3-Carboxymethylthio-1,5-diphenylformazan: a Potential Terdentate Ligand with Unusual Properties

By **Alan T. Hutton** and **Harry M. N. H. Irving**,* Department of Inorganic Chemistry, University of Cape Town, Rondebosch 7700, South Africa

3-Carboxymethylthio-1,5-diphenylformazan (8), prepared by two synthetic routes as a potential terdentate ligand, is a very weak base ($pK_1 = -0.43$) and an exceptionally weak acid ($pK_2 = 12.48$) which does not yield metal complexes. No carboxylic proton resonance appears in the n.m.r. spectrum of the yellow, monomeric solution of (8) in $CDCl_3$ until it is diluted progressively with $(CD_3)_2CO$ when equilibrium with a purple isomer occurs. Comparable phenomena occur with 3-[1 (or 2)-carboxyethyl]thio-1,5-diphenylformazan and the unusual properties of these formazans are attributed to configurations in which the carboxylic proton is strongly intramolecularly hydrogen-bonded to a quasi-aromatic formazan ring system.

It has long been known that dithizone [3-thioxo-1,5-diphenylformazan; H_2Dz; (1a) $\rightleftharpoons$ (1b)] acts as a weak monobasic acid (p*K* *ca.* 4.5) in forming chelate complexes, $M(HDz)_n$, with a number of cations (*e.g.* of Ag, Tl, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Pb, Pd, Pt, In and Bi) and with organometallic cations such as R_3Pb^+, R_2Tl^+, R_2Pb^{2+}, R_2Sn^{2+}, and RHg^+ (where R = alkyl or aryl groups). Since these complexes are highly coloured ($\varepsilon_{max.}$ 3 000—9 500 m^2 mol^{-1}), and, like the parent reagent, very sparingly soluble in water but quite soluble in immiscible organic solvents such as chloroform, tetrachloromethane, or aromatic hydrocarbons, they lend themselves to liquid–liquid extraction procedures and to absorptiometric determinations.[1]

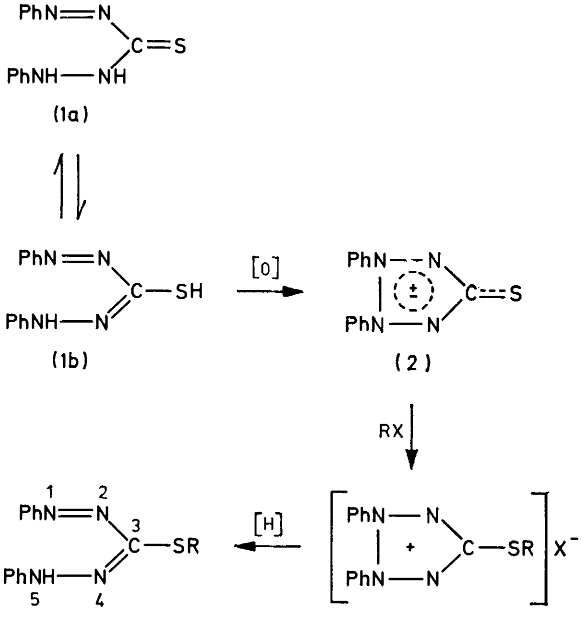
On the other hand, when the acidic function in (1b) is blocked by methylation, the product, 3-methylthio-1,5-diphenylformazan [*S*-methyldithizone; MeHDz; (7)] forms only a restricted range of metal complexes such as $M(MeDz)_2$ (M = Ni and Pd), PhHg(MeDz), and MCl(MeDz) (M = Cu and Hg) which, though highly coloured, do not lend themselves to liquid–liquid extraction procedures.[2] It seemed of interest, therefore, to examine a ligand in which the chromophoric and chelating properties of (1) and (7) were retained, while the acidic function of dithizone would be restored by the introduction of a readily ionizable proton. Such a reagent, 3-carboxymethylthio-1,5-diphenylformazan (8), which should be potentially terdentate by co-ordination through nitrogen, sulphur, and oxygen, can be prepared by the reduction of the tetrazolium salt (4) which results from the addition of chloroethanoic acid to the mesoionic compound 2,3-diphenyl-2*H*-tetrazolium-5-thiolate (2), commonly referred to as ' dehydrodithizone '.[3]

Although *S*-methyldithizone (7) is readily prepared from iodomethane and primary silver dithizonate, Ag(HDz),[4] this metal complex did not react with chloroethanoic acid or even iodoethanoic acid. However, (8) could be prepared directly and conveniently by the action of chloroethanoic acid on sodium dithizonate, Na(HDz). The homologous formazans (9) and (10) were obtained by alkaline reduction of the tetrazolium bromides (5) and (6).

In the hope of obtaining supporting evidence for the configuration (*q.v.*) postulated to explain the unexpected properties of (8), the molecular structure has been determined by *X*-ray crystallography and is reported elsewhere.[5]

EXPERIMENTAL

Preparation of 3-*Carboxymethylthio*-1,5-*diphenylformazan* (8).—(a) *From the mesoionic compound* (2). 2,3-Diphenyl-2*H*-tetrazolium-5-thiolate [' dehydrodithizone '; (2)] was prepared in 64—67% yield by the oxidation of dithizone (1 g) in chloroform (300 cm^3) by stirring (2 h) with potassium hexacyanoferrate(III) (3.2 g) and potassium carbonate (3.0 g) in water (100 cm^3). The organic layer was then removed, dried over anhydrous sodium sulphate, and the solvent was evaporated at room temperature. Recrystallization of the residue from boiling ethanol gave (2) as orange–red crystals, m.p. 173 °C (decomp.) [lit.,[1] 173 °C (decomp.)]. The same substance was also prepared using potassium permanganate (20% yield) and iodine (40% yield) as oxidants (Found: C, 61.2; H, 4.0; N, 21.9. Calc. for $C_{13}H_{10}N_4S$: C, 61.4; H, 4.0; N, 22.0%).



