Azidoacridines: Potential Nucleic Acid Mutagens

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Thermolysis of 9-azidoacridine (free base) in boiling nitrobenzene or *o*-dichlorobenzene, or photolysis in methanol, affords 9,9'-azoacridine in high yield; thermolysis in boiling decalin yields 9-aminoacridine. The formation of these products probably involves reactive nitrene intermediates. In contrast, the azide groups of 9-azidoacridine hydrochloride and 9-azido-10-methylacridinium methyl sulphate are readily displaced as hydrazoic acid by nucleophiles. The hydrochloride and methyl quaternary salt are rapidly hydrolysed to acridone and *N*-methylacridone respectively in water, and react with aromatic amines (in the former case), and with aromatic and aliphatic amines (in the latter case) to afford substituted 9-aminoacridines. 3-Azidoacridine and its salts are more stable than the 9-azido-analogues.

THE investigations of Albert and his colleagues [1,2] on the relationship between structure and biological activity of the antibacterial acridines have established that only those acridines which are substantially ionised at physiological pH (*i.e.* with $pK_a > 7{\cdot}8$) are active. Structural features in the molecule which decrease the basicity, or distort the planar character of the acridine nucleus, have a dyschemotherapeutic effect. The antibacterial and mutagenic properties of aminoacridines and related compounds are thought to be a consequence of their ability to ' intercalate ' DNA, although the exact geometry of this association remains a subject of controversy.[3–10]

Recent interest in the use of azides (or nitrenes derived therefrom) to label the binding regions of proteins [11–14] has in the present work been extended to the field of potential nucleic acid inhibitors, since the generation of a reactive nitrene intermediate within the DNA double helix might be expected to disorganise the functions of the macromolecule.[15]

9-Amino- and 3-amino-acridine are amongst the most active of the antibacterial acridines [1,2] and the prospect of comparing the biological properties of the amino-compounds and the 9- and 3-azido-analogues has motivated our examination of the chemical properties of these azides.

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[2] A. Albert, ' Selective Toxicity,' Methuen, London, 1965.
[3] A. R. Peacocke, *Chem. and Ind.*, 1969, 642.
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[6] G. Löber, *Z. Chem.*, 1969, **9**, 252.
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[9] L. S. Lerman, *J. Mol. Biol.*, 1961, **3**, 18.
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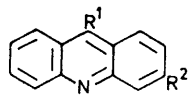
An attempt to synthesise 9-azidoacridine (Ia) from the appropriate diazonium salt and sodium azide proved unsuccessful because of difficulties experienced in the diazotisation procedure; evidently 9-aminoacridine (Ib) is not diazotised under normal conditions.[16] However, 9-hydrazinoacridine (Ic) and sodium nitrite in 2N-hydrochloric acid afforded 9-azidoacridine hydrochloride, from which the base (Ia) was recovered in high yield. A Patent [17] published during this work claimed a yield of 85% of azidoacridine (Ia) from 9-chloroacridine (Id) and sodium azide in refluxing aqueous acetone; we have verified the success of this route. 3-Azidoacridine (Ie) was prepared by diazotisation of 3-aminoacridine (If) in acetic acid, followed by treatment with excess of sodium azide.

