Structure–Carcinogenic Activity Relationships in the Benz[a]anthracene Series.[1] 1,7,12- and 2,7,12-Trimethylbenz[a]anthracenes

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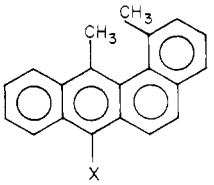
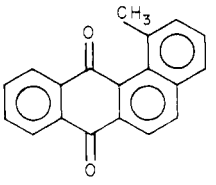
The syntheses of 1,7,12-trimethyl- and 2,7,12-trimethylbenz[a]anthracenes are described. The lack of carcinogenic activity of these compounds is discussed in relationship to the carcinogenic activity of other substituted benz[a]anthracenes.

The structure–activity relationships in carcinogenic activity of benz[a]anthracenes have been under study for many years.[3] Of the monomethyl derivatives, 7-methylbenz[a]anthracene (1) is by far the most potent. The fact that 7,12-dimethylbenz[a]anthracene (2) is more active than 1 has been explained[4] by postulating that the strain produced in the molecule by the bulk of the 12-methyl group is responsible.[5] Accordingly, we thought that if more strain were introduced more activity might result. Hence, we decided to prepare 1,7,12-trimethylbenz[a]anthracene (3) for testing because the added methyl group in the 1



4, X = H
3, X = CH_3



6

position should increase the strain.[6] However, we have been informed[7] that **3** has no carcinogenic activity.

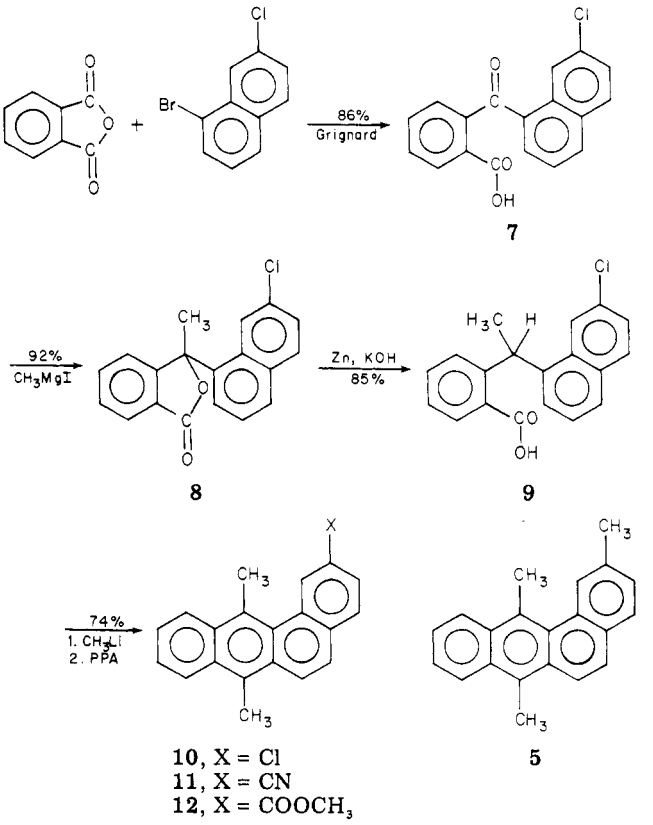
This finding may be compared to the fact that 12-methylbenz[a]anthracene has slight activity whereas 1,-12-dimethylbenz[a]anthracene (4) has none.[7,8] Recently we were informed[7] that 2,7,12-trimethylbenz[a]anthracene (5) prepared in connection with another aspect of the carcinogenic activity problem (see below) is also inactive (see also ref 5). Thus, we are faced with the fact that substitution of a methyl group in the 1,[7] 2,[8] 3,[8] and 5 position[7,8] of 7,12-dimethylbenz[a]anthracene results in inactive compounds, whereas the substitution of a methyl group in the 4,[7] 6,[7] 9,[7] or 10 position[7] yields active compounds.