(7) R = Me
(8) R = CH_2CO_2H
(9) R = $CH(Me)CO_2H$
(10) R = $(CH_2)_2CO_2H$

(3) R = Me, X = I
(4) R = CH_2CO_2H, X = Cl
(5) R = $CH(Me)CO_2H$, X = Br
(6) R = $(CH_2)_2CO_2H$, X = Br

When heated under reflux (1 h), dehydrodithizone [(2), 0.95 g] and chloroethanoic acid (0.47 g) in chloroform (60 cm^3) gave 5-carboxymethylthio-2,3-diphenyltetrazolium chloride (4), which formed snow-white crystals with m.p. 197—201 °C (decomp.) from boiling ethanol–diethyl ether (3 : 1, v/v) (Found: C, 51.5; H, 3.7; N, 16.0. Calc. for $C_{15}H_{13}ClN_4O_2S$: C, 51.6; H, 3.8; N, 16.1%). Ogilvie and Corwin [3] reported a melting point of 216—218 °C (decomp.) from n-propanol, but we did not find this a suitable solvent and the melting point could not be raised above 205 °C (decomp.) even after repeated recrystallization.[6]

The tetrazolium salt (4) (0.5 g), sodium hydroxide (0.25 g), and dextrose (2.0 g) in deionized water (100 cm^3) were kept at 80 °C (20 min) and then cooled in an ice-bath. On acidification (dilute H_2SO_4) a flocculent brown precipitate separated and was collected, dried, and recrystallized twice from aqueous ethanol to give the desired formazan (8) as fine red-brown needles [22% yield; m.p. 143—145 °C (decomp.) (Found: C, 57.3; H, 4.5; N, 17.7. Calc. for $C_{15}H_{14}N_4O_2S$: C, 57.3; H, 4.5; N, 17.8%)]. In subsequent preparations on a larger scale [2.5 g of (4)] the yield was raised to 80% by carrying out the reduction with alkaline glucose for a much shorter time, specifically until the deepest red colour appeared, followed by immediate cooling in ice, m.p. 147—149 °C (decomp.) [lit.,[3] 150—152 °C (decomp.); Nabilsi [6] reported 148—149 °C (decomp.)].

(b) *From dithizone* (1) *and chloroethanoic acid.* Dithizone (1.0 g) was added to a solution of sodium metal (0.12 g) in dry ethanol (60 cm^3) and after the addition of chloroethanoic acid (0.30 g) the whole was heated under reflux (1 h) and then poured into dilute sulphuric acid (300 cm^3, pH *ca.* 5). The fine brown suspension was taken up in chloroform and the oily residue after removal of the solvent at room temperature was taken up in the minimum amount of benzene and chromatographed on alumina with benzene-methanol to give (8), 33% yield, m.p. 143—145 °C (decomp.). The relative molecular mass was determined isopiestically by the method of Morton *et al.*[7] [Found: M_r = 295 (chloroform), 303 (acetone). Calc. for $C_{15}H_{14}N_4O_2S$: 314.4].

Preparation of 5-(2-*Carboxyethylthio*)-2,3-*diphenyltetrazolium Bromide* (6).—3-Chloropropanoic acid did not form (6) by addition to (2), and although it reacted with the sodium salt of dithizone the oily product could not be obtained crystalline. However, (6) was readily obtained from dehydrodithizone (2) (1.0 g) and 3-bromopropanoic acid (0.6 g) in chloroform (70 cm^3) by heating under reflux (1½ h). After recrystallization from boiling ethanol–diethyl ether (3 : 1) white *crystals* of (6) were obtained [87% yield; m.p. 236—239 °C (decomp.) (Found: C, 47.15; H, 3.7; N, 13.75. $C_{16}H_{15}BrN_4O_2S$ requires C, 47.2; H, 3.7; N, 13.75%)].

5-(1-*Carboxyethylthio*)-2,3-*diphenyltetrazolium bromide* (5) was prepared similarly in 73% yield, m.p. 208—210 °C (decomp.) (Found: C, 47.2; H, 3.75; N, 13.8. $C_{16}H_{15}BrN_4O_2S$ requires C, 47.2; H, 3.7; N, 13.75%).

Preparation of 3-(2-*Carboxyethylthio*)-1,5-*diphenylformazan* (10).—Reduction of the tetrazolium bromide [(6), 1.0 g] with sodium hydroxide (0.5 g) and dextrose (4.0 g) in warm distilled water (5 min) gave, on acidification, the desired *formazan* which formed fine brown needles from n-hexane–benzene (1 : 1) {63% yield; m.p. 119—121 °C (decomp.) [Found: C, 58.2; H, 4.85; N, 16.9%; M_r (isopiestic),[7] 310 (chloroform), 292 (acetone). $C_{16}H_{16}N_4O_2S$ requires C, 58.5; H, 4.9; N, 17.05%; M_r = 328.4]}.

3-(1-*Carboxyethylthio*)-1,5-*diphenylformazan* (9), prepared similarly from (5) in 68% yield, formed brown needles, m.p. 134—137 °C (decomp.) [Found: C, 58.6; H, 4.8; N, 17.0%; M_r (isopiestic),[7] 318 (chloroform), 312 (acetone). $C_{16}H_{16}N_4O_2S$ requires C, 58.5; H, 4.9; N, 17.05%; M_r = 328.4].

