Phenoxymethylpenicillin sulfoxide benzyl ester (IVb). Phenoxymethylpenicillin benzyl ester was much more resistant to oxidation with sodium periodate. A solution of 4.7 g. (0.0107 mole) of phenoxymethylpenicillin benzyl ester IIb was stirred with sodium periodate in dioxane–phosphate buffer for 10 hr. at room temperature, then for 4 days with fresh sodium periodate–dioxane–phosphate buffer, and finally for 2 days at 50° with fresh periodate–dioxane–phosphate buffer. The reaction mixture was worked up as with the benzylpenicillin sulfoxide benzyl ester. Evaporation of the chloroform solution gave an oil which crystallized after 9 days to give 2.95 g. (61%) of crude phenoxymethylpenicillin sulfoxide benzyl ester IVb, m.p. 123–125°. Recrystallization from ethyl acetate–petroleum ether gave 2.75 g. (57%) of crystalline solid, m.p. 124–125°.

Anal. Calcd. for $C_{23}H_{24}N_2O_6S$: C, 60.51; H, 5.30; N, 6.14. Found: C, 60.44; H, 5.84; N, 6.52.

Benzylpenicillin sulfoxide (VIa). Benzylpenicillin sulfoxide benzyl ester IVa, 1.76 g. (0.004 mole), was hydrogenated at atmospheric pressure with 2.6 g. of pre-reduced 10% palladium on charcoal in dry ethyl acetate. The reaction was completed in 0.5 hr. After filtering off the catalyst, the filtrate was evaporated *in vacuo* to a solid which was crystallized from ethyl acetate–petroleum ether to give 1.02 g. (73%) of product, m.p. 142–143° (with dec.).

Anal. Calcd. for $C_{16}H_{18}N_2O_5S$: C, 54.84; H, 5.18; N, 8.00. Found: C, 55.13; H, 5.28; N, 8.26.

Phenoxymethylpenicillin sulfoxide (VIb). In a similar manner 0.913 g. (0.002 mole) of phenoxymethylpenicillin sulfoxide benzyl ester (IVb) was hydrogenated to give 0.582 g. (79%) of colorless crystalline solid after recrystallization from ethyl acetate–petroleum ether, m.p. 167–168° (with dec.).

Anal. Calcd. for $C_{16}H_{18}N_2O_6S$: C, 52.45; H, 4.95; N, 7.65. Found: C, 52.42; H, 4.92; N, 7.93.

Acknowledgment. We are indebted to Mrs. D. Rolston and associates for the elemental analyses.

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

1,2,3-Benzotriazine Sulfonamides. A New Class of Oral Diuretic Agents[1]

SHREEKRISHNA M. GADEKAR AND JEAN L. FREDERICK

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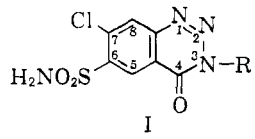
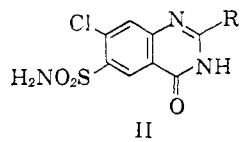
A series of 7-chloro-6-sulfamyl-1,2,3-benzotriazine-4(3*H*)-ones have been prepared from *N*-acetyl-4-chloro-5-sulfamylanthranilic acid. Some of these compounds possess diuretic activity.

During a continuing search, in these laboratories, for a better, orally active diuretic agent, we had the opportunity to examine a series of 7-chloro-6-sulfamyl-1,2,3-benzotriazine-4(3*H*)-ones (I).



I



II

On oral administration these compounds, like the quinazolinone sulfonamides[2] (II) and the chlorothiazide and hydrochlorothiazide,[3,4] types of diuretic agents, caused a pronounced natriuresis and chloruresis in experimental animals, but at the same time showed only a relatively small increase in potassium excretion.[5] Structurally they might be considered nitrogen isosteres of the quinazolinone sulfonamides II, which have been reported to be active diuretic agents. Thus, it becomes apparent that the replacement of carbon, at the 2- position, with nitrogen in the quinazolinone sulfonamide series does not cause a loss of biological activity. Substitution of hydrogen at position 3 with amino or lower alkyl groups, such as methyl and ethyl, resulted in decreased activity. A complete loss of activity occurred when this position was occupied by a benzyl or β-dimethylaminoethyl group.

