*The Synthesis of Compounds with Potential Anti-folic Acid Activity. Part II.** 5 : 7 : 9 : 10-*Tetra-aza*-1 : 2-*benzanthracene and Related Compounds.*

By D. G. I. Felton, T. S. Osdene, and G. M. Timmis.

[Reprint Order No. 5081.]

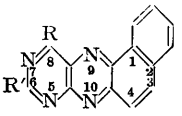
The synthesis is reported of a number of mono- and di-substituted 5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracenes by the fusion of 4-amino-5-nitrosopyrimidines with 2-naphthol. The structures are proved unequivocally by alternative methods of synthesis and interconversions. Starting from 1-nitroso-2-naphthylamine, 2-amino-5 : 6-benzoquinoxaline-3-carbonamide is prepared and this is cyclised by known methods to a number of tetra-azabenzanthracenes. A number of compounds, previously ambiguously orientated, are now allotted unequivocal structures. The reactions and stability of compounds in this series are compared with those of the related pteridine series, and the similarities and differences are commented upon briefly. A number of related compounds are described.

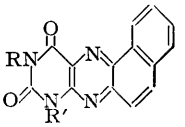
It has been shown (*J.*, 1954, 2881) that reaction between 2 : 4 : 6-triamino-5-nitrosopyrimidine and *NN*-di-2′-chloroethyl-2-naphthylamine yields 6 : 8-diamino-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = R′ = NH_2) and an excellent method of preparing this compound was found in the fusion of the triaminonitrosopyrimidine with 2-naphthol at 150°. Previous compounds of this series have been prepared by methods which do not

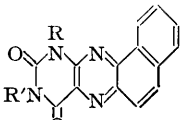
* Part I, preceding paper.

unambiguously define the structures (see *loc. cit.*). The reactions described below, however, lead to the unambiguously oriented series (I). That the products consist of one isomer only has been shown by paper chromatography, using the ascending front technique with butanol–5N-acetic acid as the mobile phase (see Albert, *Quart. Reviews*, 1952, **6**, 197, for leading references to this procedure), and detection by the ultra-violet fluorescence.

The scope of the fusion reaction was first investigated. Besides 2 : 4 : 6-triamino-5-nitrosopyrimidine which yields (I; R = R′ = NH_2), 4 : 6-diamino-2-dimethylamino-5-nitrosopyrimidine gave the analogue (I; R = NH_2, R′ = NMe_2). 2 : 4-Diamino-6-hydroxy-, 4 : 6-diamino-2-hydroxy-, and 4-amino-2 : 6-dihydroxy-5-nitrosopyrimidine did not react, probably owing to the insolubility of these compounds in molten 2-naphthol, but 4-amino-6-hydroxy-5-nitrosopyrimidine-2-thiol and the corresponding methylthio-compound reacted extremely vigorously to yield charred black masses from which no pure product was isolated. However, smooth reactions occurred between fused 2-naphthol and 4-amino-1 : 2 : 3 : 6-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 6-dioxopyrimidine, to yield 5 : 6 : 7 : 8-tetrahydro-5 : 7-dimethyl-6 : 8-dioxo-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene, m. p. 278° (II; R = R′ = Me) and between 2-naphthol and 4-amino-3 : 6-dihydro-2-hydroxy-3-methyl-5-nitroso-6-oxopyrimidine to give 5 : 6 : 7 : 8-tetrahydro-5-methyl-6 : 8-dioxotetra-azabenzanthracene, m. p. 390° (II; R = H, R′ = Me).


(I)


(II)

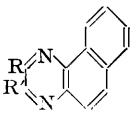
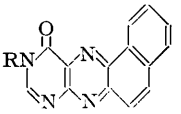

(III)

Kühling (*Ber.*, 1891, **24**, 3029) and Kuhn and Cook (*Ber.*, 1937, **70**, 761) claimed to have prepared (II; R = R′ = Me). From 1 : 2-diaminonaphthalene hydrochloride and tetramethylalloxantin in boiling water Kühling obtained a product, m. p. 285°, probably a mixture of (II; R = R′ = Me) and (III; R = R′ = Me). He further treated 1 : 2-diaminonaphthalene hydrochloride with dimethylalloxantin and gave the product, m. p. >300°, the structure (II; R = H, R′ = Me) although clearly he was unable to discriminate between this and (II; R = Me, R′ = H) or (III; R = H, R′ = Me; or R = Me, R′ = H). Kuhn and Cook, on the other hand, treated 1 : 2-diaminonaphthalene with alloxan following Kühling's procedure (*Ber.*, 1891, **24**, 2363) to obtain either (I; R = R′ = OH) or the 6 : 8 : 9 : 10-tetra-aza-analogue or a mixture of both; they also treated 1 : 2-naphthaquinone with 4 : 5-diamino-2 : 6-dihydroxypyrimidine; with diazomethane in ether these products yielded different *NN*′-dimethyl compounds (II and III; R = R′ = Me): that originating from alloxan and diaminonaphthalene had m. p. 258—260°, and the other melted at 308° (decomp.). Very probably the isomer of m. p. 258—260° corresponds to that synthesised by us. But a repetition of Kuhn and Cook's work yielded us an isomer, m. p. 318°, different from (II; R = R′ = Me) and presumably corresponding to their compound, m. p. 308° (decomp.). In the case of the reaction between alloxan and 1 : 2-diaminonaphthalene, crystallisation of the dihydroxy-compound from acetic acid to single-spot purity on a paper chromatogram, followed by treatment with diazomethane, yielded the pure isomer, m. p. 318°, directly. If the intermediate purification was omitted, or replaced by one precipitation from cold alkali by acid, then fractional crystallisation of the methylated product yielded also a more soluble fraction, which however, did not yield the pure isomer, m. p. 258—260°. Furthermore a paper chromatogram of the crude mixture of products obtained by Kühling's procedure failed to reveal the presence of the dihydroxy-compound (I; R = R′ = OH), and similar investigation of the lower-melting fractions from the diazomethylation of this demonstrated the absence of the dimethyldioxo-compound (II; R = R′ = Me). Dr. A. H. Cook (personal communication) has pointed out to us the effect of impurities upon alloxan condensations and this seems the most reasonable explanation of the discrepancy.