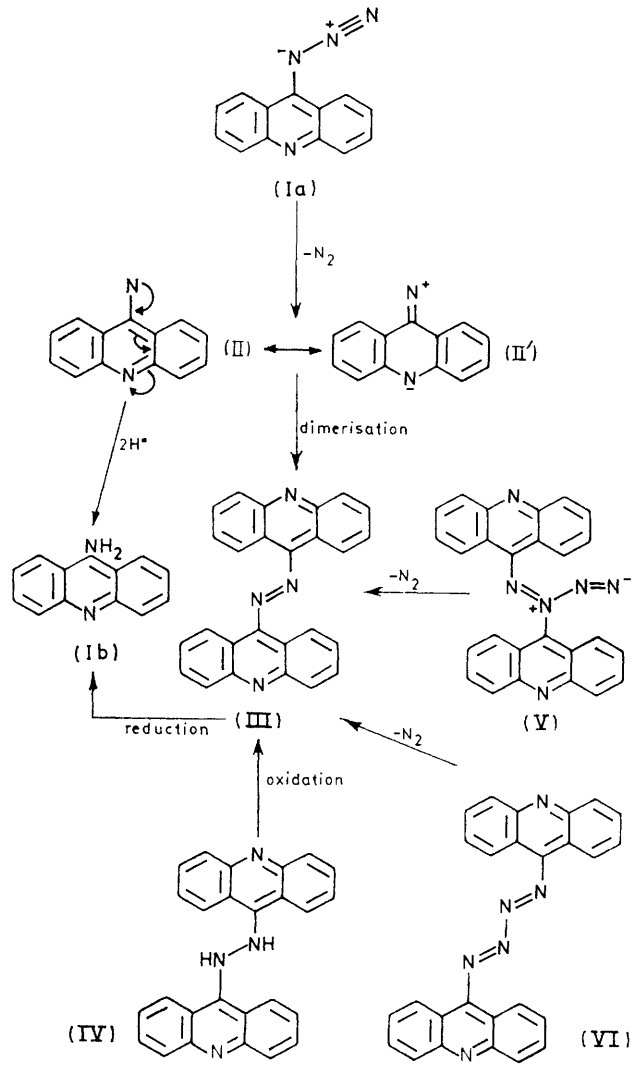


(I)

	R^1	R^2		R^1	R^2
a;	N_3	H	g;	PhNH	H
b;	NH_2	H	h;	p-MeC_6H_4·NH	H
c;	NH·NH_2	H	i;	p-ClC_6H_4·NH	H
d;	Cl	H	j;	3-Pyridylamino	H
e;	H	N_3	k;	2-Pyridylamino	H
f;	H	NH_2			

9-Azidoacridine (Ia) decomposed vigorously at 150° affording a red solid. T.l.c. of the products confirmed the presence of substantial amounts of 9,9′-azoacridine (III). Controlled decomposition in *o*-dichlorobenzene or nitrobenzene at reflux temperature gave the pure azo-compound in 70% yield. The structure of the azoacridine (III) was confirmed by its synthesis, by oxidation of 9,9′-hydrazoacridine (IV), and by its reduction to 9-aminoacridine (Scheme 1). The mass spectrum showed the appropriate molecular ion at *m/e* 384 which loses $[C_{13}H_8N_3]^{\cdot}$ to give an abundant ion of *m/e* 178, $[C_{13}H_8N]^+$; aryl carbonium ion peaks are a common feature in the mass spectra of azobenzenes.[18]

Smith [19] has stated that the formation of azo-compounds by direct dimerisation of highly reactive nitrene species formed in low concentration is improbable unless the nitrene is of sufficiently low reactivity to survive repeated collisions with solvent molecules before encountering another nitrene with which to combine. Formation of the azoacridine (III) is completely suppressed in boiling decalin; the product, 9-aminoacridine (Ib), presumably results from hydrogen abstraction by the triplet species of 9-nitrenoacridine (II). Photolysis of 9-azidoacridine in methanol also afforded the azoacridine (III). The high yield (81%) may be a consequence of co-ordination of the singlet nitrene by the unshared electron pairs in methanol, which stabilises the nitrene sufficiently to allow dimerisation.[20] Furthermore, the nitrene is prohibited from undergoing the type of intramolecular insertion at the *ortho*-position which has been invoked to explain the formation of 3*H*-azepines in the photolysis of *o*-azidobenzamides in methanol; [12,13] both *ortho*-positions are involved in ring annellation.



SCHEME 1

In contrast, photolysis of 3-azidoacridine (Ie) in methanol was not a clean reaction. In addition to substantial amounts of starting material, at least seven products were detected (t.l.c.).

