SECTION C
Organic Chemistry

Quinazoline Analogues of Folic Acid

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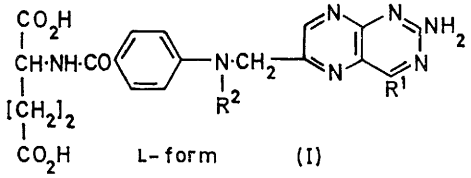
Analogues of folic acid having the pteridine ring replaced by quinazoline have been prepared by reductive condensation of appropriately substituted quinazoline-6-carbaldehydes and -carbonitriles with diesters of *N*-(*p*-aminobenzoyl)amino-acids, followed by hydrolysis.

THE folic acid (I; $R^1 = OH$, $R^2 = H$) antagonists aminopterin (I; $R^1 = NH_2$, $R^2 = H$) and methotrexate (I; $R^1 = NH_2$, $R^2 = Me$) were among the earliest known compounds to show activity against acute leukaemia; the latter is still used both for this purpose and as an immune suppressant. In view of this, the synthesis of potential antagonists derived from quinazoline (*i.e.* ' 5,8-dideazapteridine ') was undertaken.

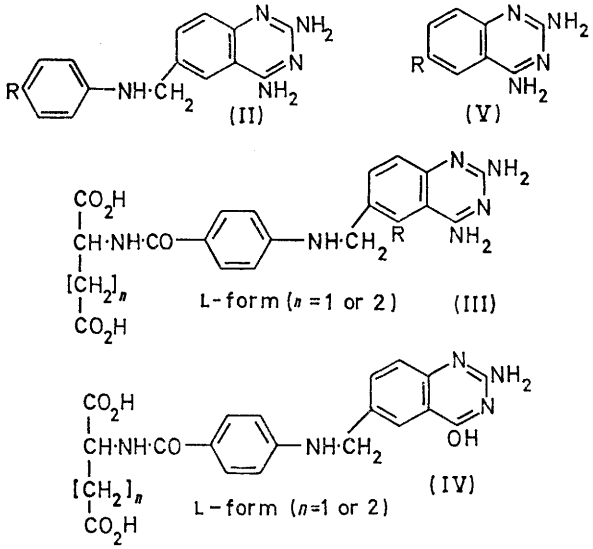


Three types of variant were required: (*a*) 2-amino-4-hydroxy- or 2,4-diamino-substituted derivatives, (*b*) derivatives containing different terminal amino-acids, and (*c*) 5-substituted derivatives, since the 5-position in folic acid is involved in its biochemical functions.

The 4-amino-4-deoxy-analogue (II; $R = CO_2H$) of pteroic acid in this series has been synthesised previously [1] by the reaction of 2,4-dibenzamido-6-bromomethylquinazoline with ethyl *p*-aminobenzoate, followed by removal of protective groups, and we initially attempted the synthesis of (III; $R = H$, $n = 2$), the quinazoline analogue of aminopterin, by using diethyl *N*-(*p*-aminobenzoyl)-L-glutamate in this synthesis. However, almost complete hydrolysis of one amino-group (presumably [2] that at position 4) occurred, during either the condensation or the subsequent hydrolysis of the protecting groups, to give principally (IV; $n = 2$).[3]

It seemed likely that a reductive condensation between a quinazoline-6-carbaldehyde or -carbonitrile and an *N*-(*p*-aminobenzoyl)amino-acid ester would proceed under milder conditions, and moreover, since heterocyclic amino-groups such as those present in 2,4-diamino-quinazoline do not readily give Schiff bases, preliminary protection of these groups would be unnecessary.

2,4-Diaminoquinazolines have usually been prepared by amination of 2,4-dichloro-compounds,[4] or from anthranilonitriles,[4] but neither route was convenient for the preparation of the required aldehyde or nitrile, and 2,4-diamino-6-nitroquinazoline (V; $R = NO_2$) proved a more suitable starting material. It was obtained in high



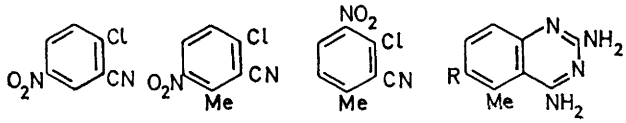
yield by three methods: (i) nitration of 2,4-diamino-quinazoline, (ii) reaction of 2-chloro-5-nitrobenzonitrile with guanidine carbonate, and (iii) reaction of 5-nitro-anthranilonitrile with guanidine. On reduction, either with tin(II) chloride or by catalytic hydrogenation, it gave 2,4,6-triaminoquinazoline (V; $R = NH_2$), of which only the 6-amino-group shows ' aromatic ' properties, so that diazotisation and reaction with cuprocyanide afforded 2,4-diaminoquinazoline-6-carbonitrile (V; R = CN). This was converted into the aldehyde (V; R = CHO) by hydrogenation in the presence of phenylhydrazine [5] followed by cleavage of the resulting phenylhydrazone.