The formulation as (II; R = R′ = Me) of the compound, m. p. 278°, obtained by us has been confirmed by two independent routes.

(*a*) The general reaction for the preparation of 7-aminopteridines (Part I, *loc. cit.*) has been extended by condensing *o*-aminonitroso-compounds with cyanoacetamide and its congeners. This very useful reaction has been employed in the synthesis of numerous fused heterocyclic systems, which will be described later. Thus 1-nitroso-2-naphthylamine and cyanoacetamide condensed in 2-ethoxyethanol in the presence of sodium 2-ethoxyethoxide, yielding 2-amino-5 : 6-benzoquinoxaline-3-carbonamide (IV; R = CO·NH_2, R′ = NH_2), and condensation with cyanoacetic acid or methyl cyanoacetate gave the corresponding 3-carboxylic acid (IV; R = CO_2H, R′ = NH_2). The acid underwent ready thermal decarboxylation to 2-aminobenzoquinoxaline, m. p. 219—220° (IV; R = H, R′ = NH_2). This amine was described but not orientated by Wolf, Beutel, and Stevens (*J. Amer. Chem. Soc.*, 1948, **70**, 2572) who hydrolysed the mixture of dihydroxy-tetra-azabenzanthracenes (benzalloxazines) obtained by Kuhn and Cook (*loc. cit.*) to the aminobenzoquinoxaline carboxylic acids. These were decarboxylated to give a mixture of aminobenzoquinoxalines, separated by fractional crystallisation into two compounds, m. p.s 150—152° and 215—217° respectively. It is now clear that the latter is 2-amino-5 : 6-benzoquinoxaline (IV; R = H, R′ = NH_2), and the former the 3-amino-isomer (IV; R = NH_2, R′ = H). Attempted hydrolysis of the amine (IV; R = H, R′ = NH_2) to the hydroxy-compound failed under both acid and alkaline conditions.

When 2-nitroso-1-naphthylamine was condensed with cyanoacetamide, the isomeric 3-amino-5 : 6-benzoquinoxaline-2-carbonamide (IV; R = NH_2, R′ = CO·NH_2) was obtained.

(IV)   (V)

The amino-amides are valuable intermediates for the synthesis of a number of compounds in which a pyrimidine ring is fused to a pyrazine ring, as in pteridine and its derivatives (Gowenlock, Newbold, and Spring, *J.*, 1948, 517; Albert, Brown, and Cheeseman, *J.*, 1951, 474; 1952, 4219; Taylor, *J. Amer. Chem. Soc.*, 1952, **74**, 1651; 1953, **75**, 1904), and this is the simplest unequivocal route to them. Prolonged heating of the aminobenzoquinoxalinecarbonamide (IV; R = CO·NH_2, R′ = NH_2) with ethyl chloroformate (Gowenlock, Newbold, and Spring, *loc. cit.*) gave an excellent yield of the urethane (IV; R = CO·NH_2, R′ = NH·CO_2Et), and this underwent ring closure in ethanol in the presence of sodium ethoxide to yield 6 : 8-dihydroxy-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = R′ = OH).

(*b*) Although 4-amino-2 : 6-dihydroxy-5-nitrosopyrimidine does not react with 2-naphthol to any noticeable extent, it is possible to produce the dihydroxytetra-azabenzanthracene (I; R = R′ = OH) by the inverse reaction, namely, the fusion of violuric acid with 2-naphthylamine.

Methylation by diazomethane of the dihydroxy-compounds obtained by both routes (*a* and *b*) yielded the *NN*′-dimethyldioxo-compound (II; R = R′ = Me), m. p. 278°, identical with the product obtained by the direct fusion method. Route (*a*), starting from a 1 : 2-disubstituted naphthalene, taken together with route (*b*) and the other typical fusion reactions involving a preformed pyrimidine ring, constitutes a complete structural proof of the series represented by (I) and (II).

With ethyl orthoformate and acetic anhydride (Albert, Brown, and Cheeseman, *J.*, 1951, 474), 2-amino-5 : 6-benzoquinoxaline-3-carbonamide (IV; R = CO·NH_2, R′ = NH_2) readily underwent ring closure to the monohydroxy-compound (I; R = OH, R′ = H) or its tautomer (V; R = H); if the period of reflux was shortened the intermediate 2-formamido-compound (IV; R = CO·NH_2, R′ = NH·CHO) was isolated, indicating the probable route by which 8-hydroxytetra-azabenzanthracene is formed. Treatment of the monohydroxy-compound with diazomethane yielded 7 : 8-dihydro-7-methyl-8-oxo-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (V; R = Me), the formulation of which as the *N*-methyl rather than the *O*-methyl compound is supported by the presence of a strong band at 1687 $cm.^{-1}$ corresponding to the vibration of a carbonyl group.

The monohydroxytetra-azabenzanthracene (I; R = OH, R′ = H) reacted with phosphorus pentasulphide in pyridine (Klingsberg and Papa, *J. Amer. Chem. Soc.*, 1951, **73**, 4988; Taylor, Carbon, and Hoff, *ibid.*, 1953, **75**, 1904) to yield the corresponding thiol (I; R = SH, R′ = H), which is of potential interest in view of the biological and clinical effects found with 6-mercaptopurine (Burchenal, Karnofsky, Murphy, Ellison, and Rhoads, *Proc. Amer. Assoc. Cancer Res.*, 1953, **1**, 7; Clarke, Philips, Sternberg, Stock, and Elion, *ibid.*, p. 9; Elion and Hitchings, *ibid.*, p. 13; Philips, Sternberg, Clarke, and Hitchings, *ibid.*, p. 42; Sugiura, *ibid.*, p. 55). An attempt was also made to pass from the monohydroxy-compound (I; R = OH, R′ = H) to the corresponding amino-compound but the necessary intermediate step of chlorination failed with phosphoryl chloride. However, the required monoamino-compound was prepared by the action of 4 : 6-diamino-5-nitrosopyrimidine on 2-naphthol, although in this case special conditions were required. If the two components were fused together in the manner described for triaminonitrosopyrimidine, a vigorous reaction occurred and the temperature rose spontaneously from 130° to 180° in a few seconds but the mixture, on being worked up at this stage, yielded only green amorphous material, suggesting that reaction was incomplete. More prolonged heating gave a little of the amino-compound (I; R = NH_2, R′ = H). The yield was improved by conducting the reaction in glacial acetic acid in the presence of sodium acetate and by careful purification.

