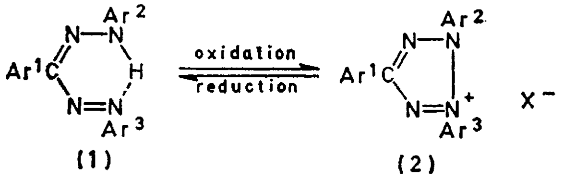
# The Oxidative Cyclization of Formazans to Tetrazolium Salts

By **Anthony F. Hegarty,*** **John H. Coy,** and **Francis L. Scott,** Chemistry Department, University College, Cork, Ireland

Bromine oxidizes 1,3,5-triarylformazans (1) quantitatively to the corresponding 2,3,5-triaryltetrazolium salts (2) in methanol or aqueous acetic acid. The reaction is ionic and is markedly aided by electron donation in the aryl rings. In methanol containing 0·1M-sodium bromide (at 25°) substituents in the *C*-aryl ring give a $\rho$ value of −1·40, while those at N-1 and -5 give $\rho$ −1·10 and −2·42. A mechanism of oxidation is proposed involving electrophilic attack by bromine at carbon and formation of a covalent intermediate, followed by unimolecular C–Br bond cleavage to yield the tetrazolium salt.

THE most widely used reaction in formazan chemistry is the ready oxidation to give tetrazolium salts (2). As such this reaction provides the only synthetically useful route to tetrazolium salts, which are widely used as biological staining agents. The salts, which are generally colourless and water soluble, may be reduced *in situ* to the highly coloured insoluble formazans (1).[1,2]

Oxidants which have been widely used for the conversion of (1) into (2) include fuming nitric acid, mercuric oxide, isopentyl nitrite under acidic conditions, lead tetra-acetate, halogeno-amides, and t-butyl hypohalite. Other oxidizing agents have been used but in some cases lead to insoluble tetrazolium salts which are inconvenient for further work. Molecular chlorine rapidly decolourizes formazan solutions but also effects nuclear chlorination.



The mechanism by which formazans are oxidized to tetrazolium salts remains unclear. Earlier studies have concentrated on the reverse reaction [conversion of (2) into (1)]. Some comparative studies were carried out by Wedekind,[3] centring on the effect of groups attached to the 3-carbon atom ($Ar^1$). Electrochemical techniques have also been applied to this reaction, mainly in the 1950s and this has been reviewed.[4] Polarography has contributed little to the question of intermediates between tetrazolium salts and formazans; however, e.s.r. measurements have identified the presence of free radical species under aerial oxidation. These radicals have been isolated and studied in some detail.[5] Studies by Maender and Russell indicate a ready one-electron oxidation of the formazan anion or reduction of the tetrazolium salt.[6]

We have examined in detail the oxidation of a wide range of formazans (1) in which the aryl groups $Ar^1$—$Ar^3$ were varied using bromine as oxidizing agent. The reactions are rapid and the disappearance both of formazan (using a spectrophotometric technique) and of bromine (electrometrically) were followed.

## RESULTS AND DISCUSSION

Triarylformazans are readily decolourized by bromine in methanolic or glacial acetic acid solution. Several quantitative experiments showed that only one mol. equiv. of bromine was consumed and that the solution was completely decolourized during reaction; the reaction followed is therefore the conversion of (1) into (2) and concomitant nuclear bromination (in $Ar^2$ or $Ar^3$) can be ruled out. The rates of bromination of a series of substituted triarylformazans (1) were investigated in methanol containing 0·1M-sodium bromide. Studies at various initial bromine and formazan concentrations showed that the reaction was second order overall, first order in both bromine and formazan (Table 1).

TABLE 1

Second-order rate constants for the oxidative bromination of 1,3,5-triphenylformazan (1; $Ar^1 = Ar^2 = Ar^3 =$ Ph) in methanol containing 0·1M-sodium bromide (at 25°)

| $10^5$[Formazan]/M | $10^5$[Bromine]/M | $k_2$/l mol$^{-1}$ s$^{-1}$ |
|---|---|---|
| 10 | 0·50 | 287 |
| 20 | 0·50 | 299 |
| 40 | 0·50 | 297 |
| 3·0 | 4·0 | 319 |
| 0·50 | 20 | 314 |
| 0·50 | 40 | 318 |
| | | Average 304 ± 10 |