Intermediates for the preparation of analogues (IV) with 2-amino-4-hydroxy-substitution (as in folic acid

[1] V. Oakes, H. N. Rydon, and K. Undheim, *J. Chem. Soc.*, 1962, 4678.
[2] R. B. Trattner, G. B. Elion, G. H. Hitchings, and D. M. Sharefkin, *J. Org. Chem.*, 1964, **29**, 2674.
[3] B.P. 1,021,196.
[4] W. L. F. Armarego, ' The Chemistry of Heterocyclic Compounds. Fused Pyrimidines. Part I. Quinazolines,' Interscience, New York, 1967, pp. 330—332.
[5] A. Gaiffe, *Chimie et Industrie*, 1965, **93**, 259.

itself) were obtained from the nitrile and aldehyde with hot dilute acid; the lability of the 4-amino-group under these conditions is well established.[2]

Unlike anthranilonitrile, its 6-chloro-derivative did not react with cyanoguanidine in dilute acid, but gave the required 2,4-diamino-5-chloroquinazoline when heated in bis-2-methoxyethyl ether with chloroformamidine hydrochloride (' cyanamide dihydrochloride '). Nitration of this, followed by reduction with tin(II) chloride, gave 2,4,6-triamino-5-chloroquinazoline (the structure of which was established by reductive dehalogenation to 2,4,6-triaminoquinazoline), which was converted into 2,4-diamino-compound. Comparison of the u.v. spectra of these with that of 2,4,6-triaminoquinazoline indicated



(VI) (VII) (VIII) (IX)

that the major component from the reaction with guanidine carbonate was the 6-nitro-compound (IX; R = NO_2), confirming the assigned structure for the

TABLE 1

Diethyl esters of (III) and (IV)

				Found (%)				Required (%)				Yield (%) from	
	R	n	M.p.	C	H	N	Formula	C	H	N	Form	Aldehyde	Nitrile
(III)	H	0	192° [a]	58·0	5·8	17·4	$C_{23}H_{26}N_6O_5,0{\cdot}5H_2O$	58·1	5·7	17·7	Needles	54	20
(III)	H	1	174—177	58·0	6·3	17·0	$C_{24}H_{28}N_6O_5,H_2O$	57·8	6·1	16·9	Beige rods	44	37
(III) [b]	H	1	173—175	58·0	6·1	16·9	$C_{24}H_{28}N_6O_5,H_2O$	57·8	6·1	16·9	Beige rods		35
(III)	Cl	0	200—202 [a]	55·2	5·0	17·0	$C_{23}H_{25}ClN_6O_5$	55·1	5·0	16·8	Cream blades		20
(III)	Cl	1	198—200	55·6	5·3	16·9 [c]	$C_{24}H_{27}ClN_6O_5$	56·0	5·3	16·3	Yellow needles		6
(III)	Cl	2	173—174	54·8	5·5	15·6	$C_{25}H_{29}ClN_6O_5,H_2O$	54·9	5·7	15·4	Needles		10
(III)	Me	0	214 [a]	60·2	5·9	17·7	$C_{24}H_{28}N_6O_5$	60·0	5·9	17·5	Tan blades		31
(III)	Me	1	211—212	60·1	6·1	16·9	$C_{25}H_{30}N_6O_5$	60·7	6·4	17·0	Yellow rods		21
(III)	Me	2	189—191	59·9	6·4	16·7	$C_{26}H_{32}N_6O_5,0{\cdot}5\,H_2O$	60·3	6·4	16·2	Powder		31
(IV)		1	209—210	59·4	6·2	14·7	$C_{24}H_{27}N_5O_6$	59·9	5·7	14·6	Blades	44	35
(IV) [d]		2	196—198	60·3	6·2	15·3	$C_{25}H_{29}N_5O_6$	60·6	5·9	14·1	Powder		28

[a] Decomp. [b] D-Isomer. [c] Found: Cl, 7·2. Required Cl, 6·9%. [d] Not obtained pure; see N analysis.

TABLE 2

Free acids and disodium salts of (III) and (IV)