When the fusion method was extended to 4-amino-6-hydroxy-5-nitrosopyrimidine in the hope of discovering a second, independent synthesis of the hydroxytetra-azabenzanthracene (I; R = OH, R′ = H), a vigorous reaction was again noted and, on one occasion, an impure specimen was isolated giving several spots on a paper chromatogram, one of the more weakly fluorescent of which corresponded with the required compound; on all other occasions, however, the product isolated was 2-amino-5 : 6-benzoquinoxaline-3-carbonamide (IV; R = CO·NH_2, R′ = NH_2). It seems probable that the fusion method yields the hydroxy-compound but that the correct conditions have yet to be discovered.

Reactions and Stability of Tetra-azabenzanthracenes.—Hydrolysis of the amino-groups of 6 : 8-diamino-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = R′ = NH_2) and its derivatives closely paralleled that of 2 : 4-diaminopteridines (Taylor and Cain, *J. Amer. Chem. Soc.*, 1949, **71**, 2538) in which use of strong acid replaces the 4-amino- by a hydroxy-group, while subsequent treatment with nitrous acid hydrolyses the 2-amino-group. A corresponding difference is shown by certain substituted aminopteridines, *e.g.*, leucopterin (Wieland, Metzger, Schöpf, and Bulow, *Annalen*, 1933, **507**, 226; Wieland and Purrmann, *ibid.*, 1940, **544**, 163), xanthopterin, and rhizopterin (Wolf, Anderson, Kaczka, Harris, Arth, Southwick, Mozingo, and Folkers, *J. Amer. Chem. Soc.*, 1947, **69**, 2753). Thus, (I; R = R′ = NH_2) with 6N-hydrochloric acid yielded an aminohydroxy-derivative, which with nitrous acid gave the dihydroxy-compound (I; R = R′ = OH), and thence with diazomethane the *NN*′-dimethyl compound (II; R = R′ = Me), thus linking the series (I) and (II) once more. That the intermediate aminohydroxy-compound was probably (I; R = OH, R′ = NH_2) was shown by hydrolysis of 8-amino-6-dimethylamino- (I; R = NH_2, R′ = NMe_2) to give 6-dimethylamino-8-hydroxy-tetra-azabenzanthracene (I; R = OH, R′ = NMe_2), this structure being confirmed synthetically by heating 4-amino-2-dimethylamino-6-hydroxy-5-nitrosopyrimidine with 2-naphthol (this reaction proceeds satisfactorily, in contrast to that with the diaminohydroxynitrosopyrimidine, presumably owing to the greater solubility of the dimethylamino-compound).

An attempt was made to use the preferential acid hydrolysis of the 8-amino-group to pass from 8-amino- (I; R = NH_2, R′ = H) to 8-hydroxy-tetra-azabenzanthracene, in order to provide corroborative evidence of the latter structure. However, the product was 2-amino-5 : 6-benzoquinoxaline-3-carboxylic acid (IV; R = CO_2H, R′ = NH_2). This result suggested that a tetra-azabenzanthracene bearing only an 8-substituent was less stable than those in which both available positions in the pyrimidine moiety were substituted and, in confirmation, it was shown that 8-hydroxytetra-azabenzanthracene was hydrolysed in a few minutes by hot N-sodium hydroxide to yield 2-amino-5 : 6-benzoquinoxaline-3-carbonamide (IV; R = CO·NH_2, R′ = NH_2). Contrariwise, the dihydroxytetra-azabenzanthracene (" benzalloxazine ") requires drastic hydrolysis to destroy the

pyrimidine ring (Wolf, Beutel, and Stevens, *loc. cit.*). This order of stability is similar to that found by Albert, Brown, and Cheeseman (*J.*, 1952, 4219) for the related pteridine compounds.

The instability of the 8-monosubstituted tetra-azabenzanthracenes explains the difficulties met in their preparation by fusion of the corresponding aminonitrosopyrimidines with 2-naphthol, since this reaction proceeds with the liberation of water at an elevated temperature.

Ultra-violet Absorption Spectra.—Throughout this work we have relied on both paper chromatography and ultra-violet absorption spectra to follow the purification of these somewhat intractable compounds. In general, absorption for the tetra-aza-1 : 2-benzanthracene is confined to two well-defined bands: one at 280—305 mμ (log ε 4·2—4·5), which may show fine structure to a degree depending on the nature of the substituents; and the other at 400—420 mμ (log ε 4·0—4·3), consisting of an inflexion and a maximum, which in one case becomes two well-defined maxima. Within these limits the spectra do not depend upon the number or nature of the substituents in the pyrimidine portion of the molecule (see Table). The spectra of *N*-methyl- and *NN'*-dimethyl-dioxotetra-azabenzanthracene closely resemble those of the diaminotetra-azabenzanthracenes. This is a little surprising since methylation of both nitrogen atoms in the one case has effectively blocked any tautomerism in the pyrimidine ring, which can no longer possess a typical aromatic structure except insofar as the dizwitterionic form (VI) can contribute to the resonance hybrid. This similarity in spectra between the diamino- and the dioxo-compound (II; R = R' = Me) must not be construed as evidence favouring a " di-imino "-structure for the former compound. In the related pteridine series the ultra-violet absorption spectra do not support " imino "-structures for the aminopteridines (Albert, Brown, and Cheeseman, *J.*, 1952, 1620, 4219) and evidence is accumulating against such forms in other heterocyclic systems (Clews and Cochran, *Acta Cryst.*, 1948, **1**, 4; 1949, **2**, 46; Broomhead, *ibid.*, 1948, **1**, 324; Angyal and Angyal, *J.*, 1952, 1461; Angyal and Werner, *ibid.*, p. 2911; Goulden, *ibid.*, p. 2939; Boarland and McOmie, *ibid.*, pp. 3716, 3722; Brown and Short, *J.*, 1953, 331). Marshall and Walker (*J.*, 1951, 1004; see also Boarland and McOmie, and Brown and Short, *locc. cit.*), working at controlled pH's—a desirable precaution which is largely precluded in our case by solubility requirements—concluded that the keto-form is favoured for hydroxypyrimidines and the same appears true of certain, at least, of the hydroxypteridines (Albert, Brown, and Cheeseman, *locc. cit.*). At the moment we wish merely to point out the possible importance of zwitterion forms such as (VI).