Interestingly, 4,7-dimethylbenz[a]anthracene is inactive[8] whereas 4,7,12-trimethylbenz[a]anthracene is active.[8] Both 4-fluoro-7-methylbenz[a]anthracene and 4-fluoro-7,12-dimethylbenz[a]anthracene are active. From these results apparently the structure–activity relationships differ in the 7-methyl- and 7,12-dimethylbenz[a]anthracene series and are also differently affected by methyl and fluorine substitution in the nonmeso positions. In each series, however, substitution of a fluorine or a methyl at the 5 position produces an inactive compound. Obviously further examples of *x*-fluoro-7,12-dimethylbenz[a]-anthracenes are needed for testing before a further discussion of structure–activity relationships is made.

The synthesis of **3** was accomplished by chloromethylation of the available 1,12-dimethylbenz[a]-anthracene[9] (4), followed by a two-step reduction of the chloromethyl group (via iodomethyl) to **3**. The structure of **3** was established by spectral data and by oxidation to the known 1-methyl-7,12-benz[a]anthraquinone[10] (**6**).

The synthesis of 2,7,12-trimethylbenz[a]anthracene (**5**) is outlined in Scheme I and is described in the Experimental Section. An alternate route which was explored after the route shown in Scheme I had been completed is

Scheme I



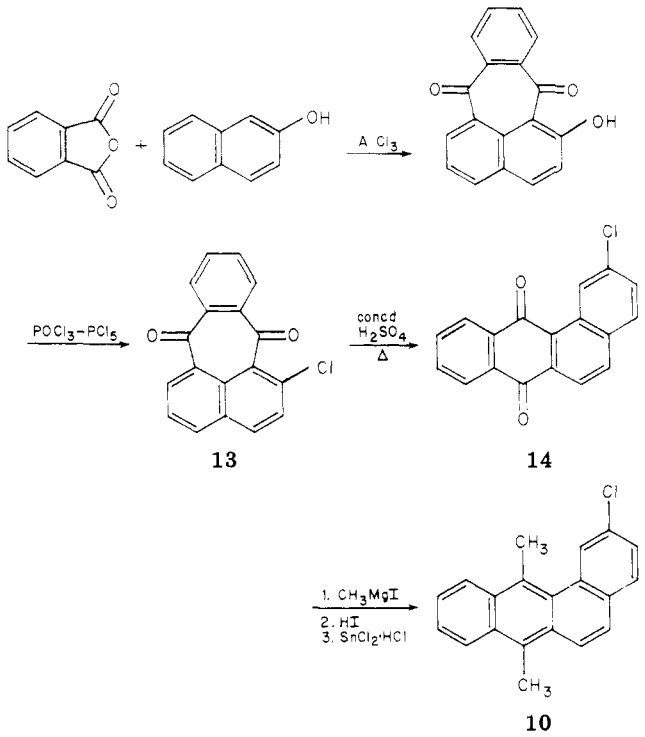
shown in Scheme II. Although insufficient development work was done to evaluate this second route, the fact that the 2-chloro-7,12-dimethylbenz[a]anthracene produced proved to be **10** establishes the structure of **14**[11] to be as shown.

Experimental Section[12]

1,12-Dimethyl-7-iodomethylbenz[a]anthracene. A solution of 0.70 g of 4 in 100 ml of HOAc was treated at room temperature with 35 ml of $ClCH_2OCH_3$ followed by 50 ml of 47% HI. After 1 h at 0 °C the bright yellow solid was collected and washed with ligroine to yield 1.06 g of crude sensitive iodomethyl compound (*m/e* 396,[13] strong ir band at 8.85 μ due to $ArCH_2X$ bending) which decomposed on heating over 120 °C. Due to instability this compound was not sent for elemental analysis.