					Found (%)				Required (%)				Yield (%)
	R	n	Deriv-ative [a]	M.p.	C	H	N	Formula	C	H	N	Form [d]	from ester
(III)	H	0	S		43·4	4·2	15·5	$C_{19}H_{16}N_6Na_2O_5,4H_2O$	43·4	4·6	16·0	N	82
(III)	H	1	A	269—271° [c]	56·7	5·2	19·9	$C_{20}H_{20}N_6O_5$	56·6	4·8	19·8	P	91 [e]
(III)	H	1	S		39·8	5·7	14·6	$C_{20}H_{18}N_6O_5,7H_2O$	40·4	5·4	14·1	P	83
(III) [b]	H	1	S		40·9	5·2	13·8	$C_{20}H_{18}N_6Na_2O_5,7H_2O$	40·4	5·4	14·1	P	94
(III)	H	2	A	241—242 [c,k]	57·0	5·0	19·4	$C_{21}H_{22}N_6O_5$	57·5	5·1	19·2	N [f]	60 [g,h]
(III) [b]	H	2	A	240—242 [c]	57·1	5·1	18·8	$C_{21}H_{22}N_6O_5$	57·5	5·1	19·2	N [f]	59 [g]
(III)	Cl	0	S		41·9	3·8	15·4	$C_{19}H_{15}ClN_6Na_2O_5,3H_2O$	42·0	3·9	15·5	N	71
(III)	Cl	1	A	250—300 [i]	48·3	5·2	16·8	$C_{20}H_{19}ClN_6O_5,2H_2O$	48·5	4·7	17·0	P	85
(III)	Cl	2	A	220—222 [c,j]	52·3	5·1	17·4	$C_{21}H_{21}ClN_6O_5,0{\cdot}5H_2O$	52·4	4·6	17·4	P	93
(III)	Me	0	S		46·3	4·7	16·0	$C_{20}H_{18}N_6Na_2O_5,3H_2O$	46·0	4·6	16·1	P	87
(III)	Me	1	S		44·4	5·5	14·7	$C_{21}H_{20}N_6Na_2O_5,5H_2O$	44·1	5·3	14·7	P	84
(III)	Me	2	S		46·5	5·5	14·8	$C_{22}H_{22}N_6Na_2O_5,4H_2O$	46·5	5·3	14·8	P	89
(IV) [l]		1	A	>250 [i]	54·3	4·7	15·9	$C_{20}H_{19}N_5O_6,H_2O$	54·2	4·8	15·8	P	88
(IV) [l]		2	A	225—240 [c,j]	54·9	5·4	15·6	$C_{21}H_{21}N_5O_6,H_2O$	55·1	5·1	15·3	P	91

[a] A = free acid, S = disodium salt. [b] D-Isomer. [c] Decomp. [d] N = Needles, P = powder. [e] From disodium salt. [f] From 50% aqueous ethanol. [g] Yield from aldehyde. [h] Yield from nitrile was 9%. [i] Decomp. without melting. [j] Sinters above 190°. [k] First obtained as an amorphous powder, m.p. 255—258°. [l] Paper chromatography of these compounds (in 1% K_2HPO_4) showed agreement with earlier samples,[3] except that the latter contained small amounts of the corresponding 2,4-diamino-derivatives.

amino-5-chloroquinazoline-6-carbonitrile by the Sandmeyer reaction.

The corresponding 5-methyl-nitrile was prepared as follows. Nitration of 6-chloro-*o*-toluonitrile gave a mixture of mononitro-derivatives (3 : 1 by g.l.c.) which could not be separated conveniently. Since *o*-chlorobenzonitrile gives only the 5-nitro-derivative (VI) under similar conditions,[6] it was provisionally assumed that the main component of the mixture was (VII), the minor component probably being (VIII). Condensation of the mixture of isomers with guanidine carbonate gave two isomeric 2,4-diamino-5-methyl-*x*-nitroquinazolines in 52 and 26% yields, each of which was reduced to a triamine (IX; R = NH_2) was then converted into the nitrile (IX; R = CN).

major component of the mixture of (VII) and (VIII). The

In model experiments on the reductive coupling of the aldehydes and nitriles with amino-compounds, it was found, surprisingly, that the aldehyde (V; R = CHO) did not give a Schiff base with aniline, and only gave a low yield of Schiff base with ethyl *p*-aminobenzoate. However, hydrogenation of the aldehyde in acetic acid in the presence of a slight excess of aniline, over Raney nickel, gave the anilinomethyl compound (II; R = H) in 57% yield. A similar reaction, with the nitrile instead

[6] H. Ph. Baudet, *Rec. Trav. chim.*, 1924, **43**, 707.

of the aldehyde, gave the same product in slightly lower yield. Use of either method with ethyl *p*-aminobenzoate afforded the pteroic acid analogue (II; R = CO_2Et) in excellent yield.

Similar methods were used to prepare the esters of the required folic acid analogues (Table 1). In general, use of the aldehyde gave higher yields than did use of the nitrile, but not always sufficiently so as to compensate for the extra step involved. However, in the preparation of compound (III; R = H, $n = 2$) and its D-isomer, in which case the ester could not be crystallised, the aldehyde was used in order to obtain a purer product.

The esters were readily hydrolysed with a slight excess of aqueous ethanolic sodium hydroxide at room temperature; in some cases the disodium salts separated directly from the solution and in the rest the free acids were precipitated by neutralisation with dilute acid. Properties of the compounds are set out in Table 2, and typical u.v. spectra are given in Table 3.

The activities of the more important of these compounds as folic acid antagonists in bacterial systems and against mouse leukaemia have been reported elsewhere.[7]

EXPERIMENTAL

Except where otherwise stated, analytical samples were dried in high vacuum at 100°. Raney nickel catalyst was the T1 type.[8]

compound (34 g.; 89% from 2,4-diaminoquinazoline). A sample crystallised from acetic acid gave pale yellow needles of an acetate; when washed with ethanol and dried these gave the free base as an orange powder, m.p. ≮360°, $\lambda_{max.}$(EtOH) 225, 263, 269, and 369 mμ (ε 26,700, 11,000, 11,200, and 13,400) (Found: C, 46·7; H, 3·5; N, 33·8. $C_8H_7N_5O_2$ requires C, 46·8; H, 3·4; N, 34·1%).

