480. *Cyclic Amidines. Part IX.* Tricycloquinazoline.*

By K. Butler and M. W. Partridge.

An unequivocal synthesis of the polyazapolycyclic carcinogen, tricycloquinazoline, is reported. Attention is drawn to the inapplicability to this compound of the hypothesis relating carcinogenic activity to electron densities of *K* and *L* regions.

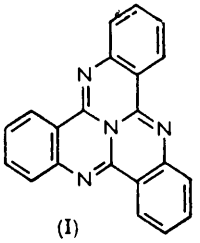
The structure assigned to tricycloquinazoline (I) has hitherto been based on its formation from a disubstituted benzene having carbon- and nitrogen-containing functional groups *ortho* to one another,[1] and on the formation of tri- and hexa-substituted derivatives.[2] Because of its carcinogenic properties,[3,4] and since, on nitration as described by Kozak and Kalmus,[2] we found it yielded a dinitro-derivative having properties similar to those described for its trinitro-derivative, we now report its unequivocal synthesis.

Anthranilamide (II), which was produced in almost quantitative yield by reduction of *o*-nitrophenyl cyanide with hydrazine and Raney nickel, reacted with *o*-nitrobenzoyl chloride to yield *o*-*o*'-nitrobenzamidobenzamide (III), together with a di-(*o*-nitrobenzoyl)-anthranilamide. Reduction of the nitro-compound (III) with stannous chloride and hydrochloric acid afforded the corresponding amine (IV) which was isolated free from tin only with considerable loss. This reduction could not be effected with iron or zinc and acetic acid, or titanous chloride,[5] or catalytically in the presence of Raney nickel or Adams catalyst.

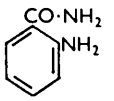
* Part VIII, *J.*, 1959, 1512.

[1] Cooper and Partridge, *J.*, 1954, 3429.
[2] Kozak and Kalmus, *Bull. Acad. polonaise*, 1933, **10**, *A*, 532.
[3] Baldwin, Butler, Cooper, Partridge, and Cunningham, *Nature*, 1958, **181**, 838.
[4] Baldwin, Cunningham, and Partridge, *Brit. J. Cancer*, in the press.
[5] Meyer, *Annalen*, 1907, **351**, 278.

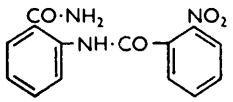
An attempted preparation of this amine (IV) by interaction of 2-*o*-aminophenyl-6-oxo-4,5-benz-1,3-oxazine [6] (V) and ammonia furnished a compound, $C_{28}H_{23}O_4N_5$, which may have structure (VI), since above its melting point it afforded dianthranilide [1] (VII).



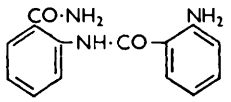
(I)



(II)

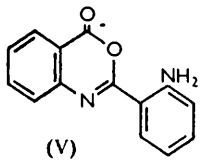


(III)

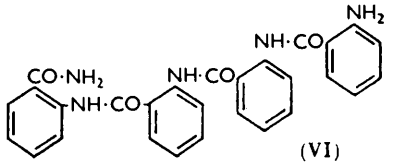


(IV)

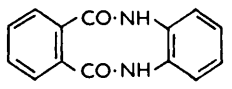
The nitro-triamide (VIII), formed by treatment of the amine (IV) with *o*-nitrobenzoyl chloride, was unstable. It decomposed when heated in 2-methoxyethanol, and afforded the diamide (IV) on hydrogenation in the presence of Adams catalyst. Reduction to the



(V)

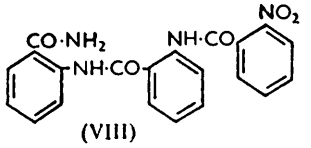


(VI)

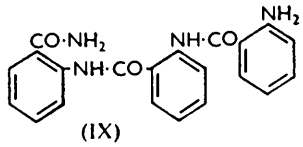


(VII)

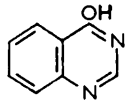
amino-triamide (IX) was carried out by stannous chloride and hydrochloric acid, the product being isolated as its stannic chloride adduct which decomposed during attempts to remove the tin. It is suggested that this adduct is of a similar type to that of 1 : 1 molecular composition formed between diacetylaniline [7] or dimethylaniline [8] and stannic chloride. An attempt to prepare the amino-triamide (IX) by interaction of the oxazine (V) and anthranilamide (II) gave only 4-hydroxyquinazoline (X).



