4-Chloro-5-sulfamylanthranilic acid. A solution of 0.20 g. (0.00073 mole) of XVII in 3 ml. of 5N sodium hydroxide was heated on a steam bath for 1.5 hr., cooled, and acidified with hydrochloric acid when 0.15 g. (83%) of a white solid melting at 267–268° dec. was obtained. The mixed melting point, infrared and ultraviolet spectra were identical to 4-chloro-5-sulfamylanthranilic acid.[2]

5-Chloro-6-sulfamylbenzimidazolone (XVIII). A suspension of 0.070 g. (0.00025 mole) of XVII in 20 ml. of xylene was refluxed for 0.5 hr. and filtered to give 0.055 g. (87%) of a white solid which did not melt <300°. (This did not have a band at 4.6μ in the infrared spectrum); λ_{max}^{NaOH} 267, 299 mμ; log ϵ 3.943, 3.978. The product was recrstalylized from a mixture of ethanol and heptane.

Anal. Calcd. for $C_7H_6ClN_3O_3S \cdot H_2O$: C, 32.9; H, 3.04; N, 15.8. Found: C, 33.0; H, 2.93; N, 15.9.

3-Chloro-6-nitro-4-sulfamylaniline (XIX). To a well-integrated mixture of 1.0 g. (0.0046 mole) of 3-chloro-4-nitroacetanilide[7] and 0.3 g. of sodium chloride, was added gradually 5 ml. of chlorosulfonic acid. The dark brown mixture was heated on a steam bath for 1 hr. and carefully poured on 50 g. of ice. The tacky solid which separated was filtered and washed with 10 ml. of ice water. This was suspended in 20 ml. of cond. ammonium hydroxide and was kept at room temperature for 18 hr. The mixture was filtered, and the product was washed with ice water. One recrystallization from hot water gave 0.1 g. (8.6%) of a shiny crystalline yellow solid decomposing at 254–255°. (Infrared spectrum–absence of the CONH–band at 5.95 μ).

Anal. Calcd. for $C_6H_6ClN_3O_4S$: C, 28.6; H, 2.39; N, 16.7 Found: C, 28.6; H, 2.80; N, 16.5.

3-Chloro-4-sulfamyl-o-phenylenediamine (XX). A solution of 0.50 g. (0.0020 mole) of XIX in 16 ml. of 1N sodium hydroxide was treated with 1.8 g. (0.0080 mole) of sodium hydrosulfite dihydrate, and the resulting mixture was warmed on a steam bath for 0.5 hr. This was diluted with 5 ml. of water, treated with activated charcoal, and filtered. On cooling, 0.16 g. (36%) of a white solid melting at 219–220° dec. precipitated from the amber filtrate.

Anal. Calcd. for $C_6H_8ClN_3O_2S$: C, 32.5; H, 3.60; N, 19.0. Found: C, 32.6; H, 3.94; N, 18.6.

5-Chloro-6-sulfamylbenzimidazolone (XVIII) from XX. Phosgene was bubbled through a solution of 0.030 g. (0.00014 mole) of XX in 2 ml. of 0.5 N sodium hydroxide until a copious white precipitate formed and the mixture was acidic to litmus paper. The mixture was filtered, and the solid was washed with ice water and dried at 62°. The product, 0.27 g. (80%) on one recrystallization from an ethanol-heptane mixture gave a material which did not melt <300° and which had the same ultraviolet and infrared spectra as the material obtained by heating the azide XVII in xylene.

Acknowledgment. We wish to thank Mr. L. Brancone and his associates for the microanalytical results.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

Investigation of the Metabolism of 3,4,9,10-Dibenzpyrene

ENVARE ÜNSEREN AND LOUIS F. FIESER

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The potently carcinogenic 3,4,9,10-dibenzpyrene when injected into mice is not metabolized but remains at the site of injection. The hydrocarbon is attacked at the 5- and 8-positions on oxidation (chromic anhydride or selenium dioxide), nitration, and acetoxylation with lead tetraacetate. Reduction with sodium and amyl alcohol gives the 1,2,6,7-tetrahydride (VII); catalytic hydrogenation gives first the 1,2-dihydride(X) and then the octahydride(XII).