(*b*) 2-Chloro-5-nitrobenzonitrile (1·83 g., 0·01 mole) and guanidine carbonate (1·80 g., 0·01 mole) were stirred under reflux in 2-ethyoxyethanol (30 ml.) for 3 hr. The nitro-compound (1·74 g.; 85%) was collected from the cooled mixture, and was identical with the product from (*a*) [i.r. spectra and reduction to the triamine (see later)].

(*c*) 5-Nitroanthranilonitrile (1·63 g., 0·01 mole) was added to a filtered solution prepared from guanidine hydrochloride (0·96 g., 0·01 mole) and sodium (0·25 g., 0·011 mole), each in absolute ethanol (10 ml.). The mixture was stirred under reflux for 5 hr. then cooled, and the nitro-compound (1·67 g.; 82%) was collected and identified as in (*b*).

2,4,6-*Triaminoquinazoline*.—(*a*) 2,4-Diamino-5-nitroquinazoline (14 g.) was added, with stirring and cooling below 30°, to tin(II) chloride dihydrate (50·6 g.) in concentrated hydrochloric acid (150 ml.). The mixture was stirred for a further 1 hr., then kept for 2 hr. at 0°. The stannichloride was collected, dissolved in hot water (100 ml.), and treated with 10N-sodium hydroxide (50 ml.). The hot solution was filtered rapidly (charcoal), and the filtrate was cooled to give the *triamine* (8·27 g.; 69%) as yellowish-brown prisms or felted needles, m.p. 255—258° (from water), $\lambda_{max.}$ (pH 6·8)

TABLE 3

U.v. absorption maxima (mμ) (10^{-3} ε in parentheses)

	R	*n*	Diester in EtOH	Disodium salt or free acid in 0·1N-HCl	Disodium salt or free acid in 0·1N-NaOH
(III)	H	1	203 (35·2), 233 (55·3), 283 (30·5), 301 (32·3), 340sh (5·6)	232 (44·3), 303 (9·2)	232 (45·2), 281 (22·8), 292 (21·3) 340sh (4·3)
(III)	Cl	1	204 (39·0), 240 (39·8), 290 (31·4), 350 (5·3)	204 (23·9), 233sh (31·0), 241 (33·1), 278 (13·8), 298 (13·7)	238 (39·6), 287 (29·1), 345sh (5·1)
(III)	Me	1	203 (34·3), 237 (41·8), 290 (28·2), 301 (29·0), 347sh (4·6)	230sh (34·9), 241 (38·6), 265sh (13·6), 304 (12·1)	235 (42·2), 285 (27·6), 294sh (25·8), 344sh (4·2)
(III)	Me	2	205 (33·8), 236 (38·0), 296 (27·5), 343sh (4·5)	207sh (22·5), 230sh (34·7), 242 (38·3), 266sh (13·2), 303 (11·8)	235 (41·7), 286 (27·4), 296sh (25·6), 350sh (4·1)
(IV)		1	227 (42·3), 275sh (20·1), 303 (26·3)	225 (44·5), 230 (43·2), 258 (12·8), 299 (9·3)	227 (45·7), 278 (22·6), 292sh (20·6)

2,4-*Diaminoquinazoline*.[9]—Anthranilonitrile (47·2 g., 0·4 mole), cyanoguanidine (33·6 g., 0·4 mole), and 2N-hydrochloric acid (200 ml.) were boiled under reflux for 2 hr. Water (1·5 l. at 95°) and 2N-sodium hydroxide (250 ml.) were added, and the solution was treated with charcoal and cooled, giving the product (48 g., 75%), m.p. 248—252° (lit., [10] 248—250°)

2,4-*Diamino*-6-*nitroquinazoline*.—(*a*) A solution of 2,4-diaminoquinazoline (30 g.) in hot water (900 ml.) was treated with concentrated nitric acid (60 ml.). The nitrate (37·2 g.) was collected from the cooled mixture, dried, and added in portions, below 10°, to a cooled, stirred mixture of fuming nitric acid (*d* 1·5; 186 ml.) and concentrated sulphuric acid (186 ml.). After 15 min. stirring with cooling, and a further 1 hr. at room temperature, the clear solution was added to crushed ice (2 Kg.) and the mixture was basified below 20° with concentrated aqueous ammonia to give the *nitro-*

[7] D. J. Hutchison, *Cancer Chemother. Rep.*, 1968, **52**, 697.
[8] X. A. Domiguez, I. C. Lopez, and R. Franco, *J. Org. Chem.* 1961, **26**, 1625.

[9] *Chem. Abs.*, 1944, **38**, 3993 (from G.P. 737,931) gives no details, but may refer to a similar preparation.
[10] A. Kötz, *J. prakt. Chem.*, 1893, [2]**47**, 303.

207, 241, 269sh, and 362 mμ (ε 10,000, 40,500, 10,900, and 4100) (Found: C, 55·3; H, 5·4; N, 39·9. $C_8H_9N_5$ requires C, 54·8; H, 5·2; N, 40·0%).

