Triazines and Related Products. Part IX.[1] Potential Irreversible Dihydrofolate Reductase Inhibitors: 2,4-Diamino-*s*-triazines with a Masked Covalent Labelling Group

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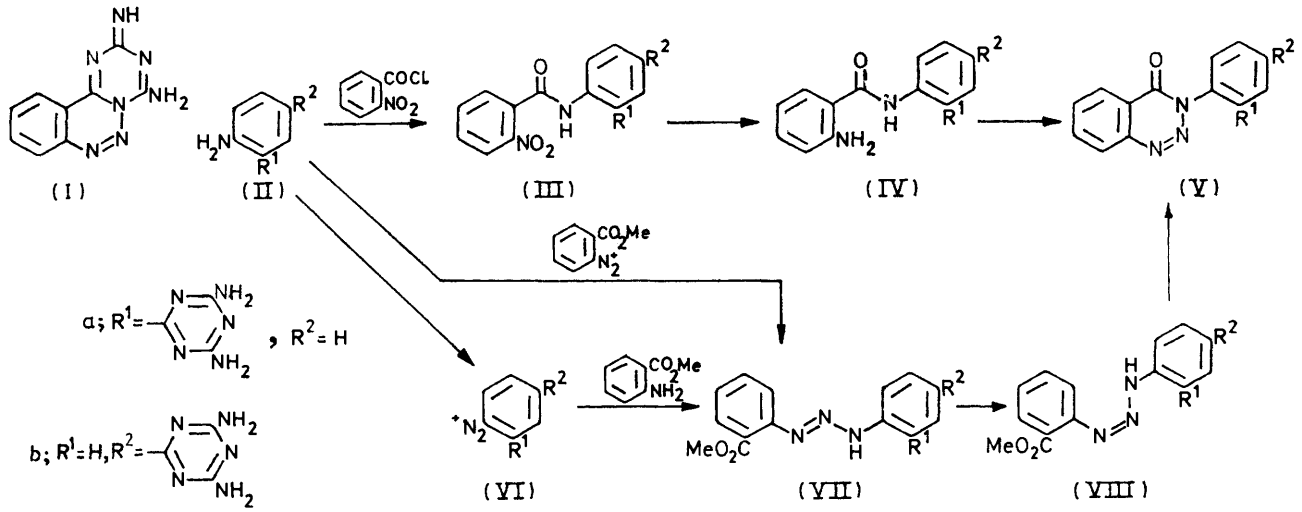
A series of 1,2,3-benzotriazin-4(3*H*)-ones carrying 3-(4,6-diamino-*s*-triazin-2-ylphenyl) substituents has been prepared by diazotisation of appropriately substituted *o*-aminobenzamides, or by cyclisation of *o*-methoxycarbonylphenyltriazenes in ethanol containing 2% piperidine. Cyclisation of 1-*p*-(4,6-diamino-*s*-triazin-2-yl)phenyl-3-*o*-cyanophenyltriazene in ethanol–piperidine affords 3-*p*-(4,6-diamino-*s*-triazin-2-yl)phenyl-3,4-dihydro-4-imino-1,2,3-benzotriazine, which in 2N-hydrochloric acid rearranges to the isomeric substituted 4-anilino-1,2,3-benzotriazine. 4-*p*-Cyanoanilino-1,2,3-benzotriazine decomposes in alcohols containing 1% potassium hydroxide to give ethers; the scope of this reaction has been investigated.

We have previously [2] described the chemical properties of 4-amino-2-imino-2*H*-*s*-triazino[1,2-*c*][1,2,3]benzotriazine (I). This compound was designed to complex reversibly to both the ionic or H-bonding locus and the hydrophobic region at the active site of the enzyme dihydrofolate reductase,[3] where irreversible inhibition of the enzyme could occur by covalent reaction with a suitably situated reactive site on the enzyme surface. Although we established that the 1,2,3-triazine ring (masked covalent labelling group) of compound (I) readily underwent heterolytic or homolytic ring cleavage with the formation of diazonium and carbonium ions or radical reactive intermediates depending on the nature of the reacting substrate,[2] this reactivity was not reflected in anti-tumour activity (against lymphoid leukaemia L-1210).