Absorption Spectra of the Formazan (8) *in Various Solvents.*—Aliquot portions (0.5 cm^3) of a 4.509×10^{-4} M-stock solution of (8) in AnalaR ethanol were transferred to 10-cm^3 volumetric flasks and the solvent removed overnight in an evacuated desiccator containing silica gel and calcium chloride. To each of the resulting known masses of dry formazan was added the appropriate spectroscopic solvent dried over molecular sieve and made up to the mark to give 2.254×10^{-5}M-solutions. Spectra were measured at 20 °C against solvent blanks on a Pye-Unicam SP1700 spectrophotometer using 1-cm matched silica cells. The results are summarized in Table 1. For experiments with mixed solvents (Figure 2) the concentration of the formazan solution was 2.092×10^{-5}M.

Determination of pK_1 *for the Formazans* (8), (9), *and* (10).—The absorption spectra for the range 200—800 nm were determined at 20 °C for solutions prepared from 0.5 cm of a stock formazan (8) solution (4.509×10^{-4}M in ethanol), x cm^3 AnalaR concentrated hydrochloric acid, and $(9.5 - x)$ cm^3 glass-distilled water against blanks comprising the same concentration of HCl in 5% ethanol.[8] A plot of $(A_{510} - A_{420})$ against values of the acidity function, H_0, given by Paul and Long [9] had a typical sigmoid form and the value of pK_1 was derived from the midpoint of the straight portion and the maximum of the first derivative curve.[10] Similar measurements were carried out with the formazans (9) and (10).

Determination of pK_2 *for the Formazans* (8), (9), *and* (10).—Solutions were prepared containing 0.5 cm^3 of stock formazan [(8); 4.509×10^{-4} in ethanol], 1M-NaCl, and varying amounts of carbonate-free sodium hydroxide made up to 10 cm^3 with glass-distilled water. The concentrations of NaOH were determined by titration of aliquot portions with standard acid after appropriate dilution. Spectra were determined at 20 °C over the range 200—800 nm against blanks of the same concentration in solutions of 1M-NaCl containing 5% ethanol. The point of inflexion of the sigmoid curve obtained by plotting $(A_{500} - A_{420})$ against pH gave pK_2 = 12.48 at I = 1.0M (NaCl).

By using equation (iii) with $K_w = 6.809 \times 10^{-15}$, ε_{HL} = 519 m^2 mol^{-1} at 500 nm, and $c = 2.25 \times 10^{-5}$M, values of pK_2 for each hydroxide ion concentration were calculated for various estimated values of ε_L. Constancy of K_2 was best achieved with ε_L = 4 400 ± 100 m^2 mol^{-1}, whence pK_2 = 12.48 as before.[8] Corresponding measurements were made with the formazans (9) and (10) and all the results are collected in Table 2.

Determination of pK_a *Values of the Tetrazolium Salts* (4), (5), *and* (6).—Potentiometric determinations were carried out at 20 °C and I = 1.0M NaCl.[8] Knowing the concentration of mineral acid [H], that of free hydrogen ions, h, and the concentration of the tetrazolium salt [Y], values of $\bar{Z}_H = \{[H] - h + (K_w/h)\}/[Y]$ were calculated and plotted against ph using the computer programs FORMAT [11] and ZPLOT.[12] Initial estimates of pK_a from Bjerrum's ½-$\bar{Z}_H$ method were refined by the computer program MINIQUAD.[13] The results are summarized in Table 3.

RESULTS AND DISCUSSION

3-Carboxymethylthio-1,5-diphenylformazan (8) forms reddish brown monoclinic crystals, space group $P2_1/n$,

$Z = 4$, with $a = 27.98(1)$, $b = 10.127\ (5)$, $c = 5.208(3)$ Å, and $\beta = 91.59(5)°$. The X-ray crystal structure (Figure 1) showed that in the solid state each molecule of (8) is remarkably planar and strongly hydrogen-bonded through its carboxy-group to the carboxy-group of a second molecule, thus forming a coplanar dimeric unit. The proton of the imino-group is internally hydrogen-bonded to N(2), effectively locking each molecule in a *syn*,*s-trans* configuration (*q.v.*). Full details are given elsewhere.[5]

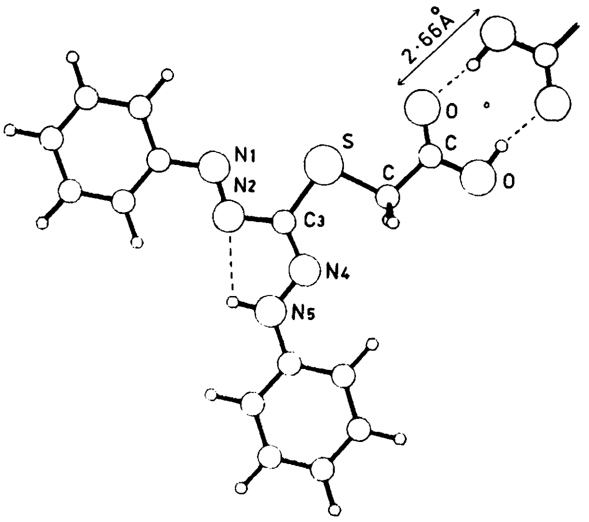


FIGURE 1 The solid-state molecular structure of the formazan (8), showing a portion of the symmetry related moiety of the dimer. The dashed lines indicate hydrogen bonds

TABLE 1

Absorption data for dehydrodithizone (2) and the formazan (8) in various solvents