The starting material for the syntheses, namely, *N*-acetyl-4-chloro-5-sulfamylanthranilic acid, has been described.[2] When this was esterified with methanol and sulfuric acid, a simultaneous loss of the acetyl group occurred, and methyl 4-chloro-5-sulfamylanthranilate (III) was obtained in 49% yield. Treatment of the ester with concentrated ammonium hydroxide at room temperature for seventy-two hours afforded the anthranilamide. When aqueous solutions of other amines were used in place of ammonia, corresponding substituted amides were formed as summarized in Table I.

Diazotizations of the amino amides with nitrous acid gave the 7-chloro-6-sulfamyl-1,2,3-benzotriazine-4(3*H*)-ones as shown in Table II.

The benzyl analog XII was prepared by diazotizing III, neutralizing the excess mineral acid with sodium hydroxide, and treating the diazo solution with benzylamine. A similar procedure has been used by Van Heyningen[6] for the syntheses

(1) Presented before the Division of Medicinal Chemistry at the 138th Meeting of the American Chemical Society, New York, September 1960.

(2) E. Cohen, B. Klarberg, and J. R. Vaughan, Jr., *J. Am. Chem. Soc.*, **81**, 5508 (1959).

(3) F. C. Novello and J. M. Sprague, *J. Am. Chem. Soc.*, **79**, 2028 (1957).

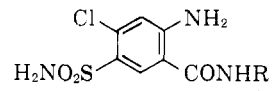
(4) G. De Stevens, L. H. Werner, A. Halamandaris, and S. Ricca, Jr., *Exper.*, **14**, 463 (1958).

(5) The information on the pharmacology of these compounds was kindly furnished by Dr. J. R. Cummings and his associates, Department of Pharmacology, Experimental Therapeutics Section, Pearl River Laboratories, N. Y.

(6) E. Van Heyningen, *J. Am. Chem. Soc.*, **77**, 6562 (1955).

TABLE I

SUBSTITUTED ANTHRANILAMIDES

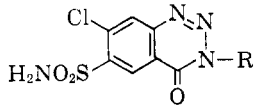


	R	%, Yield	M.P.	Formula	Calcd., % C	H	N	Found, % C	H	N
IV	H	63	272–274[a] dec.	$C_7H_8ClN_3O_3S$						
V	CH_3	93	274–276	$C_8H_{10}ClN_3O_3S$	36.4	3.83	15.9	36.4	4.17	15.9
VI	C_2H_5	73	215–217	$C_9H_{12}ClN_3O_3S$	38.9	4.32	15.1	39.1	4.70	15.3
VII	$(CH_3)_2{\cdot}N(CH_2)_2{\cdot}HCl$	31.5	269–272 dec.	$C_{11}H_{17}ClN_4O_3{\cdot}HCl$	37.0	5.07	15.7	36.9	4.69	15.5

[a] F. C. Novello, U. S. Patent **2,910,488,** reported a m.p. 277–278° dec. for this compound.

TABLE II

7-CHLORO-6-SULFAMYL-1,2,3-BENZOTRIAZINE-4(3*H*)-ONES



	R	%, Yield	M.P. dec.	Formula	Calcd. C	H	N	Found C	H	N
VIII	H	96	270–272	$C_7H_5ClN_4O_3S{\cdot}1/2\,H_2O$	31.2	2.24	20.8	31.1	2.19	20.9
IX	CH_3	75	247–250	$C_8H_7ClN_4O_3S$	35.0	2.57	20.4	35.1	2.26	20.8
X	C_2H_5	89	186–187	$C_9H_9ClN_4O_3S$	37.5	3.15	19.4	37.5	3.32	19.6
XI	$(CH_3)_2N(CH_2)_2{\cdot}HCl$	60	286–287	$C_{11}H_{14}ClN_5O_3S{\cdot}HCl$	35.9	4.10	19.0	35.9	4.40	18.6
XII[a]	$C_6H_5CH_2$	18	193–194	$C_{14}H_{11}ClN_4O_3S$	47.8	3.16	15.9	47.6	2.87	16.1
XIII[a]	$C_6H_5C(CH_3)=N$	54	237–240	$C_{15}H_{12}ClN_5O_3S$	47.9	3.21	18.5	48.0	3.64	18.7
XIV[a]	NH_2	28	230–231	$C_7H_6ClN_5O_3S$	30.5	2.20	25.4	30.8	2.19	25.6