We now describe the synthesis of related compounds which are also potential irreversible dihydrofolate reductase inhibitors, having the 1,2,3-triazine nucleus several atoms distant from the groups principally responsible for inhibitor–enzyme association.

The benzotriazinones (Va and b) were readily prepared by the routes outlined in Scheme 1. *o*-Nitrobenzoyl chloride aroylated only the arylamino-groups of the *s*-triazine derivatives (IIa and b) to afford the *o*-nitrobenzamides (IIIa and b), which were efficiently reduced to the *o*-aminobenzamides (IVa and b). Diazotisation of the amines yielded the triazinones (Va and b) in high yields, thus providing additional evidence of the utility of this general synthetic route to 1,2,3-triazines.[1,4] The same triazinones were also prepared by cyclisation of triazenes (VIIa and b). These triazenes resulted either from coupling diazotised methyl anthranilate with amines (IIa and b) in sodium acetate buffered solution, or from coupling the diazonium salts (VIa and b) with methyl anthranilate in 0·5N-hydrochloric acid. The acidic conditions suppress the competing intramolecular cyclisation of diazotised 2,4-diamino-6-(2-aminophenyl)-*s*-triazine (VIa) to the tricyclic triazine (I) which occurs readily in a basic medium.[2] Diaryltriazenes are known to exist in the crystalline state as the *trans* geometrical

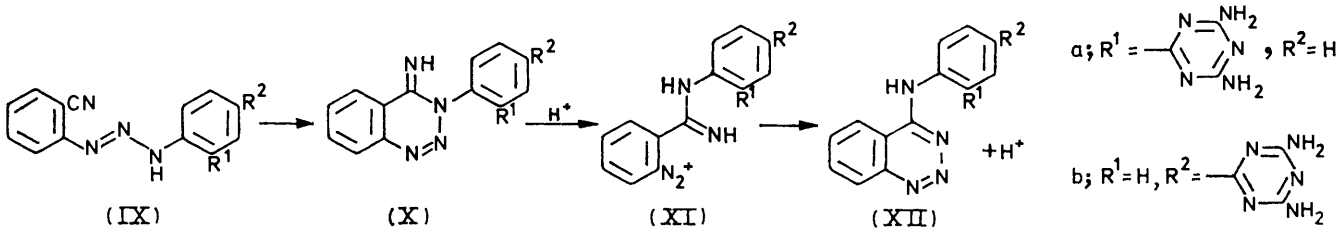


SCHEME 1

[1] Part VIII, A. C. Mair and M. F. G. Stevens, *J. Chem. Soc.* (*C*), 1971, 2317.
[2] S. M. Mackenzie and M. F. G. Stevens, *J. Chem. Soc.* (*C*), 1970, 2298.
[3] B. R. Baker, 'Design of Active-Site-Directed Irreversible Enzyme Inhibitors,' Wiley, New York, 1967.
[4] H. N. E. Stevens and M. F. G. Stevens, *J. Chem. Soc.* (*C*), 1970, 765.

isomers,[5] and cyclisation of the (presumed) *trans*-triazenes (VIIa and b) to the triazinones (Va and b) must proceed *via* the intermediate *cis*-triazines (VIIIa and b): this rearrangement and cyclisation was effected in boiling ethanol containing 2% piperidine, but not in boiling 70% aqueous ethanol (*cf.* cyclisation of related triazenes).[4,6]

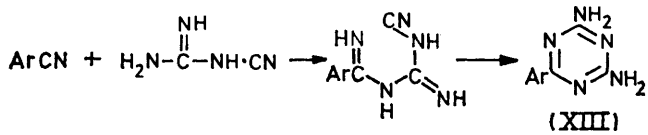
Cyclisation of *o*-cyanophenyltriazenes generally closely parallels that of the *o*-methoxyphenyl analogues.[4,7] However, the cyanotriazene (IXa) was recovered unchanged from boiling 70% aqueous ethanol, and from boiling ethanol or 2-ethoxyethanol containing 2% piperidine. The lack of reactivity of the cyanotriazene