Solvent	Z-Value [b]	Mesoionic compound (2) [a]			Formazan (8)		
		$\lambda_{max.}$/nm	$\Delta\lambda$ [c]/nm	$\varepsilon_{max.}$/m^2 mol^{-1}	$\lambda_{max.}$/nm	$\Delta\lambda$ [c]/nm	$\varepsilon_{max.}$/m^2 mol^{-1}
Water	94.6	380	100	164	424	−8	2 280
Methanol	83.6	406	74	120	418	−2	2 440
Formamide	83.3	407	73	100	428	−12	1 740
Ethanol	79.6	415	65	100	420	−4	2 000
n-Propanol	78.3	421	59	118	424	−8	2 500
n-Butanol	77.7	426	54	100	423	−7	2 090
Acetonitrile	71.3	445	35	120	422	−6	1 760
Dimethyl sulphoxide	71.1	440	40	115	443	−27	1 430
Acetone	65.7	460	20	109	420	−4	1 300
Pyridine	64.0	476	4	104	431	−15	1 790
Chloroform	63.2	468	12	117	420	−4	1 950
Ethyl ethanoate	*d*	480	0	136	416	0	1 490
Nitromethane	*d*	450	30	107	421	−5	1 690
Benzene	*d*				421	−5	2 000
Tetrachloromethane	*d*				421	−5	2 000
Diethyl ether	*d*				411	5	1 790

[a] Data from Kiwan and Irving.[15] [b] Data from Kosower [14] in kcal mol^{-1}. [c] $\Delta\lambda = \lambda_{max.}$(ethyl ethanoate) − $\lambda_{max.}$(chosen solvent). [d] Data not reported.[14]

In contrast to the presence of dimers in the solid state, 3-carboxymethylthio-1,5-diphenylformazan (8; HL) was shown by isopiestic measurements [7] to exist as a monomer in chloroform and in acetone. It is fairly soluble in most organic solvents but the position and the intensity of the absorption bands vary considerably (Table 1) and these could not be correlated with any solvent polarity parameters such as Kosower's Z-values.[14] This contrasts with the behaviour of the mesoionic dehydrodithizone (2), where an excellent linear relationship exists between the value of $\Delta\lambda = \lambda_{max.}$ (ethyl ethanoate) − $\lambda_{max.}$ (chosen solvent) and the corresponding Z-value.[15] This implies that the formazan (8) is less polar and that both ground and excited states have similar solvation energies.

The formazan (8; HL) is only sparingly soluble in water, even on heating, when it gives a yellow solution. When a solution in chloroform is equilibrated with a strong acid a red cation, H_2L^+, formed presumably by protonation of a nitrogen atom, passes into the aqueous phase. Spectrophotometric measurements on solutions containing the same amount of (8) in hydrochloric acid of different known concentrations revealed an isosbestic point at 450 nm, establishing the presence of only the two species H_2L^+ and HL in equilibrium. By using Davis and Geissman's procedure,[10] the value of $K_1 = [H^+][HL]/[H_2L^+]$ was found to be $10^{+0.43}$ at 20 °C.

On equilibrating a solution of (8) in chloroform with a strong base, the anion L^- is produced and passes into the aqueous phase to which it imparts an orange colour. The spectra of solutions of (8) in aqueous alkali of different known concentrations show an isosbestic point at 452 nm, confirming the equilibrium between the two species HL and L^-. The spectrophotometric studies led to the value $K_2 = [H^+][L^-]/[HL] = 10^{-12.48}$ at 20 °C ($I = 1.0$M-NaCl).

Since the experimental plot of $(A_{500} - A_{420})$ *versus* pH never reached a limiting value at the highest pH values accessible it was clear that the ionization of the acid HL was incomplete when $[NaOH] \leqslant 1$M and the absorption coefficient for the species L^- at $\lambda_{max.}$ 500 nm was inaccessible to direct measurement. This difficulty was overcome as follows. It can be shown that

$$\frac{A}{c} = \frac{[H^+]^2\varepsilon_{H_2L} + K_1[H^+]\varepsilon_{HL} + K_1K_2\varepsilon_L}{[H^+]^2 + K_1[H^+] + K_1K_2} \qquad \text{(i)}$$

where A is the absorbance of a solution of concentration c and ε_{H_2L}, ε_{HL}, and ε_L are the linear absorption coefficients of the species H_2L^+, HL, and L^-. When $[H_2L^+] \ll [HL] \simeq [L^-]$ equation (i) reduces to

$$\frac{A}{c} = \varepsilon_{HL} + \frac{K_2}{[H^+]}\left(\varepsilon_L - \frac{A}{c}\right) \qquad \text{(ii)}$$

$$\text{whence } K_2 = K_w\left(\frac{A}{c} - \varepsilon_{HL}\right)\Big/[OH^-]\left(\varepsilon_L - \frac{A}{c}\right) \qquad \text{(iii)}$$

Knowing $K_w = 6.809 \times 10^{-15}$ at 20 °C and $I = 1.0$M-NaCl and experimental values of A and c, as well as the linear absorption coefficient $\varepsilon_{HL} = 519$ m^2 mol^{-1} at 500 nm, values of K_2 were computed for a number of assumed values of ε_L. The correct value of ε_L will, of course, be that which gives a constant value of K_2 for all values of A. With the value $\varepsilon_L = 4\,400$ m^2 mol^{-1} the value p$K_2 = 12.48$ was constant to within 0.1%.

TABLE 2

Spectrophotometric data and pK values for the formazans (8), (9), and (10)

Formazan	L^- (orange-red)	HL (yellow)	H_2L^+ (violet-red)	pK_1	pK_2 [a]
(8)	500 [b] (4 400 ±100)	420 (2 790)	512 (4 790)	−0.43 ± 0.04	12.48 ± 0.05
(9)	502 (4 600)	423 (2 830)	516 (4 750)	−0.65 ± 0.04	12.59 ± 0.05
(10)	500 (4 810)	422 (2 880)	517 (4 910)	−0.36 ± 0.04	12.39 ± 0.05

[a] $I = 1.0$M-NaCl. [b] Wavelength/nm followed in parentheses by decadic linear absorption coefficient/m^2 mol^{-1}.