Ultra-violet absorption spectra of 5 : 7 : 9 : 10-*tetra-aza*-1 : 2-*benzanthracenes.*

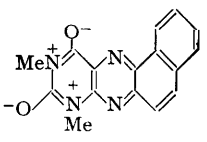
(Solvent: 4·5% formic acid, unless otherwise stated.)

	Absorption maxima					Absorption maxima			
	Band I		Band II			Band I		Band II	
Substituents	λ	log ε	λ	log ε	Substituents	λ	log ε	λ	log ε
8-Amino	290	4·32	409 *	4·11	8-Amino-6-dimethylamino	283	4·32	407	4·25
	300 †	4·26	417	4·13		289	4·33	421	4·23
8-Hydroxy	292 *	4·34	392 †	3·97		302·5	4·26		
	299	4·37	403·5	4·01	6-Dimethylamino-8-hydroxy	304	4·35	400 *	3·95
6 : 8-Diamino	281	4·26	405 *	4·17				411	4·00
	290	4·28	414	4·19	5 : 6 : 7 : 8-Tetrahydro-7-methyl-6 : 8-dioxo [2]	291	4·03	397 *	3·87
	302	4·34				302	3·98	408·5	3·91
6-Amino-8-hydroxy [1]	291	4·58	395 †	4·27	5 : 6 : 7 : 8-Tetrahydro-5 : 7-dimethyl-6 : 8-dioxo [2]	291	4·25	398 *	4·08
	300	4·57	407	4·32		302	4·22	410	4·11
6 : 8-Dihydroxy	289	4·19	395 *	4·035					
	300	4·24	404	4·06					

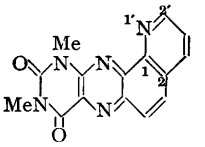
[1] Solvent: 36% formic acid. [2] Solvent: 22·5% formic acid.
* Inflexion. † Shoulder.

Related Compounds.—Some attempt has been made to extend the scope of the reaction between 4-amino-5-nitrosopyrimidines and 2-naphthol to include reaction with 1-naphthols. 1-Naphthol itself undoubtedly undergoes exothermic reaction with certain 4-amino-5-nitrosopyrimidines, *e.g.*, 2 : 4 : 6-triamino-5-nitrosopyrimidine, and crude yellow-brown products may be isolated, but these have so far resisted purification. 8-Hydroxyquinoline

may behave as a 1-naphthol in this sense and, although it does not react with the triaminonitrosopyrimidine, it combines with 4-amino-1 : 2 : 3 : 6-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 6-dioxopyrimidine to yield what is probably 5 : 6 : 7 : 8-tetrahydro-6 : 8-dimethyl-5 : 7-dioxo-1′ : 6 : 8 : 9 : 10-penta-aza-1 : 2-benzanthracene (VII). 6-Hydroxyquinoline reacts with triaminonitrosopyrimidine, but the product has not been purified.



(VI)



(VII)

Biological Results.—Through the kindness of Dr. H. O. J. Collier (Ware, Herts), a number of the substances described in this communication have been tested microbiologically as potential anti-folic or anti-folinic acid compounds. Only one, the diamino-compound (I; R = R′ = NH_2) which was weakly anti-folic, possessed significant activity.

Experimental

M.p.s were determined in electrically-heated copper blocks. Absorption spectra were measured by using a Unicam photoelectric spectrophotometer (Model SP 500). Fluorescence, both in solution and on paper chromatograms, was observed in ultra-violet light from a Hanovia lamp. All compounds were purified to constant ultra-violet absorption and/or single-spot purity on paper chromatograms on Whatman No. 1 filter paper. Analyses were by Mr. F. Oliver, Imperial College of Science and Technology, and Mr. P. R. W. Baker, Beckenham.

2-Amino-5 : 6-benzoquinoxaline-3-carbonamide (IV; R = $CO{\cdot}NH_2$, R′ = NH_2).—(*a*) 1-Nitroso-2-naphthylamine (0·86 g.), followed by cyanoacetamide (0·45 g.), was added to a solution from sodium (0·15 g.) in 2-ethoxyethanol (25 ml.), and the mixture refluxed for 5 min. while yellow crystals were precipitated. These (0·7 g.) were collected after chilling in ice and were recrystallised from 2-ethoxyethanol to yield the *carbonamide* as yellow needles, m. p. 282—283° (Found: C, 65·45; H, 4·3; N, 23·3. $C_{13}H_{10}ON_4$ requires C, 65·5; H, 4·2; N, 23·5%). The compound exhibits an intense light green fluorescence in glacial acetic acid solution and a greenish-blue fluorescence on a paper chromatogram. The *2-acetamido*-derivative, obtained by using hot acetic anhydride, formed yellow needles, m. p. 264—265°, from dimethylformamide (Found: C, 64·0; H, 4·6; N, 20·65. $C_{15}H_{12}O_2N_4$ requires C, 64·3; H, 4·3; N, 20·0%) and gave an intense green fluorescence in acetic acid.