(*b*) Hydrogenation of the nitro-compound over palladised charcoal, either at room temperature and pressure in dimethylformamide (25 ml. per g.) or at 100°/50 atmos. in dimethylformamide (5 ml. per g.) gave the triamine (64%) after crystallisation from water. The product was darker than that from (*a*), but satisfactory for further use.

2,4-*Diaminoquinazoline*-6-*carbonitrile*.— 2,4,6-Triaminoquinazoline (42 g., 0·24 mole) was dissolved in 2N-hydrochloric acid (500 ml.) and diazotised at once (before crystallisation of the hydrochloride) with sodium nitrite (17·3 g.) in water (120 ml.) at 10—15°. The clear solution was added with stirring to a warm cuprocyanide solution, prepared by adding copper(II) sulphate pentahydrate (56·4 g.) in water (180 ml.) to potassium cyanide (67·2 g.) in water (120 ml.).

The mixture was then stirred for 30 min. at 50—55°, cooled to 40°, and treated with concentrated aqueous ammonia (240 ml.). After 1 hr. stirring at room temperature, the solid was collected and extracted with boiling 15% acetic acid (650 ml.). The hot filtrate was diluted with 2-ethoxyethanol (550 ml.) and basified with concentrated aqueous ammonia (200 ml.), giving the *nitrile* (29 g., 65%) as small brown needles, m.p. ⩽360° (Found: C, 57·9; H, 3·9; N, 37·5. $C_9H_7N_5$ requires C, 58·4; H, 3·8; N, 37·8%).

2,4-*Diaminoquinazoline*-6-*carbaldehyde*.—A suspension of the nitrile (V; R = CHO) (7·40 g., 0·04 mole) in 50% acetic acid (400 ml.) was treated with phenylhydrazine (4·72 ml., 0·048 mole) and hydrogenated over Raney nickel until 0·048 mol. of hydrogen had been absorbed; further hydrogenation reduced the yield. The mixture was filtered hot and cooled, to give the *aldehyde phenylhydrazone acetate* (10·53 g., 78%) as yellow needles, m.p. 232—234° (from acetic acid) (Found: C, 60·5; H, 5·6; N, 24·9. $C_{15}H_{14}N_6,C_2H_4O_2$ requires C, 60·3; H, 5·4; N, 24·8%). A mixture of this material (7·50 g.) and *p*-nitrobenzaldehyde (3·99 g.) in 50% acetic acid (220 ml.) was heated under reflux for 2 hr., cooled, and filtered. The filtrate was treated with charcoal for 2 hr., then evaporated, and the residue was stirred with aqueous sodium carbonate, giving the *aldehyde* (3·10 g., 74%), which yielded white needles, m.p. ⩽360° (from aqueous ethanol) (Found: C, 57·8; H, 4·2; N, 29·5. $C_9H_8N_4O$ requires C, 57·4; H, 4·3; N, 29·8%).

2-*Amino*-4-*hydroxyquinazoline*-6-*carbonitrile*.— 2,4-Diaminoquinazoline-6-carbonitrile (5·55 g.) and N-hydrochloric acid (75 ml.) were boiled under reflux for 6 hr.; the mixture was filtered (charcoal) and the filtrate basified with aqueous ammonia, to give the *amino-hydroxynitrile* (5·34 g., 96%), which formed yellow blades (from aqueous 2-ethoxyethanol), m.p. 360° (decomp.) (Found: C, 57·8; H, 3·5; N, 30·2. $C_9H_6N_4O$ requires C, 58·1; H, 3·3; N, 30·1%).

2-*Amino*-4-*hydroxyquinazoline*-6-*carbaldehyde*.— 2,4-Diaminoquinazoline-6-carbaldehyde (1·14 g.), hydrochloric acid (2N exactly; 30 ml.), and ethanol (10 ml.) were boiled under reflux for 2 hr. Sodium hydroxide (2N exactly; 27 ml.) was added, and the *amino-hydroxyaldehyde* (1·11 g., 96%) was collected after cooling. It separated from 50% aqueous dimethylformamide as fine needles, which darkened above 250° but did not melt below 360° (Found: C, 56·9; H, 3·9; N, 22·1. $C_9H_7N_3O_2$ requires C, 57·1; H, 3·7; N, 22·2%).

2,4-*Diamino*-5-*chloroquinazoline*.— 6-Chloroanthranilonitrile [11] (36·8 g., 0·24 mole) and chloroformamidine hydrochloride [12] (34·5 g., 0·30 mole) in bis-2-methoxyethyl ether (240 ml.) were stirred under reflux for 2·5 hr. at 145—150° (bath). The hydrochloride was collected after cooling and additon of ether (700 ml.), and dissolved in boiling water (2·5 l.) containing excess of ammonia. The solution was treated with charcoal and cooled to give the *quinazoline* (22·5 g., 48%) as needles, m.p. 183—185° (from water) (Found: C, 48·9; H, 3·7; N, 28·8. $C_8H_7ClN_4$ requires C, 49·4; H, 3·6; N, 28·8%).