(VIII)



(IX)



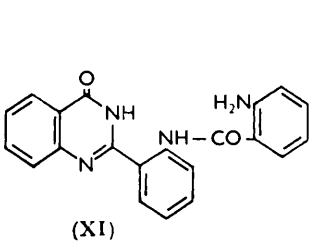
(X)

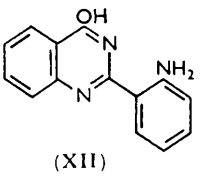
The stannic chloride adduct of (IX), when heated with phosphoric oxide in xylene, furnished tricycloquinazoline (I), identical with previously prepared specimens. Removal of one or two molecules of water from the amino-triamide (IX) could occur in several ways, but the only compound, $C_{21}H_{12}N_4$, which can feasibly be formed by removal of three molecules of water is (I). In view of the ease with which acylanthranilamides undergo cyclising dehydration to quinazolines,[9] it is probable that the first stage in the formation of tricycloquinazoline (I) involves the quinazoline (XI).

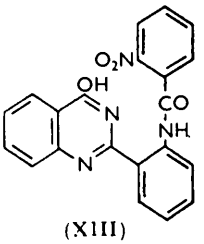
2-*o*-Aminophenyl-4-hydroxyquinazoline (XII), which was readily produced by cyclisation of the amide (IV), afforded an *o*-nitrobenzoyl derivative (XIII) which could not be induced to cyclise to the triazabenz[*a*]anthracene derivative (XIV) required for reduction and dehydration to tricycloquinazoline. An attempted synthesis in obvious steps from 4-hydroxy-2-*o*-nitrophenylquinazoline (XV), itself obtained from the amide (III), was unsuccessful since this 4-hydroxyquinazoline could not be converted into the corresponding 4-chloro-derivative.

[6] Schroeter and Eisleb, *Annalen*, 1909, **367**, 101.
[7] Dippy and Moss, *J.*, 1952, 2205.
[8] George, Mark, and Wechsler, *J. Amer. Chem. Soc.*, 1950, **72**, 3896.
[9] Meyer and Wagner, *J. Org. Chem.*, 1943, **8**, 239.

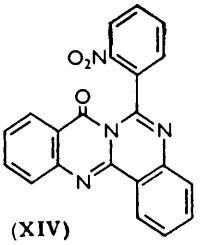
Tricycloquinazoline is a stable, very feebly basic compound which sublimes at atmospheric pressure at red heat or below without decomposition. Kozak and Kalmus [2] describe the production of 4-hydroxyquinazoline (X) by oxidation of tricycloquinazoline with chromic anhydride in dilute sulphuric acid, whereas we recovered 97% of the compound after this treatment. A similar stability to other oxidising agents was observed.

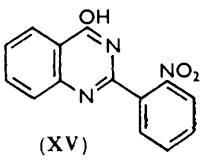

(XI)

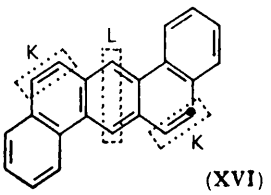

(XII)


(XIII)

Although 62% was oxidised by chromic anhydride in acetic acid at 100° during 4 days, and none was recoverable after 3 days' treatment with chromic anhydride in concentrated sulphuric acid, no recognisable oxidation product was isolated. Tricycloquinazoline does not couple with diazotised arylamines.