Alternative mechanisms for azoacridine formation are possible. Smith [19] argues that reaction of an aryl nitrene with an undecomposed molecule of azide is a statistically more probable process than direct dimerisation. Resonance interaction with the nuclear nitrogen could stabilise the singlet state [21] of 9-nitrenoacridine [(II) ⟷ (II′)], and its reaction with undissociated

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[17] F.P. 1,511,485/1968 (*Chem. Abs.*, 1969, **70**, 12,004*l*).
[18] J. H. Bowie, G. E. Lewis, and R. G. Cooks, *J. Chem. Soc.* (*B*), 1967, 621.
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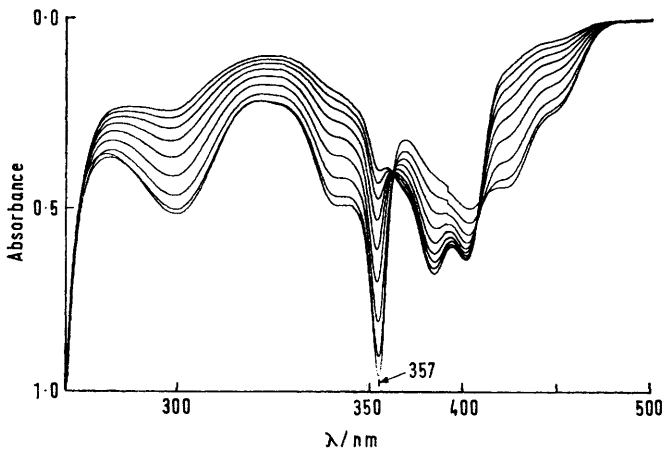
[20] L. Horner, A. Christmann, and A. Gross, *Chem. Ber.*, 1963, **96**, 399.
[21] P. A. S. Smith and J. H. Hall, *J. Amer. Chem. Soc.*, 1962, **84**, 480.

azide to give either a 1,2- (V) or a 1,4-substituted tetrazadiene (VI) can be envisaged; both tetrazadienes could subsequently give the azoacridine by loss of nitrogen.

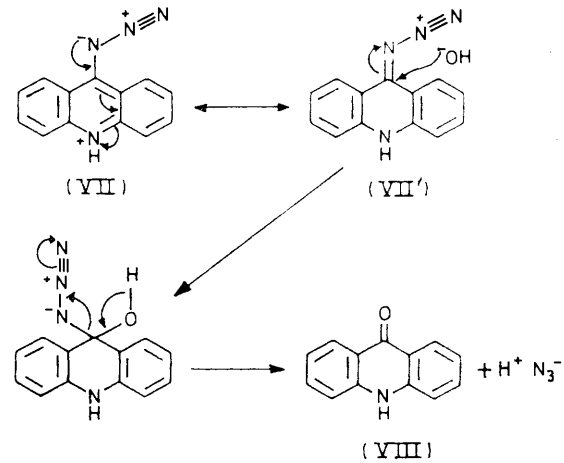
The mass spectra of 9- and 3-azidoacridines were very similar, and showed molecular ions of low intensity. The only peaks of any significance occurred at *m/e* 192 (M — 28) and 194 (M — 26), the former corresponding to those expected for loss of nitrogen to give the nitrenes. Peaks corresponding to M — 26 have been previously observed in the mass spectra of azidopyrimidines,[22] and have been shown to arise by thermal fragmentation of the azides to give nitrenes which by H abstraction give the amines in an overall process: (M — N_2) + 2H.

9-Azidoacridine free base was recovered unchanged following attempted nucleophilic displacement of the azide group by boiling water, isopropylamine or aniline. However, protonation of the endocyclic nitrogen atom in 2N-hydrochloric acid afforded a hydrochloride which was very sensitive to nucleophiles, and had properties dominated by loss of the azide group as hydrazoic acid. For example, in water 9-azidoacridine hydrochloride was hydrolysed to acridone (VIII), and the reaction was readily followed (see Figure) by observing the disappearance of the characteristic absorption at 357 nm (half-life at 60° *ca.* 120 min). Similar lability of an azido-group has been reported in the *N*-oxide and quaternary salts of 4-azidoquinoline.[23]



