Medicinal Nitro-compounds. Part II.[1] Search for *ortho*-Interactions in Tumour-inhibitory 2,4-Dinitrophenylaziridines

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The 1-(2,4-dinitrophenyl)aziridines (1) and (2) behave as alkylating agents and undergo ring-opening under a range of mild conditions. With organic acids in toluene, 1-(2,4-dinitrophenyl)aziridine (1) yields esters of 2-(2,4-dinitroanilino)ethanol (7)—(13). In strong acids (without solvent) the esters are accompanied by polymer (18). 5-(Aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) (2) similarly yields esters (14) or (15) in acetic or propionic acids, but polymer (19) in formic acid. Ring opening of the aziridine (1) in mineral acids, in alcohols containing acids, or in alcohols containing alkyl iodides is influenced by the nucleophilicity of the attacking reagent. A series of ethylenediamines (28)—(33) was prepared from the aziridines (1) and (2) and amines without a catalyst being present, but in pyridine, or 3- or 4-aminopyridine, the aziridine (1) afforded polymer (18). The aziridine (1) on acidic or basic alumina in boiling toluene afforded a product identified as bis-[2-(2,4-dinitroanilino)ethyl] ether (35) on the basis of its spectroscopic properties, whereas photolysis in methanol led to ring opening and formation of *N*-(2-methoxyethyl)-2,4-dinitroaniline (26).

1-(2,4-DINITROPHENYL)AZIRIDINE (1) [2] is one of a number of aziridines with cytotoxic activity.[3] The 5-carbamoyl derivative (2) [4] (CB 1954) shows remarkable toxicity towards the transplanted Walker 256 tumour, but is less active, or inactive, against other tumours.[4-6]

Present evidence points to CB 1954 acting *in vivo* as an alkylating agent,[7,8] and since its biochemical effects in ascites tumour cells appear identical to those of the mustard melphalan (3),[9] it has been speculatively suggested that it could be metabolised in susceptible cells to the difunctional alkylating agent (4).[10] Other forms of covalent reactivity are possible. Bio-nucleophilic substitution of the activated nucleus could lead to displacement of cytotoxic aziridine in cells. Alternatively, substitution of a nitro-group or even addition to form a Meisenheimer σ-complex, could, if they occurred subsequent to alkylation of a biologically significant macromolecule by the aziridine fragment, imply cross-linking capability for CB 1954.

A more intriguing possibility occurred to us— namely that the dinitrophenylaziridines could be bioactivated by an *ortho*-nitro-interaction.[11,12] We were encouraged to pursue this line of investigation for several reasons. (i) The cytoxicity of CB 1954 can be reversed by the purine precursor 4-aminoimidazole-5-carboxamide. This implies that CB 1954 (or a biotransformation product) could act as an antimetabolite and disorganise nucleic acid biosynthesis.[7] (ii) The *o*-nitro-substituent in (1) and (2) is essential for activity: it cannot be replaced by other electron-attracting groups.[4] The *p*-nitro-group is not essential for activity. (iii) *NN*-Disubstituted *o*-nitroanilines cyclise to 2-substituted benzimidazoles under mild conditions.[13] Perhaps significantly, 2-substituted benzimidazoles and

bibenzimidazoles exhibit antiviral activity which presumably involves interaction at the nucleic acid level.[14]

Of the two possibilities for substituent interactions, aziridinyl–nitro or carbamoyl–nitro, the former appeared more likely, and we have concentrated our search for

(1) R = H
(2) R = CO·NH₂   (3)

(4)
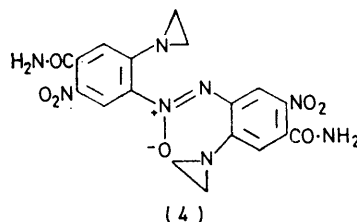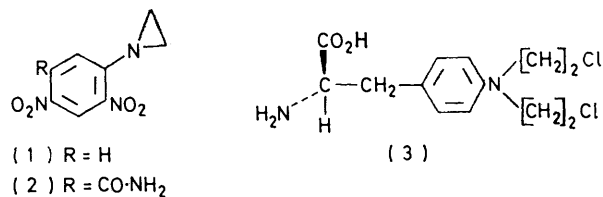
ortho-nitro-interactions on the aziridine (1), with only occasional work on CB 1954 (2).

Reactions with Acids.—The aziridine (1) in boiling toluene reacted with a range of organic acids to afford the esters (7)—(13) in high yields. These esters had characteristic spectroscopic properties: the i.r. spectrum of the acetate (8) showed $\nu_{C=O}$ and ν_{N-H} at 1727 and 3320 cm⁻¹, respectively, and the ¹H n.m.r. spectrum was simply analysed (Table); the mass spectrum showed a molecular ion of low abundance, and important peaks at m/e 43 ($CH_3C\equiv O^+$) and 196. The m/e 196 ion, formed

[7] T. A. Connors and D. H. Melzack, *Internat. J. Cancer*, 1971, **7**, 86.

[8] S. Venitt, *Chem.-Biol. Interactions*, 1971, **3**, 177.

[9] H. G. Mandel, T. A. Connors, D. H. Melzack, and K. Merai, *Cancer Res.*, 1974, **34**, 275.

[10] A. H. Khan and W. C. J. Ross, *Chem.-Biol. Interactions*, 1971, **4**, 11.

[11] J. D. Loudon and G. Tennant, *Quart. Rev.*, 1964, **18**, 398.

[12] P. N. Preston and G. Tennant, *Chem. Rev.*, 1972, **72**, 627.

[13] O. Meth-Cohn and H. Suschitzky, *Adv. Heterocyclic Chem.*, 1972, **14**, 211.

[14] W. R. Roderick, C. W. Nordeen, A. M. Von Esch, and R. N. Appell, *J. Medicin. Chem.*, 1972, **15**, 655, and references therein.

[1] Part I, B. C. Gunn and M. F. G. Stevens, *J.C.S. Perkin I*, 1973, 1682.

[2] J. A. Hendry, R. F. Homer, F. L. Rose, and A. L. Walpole, *Brit. J. Pharmacol.*, 1951, **6**, 357.

[3] O. C. Derner and G. E. Ham, ‘ Ethyleneimine and other Aziridines,’ Academic Press, New York, 1969, p. 394.