The low concentrations at which the reaction was studied and more particularly the large excess of bromide ion obviated the intervention of a more complicated kinetic pattern with kinetic terms of higher order in bromine.[7] Under the conditions used, the potential brominating species are molecular bromine and, possibly, tribromide ion. The latter was shown to be an ineffective reagent in the present instance as follows. The observed second-order rate constants for the bromination of the formazan ($k_2$) may be represented as the composite function (1)[8] where $k$ and $k^1$ are the second-order rate constants for the reaction of the formazan with bromine and tribromide ion respectively and $K$ is the equilibrium constant for the formation of tribromide ion.

$$k_2 = (k + k^1K[\mathrm{Br}^-])/(1 + K[\mathrm{Br}^-]) \qquad (1)$$

A plot (not shown) of $k_2(1 + K[\mathrm{Br}^-])$ against $[\mathrm{Br}^-]$ which was varied from 0·20 to 0·01M at constant ionic

---

[1] A. W. Nineham, *Chem. Rev.*, 1955, **55**, 404.

[2] W. D. Hooper, *Rev. Pure Appl. Chem.*, 1969, **19**, 221.

[3] E. Wedekind and A. Stavwe, *Ber.*, 1898, **31**, 1746.

[4] B. Jambor, 'Tetrazoliumsalze in der Biologie,' Fischer, Jena, 1960.

[5] F. A. Neugebauer, *Tetrahedron*, 1970, **26**, 4843.

[6] O. W. Maender and G. A. Russell, *J. Org. Chem.*, 1966, **31**, 442.

[7] P. B. D. de la Mare and J. H. Ridd 'Aromatic Substitution,' Butterworths, London, 1959, ch. 9.

[8] A. F. Hegarty and F. L. Scott, *J. Chem. Soc. (B)*, 1966, 672.

strength (maintained by sodium nitrate) gave a straight line with zero slope; this implies that $k^1 = 0$. The reaction was also studied under pseudo first-order conditions with either bromine or formazan initially in at least a 20-fold excess. The resulting rate constants ($k_2$ values, obtained at constant $[Br^-]$) were in agreement (Table 1).

The observed rate constants obtained for the bromination of two series of triarylformazans in methanol under standard conditions are summarized in Table 2.

TABLE 2

Second-order rate constants (l $mol^{-1}$ $s^{-1}$) for the bromination of (a) 1,5-diphenyl-3-arylformazans (1; $Ar^1 = XC_6H_4$; $Ar^2 = Ar^3 =$ Ph) and (b) 1-aryl-3,5-diphenylformazans (1; $Ar^1 = Ar^3 =$ Ph; $Ar^2 = YC_6H_4$) in methanol containing 0·1M-sodium bromide (at 25°)

| | Substituent (X or Y) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Substrate | 4-Me | 3-Me | H | 4-Br | 3-Br | 3-Cl | $3-NO_2$ | $4-NO_2$ |
| (a) | 726 | 544 | 304 | 209 | 146 | | 76 | |
| (b) | 643 | | 304 | 160 | | 129 | 64 | 25 |

Substituents were varied (independently) in the 1-($Ar^2$) and 3-positions ($Ar^1$). $Ar^2$ and $Ar^3$ in the formazan structure are equivalent, the actual structure of (1) being that of a rapidly equilibrating pair of tautomers.[9, 10] In fact, the rate of bromination of formazan (1) was shown to be independent of the method used for its synthesis (*e.g.* hydrazone $Ar^1CH{=}N{-}NH{-}Ar^2$ and diazonium ion $Ar^3N_2^+$ gave a formazan with the same rate of bromination as that prepared from $Ar^1CH{=}N{-}NH{-}Ar^3$ and $Ar^2N_2^+$).

Substituents in $Ar^1$ and in $Ar^2$ which are electron-withdrawing retard the conversion of (1) into (2) while electron-releasing substituents have an opposite effect. The magnitude of the substituent effect is similar in both cases. Quantitatively, a plot of log $k_2$ *versus* the $\sigma$ values of McDaniel and Brown[11] for the 3-arylformazans (1; $Ar^1 = XC_6H_4$; $Ar^2 = Ar^3 =$ Ph) gave a $\rho$ value of $-1{\cdot}40$ ($r$ 0·989).

Substituents in the 1-aryl ring ($Ar^2$) have a similar effect. A Hammett plot of log $k_2$ *versus* $\sigma$ in this case gave a $\sigma$ value of $-1{\cdot}10$ ($r$ 0·990).