1,7,12-Trimethylbenz[a]anthracene (3). A solution of 1.06 g of the above iodomethyl compound in 100 ml of dioxane containing 5 ml of concentrated HCl was added to a solution of 20 g of $SnCl_2$ in 100 ml of dioxane and 100 ml of concentrated HCl. After being held at reflux for 5 min the solution was held at 20–25 °C for 0.5 h and then diluted with 500 ml of water. The product was isolated as usual and the crude material was purified by formation and recrystallization of the 2,4,7-trinitrofluorenone[14] complex: black elongated prisms; mp 149–150 °C.[15] Anal. ($C_{34}H_{23}N_3O_7$) C, H, N. After chromatography on basic alumina and recrystallization from acetone–methanol, there was obtained 0.26 g (35% overall from 4) of **3**: yellow crystals; mp 108–109 °C; *m/e* 270; NMR τ 7.10 (s, 3, $ArCH_3$), 7.23 (s, 3, $ArCH_3$), 7.60 (s, 3, $ArCH_3$). Anal. ($C_{21}H_{18}$) C, H. In a similar series, except that

Scheme II



the iodomethyl compound was reduced with tributyl tin hydride,[16] there was obtained **3** in 44% overall yield.

1-Methyl-7,12-benz[*a*]anthraquinone (6). A mixture of 50 mg of pure **3**, 0.7 g of $K_2Cr_2O_7$, and 8 ml of HOAc was held at reflux for 5 min. After the usual work-up, the product was passed through a short column of neutral alumina and recrystallized from aqueous ethanol to yield **6**: mp 187–188 °C (lit.[10] 188.5–189.5 °C); ir (KBr) 5.97 μ (*m/e* 272); NMR τ 7.35 (s, 3, $ArCH_3$).

1-Bromo-7-chloronaphthalene. A mixture of 66 g (0.3 mol) of *N*-(7-chloro-1-naphthyl)acetamide,[17] 400 ml of CH_3OH, and 350 ml of concentrated HCl was held at reflux for 2 h. On cooling 61 g of the hydrochloride of 7-chloro-1-naphthylamine, mp 251 °C dec, was obtained and diazotized by adding 21 g of $NaNO_2$ to a stirred suspension of the solid in 300 ml of water and 600 ml of concentrated HCl at 0–5 °C. The solution thus obtained was added to a cold suspension formed by treating 108 g of $HgBr_2$ with 31 g of NaBr in 300 ml of water. The insoluble complex which separated immediately was collected by filtration and washed with water and then acetone. The air-dried complex weighed 118 g. Decomposition as described for similar cases[18] gave a distillate, bp 97–100 °C (0.05 mm), which on crystallization from 95% ethanol afforded 46.3 g (64% from amide) of colorless 1-bromo-7-chloronaphthalene, mp 67–68 °C (lit.[19] 68 °C).

2-(7-Chloro-1-naphthoyl)benzoic Acid (7*). A Grignard reagent was prepared from 48.3 g (0.2 mol) of 1-bromo-7-chloronaphthalene and 9.73 g of sublimed magnesium[20] in 500 ml of ether to which 37.6 g (0.2 mol) of ethylene dibromide was added (the entrainment method[21]). As soon as the reaction started, 500 ml of benzene was gradually added and removal of ether by distillation was started. When all of the magnesium had reacted and almost all of the ether had been distilled, this reagent was added slowly to a hot stirred solution of 29.6 g of pure phthalic anhydride in 1 l. of benzene. After 45 h[22] at reflux the reaction mixture was treated with dilute HCl and worked up as usual. From the acidic portion of the products there was obtained 53.4 g (86%) of **7**, mp 209–212 °C, suitable for the next step. The analytical sample melted at 213–214.5 °C after recrystallization (with little loss) from aqueous ethanol.

3-Methyl 3-(7-Fluoro-1-naphthyl)phthalide (8*). A Grignard reagent prepared from 4.0 g of Mg[20] and 26 g of CH_3I in 400 ml of ether was added rapidly to a stirred solution of 15.6 g of **7** in 600 ml of THF. After 40 h at reflux, the mixture was cooled, treated with dilute HCl, and worked up as usual to yield 14.2 g (92%) of **8**, mp 181–183.5 °C, from the neutral fraction of the reaction products. The analytical sample melted at 184.5–185.5 °C after recrystallization (with little loss) from ethanol–benzene.