(XIV)


(XV)


(XVI)

Its carcinogenic activity is of about the same order as that of 1,2 : 5,6-dibenzanthracene (XVI) [4] whose biological properties, together with those of other polycyclic hydrocarbons [10] and alkylated benzacridines,[11] have been interpreted in terms of the electron densities of the *K* and *L* regions. High activity is evident in certain members of these series having blocked *L* regions and *K* regions of appropriate electron density, whereas in inactive members the *K* regions have electron densities of inappropriate values, or such a region is absent. Moreover, the introduction of substituents into both *K* regions of the active carcinogen, 1,2 : 5,6-dibenzanthracene, suppresses activity.[12] Since tricycloquinazoline has no region logically identifiable as an *L* region or an unsubstituted *K* region, the interpretation of the origin of carcinogenicity in terms of the union of a *K* region with an electrophilic cell-receptor appears to be inapplicable in this case.

Experimental

Anthranilamide.—80% Hydrazine hydrate (9 ml.) was mixed with *o*-nitrophenyl cyanide (10 g.) in ethanol (100 ml.) at 45—50°. Raney nickel (3 g.) was gradually added to keep the mixture at 40—60° by the heat of reaction; if necessary, further catalyst was added until no more gas was evolved. The residue obtained by filtering and evaporating the hot solution furnished anthranilamide (9 g., 98%; m. p. 107—108°) on recrystallisation from water. Reduction by zinc dust and ammonium chloride [13] gave 40%, and hydrogenation of *o*-nitrobenzamide with Adams catalyst gave 70—85% yields.

o-o'-*Nitrobenzamidobenzamide.*—Anthranilamide (100 g.) in dry benzene (350 ml.) and

[10] Pullman and Pullman, " Advances in Cancer Research," Academic Press, New York, 1955, Vol. III, p. 117.
[11] Lacassagne, Buu-Hoï, Daudel, and Zajdela, *op. cit.*, 1956, Vol. IV, p. 315.
[12] Oliverio and Heidelberger, *Cancer Research*, 1958, **18**, 1094.
[13] Reissert and Gruber, *Ber.*, 1909, **42**, 3712.

pyridine (150 ml.) was treated with *o*-nitrobenzoyl chloride (150 g.) in benzene (600 ml.). After an hour, the benzene was evaporated from the two-phase mixture, and the residue was poured into water (3 l.). The precipitated solid furnished the *nitroamide* as pale yellow needles, m. p. 195—196° (from ethanol) (135 g.) (Found: C, 58·8; H, 3·8; N, 14·7. $C_{14}H_{11}O_4N_3$ requires C, 58·9; H, 3·9; N, 14·7%).

The ethanol-insoluble material yielded *di-*(o-*nitrobenzoyl*)*anthranilamide* (28 g.) which crystallised from acetic acid or 2-ethoxyethanol as needles, m. p. 214—216° (Found: C, 57·9; H, 3·3; N, 12·8. $C_{21}H_{14}O_7N_4$ requires C, 58·1; H, 3·3; N, 12·9%).

o-o′-*Aminobenzamidobenzamide.*—When *o-o′*-nitrobenzamidobenzamide (10 g.) in warm acetic acid (75 ml.) was stirred with stannous chloride (30 g.) in concentrated hydrochloric acid (30 ml.) for 2 hr. a solid separated. Water (150 ml.) and sufficient aqueous ammonia to bring the suspension to pH 4—5 were added and oxalic acid (40 g.) was dissolved in the mixture. Insoluble oxalate remaining after the mixture had been stirred for 1 hr. was collected, washed with aqueous oxalic acid, and basified by trituration with aqueous ammonia. The remaining tin compounds were precipitated when hydrogen sulphide was passed through a suspension of the crude product in 0·5N-sulphuric acid for 2 hr. The suspension was rapidly heated to 80°, filtered, and cooled. The precipitate afforded the pure *aminoamide* (0·98 g., 11%) as plates, m. p. 207·5—208°, from ethanol containing 1% of ammonia (Found: C, 65·4; H, 5·1; N, 16·5. $C_{14}H_{13}O_2N_3$ requires C, 65·8; H, 5·1; N, 16·5%).

Reactions of 2-o-*Aminophenyl*-6-*oxo*-4,5-*benz*-1,3-*oxazine.*—(i) Dry ammonia was passed through a solution of the oxazine [6] (4·6 g.) in ethanol (80 ml.) and after 45 min. the solution was cooled thoroughly. The crystals which separated yielded a *compound* (2·3 g.), m. p. 196—196·5° (decomp.), on recrystallisation from ethanol (Found: C, 67·8; H, 4·6; N, 14·0. $C_{28}H_{23}O_4N_5$ requires C, 68·1; H, 4·7; N, 14·2%). On being melted, this compound furnished dianthranilide,[1] m. p. and mixed m. p. 334—336° (from ethanol).