(*b*) 2-Naphthol (4·0 g.) was fused at 130° and 4-amino-6-hydroxy-5-nitrosopyrimidine (0·63 g.; Cavalieri and Bendich, *J. Amer. Chem. Soc.*, 1950, **72**, 2593) was added in one portion. When the very vigorous reaction had subsided, the cooled melt was triturated with ether, and the insoluble material removed. The ethereal filtrate was partly evaporated and then deposited a yellow solid, which after crystallisation from glacial acetic acid and then 2-ethoxyethanol gave yellow needles, m. p. 282—283°, undepressed on admixture with a specimen prepared as in (*a*).

2-Amino-5 : 6-benzoquinoxaline-3-carboxylic acid (IV; R = CO_2H, R′ = NH_2).—(*a*) 1-Nitroso-2-naphthylamine (0·6 g.) and cyanoacetic acid (0·32 g.) were added to a solution of sodium (0·2 g.) in 2-ethoxyethanol (20 ml.), and the mixture boiled under reflux for 15 min. to yield a thick precipitate (0·46 g.) which was cooled and collected. The precipitate was boiled with N-hydrochloric acid (100 ml.) and then made strongly alkaline with aqueous ammonia (*d* 0·880) to yield a solution which was clarified with charcoal and filtered. Acidification of the filtrate gave a yellow crystalline precipitate which was recrystallised from glacial acetic acid, affording the *acid* as yellow needles, m. p. 232° (effervescence) (Found: C, 65·3, 65·1; H, 3·4, 3·7; N, 18·0, 17·7. $C_{13}H_9O_2N_3$ requires C, 65·3; H, 3·8; N, 17·6%).

(*b*) 1-Nitroso-2-naphthylamine (1·72 g.) and methyl cyanoacetate (1·1 g.) were refluxed for 1½ hr. with sodium (0·25 g.) in dry 2-ethoxyethanol (25 ml.). The yellow crystalline precipitate (0·7 g.) was collected, dissolved in hot water (charcoal), filtered, and acidified with dilute hydrochloric acid to give a solid, which recrystallised from glacial acetic acid as yellow needles, m. p. 229° (effervescence), undepressed on admixture with the product described in (*a*).

2-Amino-3-methoxycarbonyl-5 : 6-benzoquinoxaline (IV; R = CO_2Me, R′ = NH_2).—2-Amino-5 : 6-benzoquinoxaline-3-carboxylic acid (1 g.), suspended in acetone (10 ml.), was treated with diazomethane (from nitrosomethylurea, 4 g.) in ether (30 ml.), vigorous evolution of nitrogen occurring. After 24 hr., the precipitate (0·8 g.) was collected and crystallised from ethanol,

affording the *methyl* ester as yellow matted silky needles, m. p. 225—226° (Found: C, 66·4, 66·4; H, 4·9, 4·7; N, 16·6, 16·55. $C_{14}H_{11}O_2N_3$ requires C, 66·4; H, 4·4; N, 16·6%).

2-Amino-5 : 6-benzoquinoxaline (IV; R = H, R′ = NH_2).—The aminobenzoquinoxaline-3-carboxylic acid (1 g.) was heated at *ca.* 250°. When evolution of carbon dioxide ceased, the mass (0·6 g.) obtained after trituration with ether was crystallised from dilute ammoniacal dimethylformamide and yielded 2-amino-5 : 6-benzoquinoxaline as microscopic rods, m. p. 219—220° (Found: C, 73·5; H, 4·8; N, 21·65. Calc. for $C_{12}H_9N_3$: C, 73·8; H, 4·65; N, 21·5%) (Wolf, Beutel, and Stevens, *J. Amer. Chem. Soc.*, 1948, **70**, 2572, give m. p.s 150—152° and 215—217° for the 2- and the 3-amino-isomer). With hot 6N-hydrochloric acid it formed the *hydrochloride*, pale yellow silky needles, m. p. 277—278° (decomp.), from glacial acetic acid (Found: C, 62·5, 62·4; H, 5·0, 5·0. $C_{12}H_9N_3$,HCl requires C, 62·2; H, 4·35%). The amine exhibits a blue fluorescence in acid solution, which is unchanged on basification.

3-Amino-5 : 6-benzoquinoxaline-2-carbonamide (IV; R = NH_2, R′ = CO·NH_2).—2-Nitroso-1-naphthylamine (1·72 g.) and cyanoacetamide (0·92 g.) were heated under reflux with a solution from sodium (0·2 g.) in ethanol (50 ml.). At the b. p. a thick precipitate of yellow needles was formed and after 1 min. the mixture was cooled and filtered. Crystallisation of the precipitate from 2-ethoxyethanol (charcoal) afforded the *carbonamide* as yellow needles, m. p. 255—256° (Found: C, 65·8; H, 4·3; N, 23·7. $C_{13}H_{10}ON_4$ requires C, 65·5; H, 4·2; N, 23·5%). The compound exhibits a bright green fluorescence in glacial acetic acid.

Tetra-aza-1 : 2-benzanthracenes.

8-Amino-6-dimethylamino-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = NH_2, R′ = NMe_2).—2-Naphthol (4 g.) was fused at 130° (internal temperature) and 4 : 6-diamino-2-dimethylamino-5-nitrosopyrimidine (1·8 g.; Roth, Smith, and Hultquist, *J. Amer. Chem. Soc.*, 1951, **73**, 2864) was added all at once with stirring. When the temperature of the melt was then slowly raised to 150° a vigorous reaction ensued, the temperature rising to 170—180°. When the reaction subsided, the melt was cooled and was extracted with ether. The yellow residue (2·24 g.), crystallised from 2-ethoxyethanol, afforded the *aminodimethylamino*-compound as clusters of orange blades with a pronounced bronze lustre, m. p. 340° (Found: C, 66·2, 66·3; H, 4·9, 4·75; N, 29·4, 29·4. $C_{16}H_{14}N_6$ requires C, 66·2; H, 4·8; N, 29·0%).