Studies of the metabolism of 1,2,5,6-dibenzanthracene[1] and 3,4-benzpyrene[2] have shown that these carcinogenic polycyclic hydrocarbons are excreted as phenols and hence that detoxification follows the pattern established for benzene and naphthalene,[3] anthracene,[4] and phenanthrene.[5] An opportunity to study the metabolic fate of 3,4,9,10-dibenzpyrene, recently recognized as a carcinogen,[6] arose through a project of F. Homburger and associates of the Bio-Research Institute[7] to supply tumor-bearing mice for chemotherapeutic studies at the Sloan-Kettering Institute. In experiments with over 10,000 inbred mice, one subcutaneous injection in the groin of 0.5 mg. of 3,4,9,-10-dibenzpyrene in tricaprylin produced 50% sarcomas at the site of injection in 14 weeks and 98% tumors in 24 weeks. Comparison with published data shows that the hexacyclic hydrocarbon ranks in potency between methylcholanthrene and 3,4-benzpyrene. The latent period is somewhat longer than with methylcholanthrene, but the dose yielding 50% tumors is one third the dose of methylcholanthrene. These results led to selection of 3,4,9,10-dibenzpyrene for routine tumor production on a large scale. A supply of hydrocarbon was synthesized by Thomas U. Hall of Arthur D. Little

(1) A. A. Levi and E. Boyland, *Chem. Ind.*, **15**, 446 (1937); E. Boyland, A. A. Levi, E. H. Mawson, and E. M. F. Roe, *Biochem. J.*, **35**, 184 (1941); K. Dobriner, C. P. Rhoads, and G. I. Lavin, *Proc. Soc. Exptl. Biol.*, **41**, 67 (1939).

(2) J. G. Chalmers and D. Crowfoot, *Biochem. J.*, **35**, 1270 (1941); I. Berenblum and R. Schoental, *Cancer Research*, **3**, 145 (1943); **6**, 699 (1946); I. Berenblum, D. Crowfoot, E. R. Holiday, and R. Schoental, *ibid.*, **3**, 151 (1943); F. Weigert and J. C. Mottram, *ibid.*, **6**, 97, 109 (1946).

(3) L. Young, *Biochem. J.*, **41**, 417 (1947); J. Booth and E. Boyland, *ibid.*, **44**, 361 (1949).

(4) E. Boyland and A. A. Levi, *Biochem. J.*, **29**, 2679 (1935); **30**, 728 (1936).

(5) L. Young, *Biochem. J.*, **41**, 417 (1947); E. Boyland and G. Wolf, *ibid.*, **47**, 64 (1950).

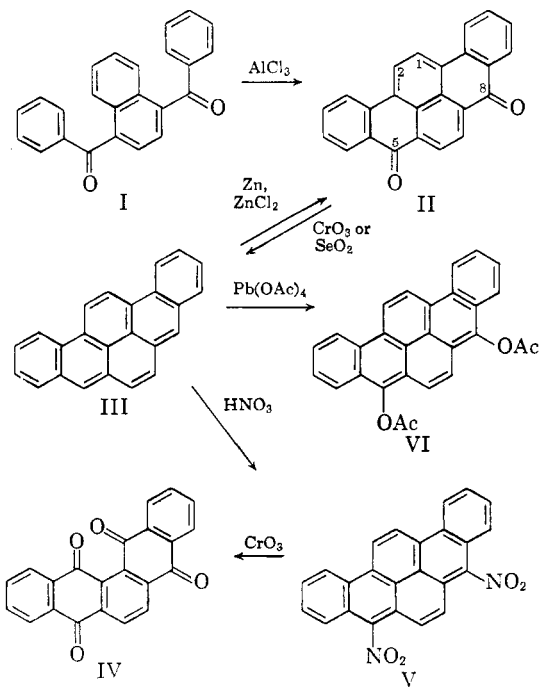
(6) N. P. Buu-Hoï and H. Chalvet, *Compt. rend.*, **244**, 273 (1957).

(7) F. Homburger and A. Tregier, *Progr. Exptl. Tumor Research*, **1**, 311 (1960).

Co. by a procedure recorded in the experimental part; the method, suggested by one of us, involved the intermediates I and II.[8] The standard dose of 0.5 mg. per mouse was injected in tricaprylin containing 3% of cholesterol, which to some extent accelerates tumor formation perhaps by increasing the solubility of the hydrocarbon.