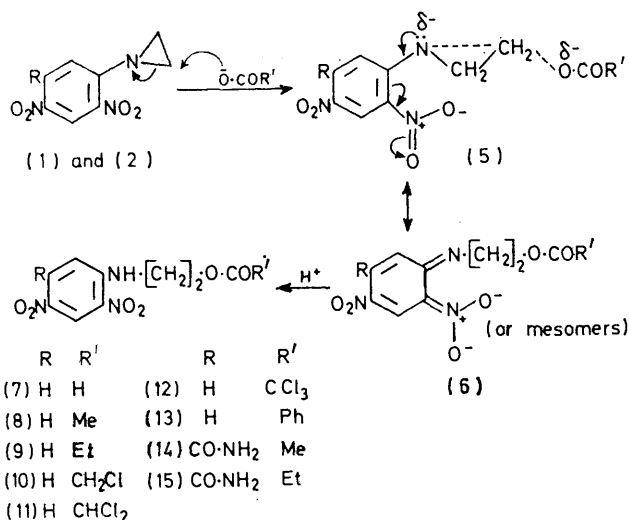[4] A. H. Khan and W. C. J. Ross, *Chem.-Biol. Interactions*, 1969, **1**, 27.

[5] L. M. Cobb, T. A. Connors, L. A. Elson, A. H. Khan, B. C. V. Mitchley, W. C. J. Ross, and M. E. Whisson, *Biochem. Pharmacol.*, 1969, **18**, 1519.

[6] M. J. Tisdale, *Chem.-Biol. Interactions*, 1971, **3**, 95.

by cleavage of the C–C bond adjacent to the amino-group proved particularly diagnostic, and was the base peak in the spectra of the majority of ring-opened derivatives (see later). The products from the aziridine (1) and succinic or toluene-p-sulphonic acid in toluene had spectroscopic properties in accord with structure (16) or (17), respectively.

In the non-polar solvent, ring opening by the acid anions may involve the non-protonated weakly basic aziridine (Scheme 1) with the $-M$ substituents stabilising the negative charge developing on the aziridine N atom [(5) ←→ (6)] in the S_N2 transition state. Similar aziridines with electron-attracting N-substituents have been described as 'activated aziridines.'[15]

When the aziridine (1) was heated with carboxylic acids alone, the same series of esters (7)—(13) was

| R	R'		R	R'
(7)	H	H	(12)	H	CCl₃
(8)	H	Me	(13)	H	Ph
(9)	H	Et	(14)	CO·NH₂	Me
(10)	H	CH₂Cl	(15)	CO·NH₂	Et
(11)	H	CHCl₂			

SCHEME 1

formed, but with the strongly acidic chloroacetic acids the esters (10)—(12) were accompanied by a high-melting insoluble brown by-product. In 98% formic acid this by-product, formed in a strongly exothermic reaction, accounted for 65% of the yield, the remainder being the formate (7). The by-product was purified by precipitation from dimethylformamide with ethanol, and is tentatively assigned the polymer structure (18) since it differed from the diphenylpiperazine (20) and had no N–H absorptions in its i.r. spectrum. However, branched or crown polymers are also possible. CB 1954 (2) similarly gave predominantly the polymer (19) in formic acid but the esters (14) and (15) in acetic or propionic acid. Hydrolysis of the acetate (8) in 2N-hydrochloric acid furnished the 2-hydroxyethylamine (21).

It is significant that only the strong acids formic (pK_a 3·75),[16] chloroacetic (2·84), dichloroacetic (1·25), and trichloroacetic (0·66) yield polymer, and these must partially protonate the aziridine, allowing unprotonated

[15] Ref. 3, p. 106.
[16] All pK_a values referred to in this paper are quoted from 'Ionisation Constants of Acids and Bases,' A. Albert and E. P. Serjeant, Methuen, London, 1962.

aziridine to compete with the nucleophilic acid anions in the ring-opening step (Scheme 2). The balance between ester and polymer formation will clearly be
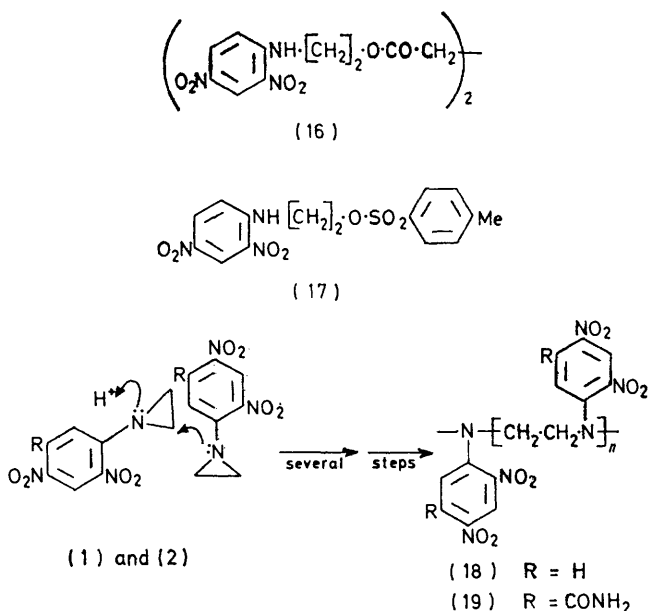
SCHEME 2

determined by such factors as the pK_a of the reacting aziridine and acid, the polarity of the solvent, and the nucleophilicity of the acid anions. This sensitivity is illustrated by the behaviour of the aziridine (1) towards mineral acids. In 2N-hydrochloric acid the product is exclusively the 2-chloroethylamine (22), which was also formed from (1) and thionyl chloride. In 2N-sulphuric acid, on the other hand, the product was the polymer (18). The differences can be attributed to the relative nucleophilicities of chloride ion (strong) and hydrogen sulphate ion (weak). Interestingly, N-(2,4-dinitrophenyl)pyrrolidine and its larger ring homologues

(20)

	R	R'		R	R'
(21)	H	OH	(25)	H	Br
(22)	H	Cl	(26)	H	OMe
(23)	CO·NH₂	Cl	(27)	H	OEt
(24)	H	I			
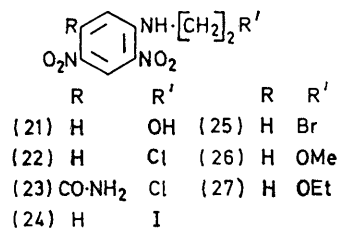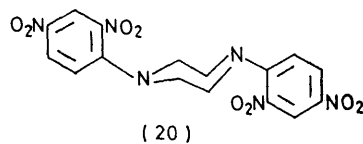
cyclise to benzimidazole N-oxides in hydrochloric acid.[17] CB 1954 (2) has also been reported to form the appropriate 2-chloroethylamine (23) with dry hydrogen

[17] R. Fielden, O. Meth-Cohn, and H. Suschitzky, J.C.S. Perkin I, 1973, 696.

chloride in acetone,[4] and further examples of the importance of the strength of the nucleophile are found in reactions of the aziridine (1) in alcohols.