2-(7-Chloro-1-naphthylethyl)benzoic Acid (9*). A stirred slurry of 30 g of Zn dust, 1.0 g of $CuSO_4$, 7.71 g of **8**, 30 g of KOH, and 300 ml of ethylene glycol was held at reflux for 24 h. The cooled reaction mixture was filtered through Celite and the filtrate was worked up in the usual way to yield 6.6 g (85%) of **9**, mp 188–190 °C, suitable for further work. The analytical sample melted at 192–193 °C after recrystallization (with little loss) from benzene–cyclohexane.

2-Chloro-7,12-dimethylbenz[*a*]anthracene (10*). A stirred solution of 6.2 g of **9** in 500 ml of ether was treated dropwise with 40 ml of 2.2 M CH_3Li.[23] After 65 h at reflux the reaction mixture was treated with dilute HCl and worked up as usual. The neutral fraction (6.5 g) was stirred with 50 g of 115% polyphosphoric acid[24] at 100 °C for 10 min. A benzene solution of organic product was passed through a short column of neutral alumina.[25] Crystallization of the product thus obtained from cyclohexane–low-boiling petroleum ether yielded 4.3 g (74% from **9**) or pure **10**, mp 99–100 °C.

2-Cyano-7,12-dimethylbenz[*a*]anthracene (11*). Treatment of 1.5 g of **10** with 2.0 g of CuCN in 20 ml of *N*-methylpyrrolidone[26] at reflux for 3 days as described[27] afforded 2.71 g (49%) of **11**, mp 116–118 °C. The analytical sample, mp 120–121 °C, was obtained by recrystallization (with little loss) from benzene–ethanol.

2-Carbomethoxy-7,12-dimethylbenz[*a*]anthracene (12*). A solution of 1.5 g of **11** in 20 ml of ethylene glycol containing 2.0 g of water and 3.0 g of KOH was held at reflux for 6 h. The crude acid thus obtained was heated with methanol saturated with HCl for 7 h to yield 1.54 g (92%) of pure **12**, mp 150–151 °C, after recrystallization from methanol.

2,7,12-Trimethylbenz[*a*]anthracene (5). A solution of 1.4 g of **12** in 150 ml of THF was held at reflux for 16 h with 3.0 g of $LiAlH_4$. After work-up as usual there was obtained 0.97 g (80%) of **5**, mp 102–104 °C. A mixture melting point with a sample of **5** supplied by Dr. R. G. Harvey, University of Chicago, was not depressed.[8]

Assay for Abilities of 3 and 5 to Induce Sarcomas in Rats.[28] In the first experiment groups of 18 male CD random-bred rats (Charles River Breeding Laboratory, Wilmington, Mass.; av wt, 260 g) received single sc injections of 0.75 or 1.5 mg of **2** or 0.5 mg of **3** in the right hind leg. None of the rats injected with **3** developed tumors; 15 rats were autopsied on termination of the experiment at 15 months and three were autopsied when they died prior to this time. By 6, 10, and 15 months 4, 8, and 14, respectively, of the rats injected with the lower dose of **2** and 7, 17, and 17, respectively, of the rats injected with the higher dose had developed sarcomas at the site of injection; three and one rats, respectively, from these two groups were alive and tumor-free at the termination of the experiment. In a second experiment groups of 12 male Fischer rats (Charles River Breeding Laboratory, av wt 180 g) received one sc injection in the right hind leg of 2.2 mg of **2** or 2.3 mg of **5** one or three times at weekly intervals. All 12 rats in each of the two groups injected with **5** survived to the termination of the experiment at 18 months and were found to be tumor-free on autopsy. Of the rats that were injected with **2** five had sarcomas at the injection site by 6 months and all had sarcomas at the injection site by 8 months.