In view of the high potency of the hydrocarbon and the small amount administered, we were glad to accept Dr. Homburger's invitation to investigate possible metabolites excreted in feces collected over a period of one month after injection of 3,4,9,-10-dibenzpyrene. However, chromatography of benzene extracts of the feces gave a series of fractions having similar ultraviolet absorption spectra showing no resemblance to the characteristic multiple-band spectrum of 3,4,9,10-dibenzpyrene or to those of products of oxidation and of reduction examined for comparison. In short, aromatic metabolites seemed to be absent. The only crystalline substance encountered proved to be cholesterol. We then examined hydrocarbon-induced tumors and found that benzene extracts a substance having a yellow fluorescence identified as 3,4,9,10-dibenzpyrene by complete correspondence of the ultraviolet absorption spectrum. Thus, the sparingly soluble 3,4,9,10-dibenzpyrene remains at the injection site and is not eliminated in the form of metabolites. The low solubility accounts for the slowness of action, as compared to methylcholanthrene, and the persistence at the injection site explains the equivalent tumor yield at one third the dose.

3,4-Benzpyrene reacts readily with lead tetraacetate[9] and with nitric acid[10] to give substances identified[11] as the 5-acetoxy and 5-nitro derivatives.



In 3,4,9,10-dibenzpyrene the 5- and 8- positions are identical and thus should both possess high reactivity. In accordance with expectations, we found that the hydrocarbon is oxidized to the 5,8-quinone II by either chromic acid in acetic acid or selenium dioxide in nitrobenzene. The product of reaction with lead tetraacetate was shown to be the 5,8-diacetoxy derivative VI by the preparation of an identical product by reductive acetylation of the quinone II and by conversion of VI into this quinone by saponification and air oxidation. Nitration of dibenzpyrene afforded the 5,8-dinitro derivative V, as shown by oxidation to the known[12] 1,2-phthaloylanthraquinone IV.

Reduction of 3,4,9,10-dibenzpyrene with sodium and amyl alcohol gave a tetrahydride which we regard as the 1,2,6,7-tetrahydride VII (Fig. 1)

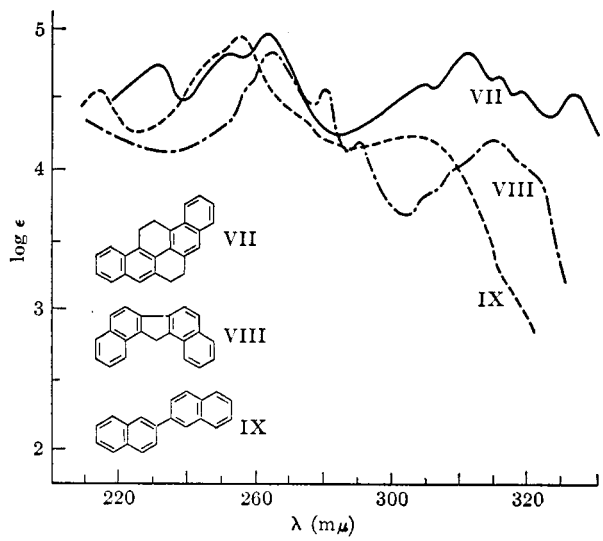


Figure 1

because the spectrum is not of the 3,4-benzpyrene type but resembles the spectra of 1,2,7,8-dibenzfluorene (VIII) and of β-dinaphthyl (IX). In ethyl acetate–acetic acid in the presence of the Adams catalyst, the hydrocarbon consumed one mole of hydrogen rapidly and afforded in 65% yield a dihydride. Fig. 2 shows that this substance corresponds in spectrum to picene (XI) and hence that it must be the 1,2-dihydride X. Hydrogenation under the same conditions but over a ten-hour period gave an octahydride of spectrum (Fig. 3). typical of pyrene (XIII) but with the peaks shifted to longer wave length from the bathochromic effect of alkyl substitution. Thus, the two terminal rings appear to have been hydrogenated, as in XII and

(8) G. Kränzlein, H. Vollmann, and M. Corell, U. S. Patents 1,876,966 (1932), 1,901,307 (1931).

(9) A. Windaus and S. Rennhak, *Z. physiol. Chem.*, **249**, 256 (1937).

(10) L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, 60, 2542 (1938).

(11) L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, 61, 1565 (1939).

(12) R. Scholl and H. Neumann, *Ber.*, 55, 118 (1922).