(ii) The oxazine [6] (0·6 g.) and anthranilamide (0·34 g.) when heated together at 100° for 8 days afforded 4-hydroxyquinazoline (0·09 g.; m. p. and mixed m. p. 216—218°) as a sublimate. Anthranilamide was recovered from an ethanolic extract of the melt.

o-o′-o″-*Nitrobenzamidobenzamidobenzamide.*—*o-o′*-Aminobenzamidobenzamide (0·25 g.) in pyridine (4 ml.) was treated with *o*-nitrobenzoyl chloride (0·2 g.) in benzene (1 ml.) and kept for 16 hr. Solvent was evaporated, and the precipitate obtained on pouring of the residue into water, when rapidly recrystallised from 2-methoxyethanol, afforded the o-*nitrobenzoyl derivative* (0·16 g.) as yellow needles, m. p. 254—255° (decomp.) (Found: C, 62·2; H, 4·1; N, 13·6. $C_{21}H_{16}O_5N_4$ requires C, 62·4; H, 4·0; N, 13·9%).

o-o′-o″-*Aminobenzamidobenzamidobenzamide.*—The foregoing nitro-compound (1 g.) in acetic acid (100 ml.) was shaken for 30 min. with stannous chloride (1·8 g.) and concentrated hydrochloric acid (4 ml.), warmed to 40°, and shaken a further 30 min. The solid which separated when the mixture was cooled to 0°, was washed with water and ethanol and crystallised from acetic acid, furnishing the crystalline *amine stannic chloride adduct* (0·83 g.), m. p. 256—257° (Found: C, 39·9; H, 3·2; N, 8·6. $C_{21}H_{18}O_3N_4,SnCl_4$ requires C, 39·7; H, 2·8; N, 8·8%).

The nitro-compound (0·84 g.) in glacial acid (25 ml.) gradually dissolved on being shaken for 30 min. with hydrogen and Adams catalyst (0·06 g.). The solid which separated when the filtrate was adjusted to pH 4—5 was *o-o′*-aminobenzamidobenzamide (0·39 g.), m. p. and mixed m. p. 205—206°.

Tricycloquinazoline.—The foregoing stannic chloride complex (0·34 g.) was boiled with phosphoric oxide (0·5 g.) in xylene (75 ml.) for 30 min. and filtered. The addition of water to the residue afforded tricycloquinazoline, m. p. and mixed m. p. 322—323°, after recrystallisation from benzene (yield, 0·15 g., 66%) (Found: C, 79·0; H, 3·6; N, 17·7. Calc. for $C_{21}H_{12}N_4$: C, 78·7; H, 3·8; N, 17·5%).

Light absorption (λ *in* mμ) *of tricycloquinazoline in chloroform.*

Synthetic specimen	$\lambda_{max.}$	245	250	285	296	310	378	400	426	455
	ε	39,400	41,200	22,400	29,200	25,600	21,500	20,300	7200	2300
Previously reported [1]	$\lambda_{max.}$	—	252	284	296	310	378	400	424	452
	ε	—	40,900	23,500	32,600	29,200	23,800	23,000	8200	2600

4-*Hydroxy*-2-o-*nitrophenylquinazoline.*—*o-o′*-Nitrobenzamidobenzamide (2 g.) was refluxed with potassium hydroxide (0·5 g.) in ethanol (20 ml.) for 40 min. After being diluted with

water, the mixture was neutralised with acetic acid; the precipitated quinazoline (1·72 g., 92%) crystallised as yellow prisms, m. p. 226—227°, from toluene (Found: C, 63·3; H, 3·2; N, 15·6. Calc. for $C_{14}H_9O_3N_3$: C, 62·9; H, 3·4; N, 15·7%). Smith and Stephen [14] record m. p. 237°.