[a] These were prepared by special methods discussed.

of 1,2,3-benzotriazines. For the preparation of the 3-amino derivative XIV, the ester III was converted to the hydrazide XV in 41% yield using hydrazine hydrate. Reaction of this with acetophenone gave the acylhydrazone XVI in 86% yield, which on diazotization in acetic acid gave XIII in 54% yield. The protecting α-methylbenzylidene group was removed by warming XIII in 1N hydrochloric acid for one hour to form XIV in 28% yield. However, when this hydrolysis was carried out in 7N hydrochloric acid, the amino azide XVII was obtained. This was shown to be 4-chloro-5-sulfamylanthranilazide by hydrolyzing it to the known 4-chloro-5-sulfamylanthranilic acid.[2] The azide was also obtained by diazotization of XV in acetic acid. The azide on heating in boiling xylene underwent a Curtius rearrangement, and a simultaneous intramolecular cyclization to give XVIII in 66% yield. This was identified as 5-chloro-6-sulfamylbenzimidazolone (XVIII) by the following independent synthesis. Chlorosulfonation of 3-chloro-6-nitroacetanilide[7] XIX followed by amidation resulted in loss of the acetyl group and gave XX in 8.6% yield. Reduction of XX with sodium hydrosulfite formed XXI in 36% yield. An 80% yield of XVIII was obtained when the diamine XXI was treated with phosgene. The imidazolone was devoid of diuretic activity.

(7) H. H. Hodgson and A. Kershaw, *J. Chem. Soc.*, 2919 (1929).

EXPERIMENTAL[8]

Methyl 4-chloro-5-sulfamylanthranilate (III). A mixture of 44 g. (0.15 mole) of *N*-acetyl 4-chloro-5-sulfamylanthranilic acid in 450 ml. of reagent methanol was cooled and treated gradually with 16 ml. of cond. sulfuric acid. This was refluxed for 24 hours and was evaporated to a slurry. Trituration first with 200 ml. of water and then with aqueous sodium bicarbonate gave 19.4 g. (49%) of white product, melting at 225–227°. A sample recrystallized from ethanol melted at 223–227°.

