of 3-methyl-1-naphthylmagnesium bromide with *o*-tolunitrile, melted (alone and mixed with the ketone formed by decarboxylation of IV) at 246–248° dec., after recrystallization from benzene–isopropyl alcohol. The infrared spectra of the two samples were also identical.

Anal. Calcd. for $C_{25}H_{20}N_4O_4$: C, 68.2; H, 4.5; N, 12.7. Found (for *m*-isomer): C, 68.1; H, 4.5; N, 12.8. Found (for *o*-isomer): C, 68.1; H, 4.5; N, 12.9.

2-(3-Methyl-1-naphthyl)methyl-6 methylbenzoic Acid (V).—A mixture of 50 g. of zinc dust activated with copper sulfate, 29.1 g. of III, and 700 ml. of 10% sodium hydroxide solution was refluxed and stirred for 36 hr. The acid fraction, isolated by the usual means, was crystallized from toluene to yield 27.4 g. (99%) of V, m.p. 162–163°.

Anal. Calcd. for $C_{20}H_{18}O_2$: C, 82.8; H, 6.2. Found: C, 82.8; H, 6.2.

When a similar mixture was heated for 20 hr., a 32% yield of the lactone of 2-(3-methyl-1-naphthyl)hydroxymethyl-6-methylbenzoic acid, m.p. 178–179°, was obtained in addition to 57% of V.

Anal. Calcd. for $C_{20}H_{16}O_2$: C, 83.4; H, 5.6. Found: C, 83.2; H, 5.7.

4,5-Dimethyl-9,10-dihydro-10-keto-1,2-benzanthracene (VI).—A solution of 25.0 g. of V in 200 ml. of anhydrous hydrogen fluoride was left to evaporate overnight in a plastic narrow-mouth bottle. After isolation in the usual way (which included a washing with 10% sodium carbonate) there was obtained 19.7 g. (87%) of VI, m.p. 103–106°. Recrystallization from benzene and methanol afforded a pure sample, m.p. 107–108°, with little loss. The infrared spectrum showed no hydroxyl absorption and a strong ketonic band at 6.02 μ (1660 cm.$^{-1}$).

Anal. Calcd. for $C_{20}H_{16}O$: C, 88.2; H, 5.9. Found: C, 88.2; H, 6.0.

4,5-Dimethyl-1,2-benzanthracene (VII).—A well-stirred mixture of 10 g. of zinc activated with copper sulfate solution, 3.0 g. of VI, m.p. 103–106°, 20 ml. of toluene, and 50 ml. of 10% sodium hydroxide solution was heated at reflux for 36 hr. The yellow color of the initial mixture faded towards the end. After the usual work-up, the crude product was purified by chromatography over alumina using petroleum ether–benzene as developing solvent. Finally, recrystallization from benzene and ethanol afforded 2.2 g. (78%) of colorless needles, m.p. 138–139°.

Anal. Calcd. for $C_{20}H_{16}$: C, 93.7; H, 6.3. Found: C, 93.7; H, 6.3.

The trinitrofluorenone derivative,[21] m.p. 227–228°, was prepared in benzene and recrystallized from xylene.

Anal. Calcd. for $C_{33}H_{21}N_3O_7$: C, 69.4; H, 3.7; N, 7.4. Found: C, 69.0; H, 3.7; N, 7.7.

4,5,10-Trimethyl-1,2-benzanthracene (VIII).—Methyllithium prepared from 23 g. of methyl iodide and excess lithium in 75 ml. of ether was forced under nitrogen into a solution of 3.2 g. of VI in 75 ml. of pure dry benzene. After refluxing for 10 hr. the mixture was poured on dilute hydrochloric acid and the organic product was isolated as usual. After chromatography over alumina as for VII there was isolated a pale yellow oil which afforded 1.2 g. (38%) of VIII on crystallization from ethanol. When first obtained a form, m.p. 87–88°, was isolated. This proved to be a low-melting polymorphic form, as a form of VIII, m.p. 105–106°, was later obtained.

Anal. Calcd. for $C_{21}H_{18}$: C, 93.3; H, 6.7. Found: C, 93.4; H, 6.8.

The trinitrofluorenone derivative, m.p. 232–234°, was obtained in and recrystallized from benzene containing about 2% excess VIII.

Anal. Calcd. for $C_{34}H_{23}N_3O_7$: C, 69.8; H, 3.9; N, 7.2. Found: C, 69.5; H, 3.8; N, 7.2.

(20) The decarboxylations and the syntheses of ketones were carried out as described for similar cases by M. S. Newman and P. G. Scheurer, *J. Am. Chem. Soc.*, **78**, 5004 (1956).

(21) M. Orchin and O. Woolfolk, *J. Am. Chem. Soc.*, **68**, 1727 (1946).

Synthesis of N^6,N^6-Bis(2-chloroethyl)-DL-lysine

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Received January 27, 1964

The synthesis of N^6,N^6-bis(2-chloroethyl)-DL-lysine, using classical procedures, is described. The starting material, 5-(4-hydroxybutyl)-3-benzylhydantoin, was converted to the 5-(4-bromobutyl) derivative. By reaction of this with diethanolamine, hydrolytic ring opening, N^2-benzoylation, esterification, chlorination, and hydrolysis, N^6,N^6-bis(2-chloroethyl)-DL-lysine was obtained as the hydrochloride. The free base was obtained from this. A method has been studied (which could be extended to other amino acids) for the purification of the intermediate and final products through their precipitation as reineckates. The free intermediates and amino acids were obtained from their reineckates by use of a cation-exchange resin.

As a result of the studies by Larionov[1] and Bergel[2] the investigation of amino acids as precursors of possible antitumor agents, has attracted the attention of numerous research workers.[3–5] A number of N-substituted bis(2-chloroethyl)amino acids were synthesized and subjected to biological testing. In particular, the synthesis of N^6,N^6-bis(2-chloroethyl)-DL-lysine was suggested,[6] and a first hint on the preparation of this compound was given by Ishidate.[7] From Ishidate's work it appears that the lysine derivative was isolated as an impure double salt of picrylsulfonic and hydrochloric acids. Larionov[8] then reported on the antitumor

(1) (a) L. F. Larionov, "Malignant Tumors," Vol. 1, Part 2, N. N. Petrov, Ed., Leningrad, 1948, p. 149; (b) L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina, V. I. Trusheikina, and A. M. Novikova, *Lancet*, **269**, 169 (1955).

(2) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954).

(3) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *ibid.*, 2386 (1953).

(4) A. P. Martinez, W. A. Skinner, W. W. Lee, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 6050 (1960).

(5) G. E. McCasland, R. Horvat, J. Korntved, and A. Furst, *J. Org. Chem.*, **23**, 1568 (1958).

(6) G. E. Lewis, *Rept. Brit. Emp. Cancer Campaign*, **33**, 455 (1955).

(7) M. Ishidate, Y. Sakurai, and I. Aiko, *Chem. Pharm. Bull.* (Tokyo), **8**, 732 (1960).

(8) L. F. Larionov and I. G. Spasskaia, *Vopr. Onkol.*, **7**, No. 11, 75 (1961); *Chem. Abstr.*, **56**, 13,512 (1962).