Similar measurements on the formazans (9) and (10) are collected in Table 2. The outstanding feature is that they are all very weak bases and quite exceptionally weak acids. The acidifying effect of a sulphur atom may be seen by comparing mercaptoacetic acid, $HS{\cdot}CH_2CO_2H$, (pK_a *ca.* 3.4) with ethanoic acid (pK_a *ca.* 4.6). The interposition of a methylene group as in 3-mercaptopropanoic acid, $HS{\cdot}CH_2CH_2CO_2H$, (pK_a *ca.* 4.3) has the expected effect of lowering the acidity almost to that of ethanoic acid.[16] Comparable effects are found with the tetrazolium salts (4), (5), and (6) (see Table 3). Here the large increase in the acidity of the carboxy-group of the tetrazolium chloride (4) (p$K_a = 2.63$) as compared with ethanoic acid (pK_a *ca.* 4.6) has been interpreted in terms of inductive and mesomeric effects which place special emphasis on canonical forms such as (11).[3]

TABLE 3

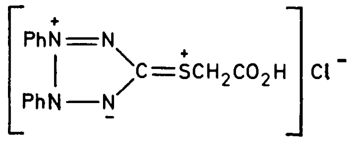
Acid dissociation constants of the tetrazolium salts (4), (5), and (6) at 20 °C ($I = 1.0$M NaCl)

Tetrazolium salt	(4)	(5)	(6)
pK_a	2.63 ± 0.01	2.70 ± 0.02	3.80 ± 0.01
R [a]	0.012	0.025	0.021

[a] Residual index, *cf.* ref. 13.

Whereas the introduction of a side-chain methyl group has the expected small acid-weakening effect [pK_a *ca.* 2.70 for (5)], the insulation of the carboxy-group from the positively charged sulphur by a methylene group in (6) reduces the acidity by 1.2 units. In contrast, no such effects of the methylene group are detectable in the acidities of (8) and (10) (pK 12.48 and 12.39) and the similar value of 12.59 for (9): the exceptional reluctance of the carboxylic proton to become ionized calls for a special explanation.

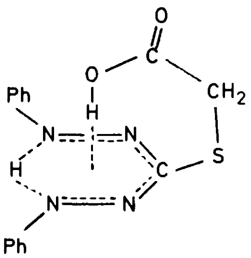
The lack of any absorption in the region 3 100—3 500 cm^{-1} in the solid-state i.r. spectrum of the formazan (8) indicates the absence of a free ⁚NH group and implies its participation in strong hydrogen bonding. Broad bands in the region 2 500—3 000 cm^{-1} are consistent with the presence of a strongly hydrogen-bonded OH group of a carboxylic acid involved in internal chelation. Whereas formazans were thought to exhibit mesomerism through hydrogen bonding by the imino-proton which locked the molecule in the form of a *syn*,s-*cis* ring system,[17] the electron delocalization would now seem to be more correctly explained by the very rapid tautomerism which has been established by n.m.r. studies using ^{15}N-labelled formazans.[18,19] This produces an equivalent effect in which the $4n + 2$ π-electrons are delocalized to form a quasi-aromatic formazan ring in which carbon and nitrogen atoms are all *sp*^{2}-hybridized. Scale models show that the carboxylic proton of the formazan (8) could itself be intramolecularly hydrogen-bonded to any one of the four nitrogen atoms so that the arrangement most favoured sterically is that in which the OH group of the side-chain SCH_2CO_2H points almost axially through the centre of the π-electron system of the formazan ring (12a, b), thus giving rise to a formally neutral species, HL, of exceptional stability. Protonation of one of the four nitrogen atoms would disrupt the quasi-aromatic ring system and furthermore prevent the formation of the intramolecular hydrogen bonding by the carboxy-group, as postulated above: hence the species HL will be a very weak base. Ionization of the carboxy-group in (8) will be opposed by the accompanying loss of intramolecular hydrogen-bonding energy, accounting for its exceptionally low acidity.



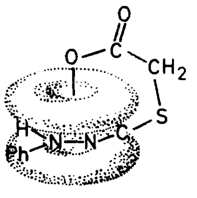
(11)

Hydrogen bonding to π-electron systems is well established,[20,21] *e.g.* between alcohols and aromatic solvents. Intramolecular hydrogen bonding has been observed *inter alia* in the case of 1-hydroxyethylferrocene (13),[22] 2-phenylethanol (14),[21] and the *cis*-isomer of biphenyl-2-ol (15),[23] but there appears to be no precedent for the proposed intramolecular hydrogen bond to the quasi-aromatic formazan ring.

Further information on the nature of the species present in solutions of the formazans (8), (9), and (10) was obtained by visible spectrophotometry and proton resonance spectroscopy.[8]



(12a)



(12b)

The addition of acetone to a solution of (8) in chloroform does not shift the position of the maximum at 420 nm but the absorbance decreases and a new band appears at 540 nm accompanied by a colour change from yellow to reddish purple. The occurrence of an isosbestic point at 475 nm (Figure 2) points to the existence of two species in conformational equilibrium, that with $\lambda_{max.}$ = 540 nm being stabilized by a carbonyl function. Similar colour changes occur in mixtures of dimethyl sulphoxide (purple solution) and water (yellow solution) with an isosbestic point at 468 nm. The homologues (9) and (10) behave in the same way.