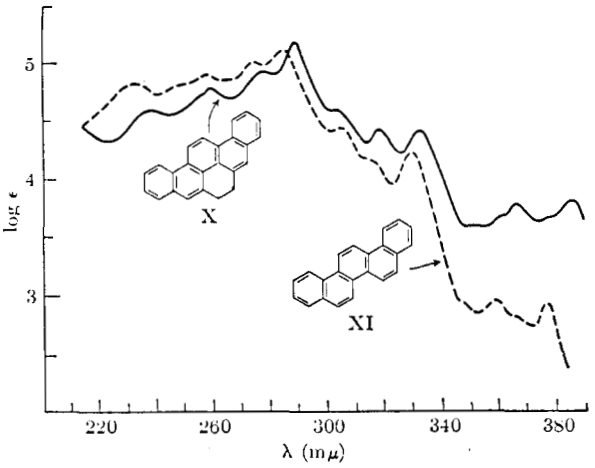


Figure 2

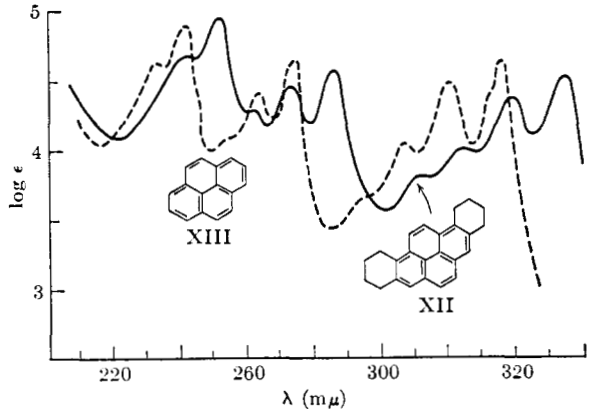


Figure 3

the substance is derived from the dihydride X not by direct hydrogenation but by a process of hydrogenation-dehydrogenation.

EXPERIMENTAL

Preparation of 3,4,9,10-dibenzypyrene (by T. U. Hall). An intimate mixture of 7 g. of 3,4,9,10-dibenzypyrene-5,8-quinone,[13] 7 g. of zinc dust, 7 g. of sodium chloride, and 35 g. of zinc chloride was heated for 1 hr. at 290–300° while being stirred with a stainless steel paddle and shaft. After cooling, 1 kg. of ice and 150 ml. of concentrated hydrochloric acid were added. When the inorganic matter had dissolved, the residue was collected, dried, and crystallized from xylene and then from benzene (Norit). The hydrocarbon (4.1 g., 60%) was obtained as lustrous golden plates, m.p. 278°.

Extraction of feces. A suspension of 120 g. of feces in 300 ml. of benzene was stirred for 10 hr., the dark green-black extract was evaporated under vacuum at room temperature to about 50 ml., centrifuged, and chromatographed on 30 g. of alumina. Elution with petroleum ether, benzene, ether, and methanol afforded 30 fractions, which were green-black, green, or yellow and which showed reddish- blue or blue fluorescence in ultraviolet light. The ultraviolet spectra of all fractions showed only one peak at 275 mμ and were nearly identical, and none showed evidence of aromatic systems. Evaporation left oily residues, and the only solid product encountered, after repeated crystallization from methanol-ether, formed colorless leaflets, m.p. 146°, and was identified as cholesterol by mixed m.p. determination, infrared comparison, and conversion to the acetate. The cholesterol gave a positive selenium dioxide test indicative of the presence of Δ^7-stenol. The late fractions eluted by methanol on distillation gave a colorless oil, b.p. 26°/20 mm.; the infrared spectrum showed a hydroxyl band.

Extraction of Tumors. A 32-g. batch of tumors was refluxed with 200 ml. of benzene for 8 hr. and the extract was concentrated at room temperature under vacuum to about 30 ml. and chromatographed on 20 g. of alumina. Elution with petroleum ether, benzene, ether, and methanol gave 25 fractions, all showing yellow fluorescence under ultraviolet light. Four fractions eluted by benzene showed spectra identical with that of 3,4,9,10-dibenzpyrene and were combined. Evaporation of the solvent left a solid which crystallized from benzene in yellow needles, but the amount was insufficient for further characterization. Cholesterol was isolated from later fractions. All other fractions showed spectra essentially the same and with a single peak at 275 mμ.

3,4,9,10-Dibenzpyrene-5,8-quinone (a) A stirred suspension of 1.017 g. of dibenzypyrene in 50 ml. of acetic acid was treated with a solution of 1.320 g. of chromic anhydride in 2 ml. of water and refluxed for 1 hr. The product which precipitated on dilution with water on crystallization from xylene afforded 0.736 g. (66%) of red needles, m.p. 370°, λ^{benzene} 276 (17,900), 306 mμ (25,800).