Reactions with Alcohols.—The aziridine (1) was stable indefinitely in refluxing methanol or ethanol, but with traces of sulphuric acid or acidic alumina the ring-opened ethers (26) and (27) were formed (*cf.* the behaviour of the 'activated' 1-ethoxycarbonylaziridine).[18] When toluene-*p*-sulphonic acid was employed as acidic catalyst, mixtures of the ethers (26) and (27) and the toluene-*p*-sulphonate (17) were isolated; with hydrochloric acid, the aziridine (1) in methanol or ethanol afforded the 2-chloroethylamine (22), following the intervention of chloride ion in the ring-opening step.

The methyl ether (26) was also formed in high yield when the aziridine (1) was boiled in methanol with either methyl or ethyl iodide. We suggest that the corresponding 2-iodoalkylamine (24) participates as an intermediate in these reactions. This is in accord with the greater propensity for iodide ion (in comparison with chloride ion) to act as a leaving group, and with the observation that reaction of the aziridine (1) with alkyl iodides in ethanol gave the 2-iodoethylamine (24) after a short period: the yield of the ethyl ether (27) increased at the expense of the iodoethylamine as reaction time was prolonged. In no cases were 'exchange' ethers detected (*i.e.* the methyl ether from the reaction of aziridine with methyl iodide in ethanol, or ethyl ether from reaction with ethyl iodide in methanol). The 2-iodoethylamine formed as an intermediate probably arises from reaction with hydriodic acid generated *in situ* from the alkyl iodides and alcohols, since the aziridine (1) does not react with the alkyl iodides alone, and reaction in the presence of alcohols is suppressed by sodium carbonate. The aziridine (1) and an excess of benzyl bromide in refluxing xylene afforded the 2-bromoethylamine (25) in substantial yield, probably from hydrobromic acid liberated in the decomposition of benzyl bromide.

Reactions with Bases.—The aziridines (1) and (2) reacted with various amines at 100° to afford the ethylenediamines (28)—(32). No catalyst was required. Indeed, when ammonium or anilinium toluene-*p*-sulphonate was employed as the base components no reaction occurred. In pyridine, the aziridine (1) reacted exothermically to yield a black solid, which after repeated crystallisation from dimethylformamide–ethanol gave a product identical (i.r.) with the polymer (18). A cleaner polymer was obtained by employing xylene or toluene as solvent. Aziridine (1) and 3- or 4-aminopyridine, with or without toluene as solvent, similarly afforded polymer.

2-Aminopyridine and the aziridine (1) gave two isomeric products in variable yield in addition to polymer. The high-melting isomer was identified as the diamine (33) because its electronic absorption spectrum [$\lambda_{max.}$ 350 nm (log ε 4·19)] was similar to that of the anilino-

analogue (28) [$\lambda_{max.}$ 349 nm (log ε 4·15)] and its mass spectrum showed ions at *m/e* 196 and 107. The unstable low-melting isomer, which was not obtained pure, was probably the imine (34) since it rearranged to the high-melting isomer in aqueous sodium hydroxide. Similar Dimroth rearrangements are a feature of the chemistry of 1-alkyl-1,2-dihydro-2-iminopyridines.[19]

Triethylamine catalysed ring opening of the aziridine (1) to the methyl ether (26) in methanol, although the process was less efficient than acid catalysis. No displacement of aziridine occurred. The aziridine (1) does however yield sodium 2,4-dinitrophenoxide quantitatively when boiled in aqueous 2N-sodium hydroxide with liberation of aziridine (or 2-aminoethanol).

Thermolysis and Photolysis.—In order to suppress the competing ring-opening reactions that occur with mineral or organic acids, and in an attempt to encourage a thermal *ortho*-nitro-interaction, the aziridine (1) was

	R	R¹	R²		R	R¹	R²
(28)	H	H	Ph	(31)	H		[CH₂]₅
(29)	H	H	Prⁱ	(32)	CO·NH₂		[CH₂]₅
(30)	H	Et	Et				

(33)

(34)

(35)

(36)
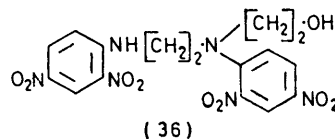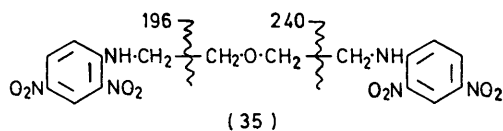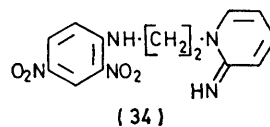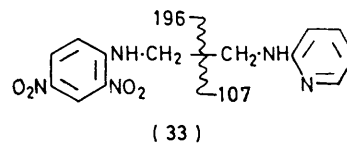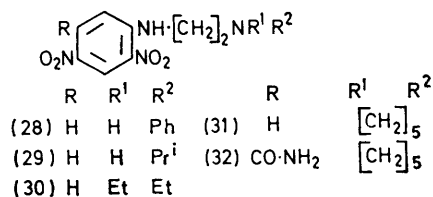
boiled in anhydrous xylene containing a ten-fold excess (w/w) of vacuum-dried acidic alumina as acidic catalyst. The yellow product (80%), $C_{16}H_{16}N_6O_9$, was tentatively assigned structure (35): although the mass spectrum did not show a molecular ion peak at *m/e* 436, this was not unexpected (see later). The spectroscopic properties of this compound were similar to those of the aforementioned ethers (26) and (27). The mass spectrum showed a base peak at *m/e* 196 and a major ion at *m/e*

[18] Ref. 3, p. 248.
[19] D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1965, 5542.