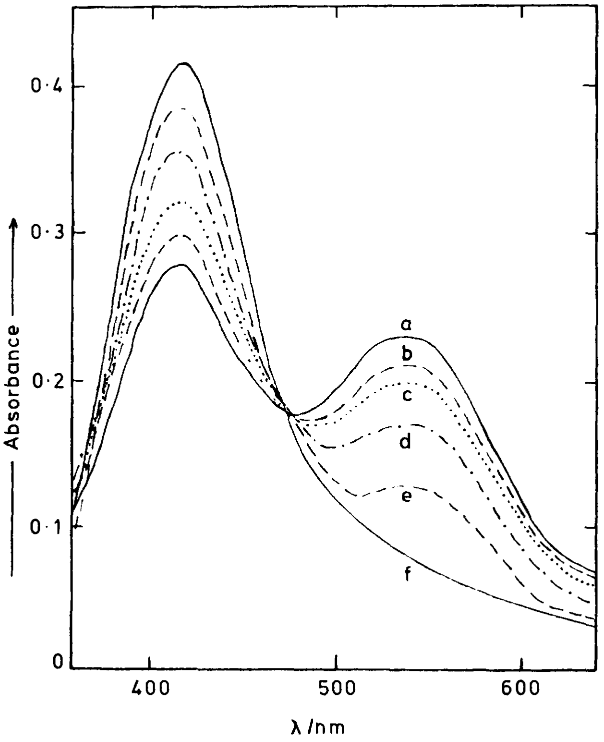
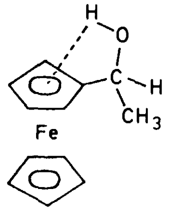
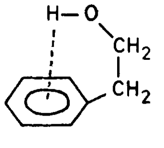


FIGURE 2 Absorption spectra for the formazan (8) in mixed solvents (cm³ acetone : cm³ chloroform) a, 10 : 0; b, 8 : 2; c, 6 : 4; d, 4 : 6; e, 2 : 8; f, 0 : 10. The formazan concentration was 2.092 × 10^{-5}M

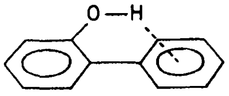
Further data on the species present in solution were obtained by n.m.r. spectroscopy, reported in Table 4. In $CDCl_3$ the methylene protons of (8) appear to be equivalent and give rise to an unsplit singlet. There is, however, a complete absence of any resonance peak that can be ascribed either to the imino-hydrogen atom or to the carboxylic proton. The former is often very broad or not observed in formazans [18,24] or it may be obscured by the aromatic protons; the absence of a carboxylic proton is consistent with structure (12). In contrast the spectrum of (8) in $(CD_3)_2CO$ shows an unambiguous carboxylic proton resonance at δ 11.22 which disappears on addition of D_2O. There are now two methylene singlets in the ratio 2 : 1 integrating for a total of two protons. Hence in addition to the yellow form that appears to be stabilized in chloroform in some conformation wherein the carboxylic proton is masked, a second



(13)



(14)



(15)

TABLE 4

Proton n.m.r. data [a] for 3-carboxymethylthio-1,5-diphenylformazan (8) and its homologues (9) and (10)

Formazan	Solvent	Shift (δ)	Multiplicity	Integration	Assignment
(8)	$CDCl_3$	3.68	s	2H	CH_2
		7.17—7.76	m	10H	ArH
	$(CD_3)_2CO$	3.80	s	2H	CH_2
		3.90	s		
		7.10—7.84	m	10H	ArH
		11.22 [b]	s	~⅔H	CO_2H
(9)	$CDCl_3$	1.56	d	3H	CH_3
		3.93	q	1H	CH
		7.14—7.76	m	10H	ArH
	$(CD_3)_2CO$	~2.1	c	c	CH_3
		4.20	m	1H	CH
		7.11—7.89	m	10H	ArH
		11.20 [b]	s	~⅔H	CO_2H
(10)	$CDCl_3$	2.68	ut	2H	CH_2CO_2H
		3.16	ut	2H	SCH_2
		7.04—7.93	bm	10H	ArH
	$(CD_3)_2CO$	2.62 * [d]	t	2H	CH_2CO_2H
		2.87 †	t		
		3.12 *	t	2H	SCH_2
		3.34 †	t		
		7.16—7.92	m	10H	ArH
		10.26 [b]	s	~⅓H	CO_2H
		11.19 [b]	s	~⅔H	

[a] ¹H n.m.r. spectra obtained with a Bruker WH–90DS spectrometer operating at 90 MHz and ambient temperature in the Fourier-transform mode. Chemical shifts (δ) measured relative to $SiMe_4$. Abbreviations used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad; u, unresolved. [b] Disappears on addition of D_2O. [c] Obscured by solvent resonance. [d] Two sets of two triplets are denoted by * and †.

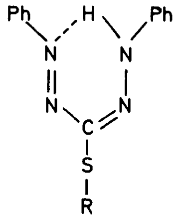
purple form with a free carboxy-group arises on the addition of acetone. The ratio of purple : yellow form in acetone solution appears to be about 2 : 1 from inspection of the two methylene singlets and since the carboxylic proton resonance integrates for only *ca.* 2/3 of a proton. Measurement of the n.m.r. spectrum of a solution of the formazan (8) in $(CD_3)_2CO$ as it is progressively diluted with $CDCl_3$ shows the progressive disappearance of the carboxylic proton resonance and the coalescence of the two methylene signals.

On the other hand, titration of a solution of the formazan (8) in $(CD_3)_2CO$ with dimethyl sulphoxide, $(CD_3)_2SO$, leaves the methylene resonances unchanged but shifts the CO_2H resonance progressively downfield with unchanged integral, due to its interaction with the more polar dimethyl sulphoxide molecules. Titration of a solution of the formazan (8) in $(CD_3)_2CO$ with D_2O results in the immediate disappearance of the carboxy-resonance and a progressive coalescence of the methylene resonances into a single line: there is a concomitant change in colour from purple-red to yellow.