Decomposition of 9-azidoacridine hydrochloride in water at 60° recorded at 20 min intervals on a Perkin-Elmer 402 spectrometer

The electronic influence of the azido-group in a benzene ring has been compared to those of the fluoro- and acylamino-groups.[24] It exerts an electron-donating electromeric effect (azidobenzene is more reactive than benzene to electrophilic attack, and the substituents enter the *o*- and *p*-positions) and an electron-attracting inductive effect. The powerful electron-attracting character of the protonated endocyclic nitrogen atom [(VII) ◄► (VII')] activates the 9-position to nucleophilic attack (Scheme 2). Thus, 9-azidoacridine hydrochloride reacted smoothly with the weakly basic aromatic amines aniline (pK_a 4·58),[25] *p*-toluidine (5·12), *p*-chloroaniline (3·98), and 3-aminopyridine (5·98) to liberate hydrazoic acid and afford high yields of the substituted



SCHEME 2

9-aminoacridines (Ig—j) as their hydrochlorides; only proton exchange occurred with the strongly basic aliphatic amines isopropylamine (pK_a 10·63) and aziridine (8·04), and 9-azidoacridine free base was recovered.

Albert [1,2] has established that the biological activity of acridine derivatives is directly related to their degree of ionisation (as cations). If it is assumed that the basicity of 9-azidoacridine is comparable to that of the parent heterocycle acridine (pK_a 5·60), the azide would be insufficiently ionised at physiological pH values to intercalate DNA (unless unknown factors intervened); quaternary salts of 9-azidoacridine on the other hand would be completely ionised.

Interaction of 9-azidoacridine (Ia) and dimethyl sulphate in anhydrous benzene yielded 9-azido-10-methylacridinium methyl sulphate (IX); the purification of this salt was thwarted by its extreme sensitivity to traces of water in the atmosphere. Attempted crystallisation from ' anhydrous ' alcoholic solvents always led to the isolation of substantial amounts of *N*-methylacridone (X); no ethers were isolated. Study of the decomposition of the salt in water by u.v. spectroscopy gave a series of absorption curves similar to those of the azide hydrochloride, and indicated a half-life of 20 min at 60° (measured at 360 nm). Eventually the spectrum was identical with that of *N*-methylacridone. The increased reactivity of the quaternised (compared to protonated) azidoacridine was reflected in its reactivity towards amines. A vigorous effervescence (hydrazoic acid) was

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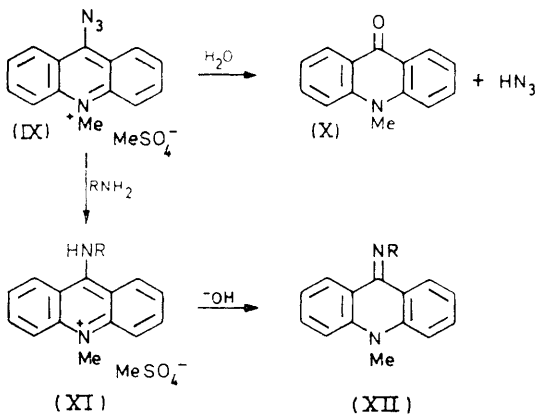
[23] S. Kamiya, *Chem. and Pharm. Bull.* (*Japan*), 1962, **10**, 669.
[24] P. A. S. Smith, J. H. Hall, and R. O. Kan, *J. Amer. Chem. Soc.*, 1962, **84**, 485.
[25] All pK_a values referred to in this paper are quoted from ' Ionisation Constants of Acids and Bases,' A. Albert and E. P. Serjeant, Methuen, London, 1962.

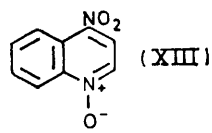
observed when the sulphate (IX) was stirred with either weak or strong bases [aniline, 2-aminopyridine (pK_a 6·86), or isopropylamine]. The quaternary salts thus formed (XI; R = Ph, 2-pyridyl, or Pr^i) were characterised as the anils (XII; R = Ph, 2-pyridyl, or Pr^i), which were deposited when the crude salts were basified with aqueous ammonia (Scheme 3).