240 both consistent with structure (35); the i.r. spectrum displayed v_{N-H} at 3400 cm^{-1} and v_{C-O} at 1140 cm^{-1}; the ^{1}H n.m.r. spectrum showed the methylene proton signal as an overlapping multiplet indicating that the flanking substituents are of comparable electronegativity. Absence of both O–H absorption in the i.r. spectrum and an ion at m/e 31 in the mass spectrum exclude the isomeric alcohol structure (36). Appropriate control experiments established that the 2-hydroxyethylamine (21) was not an intermediate in the formation of the ether (35) since the hydroxyethylamine neither reacted alone nor did it react with the aziridine (1) in the presence of alumina. Surprisingly, the same ether was obtained from the aziridine and basic alumina in xylene, and it is possible that it is formed directly from two molecules of aziridine and water tenaciously adsorbed to the catalytic surface.

The aziridine (1) exploded when heated at 200°. Controlled thermolysis in sand at 220° or in refluxing nitrobenzene (210°) led to evolution of a brown gas and formation of a complex mixture of products. None of the components has been identified. In contrast, starting material was recovered quantitatively when (1) was boiled in o-dichlorobenzene (175°), decalin (195°), or toluene (110°) for 6 h. The related diaziridine (37) undergoes an *ortho*-interaction to afford the benzotriazole 1-oxide (38) in boiling toluene in 97% yield.[20]

The reported photocyclisation of the picrylaziridine (39) to a 1-hydroxybenzotriazole (40) [21] is also analogous to the type of cyclisation for which we were searching. Irradiation of the aziridine (1) in methanol led to a puzzling result. No change in the electronic absorption spectrum of the solution was detected during 48 h illumination by a 100 W medium-pressure arc (quartz apparatus). However, when the lamp was extinguished a progressive bathochromic shift from 327 to 350 nm was observed and after 48 h the methyl ether (26) was isolated in 25% yield. The yield of ether increased with time. In control experiments it was established that the aziridine was stable in methanol in the dark, or in methanol exposed to ambient light without prior irradiation in the u.v. region. A possible explanation could be that ring opening occurs when sufficient formic acid has been generated in the oxidative u.v. photolysis of the methanol solvent. Acid-catalysed ring opening could then yield the methyl ether (26). Support for this hypothesis came from an independent experiment in which the methyl ether (26) was formed in 65% yield in methanol containing 0·1% formic acid. However, we are unable to explain the lack of ring opening under illumination. The possibility that a reversible reaction [(1) ⇌ (26)] might be involved, with irradiation favouring the cyclic species (1), was discounted, since the ether was independently shown to be photostable both in methanol alone, and in methanol containing traces of formic acid.

Crystalline samples of all the compounds examined in

the course of this work were exposed to intermittent sunlight for a minimum period of 2 years. With the exception of the cyclic tertiary amines (1), (2), and (20), which darkened slightly, no colour changes were observed.

Spectroscopic Properties.—The i.r. spectrum (KBr) of the aziridine (1) is similar to that of *N*-(2,4-dinitrophenyl)piperidine and shows NO$_2$ absorptions at 1515 and 1338 cm^{-1}. In the case of CB 1954 (2) these occur at 1510 and 1337 cm^{-1}, with $v_{C=O}$ at 1672 cm^{-1}. The i.r.
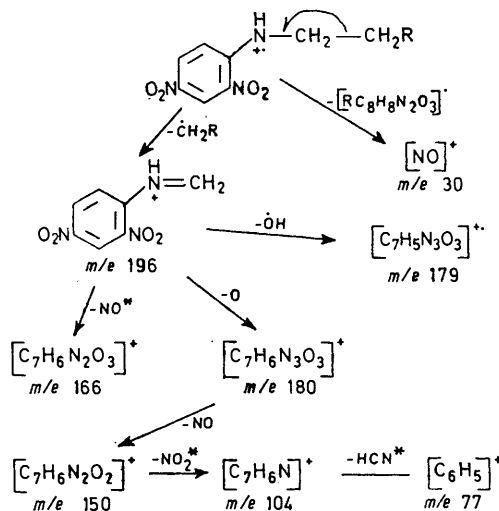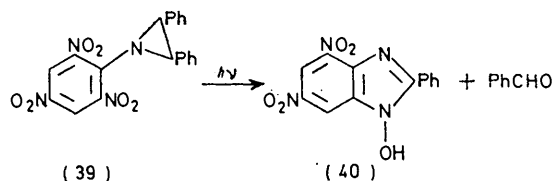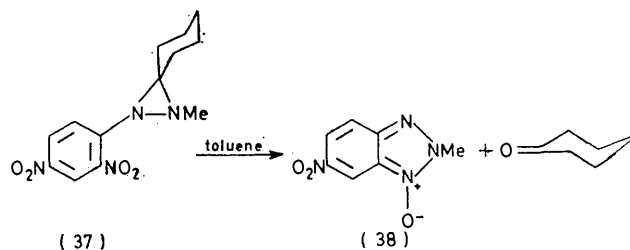
SCHEME 3

spectra of the polymers (18) and (19) were similar to that of the piperazine (20), with characteristically broad absorptions. The ring-opened derivatives all show sharp N–H absorptions in the range 3320—3360 cm^{-1} with other absorptions characteristic of their appropriate functional groups. In the series of esters, $v_{C=O}$ ranges from 1720 for formate (7) [1727 for (8), 1736 (9), 1754 (10), 1763 (11)] to 1765 cm^{-1} for the trichloroacetate (12), clearly reflecting the electronic influences of the acyl substituents.

The ^{1}H n.m.r. spectrum of aziridine (1) shows an

[20] H. W. Heine, P. G. Williard, and T. R. Hoye, *J. Org. Chem.*, 1972, **37**, 2980.

[21] H. W. Heine, G. J. Blosick, and G. B. Lowrie, *Tetrahedron Letters*, 1968, 4801.