6-Dimethylamino-8-hydroxy-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = OH, R′ = NMe_2).—This was similarly prepared from 2-naphthol (6 g.) and 4-amino-2-dimethylamino-6-hydroxy-5-nitrosopyrimidine (1·83 g.; Roth *et al.*, *loc. cit.*) at 140—180°. The yellow-brown insoluble product (2·6 g.), obtained after extraction with ether, was crystallised from aqueous ethanol and gave the *tetra-aza-benzanthracene* as golden-yellow needles, m. p. 360° (Found: C, 61·6, 61·6; H, 5·2, 4·8; N, 22·7, 22·7. $C_{16}H_{13}ON_5$,H_2O requires C, 62·1; H, 4·9; N, 22·6. Found, in a sample dried at 150° in a high vacuum: C, 65·7, 65·7; H, 4·6, 4·6; N, 24·4, 24·5. $C_{16}H_{13}ON_5$ requires C, 66·0; H, 4·5; N, 24·0%).

8-Amino-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = NH_2, R′ = H).—A variety of experimental conditions were tried for the reaction of 2-naphthol with 4 : 6-diamino-5-nitrosopyrimidine, but chromatographic examination of the products after attempted purification demonstrated the existence of several contaminants. The following procedure was eventually adopted: 2-Naphthol (4·8 g.) and 4 : 6-diamino-5-nitrosopyrimidine (4·2 g.; Cavalieri and Bendich, *J. Amer. Chem. Soc.*, 1950, **72**, 2587) were dissolved in glacial acetic acid (30 ml.), and anhydrous sodium acetate (3 g.) was added. The mixture was refluxed for 1½ hr., then diluted to 180 ml. with water. The precipitate obtained on addition of excess of concentrated aqueous ammonia was ground with concentrated hydrochloric acid (30 ml.) to form a hydrochloride, which was washed with methanol and then with ether. It was then suspended in water and made alkaline (Clayton-yellow paper) with dilute sodium hydroxide solution. The collected base was dissolved in hot 2% formic acid (charcoal) and again precipitated by the addition of dilute aqueous ammonia solution. After several crystallisations from butanol, the *amino*-compound formed yellow crystals, m. p. 343°, single-spot chromatographic purity (Found: C, 67·9; 67·9, H, 4·05, 3·9; N, 28·3, 28·0. $C_{14}H_9N_5$ requires C, 68·0; H, 3·7; N, 28·3%).

8-Hydroxy-5 : 7 : 9 : 10-tetra-aza-1 : 2-benzanthracene (I; R = OH, R′ = H).—(*a*) 2-Amino-5 : 6-benzoquinoxaline-3-carbonamide (1·2 g.; see above) was refluxed for 1 hr. with ethyl orthoformate (10 ml.) and acetic anhydride (10 ml.), and the cooled suspension (1·1 g.) was collected. The solid, crystallised from glacial acetic acid, yielded *2-formamido-5 : 6-benzoquinoxaline-3-carbonamide* as yellow needles, m. p. 398—400° (decomp.) (Found: C, 63·2; H, 4·0; N, 21·0. $C_{14}H_{10}O_2N_4$ requires C, 63·15; H, 3·8; N, 21·0%).

(*b*) The aminobenzoquinoxalinecarbonamide (6·0 g.) was refluxed with ethyl orthoformate (100 ml.) and acetic anhydride (100 ml.) for 4 hr. The precipitated crystals (5·1 g.) were collected and recrystallised from glacial acetic acid, giving the *hydroxytetra-aza-benzanthracene* as yellow needles, slowly decomposing above 340° (Found: C, 67·3, 67·65; H, 3·0, 3·2; N, 22·1, 22·0. $C_{14}H_8ON_4$ requires C, 67·7; H, 3·25; N, 22·5%). A solution of the substance in glacial acetic acid exhibited a pale yellow fluorescence.

7 : 8-*Dihydro*-7-*methyl*-8-*oxo*-5 : 7 : 9 : 10-*tetra-aza*-1 : 2-*benzanthracene* (V; R = Me).—The hydroxy-tetra-aza-compound (1 g.), suspended in acetone (40 ml.), was treated with diazomethane (from nitrosomethylurea, 4 g.) in ether (40 ml.) and after 4 days at room temperature the *product* (0·75 g.) was collected and crystallised from butanol as irregular yellow prisms, m. p. 316—317° (Found: C, 68·8, 68·6; H, 3·7, 4·0; N, 21·5, 21·6. $C_{15}H_{10}ON_4$ requires C, 68·7; H, 3·8; N, 21·4%). Infra-red absorption in Nujol mull: strong band at 1687 cm.$^{-1}$.

8-*Mercapto*-5 : 7 : 9 : 10-*tetra-aza*-1 : 2-*benzanthracene* (I; R = SH, R′ = H).—The hydroxy-tetra-aza-compound (1·25 g.), in dry pyridine (30 ml.), was treated with phosphorus pentasulphide (1·2 g.), and the mixture refluxed for 1½ hr. and then poured into hot water (100 ml.). The cooled suspension was collected and the solid (1·4 g.) crystallised several times from pyridine, to afford the *mercapto*-compound as beautiful bronze leaflets, m. p. 356° (decomp.) (Found: C, 62·9, 62·9; H, 3·55, 3·2; N, 21·6. $C_{14}H_8N_4S$ requires C, 63·6; H, 3·05; N, 21·2%). The compound gave a positive reaction with azide–iodine reagent, diagnostic for a mercapto-group (Feigl, "Qualitative Analysis by Spot Tests," Elsevier, New York, 1947, p. 353), and exhibited a pale blue fluorescence in water, which became white on addition of alkali. It did not yield a fluorescent spot on a paper chromatogram.