References and Notes

(1) This work was supported by Grant 2 R01 CA-07934 of the National Cancer Institute, NIH.
(2) Postdoctoral Research Associate.
(3) For a review, see J. C. Argos and M. F. Argus, "Chemical Induction of Cancer", Part III, Academic Press, New York, N.Y., 1974.
(4) M. S. Newman and R. F. Cunico, *J. Med. Chem.*, **15**, 323 (1972).
(5) For evidence from heats of combustion, see M. A. Frisch, C. Barker, J. L. Margrave, and M. S. Newman, *J. Am. Chem. Soc.*, **85**, 2356 (1963).
(6) See "The Biochemistry of Disease, Chemical Carcinogenesis", Part A, P. O. P. Ts'o and J. A. DiPaolo, Ed., Marcel Dekker, New York, N.Y., 1974, p 177.
(7) J. A. Miller and E. J. Miller, unpublished results, McArdle

Laboratory for Cancer Research, University of Wisconsin.
(8) J. Pataki, C. Duguid, P. W. Rabideau, H. Huisman, and R. G. Harvey, *J. Med. Chem.*, **14**, 940 (1971).
(9) M. S. Newman, W. C. Sagar, and M. V. George, *J. Am. Chem. Soc.*, **82**, 2376 (1960).
(10) (a) J. W. Cook and A. M. Robinson, *J. Chem. Soc.*, 505 (1938); (b) L. F. Fieser and A. M. Seligman, *J. Am. Chem. Soc.*, **60**, 170 (1938).
(11) L. F. Fieser and M. Fieser, *J. Am. Chem. Soc.*, **55**, 3342 (1933). See also L. F. Fieser, *ibid.*, **53**, 3546 (1931).
(12) The phrase "worked up in the usual way" means that an ether–benzene solution of the products was washed with dilute acid and/or base and with saturated NaCl solution. The organic layer was then filtered through a cone of $MgSO_4$ and the solvent removed by distillation or on a rotary evaporator. All compounds designated with an asterisk gave elemental analysis within ±0.3% of the theory for C, H, Cl, and N (where applicable).
(13) We thank Mr. R. Weisenberger for the mass spectra.
(14) M. Orchin and E. Woolfolk, *J. Am. Chem. Soc.*, **68**, 1727 (1946).
(15) All melting points and boiling points are uncorrected.
(16) H. G. Kuivila and O. F. Beumel, Jr., *J. Am. Chem. Soc.*, **83**, 1246 (1961).
(17) M. S. Newman and W. M. Hung, *J. Org. Chem.*, **38**, 4073 (1973).
(18) M. S. Newman and P. H. Wise, *J. Am. Chem. Soc.*, **63**, 2847 (1941).
(19) The present synthesis seems superior to that described by D. Horn and F. Warren, *J. Chem. Soc.*, 144 (1946).
(20) Highly pure sublimed magnesium was obtained through the courtesy of the Dow Chemical Co., Midland, Mich., and from Dr. T. E. Leontis, Battelle Memorial Institute, Columbus, Ohio.
(21) D. E. Pearson, D. Cowan, and J. D. Beckler, *J. Org. Chem.*, **24**, 504 (1959).
(22) Shorter periods of reflux, about 5–10 h, gave much poorer yields.
(23) Used as obtained from Foote Mineral Co., Exton, Pa.
(24) Used as obtained from Sigma Chemical Co., St. Louis, Mo. 63178.
(25) Neutral Woelm alumina (activity I) was used.
(26) We thank the General Aniline and Film Corp. for a generous gift of *N*-methylpyrrolidone.
(27) M. S. Newman and H. Boden, *J. Org. Chem.*, **26**, 2525 (1961).
(28) Data provided by Drs. James A. Miller and Elizabeth C. Miller, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wis.

Book Reviews

Clinical Cancer Chemotherapy. Edited by Ezra M. Greenspan. Raven Press, New York, N.Y. 1975. xvii + 414 pp. 16 × 24 cm. $16.50.