AMX pattern for the aromatic protons with chemical shifts and coupling constants similar to those of other 2,4-dinitrophenyl derivatives (Table). Physical processes which could lead to non-equivalence of the methylene protons (*i.e.* restricted rotation about the aziridine–aryl bond or slow inversion at the tertiary nitrogen) were sufficiently rapid on the n.m.r. time scale to result in the signal for these protons appearing as a sharp singlet (δ 2·43) at magnet temperature and at

As already mentioned, the molecular ions in the mass spectra of the ring-opened derivatives are either absent or of low abundance and the dominating fragmentation is cleavage of the C–C bond adjacent to the amino-group. The ion at m/e 196 is the base peak in the spectra of most of these derivatives, and, except in those cases where alternative cleavages afford favourably stabilised ions, the spectra are nearly identical below m/e 196. The constitutions of the ions in (Scheme 3)

¹H N.m.r. spectra of 2,4-dinitrophenyl derivatives {Varian HA-100D spectrometer; Me₄Si as internal (in CDCl₃) or external [in (CD₃)₂SO] standard}

Compound	Solvent	Aromatic			Other absorptions
		H-3	H-5	H-6	
(1)	CDCl₃	8·75 (d, $J_{3,5}$ 2·6 Hz)	8·23 (dd)	7·22 (d, $(J_{5,6}$ 8·9 Hz)	2·43 (s, [CH₂]₂)
(2)	(CD₃)₂SO	8·91 (s)		7·69 (s)	8·47 and 8·12 (br, s, NH₂), 2·81 (s, [CH₂]₂)
1-Chloro-2,4-dinitrobenzene	CDCl₃	8·77 (d, $J_{3,5}$ 2·7 Hz) [a]	8·45 (dd)	7·85 (d, $(J_{5,6}$ 8·81) [a]	
N-(2,4-Dinitrophenyl)-piperidine	CDCl₃	8·61 (d)	8·18 (dd)	7·08 (d)	3·27 and 1·74 (br, s, [CH₂]₅) [b]
1,-Methoxy-2,4-dinitrobenzene	CDCl₃	8·60 (d)	8·39 (dd)	7·29 (d)	4·08 (s, CH₃)
(7)	CDCl₃	9·10 (d)	8·28 (dd)	6·94 (d)	8·65br (s, NH), 8·05 (s, CHO), 4·46 (t, CH₂O), 3·70 (m, NHCH₂)
(8)	CDCl₃	9·14 (d)	8·31 (dd)	7·01 (d)	8·75br (s, NH), 4·43 (t, CH₂O), 3·74 (m, NHCH₂), 2·12 (s, CH₃)
(9)	CDCl₃	9·05 (d)	8·26 (dd)	7·04 (d)	8·72br (s, NH), 4·44 (t, CH₂O), 3·77 (m, NHCH₂), 2·40 (q, CH₂·CH₃), 1·16 (t, CH₃)
(10)	CDCl₃	9·10 (d)	8·28 (dd)	6·98 (d)	8·65br (s, NH), 4·51 (t, CH₂O), 4·06 (s, CH₂Cl), 3·76 (m, NHCH₂)
(11)	CDCl₃	9·15 (d)	8·33 (dd)	7·03 (d)	8·70br (s, NH), 5·99 (s, CHCl₂), 4·61 (t, CH₂O), 3·80 (m, NHCH₂)
(12)	CDCl₃	9·03 (d)	8·27 (dd)	7·00 (d)	8·70br (s, NH), 4·63 (t, CH₂O), 3·86 (m, NHCH₂)
(14)	(CD₃)₂SO	9·10 (s)		7·47 (s)	8·85br (s, NH), 8·38 and 8·07 (br, s, NH₂), 4·60 (t, CH₂O), 4·13 (m, NHCH₂), 2·33 (s, CH₃)
(15)	(CD₃)₂SO	9·11 (s)		7·50 (s)	8·80br (s, NH), 8·38 and 8·08 (br, s, NH₂), 4·58 (t, CH₂O), 4·13 (m, NHCH₂), 2·63 (q, CH₂CH₃), 1·21 (t, CH₃)
(20)	(CD₃)₂SO	8·97 (d)	8·64 (dd)	7·76 (d)	3·47 and 2·88 (br, s, [CH₂]₂)
(22)	CDCl₃	8·99 (d)	8·22 (dd)	6·95 (d)	8·70br (s, NH), 3·80 (m, [CH₂]₂)
(24)	CDCl₃	9·10 (d)	8·25 (dd)	6·87 (d)	8·70br (s, NH), 3·81 (m, NHCH₂), 3·37 (t, CH₂I)
(25)	CDCl₃	9·07 (d)	8·25 (dd)	6·87 (d)	8·70br (s, NH), 3·87 (m, NHCH₂), 3·58 (t, CH₂Br)
(26)	CDCl₃	9·07 (d)	8·22 (dd)	6·91 (d)	8·70br (s, NH), 3·65 (m, [CH₂]₂), 3·39 (s, CH₃)
(27)	CDCl₃	9·04 (d)	8·26 (dd)	6·95 (d)	8·80br (s, NH), 3·65 (m, [CH₂]₂ and CH₂·CH₃), 1·25 (t, CH₃)
(31)	CDCl₃	9·06 (d)	8·20 (dd)	6·84 (d)	7·23br (s, NH), 3·42 (m, NHCH₂), 2·68 (t, CH₂N<), 2·48 and 1·60 (m, [CH₂]₅)
(32)	(CD₃)₂SO	9·02 (s)		7·33 (s)	8·30 and 8·00 (br, s, NH₂), 3·83 (m, NHCH₂), 2·89 (t, CH₂N<), 2·71 and 1·75 (br, s, [CH₂]₅)
(35)	(CD₃)₂SO	9·10 (d)	8·48 (dd)	7·55 (d)	9·04br (s, NH), 4·08 (m, [CH₂]₂)

[a] Coupling constants obtained in acetone solution (S. L. Smith and A. M. Ihrig, *J. Mol. Spectroscopy*, 1967, **22**, 241). [b] No change in methylene absorptions at −70°.