SCHEME 2

in 2N-hydrochloric acid also contrasts with the cyclisation that occurs when 1-*o*-cyanophenyl-3-phenyltriazene (IX; $R^1 = R^2 = H$) is dissolved in acid.[8] The *p*-substituted triazene (IXb), however, cyclised smoothly in 95% ethanol containing 2% piperidine in a reaction that was followed spectroscopically by observing the disappearance of the u.v. absorption at 374 nm. Assignment of structure to the products of cyclisation of *o*-cyanophenyltriazenes is complicated because the 4-imino-1,2,3-benzotriazines initially formed [*e.g.* (X)] can undergo Dimroth-type rearrangement to the isomeric series [*e.g.* (XII)] *via* an intermediate diazonium species [*e.g.* (XI)]. This rearrangement proceeds spontaneously when the 4-iminobenzotriazines bear electron-attracting substituents on the 3-aryl group [*e.g.* (X; R^1 or R^2 = CN or NO_2)]; in other cases rearrangement can be accelerated with 2N-hydrochloric acid.[4] The product from the ethanol–piperidine cyclisation of the cyanotriazene (IXb) was assigned structure (Xb) because its electronic absorption spectrum was similar to spectra of related 3-substituted 4-imino-1,2,3-benzotriazines,[4] and in 2N-hydrochloric acid it quantitatively rearranged to the isomer (XIIb). The electronic absorption characteristics of this isomer (XIIb) were similar to those of 4-*p*-cyanoanilino-1,2,3-benzotriazine (XIV),[4] and the i.r. spectrum showed a strong (unassigned) absorption at 1150 cm^{-1} (*cf.* related substituted 4-anilino-1,2,3-benzotriazines, which absorb at 1145 ± 10 cm^{-1}).[4]

A further approach to the synthesis of compounds required for this work suggested itself following our success in synthesising 2,4-diamino-6-aryl-*s*-triazines (XIII) by interaction of aryl nitriles and cyanoguanidine in the presence of base (Scheme 3).[2] However, when 4-*p*-cyanoanilino-1,2,3-benzotriazine (XIV) and cyanoguanidine were boiled in 2-methoxyethanol containing 1% potassium hydroxide, rapid evolution of nitrogen was observed and the colourless product (50%) did not give the characteristic red colour in the Bamberger–Goldberger test for 1,2,3-benzotriazines.[9] An improved yield (95%) of the same compound was obtained when the cyanoguanidine was omitted. Spectroscopic examination of this compound served to establish its structure as (XVa); the i.r. spectrum confirmed the presence of aliphatic C–H, cyano-, and amino-groups; and the n.m.r. spectrum ($CDCl_3$) showed a methyl resonance as a singlet at τ 6·63, and [after exchange of the NH_2 protons (broad singlet at τ 5·60) with D_2O] a pair of triplets at τ 6·27 and 5·52 assigned to the



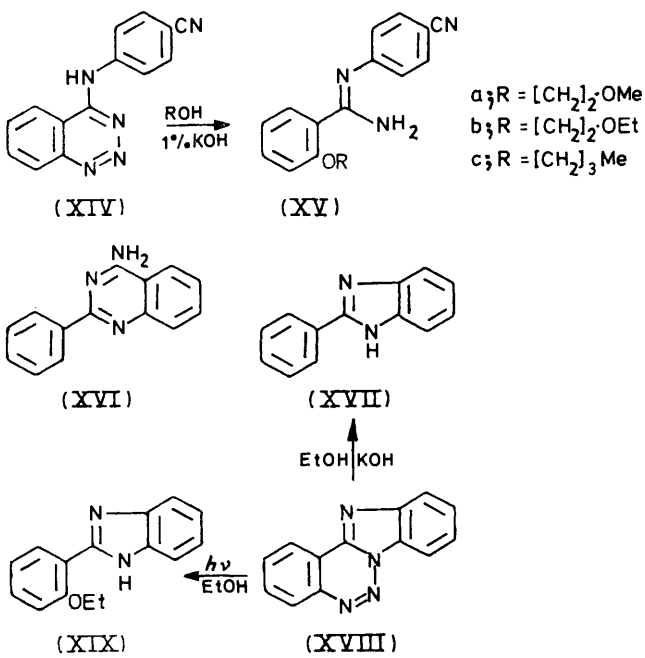
SCHEME 3

methylene protons. Triazine (XIV) decomposed similarly in 2-ethoxyethanol containing 1% potassium hydroxide to afford the related ether (XVb) in 85% yield, but the yield of the butoxy-analogue (XVc) from boiling n-butanol was less satisfactory (60%). The scope of the reaction proved less extensive than anticipated; the triazine (XIV) was recovered unchanged after treatment with 1% potassium hydroxide in refluxing methanol, ethanol, propan-2-ol, or butan-2-ol, possibly because of the lower boiling points of these solvents (65, 78, 83, and 107°, respectively) compared to those of 2-methoxy- (124°) and 2-ethoxy-ethanol (134°), and n-butanol (118°).