2-o-*Aminophenyl*-4-*hydroxyquinazoline*.—(i) *o-o'*-Aminobenzamidobenzamide (1 g.) was boiled with potassium hydroxide (0·2 g.) in ethanol (20 ml.) for 20 min. The precipitate obtained on dilution and neutralisation of the mixture gave the required quinazoline (0·66 g.) as needles, m. p. 236—238° (from ethanol). Mohr and Köhler [15] give m. p. 237°. Its *hydrochloride* crystallised as pale yellow needles, m. p. 278—280°, from 2N-hydrochloric acid (Found: N, 14·5; Cl, 12·4. $C_{14}H_{12}ON_3Cl,H_2O$ requires N, 14·4; Cl, 12·2%). Stephen and Stephen [16] state that the base crystallises from dilute hydrochloric acid. The acetyl derivative had m. p. 274—275° (decomp.) (Found: N, 14·9. Calc. for $C_{16}H_{13}O_2N_3$: N, 15·0%). Mohr and Köhler [15] record m. p. 278° (decomp.); Jacini [17] gives m. p. 276°, but Stephen and Stephen [16] record m. p. 177°.

(ii) The same quinazoline derivative was obtained in 43% yield by reduction of 4-hydroxy-2-*o*-nitrophenylquinazoline with stannous chloride and hydrochloric acid.

4-*Hydroxy*-2-(o-o'-*nitrobenzamidophenyl*)*quinazoline*.—2-*o*-Aminophenyl-4-hydroxyquinazoline (1 g.) was refluxed with *o*-nitrobenzoyl chloride (3 g.) in benzene (12 ml.) and pyridine (30 ml.) for 2½ hr. The mixture was poured into water, benzene was distilled off, and the alkali-soluble fraction of the insoluble material, after recovery, furnished the *nitrobenzoyl derivative* as yellow needles, m. p. 272·5—273°, from 2-methoxyethanol (Found: C, 65·1; H, 3·7; N, 14·6. $C_{21}H_{14}O_4N_4$ requires C, 65·3; H, 3·7; N, 14·5%). This compound was unchanged after 24 hours' boiling with 2N-hydrochloric acid or 2N-sodium hydroxide. With 80% sulphuric acid at 100° for 4 hr., it afforded 2-*o*-aminophenyl-4-hydroxyquinazoline (61%). Attempts to effect cyclisation with phosphoric oxide or zinc chloride in xylene, polyphosphoric acid at 180°, or aluminium chloride in benzene were unsuccessful.

Dinitrotricycloquinazoline.—Tricycloquinazoline (1 g.) in glacial acetic acid (10 ml.) was heated with fuming nitric acid (5 ml.) at 100° for 2 hr. The solid (0·7 g., 55%) which separated furnished *dinitrotricycloquinazoline* (0·25 g.) as elongated orange prisms, m. p. about 380° (decomp.), on recrystallisation from nitrobenzene (Found: C, 61·3; H, 2·7; N, 20·5. $C_{21}H_{10}O_4N_6$ requires C, 61·5; H, 2·4; N, 20·5%). The same compound was obtained (28% yield) when nitration was similarly effected with nitric acid (*d* 1·32).[2] The amine formed on reduction of the foregoing nitro-compound could not be characterised.

Oxidation of Tricycloquinazoline.—No identifiable product was isolated in the following attempted oxidations (recoveries of tricycloquinazoline are given in parentheses): chromic anhydride and dilute sulphuric acid [2] (97%). 33% Hydrogen peroxide in glacial acetic acid at 100° for 5 days (96%). Refluxing alkaline potassium permanganate (9 equiv.) for 45 min. (93%). 5N-Nitric acid for 2 days (96%). Chromic anhydride (9 equiv.) in glacial acetic acid at 100° for 4 days (38%). Chromic anhydride in concentrated sulphuric acid at 20° for 3 days (nil).

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[14] Smith and Stephen, *Tetrahedron*, 1957, **1**, 38.
[15] Mohr and Kohler, *J. prakt. Chem.*, 1909, **80**, 521.
[16] Stephen and Stephen, *J.*, 1956, 4178.
[17] Jacini, *Gazzetta*, 1943, **73**, 306.