2,4-*Diamino*-5-*chloro*-6-*nitroquinazoline*.—The 5-chlorodiamine (50 g.) was added in portions, below 20°, to a cooled, stirred mixture of fuming nitric acid (*d* 1·5; 270 ml.) and concentrated sulphuric acid (270 ml.). After 18 hr. at room temperature, the solution was added to crushed ice (3 kg.) and the mixture was basified with concentrated aqueous ammonia to give the *nitro-compound* (56·6 g.; 92%), which gave yellow prisms (from aqueous methanol), decomp. 225—227° (Found: C, 40·1; H, 2·5; N, 29·0. $C_8H_6ClN_5O_2$ requires C, 40·1; H, 2·5; N, 29·2%).

2,4,6-*Triamino*-5-*chloroquinazoline*.—The 6-nitro-compound (22·1 g.) was added, with stirring and cooling below 30°, to tin(II) chloride dihydrate (65 g.) in concentrated hydrochloric acid (350 ml.) and acetic acid (92 ml.). The mixture was stirred for 18 hr. and the solid was collected and suspended in ice–water (500 ml.). Basification with 10N-sodium hydroxide and crystallisation of the resulting solid from water gave the *triamine* (13·5 g., 64%) as fawn needles, m.p. 200—203° [Found (material dried at room temperature): C, 42·7; H, 4·8; N, 30·7. $C_8H_8ClN_5,H_2O$ requires C, 42·2; H, 4·4; N, 30·8%].

This material, on hydrogenation over palladised charcoal in aqueous ethanol containing a slight excess of sodium hydroxide, gave 2,4,6-triaminoquinazoline (64%), identical with authentic material.

2,4-*Diamino*-5-*chloroquinazoline*-6-*carbonitrile*.—2,4,6-Triamino-5-chloroquinazoline (13·65 g., 0·06 mole) was treated with 2N-hydrochloric acid (125 ml.), and the resulting crystalline paste of hydrochloride was diazotised and converted into the nitrile as described for 2,4-diaminoquinazoline-6-carbonitrile. The 5-*chloro-nitrile* (9·02 g., 68%) formed a brown powder; crystallisation from acetic acid (charcoal) gave the acetate as brown laths, which when dried reverted to the free base, m.p. 287° (decomp.) (Found: C, 47·2; H, 2·8; N, 30·3. $C_9H_6ClN_5,0·5H_2O$ requires C, 47·3; H, 3·1; N, 30·6%).

6-*Chloro*-3-*nitro*-o-*toluonitrile*.— 6-Chloro-*o*-toluonitrile [13] (33 g.) was added, with stirring and cooling below 0°, to fuming nitric acid (*d* 1·5; 165 ml.). After 24 hr. the solution was added to ice–water and the product (42·8 g., 100%) (skin-irritant) gave needles (35·8 g.), m.p. 75–80° (from aqueous ethanol), unchanged by fractional distillation (Found: C, 48·9; H, 2·6; N, 14·2. $C_8H_5ClN_2O_2$ requires C, 48·9; H, 2·6; N, 14·2%). G.l.c. showed this to consist of two components, 6-*chloro*-3-*nitro*-o-*toluonitrile* (see later) (75%) and an isomer (25%), probably 6-chloro-5-nitro-*o*-toluonitrile.

2,4-*Diamino*-5-*methyl*-6-(*and* 8-?)*nitroquinazolines*.—The foregoing mixture of isomers (10 g.) and guanidine carbonate (18·3 g.) in 2-ethoxyethanol (500 ml.) were boiled under reflux for 3 hr., then evaporated; the residue was triturared with water (100 ml.) and the product was collected. A solution of this material in 80% acetic acid (100 ml.) was treated with charcoal and partly neutralised with 6N-ammonia (40 ml.) to give 2,4-*diamino*-5-*methyl*-6-*nitroquinazoline acetate* (7·32 g., 52%) as brown cubes, m.p. 288° (decomp.) after loss of acetic acid above 220° [Found (material dried at room temperature): C, 47·5; H, 5·3; N, 25·6. $C_9H_9N_5O_2,C_2H_4O_2$ requires C, 47·3; H, 4·7; N, 25·1%]. Addition of 2N-sodium hydroxide to a solution of the acetate in dimethylformamide gave the *free base* as yellow needles (from dimethylformamide), m.p. 293° (decomp.) (Found: C, 49·4; H, 4·0; N, 31·6. $C_9H_9N_5O_2$ requires C, 49·3; H, 4·1; N, 32·0%).

The filtrate from the acetate was made strongly basic with ammonia to give 2,4-*diamino*-5-*methyl*-8(?)-*nitroquinazoline* (2·91 g., 26%), which yielded tan needles, m.p. 255° (decomp.), after two recrystallisations from ethanol (Found:

[11] H. Koopman, *Rec. Trav. chim.*, 1961, **80**, 1075.
[12] A. Hantzch and A. Vagt, *Annalen*, 1900, **314**, 339; *cf.* T. B. Johnson and J. M. Sprague, *J. Amer. Chem. Soc.*, 1939, **61**, 176.
[13] M. Haring, *Helv. Chim. Acta*, 1960, **43**, 104.