Our interest in the chemistry of triazenes and triazines stemmed originally from the observation that 1,3-di-*o*-cyanophenyltriazene (IX; R^1 = CN, R^2 = H) decomposes in ethanol to 4-amino-2-phenylquinazoline (XVI),[8] and recent work [4,10,11] has established that 4-*o*-cyanoanilino-1,2,3-benzotriazine (XII; R^1 = CN, R^2 = H) is

[5] Yu. D. Kondrashev, *Kristallografiya*, 1961, **6**, 515.
[6] H. Mehner, *J. prakt. Chem.*, 1901, [2], **63**, 241.
[7] H. N. E. Stevens and M. F. G. Stevens, *J. Chem. Soc.* (*C*), 1970, 2308.
[8] M. W. Partridge and M. F. G. Stevens, *J. Chem. Soc.*, 1964, 3663.
[9] M. S. Gibson, *J. Chem. Soc.*, 1963, 3539.
[10] M. F. G. Stevens, *J. Chem. Soc.* (*C*), 1967, 1096.
[11] H. N. E. Stevens and M. F. G. Stevens, *J. Chem. Soc.* (*C*), 1970, 2289.

an intermediate in this reaction sequence. Attempts to intercept this intermediate in boiling 2-methoxyethanol–potassium hydroxide were unsuccessful: none of the expected ether, 4-amino-2-[2-(2-methoxy)ethoxyphenyl]-



quinazoline was formed, and only a 17% yield of the corresponding arene (XVI) was isolated. 3-*p*-Cyanophenyl-1,2,3-benzotriazin-4(3*H*)-one (V; $R^1 = H$, $R^2 = CN$), prepared by cyclisation of 1-*p*-cyanophenyl-3-*o*-methoxycarbonylphenyltriazene (VII; $R^1 = H$, $R^2 = CN$) in 70% aqueous ethanol also partially decomposed in boiling ethanolic potassium hydroxide to afford a low yield of the arene, N-*p*-cyanophenylbenzamide.

In their sensitivity to alcohols therefore, 1,2,3-benzotriazines show reactivity characteristic of diazonium compounds,[12] and this behaviour is particularly marked when the 1,2,3-benzotriazine system is fused to another heterocyclic system. Both arene formation and ether formation have been observed in the decomposition of the benzimidazobenzotriazine (XVIII); in ethanolic potassium hydroxide the product is 2-phenylbenzimidazole (XVII), whereas photolysis in ethanol gives, in addition to the arene (XVII), the ether (XIX) [13] in low yield. Even more reactive is the *s*-triazinobenzotriazine (I), which undergoes reductive elimination of nitrogen in the presence of cold ethanol to afford 2,4-diamino-6-phenyl-*s*-triazine.[2]

EXPERIMENTAL

N-o-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-2-*nitrobenzamide* (IIIa).—2,4-Diamino-6-(2-aminophenyl)-*s*-triazine [14] (2·02 g) in pyridine (3 ml) was added dropwise to *o*-nitrobenzoyl chloride (1·86 g) in benzene. The mixture was refluxed for 5 min and poured on ice. The *nitro-amide* (80%) was collected and crystallised from aqueous acetic acid as cream needles, m.p. 279—280° (Found: C, 54·6; H, 3·7; N, 27·7. $C_{16}H_{13}N_7O_3$ requires C, 54·7; H, 3·7; N, 27·9%).

N-p-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-2-*nitrobenzamide* (IIIb).—Prepared (70%) from *o*-nitrobenzoyl chloride and 2,4-diamino-6-(4-aminophenyl)-*s*-triazine,[14] this *amide* crystallised from ethanol as cream needles, m.p. 285—290° (Found: C, 54·3; H, 3·8; N, 27·8%).