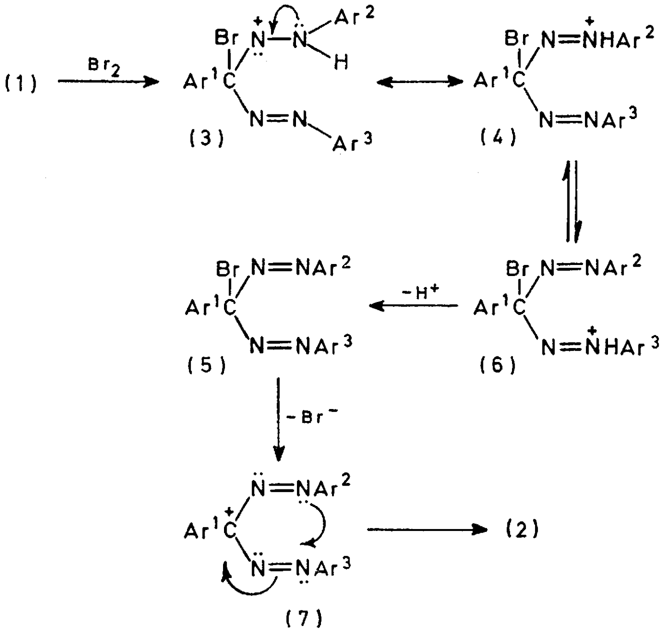
The magnitudes of the two $\rho$ values obtained (see also below) argue in favour of an ionic mechanism for the oxidative bromination. Since there is considerable build-up of charge on $Ar^1$ and $Ar^2$ the whole molecule (*i.e.* including $Ar^3$ and the nitrogen chains) must bear something close to a full positive charge in the transition state. The clean and reproducible second-order kinetics also disfavour a free radical or chain mechanism. Moreover, the same rate constants were obtained when the reaction media were irradiated (u.v. or visible light) or when the reactions were carried out in the dark. The rates of oxidative bromination are also sensitive to the ionizing power of the medium. Thus, the rates of cyclization of (1) to (2) increase, on average, 14-fold when the medium is changed from methanol to 70 : 30 acetic acid–water (Table 3). It has been demonstrated that the electrophilic addition of bromine to alkenes is characterized by Grunwald–Winstein *m* values of $\geqslant 1{\cdot}0$; that is, the reaction is about as sensitive to the ionizing power of the medium as an $S_N1$ reaction.[12]

TABLE 3

Second-order rate constants (l $mol^{-1}$ $s^{-1}$) for the bromination of 1,5-diphenyl-3-arylformazans (1; $Ar^1 = XC_6H_4$; $Ar^2 = Ar^3 =$ Ph) in 70 : 30 acetic acid–water containing 0·1M-potassium bromide at 25°

| X | 4-Me | H | 4-Br | $4-NO_2$ |
|---|---|---|---|---|
| $k_2$ | 3600 | 4300 | 900 | 800 |

The mechanism of Scheme 1 is proposed to account for these observations. Initial electrophilic attack at the carbon of the formazan occurs to give the stabilized ions (3)—(5). The relatively large contribution of substituents on $Ar^2$ to the overall stabilization of the intermediate indicates that (4) and (6) are the important structures. Proton loss gives the bisazo material (5). Formation of the tetrazolium salt from (5) can be envisaged by the following route: loss of bromide ion gives the stabilized azocarbocation (7) which on cyclization gives (2).



SCHEME 1

The initial bromination step (1) ⟶ (3) is analogous to the rate-determining step in the bromination of hydrazones (8) (Scheme 2). In this case substituents in $Ar^2$ have a larger effect ($\rho$ $-2{\cdot}2$) on reactivity than those in $Ar^1$ ($\rho$ $-0{\cdot}62$).[8] However, there is another possibility for charge delocalization in the formazan

---

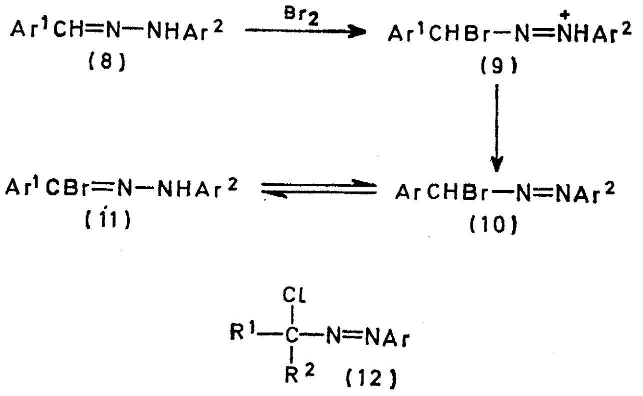
[9] G. V. Tiers, S. Plovan, and S. Searles, jun., *J. Org. Chem.*, 1960, **25**, 285.

[10] P. B. Fischer, B. L. Kaul, and H. Zollinger, *Helv. Chim. Acta*, 1968, **51**, 1449.