C, 49·4; H, 4·3; N, 31·5. $C_9H_9N_5O_2$ requires C, 49·3; H, 4·1; N, 32·0%).

2,4,6-*Triamino-5-methylquinazoline*.— 2,4-Diamino-5-methyl-6-nitroquinazoline acetate (9 g.) in ethanol (500 ml.) was hydrogenated at 45° over palladised charcoal. The *triamine* (5·65 g., 95%) separated on evaporation to 50 ml., and gave yellow-brown needles (from water) which partly melted at 60—70°, then resolidified, and melted finally at 220—224°, $\lambda_{max.}$ (pH 6·8) 244 and 365 mμ (ε 35,400 and 4100) (Found: C, 56·9; H, 5·9; N, 36·8. $C_9H_{11}N_5$ requires C, 57·1; H, 5·9; N, 37·0%).

2,4,8(?)-*Triamino-5-methylquinazoline*.—Similarly prepared from 2,4-diamino-5-methyl-8(?)-nitroquinazoline, the *triamine* separated from water as light-brown feathery crystals, m.p. 215—217°, $\lambda_{max.}$ (pH 6·8) 213sh, 226, 258, and 352 mμ (ε 21,200, 24,400, 21,800 and 3800) (Found: C, 56·8; H, 5·9; N, 36·8. $C_9H_{11}N_5$ requires C, 57·1; H, 5·9; N, 37·0%).

2,4-*Diamino-5-methylquinazoline-6-carbonitrile*.— 2,4,6-Triamino-5-methylquinazoline (3·78 g.) was converted by the method used for 2,4-diaminoquinazoline-6-carbonitrile into the 5-*methyl-nitrile* (1·90 g., 46%), which formed pale-yellow laths (from ethanol), decomp. between 260 and 310° (Found: C, 58·5; H, 4·6; N, 34·0. $C_{10}H_9N_5,0·33H_2O$ requires C, 58·5; H, 4·7; N, 34·1%).

2,4-*Diamino-6-anilinomethylquinazoline*.—(*a*) A solution of 2,4-diaminoquinazoline-6-carbaldehyde (1·88 g., 0·01 mole) in acetic acid (60 ml.) was cooled and treated with aniline (1·11 g., 0·012 mole). On hydrogenation with Raney nickel, 1·01 mol. of hydrogen was absorbed. The product was recrystallised from water and then from 30% ethanol containing ammonia to give the *anilinomethyl compound* (1·50 g., 57%) as leaflets, m.p. 190—197° (slow heating), $\lambda_{max.}$ (EtOH) 234, 270, 279, and 341 mμ (ε 51,600, 14,100, 14,100, and 4400) (Found: C, 67·6; H, 5·8; N, 26·2. $C_{15}H_{15}N_5$ requires C, 67·9; H, 5·7; N, 26·4%).

(*b*) In a similar condensation with 2,4-diaminoquinazoline-6-carbonitrile, and 50% acetic acid as solvent, 1·81 mol. of hydrogen was absorbed, and the same anilinomethyl compound (52%) was obtained.

Ethyl p-(2,4-*Diaminoquinazolin-6-ylmethyleneamino*)-*benzoate*.—2,4-Diaminoquinazoline-6-carbaldehyde (0·94 g., 5 mmoles) and ethyl *p*-aminobenzoate (0·83 g., 5 mmoles) in acetic acid (10 ml.) were boiled under reflux for 2 hr. Ether (35 ml.) was added slowly to the cooled solution, and the precipitated acetate was stirred for 1 hr. with 2N-sodium carbonate (30 ml.). Crystallisation of the resulting solid from ethanol gave the *Schiff base* (0·42 g., 25%) as yellow laths, m.p. $<360°$, $\lambda_{max.}$ (EtOH) 233, 262, and 350 mμ (ε 29,300, 22,700, and 32,200) (Found: C, 64·5; H, 5·1; N, 20·9. $C_{18}H_{17}N_5O_2$ requires C, 64·5; H, 5·1; N, 20·9%).

On hydrogenation in acetic acid over Raney nickel this afforded the reduced product (59%), identical with the material obtained in the following experiments.

Ethyl p-(2,4-*Diaminoquinazolin-6-ylmethylamino*)-*benzoate*.—(*a*) The diamino-aldehyde and ethyl *p*-aminobenzoate were reductively condensed as described for the anilinomethyl compound. A solution of the material obtained in hot ethanol was basified with ammonia, to give the *product* (83%) as colourless rectangular plates, m.p. 224—225° (from ethanol), $\lambda_{max.}$ (EtOH) 202, 234, 284sh, 307, and 339sh mμ (ε 26,000, 49,200, 21,000, 31,300, and 4900) (Found: C, 62·5; H, 6·1; N, 20·6. $C_{18}H_{19}N_5O_2,0·5H_2O$ requires C, 62·4; H, 5·8; N, 20·2%) [lit.,[1] m.p. 162—164° for material with analytical figures (C, H only) corresponding to anhydrous compound; a sample of this supplied by Professor Rydon melted at 209—212° on slow heating, and at 162—164° when inserted at 150°. I.r. and u.v. spectra were in close agreement with those of our material, and the reason for the difference in m.p. behaviour is not clear].