SCHEME 3

In contrast to the instability of the 9-azido-isomer, 3-azidoacridine hydrochloride was recovered (as the free base) from boiling water (3 h), or after being heated with aniline or 2-aminopyridine. 3-Azido-10-methylacridinium methyl sulphate likewise appeared substantially more stable than the 9-azido-isomer, and could be purified by crystallisation from ethanol–ether without decomposition.

Although initially we were interested in examining the possibility of interposing reactive nitrene intermediates into nucleic acids, the lability of the azide group of 9-azido-10-methylacridinium methyl sulphate towards nucleophiles suggests that this compound could prove to be a nucleic acid mutagen operating by a different mechanism. If the quaternary salt could successfully intercalate DNA, it should subsequently act as an 'hetarylating agent' by reaction with a suitably positioned nucleophile. The biological consequences of this covalent interaction could be similar to those elicited by the cytotoxic 4-nitroquinoline *N*-oxide (XIII) which is bound to DNA [26] and is also sensitive to nucleophiles.[27]



(XIII)

The results of a comparative study of the biological activities of the compounds described here against bacterial cultures and murine tumours will be published elsewhere.

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[27] T. Kurihara, H. Ichimura, T. Igaki, and A. Ohta, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 37.

EXPERIMENTAL

9-Azidoacridine.—9-Hydrazinoacridine [28] (2·7 g) in 2N-hydrochloric acid (50 ml) was diazotised at 0° with aqueous sodium nitrite (1·5 g in 10 ml). The yellow *hydrochloride* (2·5 g) was collected, washed with ether, and crystallised from ethanol–ether to give rosettes, m.p. 270° (decomp.); $\nu_{max.}$ (KBr) 2140 cm^{-1} (N_3) (Found: C, 60·8; H, 3·6; N, 21·9. $C_{13}H_8N_4$,HCl requires C, 60·8; H, 3·5; N, 21·8%). The *free base* crystallised from light petroleum (b.p. 40—60°) as pale yellow plates, m.p. 85—87° (with effervescence to give a red solid) (Found: C, 70·6; H, 3·7; N, 25·8. $C_{13}H_8N_4$ requires C, 70·9; H, 3·6; N, 25·5%). The base had an i.r. spectrum [$\nu_{max.}$ (KBr) 2140 cm^{-1} (N_3)] identical with that of the compound prepared by treating 9-chloroacridine with sodium azide in refluxing aqueous acetone.[17]

Properties of 9-Azidoacridine.—(i) The azide (0·5 g) dissolved in *o*-dichlorobenzene (10 ml) was added dropwise to refluxing *o*-dichlorobenzene (15 ml). The solution was boiled for 1 h, then cooled, and a solid was collected. Crystallisation from nitrobenzene afforded red needles of 9,9′-azoacridine (0·3 g), m.p. 320—322°, the mass spectrum of which showed a molecular ion at *m/e* 384 (required *M*, 384), and was identical with that of the product formed by oxidising 9,9-hydrazoacridine [28] with potassium permanganate in ethanol.[29] Reduction of the azo-compound with zinc powder in refluxing acetic acid afforded 9-aminoacridine (80%).

(ii) Thermolysis of 9-azidoacridine in refluxing nitrobenzene (1 h) gave 9,9′-azoacridine (70%).

(iii) Photolysis of 9-azidoacridine (1·7 g) in methanol (1 l) with an unfiltered medium-pressure arc in a Hanovia photochemical reactor led to the immediate evolution of nitrogen and the deposition of a brown solid. When gas evolution was complete (2 h) the solid was collected and crystallised from nitrobenzene to afford red needles of 9,9′-azoacridine (1·3 g). The same product (60%) was formed when 9-azidoacridine was photolysed in benzene (10 h).