6 : 8-*Dihydroxy*-5 : 7 : 9 : 10-*tetra-aza*-1 : 2-*benzanthracene* (I; R = R′ = OH).—(a) *From* 2-*aminobenzoquinoxaline*-3-*carbonamide*. A suspension of the amide (1 g.) in ethyl chloroformate (25 ml.) was refluxed for 40 hr., during which hydrogen chloride was slowly evolved. The ester was removed under reduced pressure and the residue (1·3 g.) crystallised from ethanol, to afford 2-*ethoxycarbonylamino*-5 : 6-*benzoquinoxaline*-3-*carbonamide* (IV; R = CO·NH$_2$, R′ = NH·CO$_2$Et) as sheaves of flat yellow prisms, m. p. >360° (Found: C, 62·05, 62·2; H, 4·7, 4·5; N, 18·1, 17·9. $C_{16}H_{14}O_3N_4$ requires C, 61·9; H, 4·55; N, 18·1%). The compound shows a bluish-white fluorescence in acetic acid. This urethane (0·5 g.) was added to a solution of sodium (0·5 g.) in dry ethanol (50 ml.), and the mixture refluxed for 3 hr., during which a precipitate separated. The solvent was evaporated under reduced pressure, the residue was dissolved in hot 2% sodium hydroxide solution, and the solution was filtered from a small amount of insoluble matter and acidified to yield a yellow precipitate (0·26 g.), m. p. >400°. This was identified by methylation by diazomethane (see below).

(b) *From violuric acid and* 2-*naphthylamine. The "inverse" fusion reaction.* 2-Naphthylamine (6 g.) was fused and violuric acid (1·6 g.) was added when the internal temperature was 130°. A vigorous reaction occurred when the temperature reached 150° and after a further ½ hr. at this temperature the melt was allowed to cool and was extracted successively with ethanol and warm 2N-sodium hydroxide. The filtered alkaline extract was acidified with dilute acetic acid, and the yellow precipitate was crystallised from 80% formic acid to yield the dihydroxy-compound as flat yellow needles, m. p. >400° (Found: C, 60·1, 60·0; H, 3·4, 3·6; N, 19·8, 19·9. $C_{14}H_8O_2N_4$,1H_2O requires C, 59·6; H, 3·6; N, 19·85. Found, in a sample dried at 150° in a high vacuum: C, 63·6, 63·7; H, 3·2, 3·2; N, 21·1, 21·2. Calc. for $C_{14}H_8O_2N_4$: C, 63·6; H, 3·05; N, 21·2%). The compound gives a pure blue fluorescence both in solution and on paper chromatograms.

5 : 6 : 7 : 8-*Tetrahydro*-5 : 7-*dimethyl*-6 : 8-*dioxo*-5 : 7 : 9 : 10-*tetra-aza*-1 : 2-*benzanthracene* (II; R = R′ = Me).—(a) *By direct condensation.* 4-Amino-1 : 2 : 3 : 6-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 6-dioxopyrimidine (1·84 g.; Traube, *Ber.*, 1900, **33**, 3035) was added to fused 2-naphthol at 130° and, after the vigorous reaction had subsided, the melt was extracted with ethanol and ether to leave a yellow powder (1·82 g.). This was crystallised from 80% formic acid to give the *NN′*-dimethyl compound as pale yellow needles, m. p. 278° (Found: C, 66·2, 66·2; H, 4·1, 4·0; N, 19·1, 19·5. Calc. for $C_{16}H_{12}O_2N_4$: C, 65·8; H, 4·1; N, 19·2%). The compound gave a pure blue fluorescence in acetic acid solution and on paper. Kuhn and Cook (*loc. cit.*) give m. p. 258—260°.

(b) *By methylation of the dihydroxy-compound.* 6 : 8-Dihydroxy-5 : 7 : 9 : 10-tetra-aza-benzanthracene (0·5 g.; obtained by several routes) was suspended in acetone (20 ml.) and treated at room temperature with diazomethane (from nitrosomethylurea, 2 g.) in ether (20 ml.), and the precipitate (0·35 g.) collected after 2 or 3 days. Crystallisation from 80% formic acid gave pale yellow needles, m. p. and mixed m. p. 276—278°.

5 : 6 : 7 : 8-*Tetrahydro*-5-*methyl*-6 : 8-*dioxo*-5 : 7 : 9 : 10-*tetra*-*aza*-1 : 2-*benzanthracene* (II; R = H, R′ = Me).—The fusion reaction between 2-naphthol (4 g.) and 4-amino-3 : 6-dihydro-2-hydroxy-3-methyl-5-nitroso-6-oxopyrimidine (1·7 g., Traube, *loc. cit.*) was carried out at 150° for ½ hr. The residue (2·7 g.), isolated in the usual way, crystallised from a large volume of 80% formic acid to give the *monomethyl* derivative as pale yellow flat needles, m. p. 390° (Found: C, 64·6, 64·8; H, 3·7, 3·7; N, 20·6, 20·6. $C_{15}H_{10}O_2N_4$ requires C, 64·8; H, 3·6; N, 20·2%). This compound is very much less soluble than the related dimethyl compound. It too gives a pure blue fluorescence both in glacial acetic acid and on paper.