(*b*) Use of the nitrile, as described for an *anilino*methyl compound, instead of the aldehyde, gave the same material in 45% yield.

Diethyl Esters of N-(p-*Aminobenzoyl*)*amino-acids*.—Diethyl (*p*-aminobenzamido)malonate was prepared by the reported method.[14] The preparation of diethyl N-(*p*-aminobenzoyl)-L-aspartate is given below; its D-isomer was similarly prepared. Diethyl N-(*p*-aminobenzoyl)-D-glutamate was prepared from diethyl D-glutamate as described for its L-isomer.[15]

Diethyl N-(p-*Nitrobenzoyl*)-L-*aspartate*.—To a solution of diethyl L-aspartate hydrochloride (13·53 g., 0·06 mole) and *p*-nitrobenzoyl chloride (12·50 g., 0·066 mole) in dry 1,2-dichloroethane (120 ml.) was added triethylamine (12·1 g., 0·12 mole) in four portions, with shaking and cooling in ice–water. After 18 hr. chloroform (100 ml.) was added and the solution was washed with water and aqueous sodium hydrogen carbonate, dried, and evaporated to give the crystalline *nitro-compound* (100%), which was used directly for the next stage. A sample gave needles (from aqueous ethanol), m.p. 87—89° (Found: C, 53·8; H, 5·3; N, 8·5. $C_{15}H_{18}N_2O_7$ requires C, 53·5; H, 5·4; N, 8·3%).

Diethyl N-(p-*Aminobenzoyl*)-L-*asparate*.—Hydrogenation of the foregoing nitro-compound in ethanol, over palladised charcoal, gave the *amino-compound* (73%), as needles (from ethanol), m.p. 116—117° (Found: C, 58·6; H, 6·6; N, 8·8. $C_{15}H_{20}N_2O_5$ requires C, 58·4; H, 6·5; N, 9·1%).

Diethyl Esters of Folic Acid Analogues.—The quinazoline aldehyde or nitrile (0·01 mole) was dissolved by heating in acetic acid (70 ml.) (for 2,4-diaminoquinazoline-6-carbaldehyde) or in 70% acetic acid (50—100 ml.) (for the other compounds). To the cooled solution was added the appropriate *p*-(aminobenzoyl)amino-acid diethyl ester (0·012 mole), and the mixture was hydrogenated over Raney nickel at room temperature and pressure until uptake ceased (2—6 hr.; uptake 0·9—1·0 mol. for aldehydes and 1·4—1·9 mol. for nitriles). The product was triturated with 2N-sodium carbonate and set aside overnight. If solidification did not occur, the supernatant liquid was decanted and a solution of the crude material in a little ethanol added to excess of 2N-sodium carbonate; this method was also used when the crude material retained acetic acid tenaciously, and as a result failed to crystallise satisfactorily. The diethyl esters of (II; R = H, $n = 2$) could not be crystallised and were hydrolysed directly; the remaining compounds were washed with water and recrystallised from ethanol. Properties are given in Tables 1 and 3.

Folic Acid Analogues.—The diesters of (II; R = H, $n = 0$ or 1; R = Cl, $n = 0$; R = Me, $n = 0$, 1, or 2) were dissolved in hot ethanol (20—120 ml./g.), cooled rapidly to *ca.* 35°, and treated with 2N-sodium hydroxide (2·2 equiv.). The disodium salts were collected after 18 hr. and washed with ethanol. In one case (R = Cl, $n = 0$) hydrolysis was incomplete, and the separated solid (0·82 g.) was dissolved in water (2 ml.), treated with 2N-sodium hydroxide (1 ml.),

[14] W. B. Wright, jun., D. B. Cosulich, M. J. Fahrenbach, C. W. Waller, J. M. Smith, jun., and M. E. Hultquist, *J. Amer. Chem. Soc.*, 1949, **71**, 3014.

[15] F. E. King, R. M. Acheson, and P. C. Spensley, *J. Chem. Soc.*, 1949, 1401.

and kept for 18 hr.; the separated solid was collected after the addition of ethanol. Similar hydrolysis of the diester of (II; R = Cl, $n = 2$) gave a gummy sodium salt; this was dissolved by addition of water and the free acid was precipitated with the calculated amount of N-hydrochloric acid.

The crude diester of (II; R = H, $n = 2$), and its D-isomer, were dissolved in ethanol (25 ml. for a 0·01M) and treated with 2N-sodium hydroxide (18 ml.). After 18 hr., the pH was checked to be greater than 11, and an equivalent amount of N-hydrochloric acid was added.

The diester of (II; R = Cl, $n = 1$) was stirred with 50% ethanol (20 ml./mmole) containing 2N-sodium hydroxide (3 equiv.) until it dissolved (*ca.* 24 hr.), then acidified with the calculated amount of N-hydrochloric acid; compounds (III; $n = 1$ or 2) were similarly prepared, using 3·3 equiv. of sodium hydroxide. Properties are given in Tables 2 and 3.

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