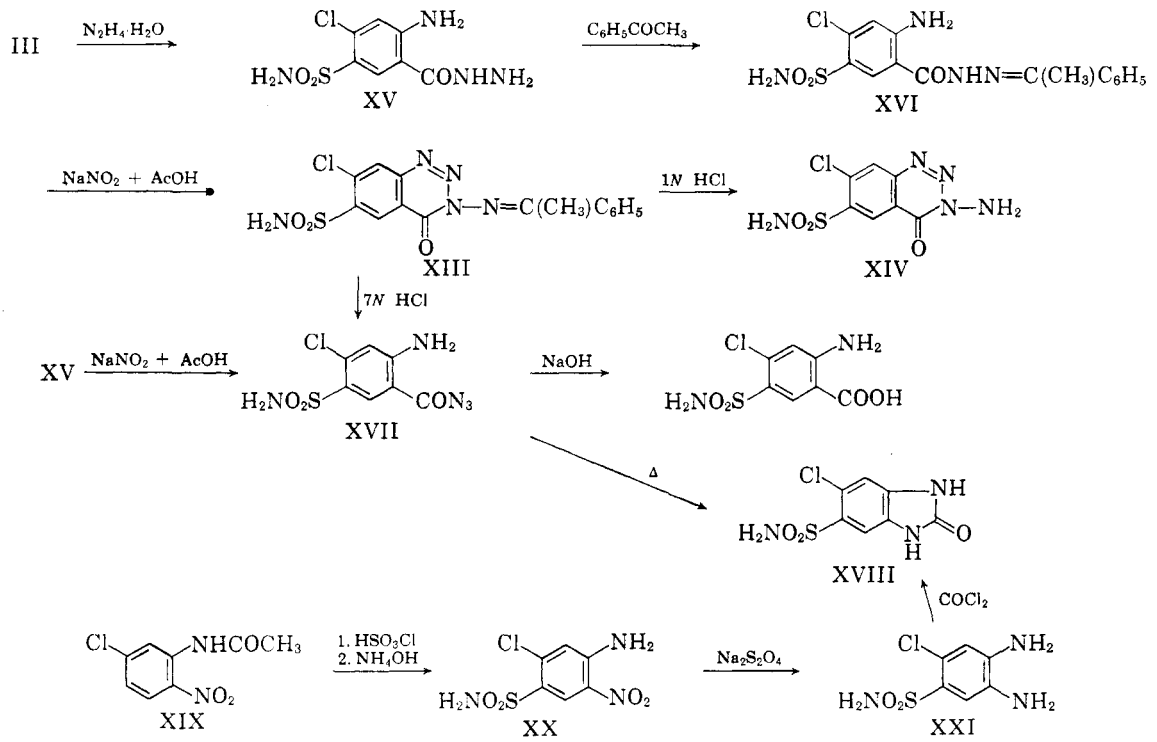
Anal. Calcd. for $C_8H_9ClN_2O_4S$: C,36.4; H, 3.44; Cl, 13.9; N, 10.6; S, 12.1. Found: C, 36.7; H, 3.75; Cl, 13.6; N, 10.8; S, 11.9.

The amides, V, VI and VII, were prepared by the general procedure outlined for IV.

4-Chloro-5-sulfamylanthranilamide (IV). A flask containing a solution of 0.20 g. (0.00076 mole) of methyl 4-chloro-5-sulfamylanthranilate in 2 ml. of methanol and 10 ml. of concd. ammonium hydroxide was stoppered and was left at room temperature for 72 hr. The solution was evaporated under reduced pressure to dryness and the residue was triturated with 2 ml. of aqueous sodium bicarbonate. The insoluble solid was filtered and washed with cold water to give 0.12 g. of the amide. This was recrystallized from ethanol in which it was sparingly soluble.

7-Chloro-6-sulfamyl-1,2,3-benzotriazine-4(3H)-one (VIII). A stirred suspension of 2.5 g. (0.010 mole) of the amide IV in 5 ml. of concd. hydrochloric acid and 30 ml. of water was cooled to 5° and then treated with a solution of 1.0 g. (0.015

(8) All melting points are uncorrected.



mole) of sodium nitrite in 20 ml. of water during 10 min. The reaction mixture cleared to a straw-colored liquid, and soon a precipitate began to appear. At this point the mixture was rendered basic with 10N sodium hydroxide and then reacidified immediately with cond. hydrochloric acid. The reaction temperature during all these operations was maintained between 5–10°. The solid that formed was filtered, washed with cold water and air-dried; wt. 2.5 g. This was recrystallized from hot water.

Compounds IX, X, and XI were prepared similarly, except that in these preparations, the base-acid treatment after the diazotization was eliminated. The products were recrystallized from aqueous ethanol, instead of water.

3-Benzyl-7-chloro-6-sulfamyl-1,2,3-benzotriazine-4(3H)-one (XII). To a stirred suspension of 4.9 g. (0.020 mole) of III in 50 ml. of 3N hydrochloric acid was added 1.4 g. (0.022 mole) of sodium nitrite in 10 ml. of water, maintaining the temperature below 10°. Stirring was continued for an additional 20 min. The mixture was neutralized by a dropwise addition of 25 ml. of 5 N sodium hydroxide followed by an addition of 4 ml. (0.04 mole) of benzylamine, taking care that the mixture remained between 5–10°. After 1 hr. of stirring the undissolved solid (1.2 g.) was filtered and identified as the starting ester III (m.p. 223–226°). The filtrate was extracted exhaustively with ether. Evaporation of the ether layer gave 1.2 g. of the desired product which was recrystallized from ethanol.