[11] D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, 1958, **23**, 420.

[12] F. Garnier, R. H. Donnay, and J. E. Dubois, *Chem. Comm.*, 1971, 830.

reaction (Scheme 1). Because of the symmetry of the formazan structure, charge may be delocalized towards Ar$^2$ and/or Ar$^3$. The major contributing structure [(4) or (6)] will depend on the relative abilities of Ar$^2$ and Ar$^3$ to stabilize positive charge. When Ar$^2$, therefore, has an electron-withdrawing group and Ar$^3$ is phenyl, (6) will be the major structure and consequently the electron-withdrawing group in Ar$^2$ will have a relatively smaller effect on reactivity. When both Ar$^2$ and Ar$^3$ have electron-withdrawing groups then a more accurate estimate of the charge build-up on the *N*-aryl rings may be made. We have measured the rate of oxidation of 1,5-bis-(*m*-chlorophenyl)-3-phenylformazan (1; Ar$^1$ = Ph, Ar$^2$ = Ar$^3$ = *m*-ClC$_6$H$_4$) as 19·5 l mol$^{-1}$ s$^{-1}$ (at 25° in methanol; 0·1M-sodium bromide). The introduction of the second *m*-chloro-substituent has consequently a far greater effect in decreasing the rate of oxidation than does the first. On this basis a ρ value of $-2{\cdot}42$ can be calculated for substitution in the Ar$^3$ ring by electron-withdrawing substituents. The relative charge distribution along the formazan is, therefore, very similar to that observed for bromination of hydrazones.[8]



SCHEME 2

The bromo-azo-compound (10) formed as an intermediate during bromination of aldehydic hydrazones (Scheme 2) normally undergoes rapid tautomerism to the more stable hydrazonyl bromide (11). Intermediates such as (10) have not been isolated in protic solvents [because of their rapid conversion into (11)] but they have been formed from aldehydic [13] and ketonic hydrazones in non-aqueous solvents; their high reported [13-15] reactivity is consistent with their involvement in the reaction (Scheme 2).

The intermediate (5) cannot tautomerize; however, it is expected to undergo rapid unimolecular loss of bromide ion in the solvents used. Thus, the model compound (12; R$^1$ = Me, R$^2$ = Ph, R$^3$ = 2,4,6-Cl$_3$C$_6$H$_2$) has $t_{\frac{1}{2}}$ 4 s in 60 : 40 dioxan–water at 25° for loss of chloride ion. On this basis (5) would be solvolysed even more rapidly because of the better leaving group (bromide ion rather than chloride) and because of the presence of *two* azo-groups to stabilize the carbocation. One can also envisage the possibility that one of the neighbouring azo-functions has an anchimeric effect in the expulsion of Br$^-$ from (5), leading to the tetrazolium salt (2) directly. Thus, (5), if formed on the reaction pathway, would have a relatively short lifetime. Consistent with this, we have found that there is no build-up of relatively long lived intermediate in the conversion (1) ⟶ (2) (as shown by tight isosbestic points in repetitive scans of the u.v. and visible spectra of the reaction mixture). It might be possible to slow the conversion (5) ⟶ (2) by reducing the ionizing power of the medium. However, the bromination step (1) ⟶ (5) would also be slowed and more importantly, the ionic nature of the process might be in doubt.

The use of bromine in an ionic medium thus constitutes an efficient method for the oxidation of formazans. Indeed, because of the 1 : 1 nature of the reaction, electrogenerated bromine can be used as a convenient method for the estimation of formazans. Although an ionic (rather than free radical) mechanism has been proposed in the present instance using bromine, it is possible that use of other agents, *e.g.* aerial oxidation might involve a free radical pathway.

## EXPERIMENTAL

All inorganic materials used were AnalaR grade. The methanol used for the kinetic experiments was AnalaR grade which had been left for 24 h with a small amount of bromine, then refluxed until all traces of bromine had disappeared, and finally distilled twice, with retention of only the central fraction of distillate. AnalaR acetic acid was twice distilled from chromium trioxide and had b.p. 117—118°.