−30°, with only slight broadening at −70°. The methylene absorptions of other activated aziridines are similarly uninfluenced at temperatures above −70°.[22] The chemical shifts of the methylene protons in the ring-opened derivatives give information on the electronic nature of the attached groups, and are a valuable aid in structure determination (Table). These signals are well separated in the esters but overlap to greater or lesser extents in the ethers, 2-halogenoethyl-amines, and ethylenediamines.

have been corroborated by high-resolution mass measurements, and fragmentations marked with an asterisk are supported by appropriate metastable ions. Deviations from this pattern occur in the acetate (8), propionate (9), and benzoate (13) where the base peaks are the acylium ions at m/e 43, 57, and 105, respectively, and

[22] A. T. Bottini and J. D. Roberts, *J. Amer. Chem. Soc.*, 1958, **80**, 5203; see however, H. Nakanishi and O. Yamamoto, *Tetrahedron*, 1974, **30**, 2115 on the influence of traces of moisture on the n.m.r. spectra of aziridines.

in the ethylenediamine derivatives (28)—(33) where the ion at m/e 196 accounts for <1% abundance. The base peaks in these latter examples are formed by C–C bond fission giving ions with charge residing on the alternative amino-group rather than on the dinitroanilino-fragment.

Conclusion.—The work described here lends further support to the conclusions of the Chester Beatty workers regarding the alkylating activity of the aziridines (1) and (2). No chemical evidence for *ortho*-nitro-interactions was discerned, although it is possible that such reactions could be mediated by enzymes within susceptible cells.

Most of the ring-opened derivatives prepared in the course of this work were screened for tumour-inhibitory activity against lymphoid leukaemia (L-1210) in mice. None of the derivatives was active.

EXPERIMENTAL

I.r. spectra were recorded for potassium bromide discs on a Perkin-Elmer 257 spectrometer (slow scan speed). Photolyses were conducted with a 100 W medium-pressure lamp in a Hanovia photochemical reactor. ' Acidic ' or ' basic ' alumina refers to B.D.H. Aluminium Oxide Active (Grade I).

Reactions in Acids.—2-(2,4-*Dinitroanilino*)*ethyl formate* (7). A solution of 1-(2,4-dinitrophenyl)aziridine (2·07 g) [4] and formic acid (98%; 1·1 mol. equiv.) in toluene (20 ml) was boiled for 3 h. The concentrated solution afforded the *formate* (90%), which crystallised from toluene–light petroleum as yellow needles, m.p. 133—134° (Found: C, 42·5; H, 3·6; N, 16·6. $C_9H_9N_3O_6$ requires C, 42·4; H, 3·5; N, 16·5%), $\nu_{max.}$ 3328 (N–H) and 1720 cm^{-1} (C=O).

2-(2,4-*Dinitroanilino*)*ethyl acetate* (8). This *acetate* was prepared from 1-(2,4-dinitrophenyl)aziridine and acetic acid (1·1 mol. equiv.) in toluene as above (89%), or from the aziridine in boiling acetic acid (95%) or acetic anhydride (90%). It crystallised from toluene–light petroleum as yellow needles, m.p. 131—132° (Found: C, 44·4; H, 4·1; N, 15·5. $C_{10}H_{11}N_3O_6$ requires C, 44·6; H, 4·1; N, 15·6%), $\nu_{max.}$ 3320 (N–H) and 1727 cm^{-1} (C=O).

Hydrolysis of the acetate in boiling 2N-hydrochloric acid (2 h) afforded 2-(2,4-dinitroanilino)ethanol (95%), m.p. 85—86° (lit.,[23] 87—88°).

2-(2,4-*Dinitroanilino*)*ethyl propionate* (9). Prepared from 1-(2,4-dinitrophenyl)aziridine and propionic acid alone (78%), the *propionate* crystallised from toluene–light petroleum as yellow needles, m.p. 63—64° (Found: C, 46·5; H, 4·6; N, 14·8. $C_{11}H_{13}N_3O_6$ requires C, 46·6; H, 4·6; N, 14·8%), $\nu_{max.}$ 3360 (N–H) and 1736 cm^{-1} (C=O).

Similarly prepared from 1-(2,4-dinitrophenyl)aziridine and acids (1·1 mol. equiv.) in boiling toluene were the following 2-(2,4-dinitroanilino)ethyl esters: *chloroacetate* (10) (75%), m.p. 104—105° (Found: C, 39·7; H, 3·3; N, 13·9. $C_{10}H_{10}ClN_3O_6$ requires C, 39·5; H, 3·3; N, 13·8%), $\nu_{max.}$ 3330 (N–H) and 1754 cm^{-1} (C=O); *dichloroacetate* (11) (70%), m.p. 120—121° (Found: C, 35·6; H, 2·8; N, 12·5. $C_{10}H_9Cl_2N_3O_6$ requires C, 35·5; H, 2·15; N, 12·4%), $\nu_{max.}$ 3350 (N–H) and 1763 cm^{-1} (C=O); *trichloroacetate* (12) (66%), m.p. 95—96° (Found: C, 32·4; H, 2·2; N, 11·4. $C_{10}H_8Cl_3N_3O_6$ requires C, 32·2; H, 2·2; N, 11·3%), $\nu_{max.}$ 3360 (N–H) and 1765 cm^{-1} (C=O); *benzoate* (13) (78%), m.p. 116—118° (from ethanol) (Found: C, 54·5; H, 4·0;

N, 12·8. $C_{15}H_{13}N_3O_6$ requires C, 54·4; H, 3·9; N, 12·7%), $\nu_{max.}$ 3358 (N–H) and 1713 cm^{-1} (C=O); *succinate* (diester) (16) (59%), m.p. 147—148° (from acetone) (Found: C, 44·6; H, 3·5; N, 15·8. $C_{20}H_{20}N_6O_{12}$ requires C, 44·8; H, 3·7; N, 15·7%), $\nu_{max.}$ 3315 (N–H) and 1737 cm^{-1} (C=O); *toluene*-p-*sulphonate* (17) (75%), m.p. 162—163° (from acetone) (Found: C, 47·3; H, 4·0; N, 11·0. $C_{15}H_{15}N_3O_7S$ requires C, 47·2; H, 4·0; N, 11·0%).

2-(5-*Carbamoyl*-2,4-*dinitroanilino*)*ethyl acetate* (14). A solution of 5-(aziridin-1-yl)-2,4-dinitrobenzamide (2·0 g) [4] in acetic acid (20 ml) was refluxed for 30 min and diluted with water (30 ml). The precipitated *acetate* (78%) crystallised from butan-1-ol as yellow rosettes, m.p. 195—197° (Found: C, 42·5; H, 4·1; N, 17·7. $C_{11}H_{12}N_4O_7$ requires C, 42·3; H, 3·9; N, 17·9%); $\nu_{max.}$ 1729 (C=O ester) and 1660 cm^{-1} (C=O amide).