4-chloro-5-sulfamylanthranilhydrazide (XV). A solution of 0.50 g. (0.0020 mole) of III in 10 ml. of hydrazine hydrate was kept at room temperature for 90 hr. After evaporating to dryness *in vacuo*, the residue was triturated with 15 ml. of a 5% sodium bicarbonate solution, and the solid was filtered and washed thoroughly with water. Recrystallization from ethanol gave 0.21 g. (41%) of the desired compound, melting at 238–241° dec.

Anal. Calcd. for $C_7H_9ClN_4O_3S$: C, 31.8; H, 3.44; N, 21.3. Found: C, 31.9; H, 3.64; N, 21.4.

N^1-4-Chloro-5-sulfamylanthranoyl-N^2-(α-methylbenzylidene)hydrazine (XVI). A mixture containing 1.8 g. (0.0068 mole) of XV, 0.79 ml. (0.0068 mole) of acetophenone, 0.15 ml. of acetic acid and 150 ml. of ethanol was refluxed for 1 hr. Since all of the hydrazide failed to go into solution, an additional 100 ml. of ethanol was added and refluxing was continued for three more hours. On cooling, a light yellow solid precipitated. An additional amount of this solid was obtained on partial evaporation of the filtrate; total yield 2.2 g. (86%). A sample recrystallized from ethanol melted at 258–261° dec.

Anal. Calcd. for $C_{15}H_{15}ClN_4O_3S \cdot {}^1/_4H_2O$: C, 48.6; H, 4.21; N, 15.1. Found: C, 48.8; H, 4.21; N, 14.9.

7-Chloro-3-(α-methylbenzylidene)amino-6-sulfamyl-1,2,3-benzotriazine-4(3H)-one (XIII). A suspension of 3.7 g. (0.010 mole) of XVI in 24 ml. of acetic acid and 7.5 ml. of water was stirred and cooled to 0°, while a solution of 0.83 g. (0.012 mole) of sodium nitrite in 5 ml. of water was added dropwise rapidly. The mixture was stirred at 5° for 6 hr., and the precipitated yellow solid was filtered and washed with cold water. Recrystallization from ethanol gave 2.1 g. of the desired product.

3-Amino-7-chloro-6-sulfamyl-1,2,3-benzotriazine-4(3H)-one (XIV). A suspension of 0.70 g. (0.0019 mole) of XIII in 15 ml. of 1*N* hydrochloric acid was heated on a steam bath for 1 hr. The mixture was filtered, and the solid was washed thoroughly with ether. Recrystallization from ethanol gave 0.14 g. of the desired compound.

4-Chloro-5-sulfamylanthranilazide (XVII). *Method A.* A mixture of 0.30 g. (0.0078 mole) of XIV in 3.5 ml. of 7N hydrochloric acid was stirred and warmed over steam for 15 min. The mixture was cooled, and the brown solid was filtered and washed with ether. The filtrate was extracted with ether, and the aqueous layer was made basic with a sodium bicarbonate solution when an additional amount of the same product was obtained; total wt. 0.16 g. (76%). A sample recrystallized from ethanol decomposed at 155° to a white solid which did not melt <300°. (Infrared spectrum; band at 4.6μ assigned to the azido group).

Anal. Calcd. for $C_7H_6ClN_5O_3S$: C, 30.6; H, 2.19; N, 25.4. Found: C, 30.9; H, 2.11; N, 25.4.

Method B. A suspension of 1.3 g. (0.0050 mole) of XV in 10 ml. of 50% aqueous acetic acid was cooled to 5° and was treated with a solution of 0.34 g. (0.0050 mole) of sodium nitrite in 13 ml. of water. After stirring for 1 hr. at 10° the resultant brown solid was filtered and recrystallized from ethanol to give 1.0 g. (72%) of the product which had the same decomposition point and infrared spectrum as the compound obtained by Method A.

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.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

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

ENVARE ÜNSEREN AND LOUIS F. FIESER

Received Nov. 24, 1961

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

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(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).