2-*Amino*-N-o-(4,6-*diamino*-s-*triazin*-2-*yl*)*phenylbenzamide* (IVa).—Catalytic hydrogenation of the nitro-amide (IIIa) in ethanol over Adams catalyst at 25° and atmospheric pressure afforded the *amino-amide* (75%), as cream needles, m.p. 240—243° (from aqueous ethanol) (Found: C, 59·4; H, 4·7; N, 30·5. $C_{16}H_{15}N_7O$ requires C, 59·8; H, 4·7; N, 30·5%).

2-*Amino*-N-p-(4,6-*diamino*-s-*triazin*-2-*yl*)*phenylbenzamide* (IVb).—The precipitate formed when the nitro-amide (IIIb) (0·9 g), tin(II) chloride dihydrate (1·7 g), and 10N-hydrochloric acid (30 ml) were stirred for 3 h at 0° was basified to pH 11 with aqueous sodium hydroxide. The precipitated *amine* (50%) afforded cream needles, m.p. 240—250° (from aqueous ethanol) (Found: C, 59·7; H, 4·6; N, 30·1%).

3-o-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-1-o-*methoxycarbonylphenyltriazene* (VIIa).—(i) Methyl anthranilate (9·06 g) was diazotised in 10N-hydrochloric acid (40 ml) and ice (10 g) with sodium nitrite (4·2 g) in water (7 ml). The clear diazonium solution was neutralised with sodium acetate and mixed with finely powdered 2,4-diamino-6-(2-aminophenyl)-*s*-triazine (12·2 g). The mixture was stirred at 0° for 24 h and the orange solid was then collected and dried over silica gel. Crystallisation from benzene afforded the *triazene* (70%) as yellow needles, m.p. 186—189° (efferv.) (Found: C, 56·1; H, 4·4; N, 31·0. $C_{17}H_{16}N_8O_2$ requires C, 56·0; H, 4·4; N, 30·8%).

(ii) 2,4-Diamino-6-(2-aminophenyl)-*s*-triazine (6·06 g) was diazotised at 0° in 0·5N-hydrochloric acid (160 ml) with sodium nitrite (2·07 g). Methyl anthranilate (4·54 g) was added to the acidic solution and the mixture was stirred at 0° for 24 h. The triazene thus formed (50%) was identical with the aforementioned sample (i.r.).

3-p-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-1-o-*methoxycarbonylphenyltriazene*(VIIb).—(i) Diazotised methyl anthranilate and 2,4-diamino-6-(4-aminophenyl)-*s*-triazine (1 mol. equiv.) were coupled in ice-cold, neutralised (sodium acetate) solution during 24 h. The precipitated *triazene* crystallised (50%) from toluene as yellow needles, m.p. 233—235° (efferv.) (Found: C, 55·8; H, 4·5; N, 30·8%).

(ii) A solution of diazotised 2,4-diamino-6-(4-aminophenyl)-*s*-triazine in 0·5N-hydrochloric acid was mixed with methyl anthranilate (1 mol. equiv.) and the product was stirred at 0° for 24 h. The precipitate (55%) was identical with the triazene prepared in (i) (i.r.).

3-o-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-1,2,3-*benzotriazin*-4(3H)-*one* (Va).—(i) Diazotisation of the amino-amide (IVa) at 0° in 2N-hydrochloric acid yielded, after neutralisation with sodium acetate, a precipitate of the *benzotriazinone* (80%), which crystallised from aqueous ethanol as cream prisms, m.p. 303—305° (efferv.) (Found: C, 57·8; H, 3·8; N, 34·0. $C_{16}H_{12}N_8O$ requires C, 57·8; H, 3·6; N, 33·7%).

(ii) The triazene (VIIa) (2 g) and piperidine (0·6 ml) were

[12] H. Zollinger, ' Diazo and Azo Chemistry,' Interscience, New York, 1961.

[13] R. H. Spector and M. M. Joullié, *J. Heterocyclic Chem.*, 1969 **6**, 605.

[14] B.P. 908,301/1962.

boiled (3 h) in ethanol. On cooling the solution deposited yellow prisms (85%) of the same benzotriazinone (i.r.).