*Substrates*.—The triarylformazans were prepared by treating the appropriate hydrazone with the appropriate diazonium salt in the presence of pyridine.[16] The hydrazones were prepared by treating the corresponding arylhydrazine with the appropriate aromatic aldehyde. Most arylhydrazines used were commercially available. *m*- and *p*-Chlorophenylhydrazines were prepared by the method of Fischer.[17] All aldehydes used were commercially available. The 1,3,5-triarylformazans had the following m.p.s and analytical data on recrystallization from aqueous ethanol: 1,3,5-triphenyl, m.p. 178° (lit.,[18] 173·5°); 1,5-*diphenyl*-3-p-*tolyl*, m.p. 170° (Found: C, 76·4; H, 5·8; N, 17·8. $C_{20}H_{18}N_4$ requires C, 76·4; H, 5·75; N, 17·8%); 1,5-*diphenyl*-3-m-*tolyl*, m.p. 156° (Found: C, 76·5; H, 6·15; N, 17·4%); 1,5-diphenyl-3-*m*-nitrophenyl, m.p. 192° (lit.,[19] 185°); 1,5-*diphenyl*-3-p-*bromophenyl*, m.p. 207° (Found: C, 59·95; H, 3·85; N, 14·5; Br, 20·85. $C_{19}H_{15}BrN_4$ requires C, 60·15; H, 4·0; N, 14·75; Br, 21·1%); 1,5-*diphenyl*-3-m-*bromophenyl*, m.p. 173° (Found: C, 60·0; H, 4·05; N, 15·15; Br, 21·0%); 1,3-diphenyl-5-*p*-tolyl, m.p. 156° (lit.,[18,20] 155·5°); 1,3-diphenyl-5-*p*-nitrophenyl, m.p. 192° (lit.,[6] 195°); 1,3-diphenyl-5-*p*-bromophenyl, m.p. 191° (lit.,[20] 191°); 1,3-diphenyl-5-*m*-nitro-

---

[13] J. M. J. Tronchet, B. Baehler, N. Lehong, and P. F. Livio, *Helv. Chim. Acta*, 1971, **54**, 921.

[14] M. W. Moon, *J. Org. Chem.*, 1972, **37**, 386, 2005.

[15] A. F. Hegarty and J. A. Kearney, in preparation.

[16] J. N. Ashley, B. M. Davis, A. W. Nineham, and R. J. Stack, *J. Chem. Soc.*, 1953, 3887.

[17] E. Fischer, *Annalen*, 1887, **190**, 71.

[18] H. von Pechmann, *Ber.*, 1898, **31**, 1746.

[19] M. Busch and H. Pfeiffer, *Ber.*, 1926, **59**, 1162.

[20] L. Hunter and C. B. Roberts, *J. Chem. Soc.*, 1941, 820.

phenyl, m.p. 180° (lit.,[3] 180°); 1,3-*diphenyl*-5-m-*chlorophenyl*, m.p. 168° (Found: C, 68·1; H, 4·7; N, 17·25; Cl, 10·15. $C_{19}H_{15}ClN_4$ requires C, 68·15; H, 4·5; N, 16·75; Cl, 10·6%); 1-*p*-nitrophenyl-3,5-diphenyl, m.p. 192° (lit.,[6] 195°); 1,5-*bis*-(*p*-*bromophenyl*)-3-*phenyl*, m.p. 178° (Found: C, 50·0; H, 3·35; N, 12·25; Br, 34·75. $C_{19}H_{14}Br_2N_4$ requires C, 49·8; H, 3·1; N, 12·25; Br, 34·9%); 1,5-bis-(*m*-chlorophenyl)-3-phenyl, m.p. 156° (lit.,[21] 160°); 1,5-di-(*p*-tolyl)-3-phenyl, m.p. 166° (lit.,[22] 166°).

*Kinetic Measurements*.—The rates of oxidation of formazans to tetrazolium salts were followed by measuring the rate of disappearance of bromine from the solution by an electrometric technique which has already been described in detail.[8,23] The reactions were studied in the main under pseudo first-order conditions with a formazan : bromine concentration ratio at least 20 : 1. Some rates were also followed under second-order conditions with a bromine : formazan concentration ratio of 3 : 2. The rate constants quoted in Table 3 are average values for several replicate runs using variable initial concentrations of both bromine and formazan and are accurate to ±4%.

The rates of oxidation were also obtained by measuring the change in optical density of the formazan at a suitable wavelength (usually *ca.* 480 nm) using spectrophotometric apparatus which has already been described in detail.[24] The reactions in this case were studied under pseudo first-order conditions with a bromine : formazan concentration ratio at least 10 : 1.

[4/1468 *Received*, 17*th July*, 1974]

---

[21] D. Jerchel and H. Fischer, *Annalen*, 1954, **590**, 216.

[22] H. von Pechmann, *Ber.*, 1894, **27**, 320.

[23] A. F. Hegarty, P. J. Moroney, A. Moynihan, and F. L. Scott, *J.C.S. Perkin II*, 1972, 1892.

[24] A. F. Hegarty and F. L. Scott, *J.C.S. Perkin II*, 1973, 1719.