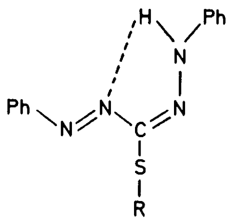
The n.m.r. spectrum of the corresponding tetrazolium salt (4) in D_2O shows the expected two signals, at δ 4.25 (singlet) and δ 7.65 (complex) of integrated area 1 : 5, accounting for the methylene and aromatic protons respectively.

The yellow species that predominates in a chloroform solution must be the internally hydrogen-bonded species (12) while in acetone the visible and n.m.r. spectra show the presence of an additional purple isomer with an exposed carboxy-group. Now the formazan ring is known to be capable of existing in a variety of conformations in solution and the kinetics of interconversion have received much attention.[25] Taking into account *syn–anti* isomerism about the :C=N– bond and *cis–trans* isomerism about the –N=N– bond the configurations (16)—(23) are possible. The C–N single bond linking the phenylazo-group to the 3-carbon atom is similar to the central bond in buta-1,3-diene and the various forms can be further distinguished as s-*cis*- and *trans*-isomers.[24,26] Serious crowding would appear to eliminate isomers with *cis*-configurations about the azo-double-bond and the proximity of the lone pairs in (18) would seem to rule this out leaving (16), (17), and (19) as possible configurations for the purple isomer. The *syn*, s-*trans*, isomer [as (17)] stabilized by an intramolecular hydrogen bond seems the most likely conformation for the purple isomer. This is the configuration found in the purple-red solid *S*-methyldithizone (7) in the solid state[27] and assigned to the solute in its freshly prepared solution in chloroform ($\lambda_{max.}$ 550 nm).[6,24] It is also the configuration adopted by the formazan (8) in the solid state (see Figure 1).[5]

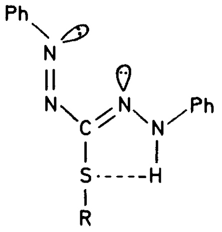
Further evidence for configurational equilibria was obtained from the formazan (10) which has the side-chain $SCH_2CH_2CO_2H$ in the 3-position. At room temperature the yellow solution in $CDCl_3$ showed neither :N*H* nor CO_2H signals and the phenyl resonances were broad, suggesting mutual interaction by way of the closed formazan ring in the *syn*-s-*cis*-form with intramolecular hydrogen bonding [as (12) and (16)]. The methylene protons appear as two unresolved triplets, the low-field triplet being assigned to SCH_2 and the high field one to CH_2CO_2H.[28] In $(CD_3)_2CO$ these two triplets split into two sets of two triplets integrating for four protons. There are also two peaks assignable to carboxylic proton resonances and integrating between them for one proton: both disappear on the addition of D_2O. Titration with $CDCl_3$ results in the progressive disappearance of these carboxylic proton peaks and the collapse of the methylene resonances back to two unresolved triplets. Increasing the temperature of a $CDCl_3$ solution increases the resolution of these triplets and at 50 °C they are fully resolved and there is a concomitant sharpening of the aromatic



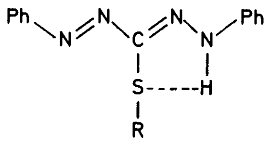
(16) *syn*, s–*cis*, *trans*



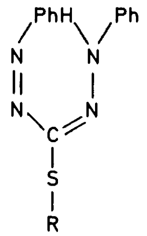
(17) *syn*, s–*trans*, *trans*



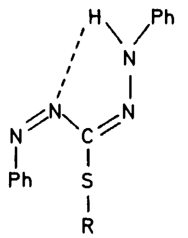
(18) *anti*, s–*cis*, *trans*



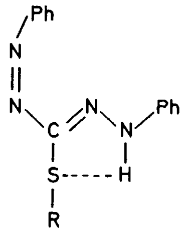
(19) *anti*, s–*trans*, *trans*



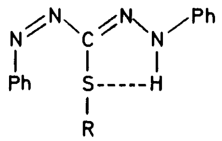
(20) *syn*, s–*cis*, *cis*



(21) *syn*, s–*trans*, *cis*



(22) *anti*, s–*cis*, *cis*



(23) *anti*, s–*trans*, *cis*

resonances. Lowering the temperature of a solution of (10) in $CDCl_3$ broadens all the lines and, more significantly, at −35 °C two carboxylic proton resonances appear. The peak at δ 10.28 integrating for *ca.* 1/3 H corresponds to the peak at δ 10.26 (*ca.* 1/3 H) in the spectrum in $(CD_3)_2CO$ at ambient temperature. It can be ascribed to the carboxylic proton resonance of the *syn*,s-*cis* (yellow) isomer with the carboxylic proton intramolecularly hydrogen-bonded to the π-electron system (as 12). On the other hand the peak at δ 9.80 in $CDCl_3$ at −35 °C (*ca.* 2/3 H) corresponds to the line at δ 11.19 in pure $(CD_3)_2CO$ at ambient temperature (*ca.* 2/3 H). The difference in chemical-shift values can be ascribed to differences in dielectric constant and solvent polarity and the resonances can be assigned to the carboxylic proton resonance of the open-chain purple *syn*,s-*trans*-isomer where this proton is able to interact with solvent molecules [as (17)].

The remaining feature of the spectrum of (10) in $CDCl_3$ at −35 °C is the appearance of a broad resonance integrating for *ca.* 1/2 H at δ 5.3 and this is the only indication of the hydrogen-bonded imino-proton in the spectra of (8), (9), or (10).

These observations can be interpreted by postulating the existence of two isomers (yellow *syn*,s-*cis*, and purple *syn*,s-*trans*) existing in acetone in slow exchange relative to the n.m.r. time scale and in the proportion estimated as 1 : 2. The *syn*,s-*trans*-isomer is clearly stabilized in acetone. The rate of interconversion is rapid and only the broad time-averaged resonances are observed at room temperature. An increase of temperature sharpens the resonances as the rate of interconversion increases. Lowering the temperature takes the equilibrium into the region of slow exchange where features of the room temperature spectrum of (10) in acetone begin to appear and the imino-proton resonance begins to ' freeze out '.