(iv) A suspension of 9-azidoacridine (2·0 g) in cold decalin (50 ml) was added dropwise (20 min) to refluxing decalin (20 ml). The mixture was boiled for a further 1 h and cooled. The precipitated solid (1·2 g), when crystallised from acetone was identical (i.r.) with an authentic sample of 9-aminoacridine.

(v) Reduction of 9-azidoacridine (1·0 g) in ethanol (50 ml) at 60—70° with Raney nickel (1 g) and hydrazine hydrate (5 ml; in portions) for 2 h yielded 9-aminoacridine (0·7 g).

(vi) Acridone (0·8 g) was deposited when a solution of 9-azidoacridine (1·0 g) in 2N-hydrochloric acid (20 ml) was boiled (1 h) and then cooled.

(vii) 9-Azidoacridine was recovered unchanged when boiled with water (4 h), or heated with aniline or isopropylamine in refluxing tetrahydrofuran.

Properties of 9-Azidoacridine Hydrochloride.—(i) The hydrochloride was efficiently prepared by suspending 9-azidoacridine in ether, adding sufficient absolute ethanol to produce a clear solution, and passing dry hydrogen chloride through it. When the hydrochloride thus formed was boiled in water (30 min) acridone was quantitatively formed.

(ii) The hydrochloride was refluxed in excess of iso-

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[29] G. Cauquis and G. Fauvelot, *Bull. Soc. chim. France*, 1964, **8**, 2014.

propylamine or aziridine (1 h). 9-Azidoacridine (free base) was recovered when the mixture was evaporated.

9-*Anilinoacridine*.—Hydrazoic acid was evolved when 9-azidoacridine hydrochloride (0·2 g) and aniline (0·5 ml) were heated (10 min) on a steam-bath. 9-Anilinoacridine hydrochloride (0·30 g) was collected and converted into the base with aqueous ammonia. 9-Anilinoacridine thus formed (0·23 g) crystallised from ethanol as yellow needles, m.p. 224—226° (lit.,[16] 224°).

9-p-*Methylanilinoacridine Hydrochloride*.— Prepared (95%) by heating 9-azidoacridine hydrochloride and *p*-toluidine, the hydrochloride crystallised from ethanol as yellow needles, m.p. 282—284° (lit.,[30] 285°).

9-p-*Chloroanilinoacridine Hydrochloride*.—Prepared from 9-azidoacridine hydrochloride and *p*-chloroaniline (1 mol. equiv.) at 100°, this hydrochloride (95%) crystallised from ethanol as brown needles, m.p. 238—240° (lit.,[31] 275—277°), identical to the product formed by heating 9-chloroacridine and *p*-chloroaniline at 150°.

9-(3-*Pyridylamino*)*acridine Hydrochloride*.—Formed by heating 9-azidoacridine hydrochloride (0·85 g) and 3-aminopyridine (0·4 g) on a steam-bath (10 min), the *hydrochloride* crystallised from ethanol–ether as orange-red rosettes (1·1 g), m.p. 288—290° (Found: C, 69·9; H, 4·5; N, 13·5. $C_{18}H_{13}N_3$,HCl requires C, 70·2; H, 4·5; N, 13·6%).

9-*Azido*-10-*methylacridinium Methyl Sulphate*.—A solution of 9-azidoacridine (2·5 g) in anhydrous benzene (100 ml) was shaken with dimethyl sulphate (3 ml) for 4 days at room temperature. The *sulphate* (2·0 g) was deposited as a brown crystalline solid, m.p. 120° (decomp.), $\nu_{max.}$ (KBr) 2140 cm^{-1} (N_3).

Properties of 9-*Azido*-10-*methylacridinium Methyl Sulphate*.—(i) The sulphate was insoluble in the non-polar solvents benzene, toluene, and cyclohexane; attempted crystallisation from carefully dried polar solvents, (ethanol, n-butanol, propan-2-ol, propan-1-ol, or dimethyl sulphoxide) produced substantial amounts of *N*-methylacridone.

(ii) When the sulphate (1·0 g) was refluxed (15 min) in water (20 ml), *N*-methylacridone (0·8 g) was deposited.