3-p-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-1,2,3-*benzotriazin*-4(3H)-*one* (Vb).—This *benzotriazinone* was prepared either by diazotisation of the amino-amide (IVb) (75% yield) or by cyclisation of the triazene (VIIb) in ethanol containing 2% piperidine (50% yield), and crystallised from aqueous ethanol as cream needles, m.p. 324—326° (Found: C, 57·5; H, 3·6; N, 33·4%).

3-o-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-1-o-*cyanophenyltriazene* (IXa).—2-Aminobenzonitrile (6·8 g) in 10N-hydrochloric acid (40 ml) was diazotised at 0° with sodium nitrite (4·2 g) in water (10 ml). The clear diazonium solution was neutralised (sodium acetate) and stirred (3 h at 0°) with finely powdered 2,4-diamino-6-(2-aminophenyl)-*s*-triazine (12·2 g). The orange *triazene* (84%) crystallised from methanol with m.p. 185—187° (efferv.) (Found: C, 57·8; H, 4·3; N, 37·7. $C_{16}H_{13}N_9$ requires C, 58·0; H, 3·9; N, 38·1%), $\nu_{max.}$ (KBr) 2230 cm^{-1} (C≡N), $\lambda_{max.}$ (EtOH) 366 nm.

The triazene was recovered unchanged after being boiled in 95% ethanol containing 2% piperidine (4 h), from boiling 2-ethoxyethanol–piperidine (3 h), and after being stirred in 2N-hydrochloric acid (at 0°) for 3 days.

3-p-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-1-o-*cyanophenyltriazene* (IXb).—Interaction between diazotised 2-aminobenzonitrile and 2,4-diamino-6-(4-aminophenyl)-*s*-triazine (1 mol. equiv.) at 0° (3 h) afforded the yellow *triazene* (85%), m.p. 195—197° (efferv.) (from methanol) (Found: C, 58·1; H, 4·2; N, 38·0%), $\nu_{max.}$ (KBr) 2230 cm^{-1} (C≡N), $\lambda_{max.}$ (EtOH) 374 nm (log ε 4·41).

3-p-(4,6-*Diamino*-s-*triazin*-2-*yl*)*phenyl*-3,4-*dihydro*-4-*imino*-1,2,3-*benzotriazine* (Xb).—The triazene (IXb) cyclised in boiling 95% ethanol (2 g in 100 ml) containing piperidine (2 ml). The 4-*iminobenzotriazine* (60%) produced after 3 h when the solution was diluted with water crystallised from aqueous dimethylformamide as cream rosettes, m.p. 214—215° (efferv.) (Found: C, 57·7; H, 3·8; N, 37·8%).

4-p-(4,6-*Diamino*-s-*triazin*-2-*yl*)*anilino*-1,2,3-*benzotriazine* (XIIb).—The 4-iminobenzotriazine (Xb) (0·2 g) was stirred in 2N-hydrochloric acid at 0° for 4 days. The *anilino*-1,2,3-*benzotriazine* (0·2 g, 100%) precipitated on basification crystallised from a large volume of ethanol as cream flakes, m.p. 259—260° (efferv.) (Found: C, 58·1; H, 4·2; N, 37·7%), $\nu_{max.}$ (KBr) 1150 cm^{-1}, $\lambda_{max.}$ (EtOH) 278 and 342 nm (log ε 4·21 and 4·30).

N-p-*Cyanophenyl*-2-(2-*methoxyethoxy*)*benzamidine* (XVa).—Potassium hydroxide (0·1 g) and 4-*p*-cyanoanilino-1,2,3-benzotriazine (1·0 g) [4] were boiled in 2-methoxyethanol (10 ml) for 2 h, and the solution was poured on crushed ice. The precipitate (1·1 g) crystallised from benzene–light petroleum to afford the *benzamidine*, as pale yellow needles, m.p. 98—100° (Found: C, 69·3; H, 5·5; N, 13·9. $C_{17}H_{17}N_3O_2$ requires C, 69·1; H, 5·8; N, 14·2%), $\nu_{max.}$ (KBr) 3444 and 3370 (NH_2), 2820—2980 (aliphatic CH), and 2229 cm^{-1} (C≡N).