Steady progress in clinical cancer chemotherapy during the last few years has made it increasingly difficult for physicians in private practice to stay abreast of the developments in this field. Several short books on cancer chemotherapy have appeared since 1970 in order to fill this gap and also to provide short, economically priced textbooks for students in general medicine. The latest entry, edited by E. M. Greenspan of the Mount Sinai School of Medicine, consists of 20 short chapters arranged according to tumor type. All the authors are physicians and the overall result is a strong emphasis on the clinical aspects of cancer chemotherapy, though an effort is made to provide at least a brief background in respect to the biochemical and pharmacologic basis of action of antitumor drugs. There is also an interesting appendix consisting of 273 multiple choice, true and false, and matching questions that medical students as well as graduate physicians will find useful in studying for specialty board exams in oncology.

Despite numerous excellent qualities this book suffers from some inadequacies, most of them unfortunately having to do with the "background fundamentals". In Chapter One, for example, folic acid is said vaguely to be a "tumor antagonist" in MTX-resistant L1210 leukemia (p 8). Without amplification this is difficult to understand, especially since the supporting reference by Law is not a published article but a personal communication. Again in the opening chapter, actinomycin D, mitomycin, and the anthracyclines are described rather imprecisely as "protein inhibitors" (Table 1-3, p 10), whereas cyclophosphamide is described as being "atypical phosphamidase activated". On p 11 the phases of the cell cycle are enumerated as "S_1, G_1, S_2 (sic), and G_2". In Chapter Three, chlorambucil is incorrectly named "1-(di-2-chloroethyl)aminophenylbutyric acid" (p 16); the mechanism of activation of cyclophosphamide is said to involve phosphamidase enzymes, but no mention at all is made of the fact that oxidative cleavage of the C–N bond must first take place (p 38); the discussion of 6-MP mentions nothing about the active metabolites of this compound or their multiple sites of inhibition of purine biosynthesis (pp 43–45); and the action of methotrexate and aminopterin is described in a quite unorthodox way as resulting from sequential blockade of dihydrofolate reductase and thymidylate synthetase (pp 45, 46).

There are also a number of small but irritating inaccuracies. For example, vincristine is said to contain a methyl group in place of a formyl group (p 89), whereas in fact it is vinblastine that has the methyl group. Rubidazone is called "rubindazone" (p 90), L-phenylalanine mustard is referred to on one occasion as L-phenylalanine (p 167), and several well-known authors have had their names spelled incorrectly ("Workheiser" on p 68, "Hutchings" on p 88, and "Djirassi" on p 201).

Though medicinal chemists will no doubt derive more benefit from other reference sources, this book still makes worthwhile reading because it provides knowledgeable and sympathetic insight into the complexities that every new drug encounters once it reaches the real world of the clinic. It is also a healthy reminder that, although dramatic gains have been made in the field of cancer chemotherapy during the past 30 years, in some areas the rate of progress has been discouragingly slow.

For those who like to "comparison shop", it should be mentioned that a competitive volume appeared this year in the form of a second edition of "Cancer Chemotherapy" by Cline and Haskell (W. B. Saunders, Philadelphia, Pa.). The two books are of comparable length and both are printed on high-quality glossy paper, though the latter lacks a question and answer appendix.

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Radioimmunoassay in Clinical Biochemistry. Edited by C. A. Pasternak. Heyden & Son Ltd., London. 1975. 299 pp. 16.5 × 25 cm. $29.50.

This volume represents a selection of papers presented at a symposium on Radioimmunoassay and Related Topics in Clinical Biochemistry held at Oxford in 1974. The main subject divisions are General Methodology, Drugs, Steroids, Thyroid Hormones, Protein Hormones, and Antibodies and Other Proteins. Most of the 29 papers are referenced and there is an index.

Several of the papers involved discussions of radioimmunoassay of drugs. Morphine, digoxin, and tetrahydrocannabinol assays are included as well as discussions of radioiodination tags for drugs and immunoreactivity of drug–protein conjugates.