The n.m.r. spectra in $CDCl_3$ of the formazan (9) with the side-chain $S{\cdot}CH(CH_3)CO_2H$ again shows no signs of imino or carboxylic proton resonances. Irradiation of the methine proton quartet at δ 3.93 caused the methyl proton doublet (J = 7.40 Hz) to collapse to a singlet. In $(CD_3)_2CO$ the carboxylic proton again makes its appearance at δ 11.20 while the methine quartet becomes a broad multiple resonance with seven distinguishable lines: two superimposed quartets would be predicted. On addition of $CDCl_3$ the carboxylic resonance progressively disappears, the methine multiplet reduces to a quartet, and the colour changes from red–purple to yellow, as in the case of the formazans (8) and (10).

So far we have failed to prepare any solid metal complexes of (8) with copper(II), nickel(II), mercury(II), or platinum(II) using a variety of solvents (*e.g.* ethanol, acetone, and dimethyl sulphoxide) with or without the addition of bases such as sodium acetate, sodium ethoxide, pyridine, and triethylamine. These observations support the hypothesis advanced above that the carboxylic proton is rendered ineffective through its participation in the very stable conformation (12).

Qualitative tests at various concentrations of metal (0.01, 0.1, and 1.0M) in alkaline, neutral, and acidic solution were carried out (spot plates) with K^+, Be^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, Ga^{3+}, Ru^{3+}, Cd^{2+}, In^{3+}, Sb^{3+}, Pb^{2+}, Bi^{3+}, Tl^+, and La^{3+} ions producing no colour change in the yellow standard formazan solution. Small colour changes were observed on the addition of Cu^{2+} (brown), Ag^+ (pink), Pt^{2+} (orange), Pd^{2+} (pink), Hg^{2+} (orange), and Sn^{4+} (orange) but further spectrophotometric studies indicated only very weak complex formation in aqueous solution. No extractable complexes were formed. Of all the cations tested Tl^{3+} and Au^{3+} were exceptional in that they completely and quantitatively oxidized solutions of the red formazan (8) to the colourless tetrazolium salt (4). These reactions are being exploited for the quantitative and selective determination of traces of these ions.

We are indebted to Mr. K. R. Koch for assistance with the n.m.r. work and to Dr. A. H. Nabilsi for preliminary measurements. The University of Cape Town and the C.S.I.R. (Pretoria) are thanked for their financial assistance.

[9/303 *Received, 26th February*, 1979]

REFERENCES

[1] H. M. N. H. Irving, ' Dithizone,' Analytical Sciences Monographs No. 5, The Chemical Society, London, 1977.

[2] H. M. N. H. Irving, A. H. Nabilsi, and S. S. Sahota, *Analyt. Chim. Acta*, 1973, **67**, 135.

[3] J. W. Ogilvie and A. H. Corwin, *J. Amer. Chem. Soc.*, 1961, **83**, 5023.

[4] H. Irving and C. F. Bell, *J. Chem. Soc.*, 1954, 4253.

[5] A. T. Hutton, H. M. N. H. Irving, L. R. Nassimbeni, and G. Gafner, *Acta Cryst.*, 1979, **B35**, 1354.

[6] A. H. Nabilsi, Ph.D. Thesis, University of Leeds, 1972.

[7] J. E. Morton, A. D. Campbell, and T. S. Ma, *Analyst*, 1953, **78**, 722.

[8] A. T. Hutton, M.Sc. Thesis, University of Cape Town, 1978.

[9] M. A. Paul and F. A. Long, *Chem. Rev.*, 1957, **57**, 1.

[10] C. T. Davis and T. A. Geissman, *J. Amer. Chem. Soc.*, 1954, **76**, 3507.

[11] G. E. Jackson, University of Cape Town, personal communication.

[12] D. R. Williams, *J.C.S. Dalton*, 1973, 1064.

[13] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.

[14] E. M. Kosower, *J. Amer. Chem. Soc.*, 1958, **80**, 3253.

[15] A. M. Kiwan and H. M. N. H. Irving, *J. Chem. Soc.* (*B*), 1971 898.

[16] ' Stability Constants of Metal-Ion Complexes,' eds. L. G. Sillén and A. E. Martell, Special Publication No. 17 and No. 25, The Chemical Society, 1964 and 1971.

[17] A. W. Nineham, *Chem. Rev.*, 1955, **55**, 380.

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

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

[20] G. C. Pimentel and A. L. McClellan, ' The Hydrogen Bond, W. H. Freeman, London, 1960, p. 190.

[21] M. D. Joesten and L. J. Schaad, ' Hydrogen Bonding,' Marcel Dekker, New York, 1974.

[22] D. S. Trifan, J. L. Weinmann, and L. P. Kuhn, *J. Amer. Chem. Soc.*, 1957, **79**, 6566.

[23] T. Kinugasa, M. Nakamura, and S. Ueji, *J.C.S. Perkin II*, 1976, 1663.

[24] C. H. Carlin, Ph.D. Thesis, The John Hopkins University, Baltimore, 1966.

[25] R. Kuhn and H. M. Weitz, *Chem. Ber.*, 1953, **86**, 1199.

[26] F. Ramiriz and A. Kirby, *J. Amer. Chem. Soc.*, 1954, **76**, 1037.

[27] J. Preuss and A. Gieren, *Acta Cryst.*, 1975, **B31**, 1276.

[28] L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon, Oxford, 1969, 2nd edn., p. 164.