Anal. Calcd. for $C_{24}H_{12}O_2$: C, 86.73; H, 3.64. Found: C, 86.44; H, 3.38.

(b) A solution of 1.010 g. of dibenzypyrene in 300 ml. of nitrobenzene was treated with 1.105 g. of selenium dioxide and heated at 150–160° with stirring for 3 hr. The color changed immediately to dark red and then metallic selenium began to separate. The mixture was cooled to about 50°, filtered, and the residue washed with benzene. The filtrate was treated with 0.5 g. of powdered silver, let stand overnight filtered, and concentrated to the point of crystallization. The shiny black crystals on recrystallization from xylene (Norit) afforded 482 mg. (43%) of red needles, m.p. and mixed m.p. 370°.

5,8-Diacetoxy-3,4,9,10-dibenzpyrene. (a) A mixture of 410 mg. of 3,4,9,10-dibenzpyrene-5,8-quinone, 470 mg. of sodium acetate, and 40 ml. of acetic anhydride was heated to boiling under a reflux condenser and 2 g. of zinc dust was added in portions in 45 min. The red color of the mixture changed to yellow as the quinone dissolved. Acetic acid in equal volume was added, the solution was decanted, and the zinc was washed with more solvent. Water was added cautiously and then to the point of saturation. Crystallization of the product from xylene gave 516 mg. (90%) of fine yellow needles, m.p. 332°, λ^{benzene} 225 (30,100), 277 (33,900), 287 (47,600), 301 (65,300), 322 (23,400), 338 (23,100), 364 (21,800), 383 mμ (49,900).

Anal. Calcd. for $C_{28}H_{18}O_4$: C, 80.37; H, 4.34. Found: C, 80.16; H, 4.19.

(b) Solutions of 1.2 g. of lead tetraacetate in 50 ml. of acetic acid and of 740 mg. of dibenzpyrene in 300 ml. of benzene were mixed and allowed to stand overnight at room temperature. The solution was reduced to about half its volume by distillation and the product that separated on cooling or recrystallization from xylene gave 765 mg. (75%) of yellow needles, m.p. 332°.

Anal. Calcd. for $C_{28}H_{18}O_4$: C, 80.37; H, 4.34. Found: C, 80.52; H, 4.29.

A suspension of 170 mg. of 5,8-diacetoxy-3,4,9,10-dibenzpyrene in 30 ml. of methanol containing 200 mg. of potassium hydroxide was refluxed for 1 hr. The color changed to green, black, and then red, and a red product separated. Crystallization from xylene gave 125 mg. of 3,4,9,10-dibenzpyrene-5,8-quinone, m.p. 370°.

5,8-Dinitro-3,4,9,10-dibenzpyrene (V). A solution of 1.205 g. of dibenzpyrene in 300 ml. of benzene was diluted with 200 ml. of acetic acid and a solution of 1 ml. of concentrated nitric acid in 25 ml. of acetic acid was added with stirring. The mixture was allowed to stand overnight at room temperature

(13) Available under the name isodibenzpyrenequinone from Otto B. May, Inc., Newark, N. J.

and then cooled in ice. The product that separated on crystallization from nitrobenzene afforded 1.313 g. (84%) of orange-red needles, m.p. 385°, $\lambda^{benzene}$ 277 (15,600), 289 (23,500) 300 mμ (32,500).

Anal. Calcd. for $C_{24}H_{12}O_4N_2$: C, 73.47; H, 3.08; N, 7.14. Found: C, 73.63; H, 2.97; N, 7.22.

1,2-Phthaloylanthraquinone (IV). A suspension of 420 mg. of 5,8-dinitrodibenzpyrene in 40 ml. of acetic acid was treated gradually with stirring with a solution of 600 mg. of chromic anhydride in 1.2 ml. of water and the mixture was heated for 1 hr. in an oil bath at 115–120° under reflux. The solution was cooled to about 50°, diluted with 80 ml. of hot water, and let cool. The product, crystallized from xylene, afforded 220 mg. (61%) of yellow needles, m.p. 330° (lit.[12] m.p. 325), $\lambda^{benzene}$ 250 (23,100), 276 mμ (46,200).

Anal. Calcd. for $C_{22}H_{10}O_4$: C, 78.10; H, 2.98. Found: C, 78.31; H, 2.89.