5 : 6 : 7 : 8-*Tetrahydro*-6 : 8-*dimethyl*-5 : 7-*dioxo*-6 : 8 : 9 : 10-*tetra*-*aza*-1 : 2-*benzanthracene* (III; R = R′ = Me).—(*a*) 1 : 2-Diaminonaphthalene hydrochloride and alloxan were condensed together, following Kühling's experimental conditions (*loc. cit.*, p. 2363), and the crude product purified by dissolving it in dilute sodium hydroxide solution (charcoal) and acidifying the filtrate with acetic acid. The precipitate (0·6 g.) was treated at room temperature with diazomethane (from nitrosomethyl-urea, 2 g.) in ether (20 ml.), and the product was collected after 2 days. It was dissolved in 50% acetic acid (150 ml.), and the insoluble residue crystallised from 80% acetic acid to yield the pure *NN*′-dimethyl compound as bright yellow needles, m. p. 318° (Found: C, 65·75, 65·9; H, 4·0, 4·3; N, 19·1, 19·3. Calc. for $C_{16}H_{12}O_2N_4$: C, 65·8; H, 4·1; N, 19·2%). This isomer exhibits a yellowish-green fluorescence in acetic acid, and a nearly pure green fluorescence on paper. The original mother-liquors (50% acetic acid) were cooled and filtered from deposited solid (m. p. 314°), and the filtrate was evaporated. The residue so obtained was crystallised twice from small volumes of 50% acetic acid: the product had m. p. 226°. Paper chromatography showed the presence of the isomer of m. p. 318° in large amounts (to judge from the intensity of fluorescence of the spot), but failed to yield a spot corresponding in position or colour of fluorescence with the isomer of m. p. 278°.

(*b*) 1 : 2-Naphthaquinone was condensed with 4 : 5-diamino-2 : 6-dihydroxypyrimidine, following Kuhn and Cook's directions (*loc. cit.*), and the crude product was purified by crystallisation from glacial acetic acid. Methylation of this compound with diazomethane, followed by crystallisation of the product from glacial acetic acid, afforded the isomer, m. p. and mixed m. p. 318°.

5 : 6 : 7 : 8-*Tetrahydro*-6 : 8-*dimethyl*-5 : 7-*dioxo*-1′ : 6 : 8 : 9 : 10-*penta*-*aza*-1 : 2-*benzanthracene* (VII).—4-Amino-1 : 2 : 3 : 6-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 6-dioxopyrimidine (1·84 g.) was added to fused 8-hydroxyquinoline (6 g.), and the melt was maintained at 180° for ½ hr. The cooled mass was thoroughly extracted with ethanol and ether, and the residue (1·06 g.) was repeatedly crystallised from a large volume of boiling water (charcoal), to yield the *penta-azabenzanthracene* as yellow needles, m. p. 360° (Found: C, 60·5, 60·5; H, 3·6, 3·8; N, 23·3, 23·4. $C_{15}H_{11}O_2N_5,\frac{1}{2}H_2O$ requires C, 60·6; H, 3·9; N, 23·5%).

Alkaline Hydrolysis of 8-*Hydroxytetra-azabenzanthracene*.—The hydroxy-compound (0·25 g.) was boiled under reflux for 10 min. with N-sodium hydroxide (10 ml.), and the mixture was then allowed to cool and was filtered. The residue (0·125 g.) crystallised from 2-ethoxyethanol to form yellow needles, m. p. 278°, undepressed on admixture with 2-amino-5 : 6-benzoquinoxaline-3-carbonamide (m. p. 282°).

Acid Hydrolysis of 8-*Aminotetra-azabenzanthracene*.—The amino-compound (0·19 g.) was refluxed with 6N-hydrochloric acid (20 ml.) for 6 hr. and the suspension was collected after refrigeration. From glacial acetic acid it formed yellow crystals, m. p. 232°, undepressed on admixture with authentic 2-amino-5 : 6-benzoquinoxaline-3-carboxylic acid.

Acid Hydrolysis of 6 : 8-*Diaminotetra-azabenzanthracene*.—The diaminotetra-azabenzanthracene (0·7 g.; Felton and Timmis, *J.*, 1954, 2881) was refluxed with 6N-hydrochloric acid (60 ml.) for 9 hr. and the solid was then collected and extracted with 2N-sodium hydroxide. The filtered extract was acidified and gave 6-*amino*-8-*hydroxy*-5 : 7 : 9 : 10-*tetra*-*aza*-1 : 2-*benzanthracene*, which on crystallisation from 80% formic acid afforded lemon-yellow crystals, m. p. >400° (Found: C, 60·2; H, 3·95; N, 25·3. $C_{14}H_9ON_5,H_2O$ requires C, 59·8; H, 3·9; N, 24·95%). The compound exhibited an intense green fluorescence in acetic acid solution and a bluish-green fluorescence on paper.

Hydrolysis of 6-*Amino*-8-*hydroxytetra-azabenzanthracene by Nitrous Acid*.—The amino-hydroxy-compound (0·2 g.) was dissolved in concentrated sulphuric acid (7 ml.) and water (2 ml.), and the solution was cooled to −5° and decomposed by the addition of excess of solid sodium nitrite in small portions. After the pasty mass had been kept at room temperature for 15 min., it was heated on a steam-bath till effervescence ceased and then poured into ice water. The yellow solid precipitated was collected and crystallised from 80% formic acid, to yield yellow needles, m. p. >400°, which were identified as the dihydroxytetra-azabenzanthracene by comparison with an authentic specimen on a paper chromatogram and by treatment with

excess of diazomethane to give the tetrahydrodimethyldioxo-compound, m. p. and mixed m. p. 278°.

Acid Hydrolysis of 8-Amino-6-dimethylaminotetra-azabenzanthracene.—8-Amino-6-dimethylaminotetra-azabenzanthracene (1·15 g.) was refluxed with 6N-hydrochloric acid (80 ml.) for 7 hr. When cold, the solution was filtered and the insoluble residue (0·85 g.) was dissolved in hot dilute sodium hydroxide solution (charcoal), filtered, and acidified with dilute acetic acid. The yellow crystalline precipitate was recrystallised from aqueous ethanol and yielded the 6-dimethylamino-8-hydroxy-compound as golden-yellow needles, m. p. and mixed m. p. 357°.

The authors thank Professor F. Bergel for his interest in this work, and are indebted to Mr. S. F. D. Orr, M.A., for the infra-red spectrum, Mrs. K. Tussler for ultra-violet absorption measurements, and Messrs. M. Baker and J. M. Johnson for technical assistance.

INSTITUTE OF CANCER RESEARCH : THE ROYAL CANCER HOSPITAL,
FULHAM ROAD, LONDON, S.W.3. [*Received, February 1st,* 1954.]