2-(5-*Carbamoyl*-2,4-*dinitroanilino*)*ethyl propionate* (15). This *propionate* (60%), similarly prepared from propionic acid, crystallised from butan-1-ol as yellow needles, m.p. 160—161° (Found: C, 44·2; H, 4·3; N, 17·1. $C_{12}H_{14}N_4O_7$ requires C, 44·2; H, 4·3; N, 17·2%); $\nu_{max.}$ 1729 (C=O ester) and 1660 cm^{-1} (C=O amide).

Poly-(2,4-*dinitrophenyliminoethylene*) (18). An orange solid was rapidly deposited when 1-(2,4-dinitrophenyl)-aziridine (1·0 g) was heated with formic acid (98%; 10 ml) at 100° (30 min). An ethanol-soluble fraction (0·15 g) was identical with 2-(2,4-dinitroanilino)ethyl formate (7). The ethanol-insoluble ochre *polymer* (0·8 g), dissolved in dimethylformamide and reprecipitated with ethanol, had m.p. 240° (variable) [Found: C, 45·7; H, 3·3; N, 19·7. $(C_8H_7N_3O_4)_n$ requires C, 45·9; H, 3·4; N, 20·1%].

The same polymer (identical i.r. spectrum) was formed (95%) when the aziridine was boiled in 2N-sulphuric acid (1 h), and as a by-product in the reactions of 1-(2,4-dinitrophenyl)aziridine with chloroacetic, dichloroacetic, and trichloroacetic acids (10, 18, and 35% yields, respectively).

Poly-(5-*carbamoyl*-2,4-*dinitrophenyliminoethylene*) (19). This *polymer* (95%) was deposited when 5-(aziridin-1-yl)-2,4-dinitrobenzamide (1·0 g) was heated in 98% formic acid (10 ml) for 30 min at 100°. It was precipitated from dimethylformamide with ethanol as a brown solid, m.p. 240° (indef.). No consistent microanalyses were obtained.

1-(2-*Chloroethylamino*)-2,4-*dinitrobenzene* (22). The chloroethylamine (98%) was formed when 1-(2,4-dinitrophenyl)aziridine (2·0 g) was boiled in 2N-hydrochloric acid (50 ml) for 1 h, and had m.p. 86—87° (lit.,[23] 87·5—88°). Reaction of the aziridine (2·0 g) in boiling thionyl chloride (20 ml) also afforded the chloroethylamine (92%) after 1 h.

When 1-(2,4-dinitrophenyl)aziridine (2·0 g) and 2N-hydrochloric acid (8 ml) were boiled in either methanol or ethanol (50 ml) for 2 h, the product was the same 2-chloroethylamine (60 and 73% yield, respectively).

1-(2-*Bromoethylamino*)-2,4-*dinitrobenzene* (25). The aziridine (1) (1·0 g) was refluxed in xylene (10 ml) containing benzyl bromide (4 ml) for 24 h. Vacuum distillation left a brown oil which was chromatographically fractionated in benzene on an alumina column. The yellow band afforded the bromoethylamine (0·6 g), m.p. 94—95° (lit.,[4] 98°).

Reactions in Alcohols.—1-(2-*Methoxyethylamino*)-2,4-*dinitrobenzene* (26). A solution of 1-(2,4-dinitrophenyl)-aziridine (1·0 g) in boiling methanol (30 ml) rapidly deposited the methoxy-derivative (78%) on addition of

[23] H. Hippchen, *Chem. Ber.*, 1947, **80**, 263.

2N-sulphuric acid (1 ml). The product, collected after 3 h, had m.p. 145—147° (lit.,[24] 145·5°).

The same ether was formed (80%) from the aziridine (1·0 g) in boiling methanol (30 ml) containing acidic alumina (5·0 g).

When toluene-p-sulphonic acid (1·0 g) was employed as the acidic catalyst under the same conditions, the products were the ether (75%) and 2-(2,4-dinitroanilino)ethyl toluene-p-sulphonate (17) (15%).

The same ether (80 and 86% yields, respectively) was formed when the aziridine (1·0 g) was boiled in methanol (30 ml) containing either methyl iodide or ethyl iodide (2·0 ml). There was no reaction when the aziridine was boiled with methanol, or methyl or ethyl iodide alone for 24 h.

Reaction of the aziridine (1·0 g) in boiling methanol (10 ml) containing triethylamine (2·0 ml) for 72 h gave a yellow semi-solid when the excess of solvent was removed by vacuum evaporation. N.m.r. analysis of this solid in CDCl$_3$ revealed the presence of unchanged aziridine and the above methyl ether (ca. 5%).

1-(2-*Ethoxyethylamino*)-2,4-*dinitrobenzene* (27). When 1-(2,4-dinitrophenyl)aziridine (1·0 g) was boiled in ethanol (20 ml) containing 2N-sulphuric acid (1 ml) for 1 h, the *ethoxyethylamine* (65%), m.p. 86—87° (from ethanol), was collected from the concentrated mixture (Found: C, 50·2; H, 5·4; N, 17·5. C$_{10}$H$_{13}$N$_3$O$_4$ requires C, 50·2; H, 5·5; N, 17·6%). When 2N-hydrochloric acid (4 ml) was employed as the acidic catalyst, the product was the 2-chloroethylamine (22) (74%); with toluene-p-sulphonic acid (1·0 g), the products were the ethyl ether (30%) and 2-(2,4-dinitroanilino)ethyl toluene-p-sulphonate (55%).

Reaction of the aziridine (1·0 g) in refluxing ethanol (20 ml) containing methyl iodide (4·0 ml) afforded 1-(2-iodoethylamino)-2,4-dinitrobenzene (0·55 g), m.p. 125—126° (lit.,[4] 127°), after 4 h. Under the same conditions, but with a 24 h reaction time, the product was 1-(2-ethoxy-ethylamino)-2,4-dinitrobenzene (75%).