1,2,6,7-Tetrahydro-3,4,9,10-dibenzpyrene (VII). A solution of 650 mg. of dibenzpyrene in 180 ml. of isoamyl alcohol was refluxed and treated with a total of 8 g. of sodium, added in the course of 4 hr. The solution was cooled, water was added, and the organic layer was diluted with ether and washed repeatedly with water and dilute acid. After removal of solvent in vacuum, the residue was dissolved in benzene and chromatographed on alumina. Some unchanged dibenzpyrene was recovered. The product eluted by benzene on crystallization from cyclohexane gave 276 mg. (40%) of yellow leaflets, m.p. 248°, $\lambda^{ethanol}$ 229 (58,500), 250 (70,300), 261 (100,000), 309 (41,800), 322 (62,900), 328 (50,500) 336 (39,200), 352 mμ (36,100).

Anal. Calcd. for $C_{24}H_{18}$: C, 94.08; H, 5.92. Found: C, 93.74; H, 5.95.

1,2-Dihydro-3,4,9,10-dibenzpyrene (X). A solution of 200 mg. of dibenzpyrene in 200 ml. of ethyl acetate and 50 ml. of acetic acid when treated with 70 mg. of platinum oxide and shaken with hydrogen absorbed 1 mole of gas in 2 hr. Chromatography of the product gave, in the benzene eluates, a substance which on crystallization from cyclohexane afforded 130 mg. (65%) of light greenish yellow leaflets, m.p. 224–225°, $\lambda^{ethanol}$ 233 (40,400), 256 (63,000), 274 (86,300), 286 (114,100), 302 (40,200), 317 (29,200), 331 mμ (27,800).

Anal. Calcd. for $C_{24}H_{16}$: C, 94.70; H, 5.30. Found: C, 94.61; H, 5.48.

3,4,9,10-Di-(tetrahydrobenz)-pyrene (VII). A solution of 341 mg. of dibenzpyrene in 300 ml. of ethyl acetate and 50 ml. of acetic acid in the presence of 80 mg. of platinum oxide absorbed 4 moles of hydrogen in 10 hr. Crystallization of the product from cyclohexane gave pinkish white needles, m.p. 190–193°. Chromatography and recrystallization gave 212 mg. (62%) of bluish white needles, m.p. 193–194°, $\lambda^{ethanol}$ 242 (47,700), 250 (98,900), 262 (21,300), 272 (29,500), 284 (40,900), 310 (6,700), 323 (11,000), 338 (24,900), 354 mμ (34,100).

Anal. Calcd. for $C_{24}H_{22}$: C, 92.86; H, 7.14. Found: C, 92.89; H, 7.10.

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[CONTRIBUTION FROM THE LIFE SCIENCES DIVISION, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] LXX. Some Simple Derivatives of the Actinomycins

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A new and simpler preparative route is described for 3-(benzyloxy)-2-nitro-*p*-toluic acid (III), a key intermediate in the synthesis of the actinocinyl-L-threonine peptides (II, XXIII, and XXIV). In contrast to previously reported results, the peptide ester (II), obtained by synthesis, is microbiologically inactive against *Staph. aureus*.

The actinomycins are a group of closely related compounds produced by certain species of *Streptomyces* and possessing antibiotic and cytostatic activity.[4] The involved chemistry of these materials has been elucidated chiefly by Brockmann and Johnson during the past decade.[5] The work in this area was crowned recently by the total synthesis of Actinomycin C_3 (I).[6]

The statement that the dimethyl ester of actinocinyl-bis-L-threonine (II) was active against *Staph. aureus* to a dilution of 1:700,000[7] suggested that the

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(4) For leading references, see "The Actinomycins and Their Importance in the Treatment of Tumors in Animals and Man," S. A. Waksman, ed., The New York Academy of Sciences, New York, 1960; and S. Farber in "Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity," Little, Brown and Company, Boston, 1958, p. 138.

(5) For pertinent reviews see H. Brockmann, *Angew. Chem.*, **72**, 939 (1960), and H. Brockmann, *Fortschr. Chem. Org. Naturstoffe*, **18**, 1 (1960).

(6) H. Brockmann and H. Lackner, *Naturwissenschaften*, **47**, 230 (1960).

(7) H. Brockmann and H. Grove, Ger. Patent **1,001,685** (July 11, 1957); *Chem. Abstr.*, **54**, 1345 (1960).