N-p-*Cyanophenyl*-2-(2-*ethoxyethoxy*)*benzamidine* (XVb).—This *benzamidine* (85%), similarly prepared from 4-*p*-cyanoanilino-1,2,3-benzotriazine, and 1% potassium hydroxide in boiling 2-ethoxyethanol, crystallised from light petroleum as cream plates, m.p. 85—86° (Found: C, 70·0; H, 6·0; N, 13·6. $C_{18}H_{19}N_3O_2$ requires C, 69·9; H, 6·1; N, 13·6%), $\nu_{max.}$ (KBr) 3449 and 3363 (NH_2), 2840—2975 (aliphatic CH), and 2225 cm^{-1} (C≡N).

2-*Butoxy*-N-p-*cyanophenylbenzamidine* (XVc).—Similarly prepared in n-butanol, this *benzamidine* (60%) had m.p. 87—90° (yellow needles, from light petroleum) (Found: C, 73·6; H, 6·3; N, 14·4. $C_{18}H_{19}N_3O$ requires C, 73·7; H, 6·5; N, 14·3%), $\nu_{max.}$ (KBr) 3472 and 3392 (NH_2), 2870—2960 (aliphatic CH), and 2227 cm^{-1} (C≡N).

4-*p*-Cyanoanilino-1,2,3-benzotriazine was recovered unchanged from boiling methanol (6 h), ethanol (4 h), propan-2-ol (2 h), or butan-2-ol (2 h), each containing 1% potassium hydroxide.

Decomposition of 1,3-*Di*-o-*cyanophenyltriazene in* 2-*Methoxyethanol containing Potassium Hydroxide*.—The solution turned dark brown and there was effervescence of nitrogen when 1,3-di-*o*-cyanophenyltriazene (2 g) was boiled (2 h) in 2-methoxyethanol (20 ml) containing potassium hydroxide (0·2 g). The solution was poured into water (100 ml) and organic material was extracted into chloroform. The extract was evaporated and the residue was purified by extraction with 2N-hydrochloric acid and reprecipitation with aqueous ammonia. A benzene solution of the solid thus formed was chromatographed in benzene on an alumina column. Evaporation of the eluate from a pale yellow band afforded 4-amino-2-phenylquinazoline (0·3 g), m.p. and mixed m.p. 147—148°, identical (i.r. spectrum) with an authentic sample.[8]

1-p-*Cyanophenyl*-3-o-*methoxycarbonylphenyltriazene*.— 4-Aminobenzonitrile (7·08 g) was diazotised at 0° in 10N-hydrochloric acid (40 ml) and ice (10 g) with sodium nitrite (4·2 g) in water (7 ml). The diazonium solution was filtered and neutralised with sodium acetate. Methyl anthranilate (9·06 g) was run in dropwise and the mixture was stirred at 0° for 3 h. The orange *triazene* (85%) was collected and crystallised from benzene, m.p. 174—176° (efferv.) (Found: C, 78·2; H, 5·1; N, 24·5. $C_{15}H_{12}N_4O_2$ requires C, 78·3; H, 5·2; N, 24·3%).

3-p-*Cyanophenyl*-1,2,3-*benzotriazin*-4(3H)-*one*.— The aforementioned triazene (5·0 g) was boiled (2 h) in 70% aqueous ethanol (300 ml). The cooled solution yielded the *benzotriazinone* (95%), which crystallised from benzene as long white needles, m.p. 230—232° (efferv.) (Found: C, 67·9; H, 3·2; N, 22·6. $C_{14}H_8N_4O$ requires C, 67·7; H, 3·3; N, 22·6%).

The triazinone (1·0 g) was boiled (13 h) in 2-methoxyethanol (5 ml) containing potassium hydroxide (0·1 g). Unchanged starting material (0·3 g) crystallised from the solution on cooling. Dilution of the mother liquor afforded N-p-*cyanophenylbenzamide* (15%), m.p. 170—172° (from aqueous ethanol) (Found: C, 75·6; H, 4·6; N, 12·9. $C_{14}H_{10}N_2O$ requires C, 75·7; H, 4·5; N, 12·6%), identical with a sample prepared from benzoyl chloride and 4-aminobenzonitrile in pyridine.

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