9,10-*Dihydro*-10-*methyl*-9-*phenyliminoacridine*.— 9-Azido-10-methylacridinium methyl sulphate (0·3 g) and aniline (0·2 g) were warmed (50°) for 30 min. The mixture was basified with aqueous ammonia and products extracted into chloroform. The dried (Na_2SO_4), evaporated chloroform extract furnished a gum which crystallised from aqueous ethanol as golden needles (0·3 g), m.p. 158—160° (lit.,[32] 162°).

9,10-*Dihydro*-10-*methyl*-9-(2-*pyridylimino*)*acridine*.— This *iminoacridine* (90%), similarly prepared from the sulphate and 2-aminopyridine (1 mol. equiv.), crystallised from light petroleum as orange needles, m.p. 153—154° (Found: C, 79·8; H, 5·3; N, 15·0. $C_{19}H_{15}N_3$ requires C, 79·8; H, 5·3; N, 15·0%).

9,10-*Dihydro*-9-*isopropylimino*-10-*methylacridine*.— Similarly prepared from the sulphate and isopropylamine, the *acridine* crystallised from light petroleum as white prisms (70%), m.p. 86—87° (Found: C, 81·8; H, 7·2; N, 11·2. $C_{17}H_{18}N_2$ requires C, 81·6; H, 7·2; N, 11·2%).

3-*Azidoacridine*.—A solution of 3-aminoacridine [33] (3·0 g) in acetic acid (30 ml) was diazotised at 0° with aqueous sodium nitrite (1·2 g in 5 ml). Sodium azide (2·0 g) was added to the mixture during 30 min and stirring was continued (3 h). The red precipitate formed when the mixture was poured into excess of aqueous ammonia, was collected and crystallised from light petroleum (b.p. 60—80°). The *azide* (1·5 g), m.p. 113—115°, formed yellow needles, $\nu_{max.}$ (KBr) 2110 cm^{-1} (N_3) (Found: C, 70·5; H, 3·9; N, 25·8. $C_{13}H_8N_4$ requires C, 70·9; H, 3·6; N, 25·5%). The base crystallised from 10N-hydrochloric acid to give a *hydrochloride monohydrate* (Found: C, 56·6; H, 4·0; N, 20·4. $C_{13}H_8N_4$,HCl,H_2O requires C, 56·8; H, 4·0; N, 20·3%). An unsolvated *hydrochloride*, formed when hydrogen chloride was passed into an ethereal solution of the base, crystallised from methanol–ether as brown prisms, m.p. 192—194° (decomp.) (Found: C, 60·7; H, 3·5; N, 21·6. $C_{13}H_8N_4$,HCl requires C, 60·8; H, 3·5; N, 21·8%).

Properties of 3-*Azidoacridine* (*Base and Hydrochloride*).—(i) 3-Azidoacridine base (1·0 g) was photolysed in methanol (1 l) for 72 h. The azide (0·3 g) was recovered, together with a brown solid (0·4 g). T.l.c. of this solid indicated that it contained at least seven components.

(ii) 3-Azidoacridine hydrochloride (0·3 g) was boiled in water (10 ml) for 3 h. 3-Azidoacridine base (0·25 g) was obtained when the solution was basified with aqueous ammonia.

(iii) 3-Azidoacridine hydrochloride was heated (2 h at 100°) with 2-aminopyridine or aniline (1 mol. equiv.). Extraction of the mixtures with light petroleum afforded 3-azidoacridine base, quantitatively.

3-*Azido*-10-*methylacridinium Methyl Sulphate*.—This *sulphate* (70%), prepared by the procedure used for the synthesis of the 9-azido-analogue, crystallised from ethanol–ether as brown rosettes, m.p. 156—158° (decomp.), $\nu_{max.}$ (KBr) 2140 cm^{-1} (N_3) (Found: C, 51·8; H, 4·2; N, 16·0. $C_{15}H_{14}N_4O_4S$ requires C, 52·0; H, 4·1; N, 16·1%).

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[33] A. Albert, *J. Chem. Soc.*, 1948, 1225.