Reactions in Bases.—N-(2,4-*Dinitrophenyl*)-N′-*phenyl-ethylenediamine* (28). A red melt was formed when 1-(2,4-dinitrophenyl)aziridine (1·0 g) and aniline (0·6 g) were heated on a steam-bath (1 h). The ethylenediamine (70%), collected when the melt was triturated with light petroleum, had m.p. 139—141° (orange flakes, from benzene–light petroleum) (lit.,[25] 142·6—143·2°).

Similarly prepared from the appropriate aziridine and an amine (1·1 mol. equiv.) at 100° were: N-(2,4-*dinitrophenyl*)-N′-*isopropylethylenediamine* (29) (60%), m.p. 77—78° (from benzene–light petroleum) (Found: C, 49·2; H, 6·1; N, 20·7. C$_{11}$H$_{16}$N$_4$O$_4$ requires C, 49·3; H, 6·0; N, 20·0%); N′-(2,4-*dinitrophenyl*)-NN-diethylethylenediamine (30) (65%), m.p. 93—94° (lit.,[26] 94°); N-(2-piperidinoethyl)-2,4-dinitroaniline (31) (65%), m.p. 102—103° (lit.,[26] 103—105°); 2,4-*dinitro*-5-(2-*piperidinoethylamino*)*benzamide* (32) (68%), m.p. 149—150° (from ethanol) (Found: C, 49·6; H, 5·6; N, 20·6. C$_{14}$H$_{19}$N$_5$O$_5$ requires C, 49·9; H, 5·9; N, 20·8%).

N-(2,4-*Dinitrophenyl*)-N′-(2-*pyridyl*)*ethylenediamine* (33). A mixture of 2-aminopyridine (1·0 g) and 1-(2,4-dinitrophenyl)aziridine (1·0 g) was heated on a steam-bath for 2 h, and then boiled with ethanol (50 ml). The ethanol-insoluble *pyridylethylenediamine* (0·4 g) crystallised from

aqueous dimethylformamide with m.p. 246—248° (Found: C, 48·8; H, 4·7; N, 22·0. C$_{13}$H$_{13}$N$_5$O$_4$,H$_2$O requires C, 48·5; H, 4·7; N, 21·9%). An unstable red solid (0·8 g), m.p. 105—108° (efferv.), slowly precipitated from the ethanolic solution. This red solid, which is probably the imine (34), rearranged to the pyridylethylenediamine in cold 2N-sodium hydroxide (24 h).

The 1-(2,4-dinitrophenyl)aziridine (1·0 g) reacted vigorously with pyridine (1 ml) at 100° (1 h) to afford a black solid. When this solid was dissolved in dimethylformamide, addition of an excess of ethanol precipitated a brown solid (1·0 g), identical (i.r.) with the polymer (18). The same polymer was formed from the aziridine and pyridine (1 mol. equiv.) in boiling benzene (12 h), boiling toluene (12 or 24 h), or boiling xylene (4 or 8 h) in 5, 80, 95, 60, or 100% yield, respectively.

Reaction of 1-(2,4-dinitrophenyl)aziridine and 4-amino-pyridine (1 mol. equiv.) in boiling butan-1-ol (4 h) or toluene (12 h) afforded the same polymer in 75 or 80% yield, respectively.

1-(2,4-dinitrophenyl)aziridine and 3-aminopyridine (1 mol. equiv.) in boiling acetone (4 h), butan-1-ol (8 h), or toluene (8 h) yielded polymer in 20, 50, or 60% yield, respectively.

Ammonium and anilinium toluene-p-sulphonates were recovered unchanged after being heated with 1-(2,4-dinitrophenyl)aziridine at 100° for 1 h.

Sodium 2,4-*dinitrophenoxide*. The brown solution formed when 1-(2,4-dinitrophenyl)aziridine (2·0 g) was boiled in 2N-sodium hydroxide (50 ml) for 1 h deposited a yellow precipitate of the phenoxide (1·5 g), identical (i.r.) with an authentic sample.

Thermolysis and Photolysis.—Bis-[2-(2,4-*dinitrophenyl*)-*ethyl*] *ether* (35). The brown suspension formed when 1-(2,4-dinitrophenyl)aziridine (1·0 g) was boiled with acidic alumina (10·0 g) in anhydrous xylene (40 ml) for 4 h was filtered to remove alumina. The yellow solid (0·2 g) deposited was repeatedly crystallised from xylene and afforded the *ether*, m.p. 181—183° (Found: C, 44·4; H, 4·0; N, 19·2. C$_{16}$H$_{16}$N$_6$O$_9$ requires C, 44·1; H, 3·7; N, 19·3%). Extraction of the alumina with refluxing acetone afforded more of the ether (0·5 g) when the acetone solution was diluted with water. The same ether (60 and 72% yields, respectively) was formed when the aziridine was boiled with acidic alumina in acetone or toluene, or basic alumina in xylene. The aziridine was recovered unchanged after 6 h boiling in toluene, xylene, o-dichlorobenzene, or decalin.

1-(2,4-Dinitrophenyl)aziridine (2·0 g) in methanol (1 l) was irradiated at 25° for 48 h. No visible change was observed in the solution, and the position and intensity of the long-wavelength band (327 nm) did not change. The lamp was extinguished and the solution was exposed to laboratory light at 25° for 48 h. Evaporation of a 500 ml sample of the photolysate afforded 1-(2-methoxyethyl-amino)-2,4-dinitrobenzene (25%), the remainder being unchanged aziridine; after 30 days, the yield of the methoxy-ethylamine had increased to 60%.

A solution of the aziridine (2·0 g) in methanol (1 l) containing 98% formic acid (1 ml) was exposed to laboratory light for 48 h. A sample of the solution was irradiated in a quartz cuvette for 48 h. There was no change in the electronic absorption spectrum of the sample (λ_{max} 350 nm)

[24] A. T. James and R. L. M. Synge, *Biochem. J.*, 1951, **50**, 109.
[25] E. F. Milliaresi, V. A. Izmail'skiv, and V. V. Efremov, *Zhur. obshchei Khim.*, 1967, **37**, 1055.

[26] A. Hunger, J. Kebrle, A. Rossi, and K. Hoffmann, *Helv. Chim. Acta*, 1960, **43**, 1032.

during this irradiation. The remainder of the original solution was concentrated to 50 ml; crystals of 1-(2-methoxyethylamino)-2,4-dinitrobenzene (65%) were deposited.