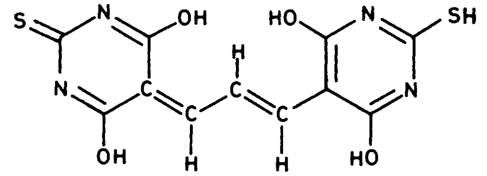
Structure and Properties of the Thiobarbituric Acid–Malonaldehyde Chromogen†

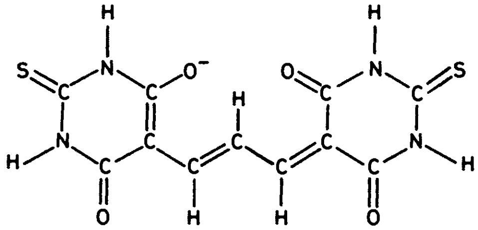
Gordon Read * **and Ruth Randal**
Department of Chemistry, University of Exeter, Exeter EX4 4QD
Michael B. Hursthouse * **and Richard Short**
Chemistry Department, Queen Mary College, London E1 4NS

An *X*-ray crystallographic study supported by ^1H n.m.r. provides unequivocal evidence that the chromogen formed from the reaction of malonaldehyde with 4,6-dihydroxy-2-mercaptopyrimidine (thiobarbituric acid) is the highly stable delocalised anion (**2**) from 5,5′-(propane-1,3-diylidene)bis-(2-thioxopyrimidine-4,6-dione).

The strongly absorbing chromogen ($\lambda_{max.}$ 535 nm; $\varepsilon_{max.}$ 1.5×10^{-3})[1] formed when thiobarbituric acid and malonaldehyde react under acidic conditions was first utilised by the food industry as a sensitive analytical measure of rancidity in fish oils and animal fats.[2] More recently this so called 'thiobarbituric acid' test has been used as an analytical tool in numerous pharmacological studies of medical conditions in which lipid peroxidation associated with cell necrosis is thought to participate, *e.g.* studies of cerebrovascular disorders,[3] cardiac diseases,[4] tumour tissues,[5] and plasma changes during cardiopulmonary bypass operations.[6] The test has been subjected to many modifications. Under some conditions compounds as diverse as hydroperoxy-dienes,[7] dienals,[8] 2-substituted pyrimidines,[9] and cyclic peroxides[10] give positive results. In such cases it is assumed that malonaldehyde is produced by substrate breakdown under the test conditions, but there is negligible chemical evidence for this. Furthermore, the extensive literature contains conflicting claims both for substrates[11] and for conditions which give positive results.[7,12] Thus, whilst the sensitivity of the test for malonaldehyde is accepted, its value in medical research is currently limited because the nature and concentration(s) of the active species are often open to question.

As part of a wider study into the influence of molecular oxygen on cell necrosis, in which the formation of hydroperoxy-dienes from polyunsaturated lipid components and their breakdown products have important roles,[1] we (G. R. and R. R.) are examining methods which would allow quantitative measurement of the individual species which contribute to the amount of chromogen formed in the test. As a prelude to this study it has seemed important to establish unequivocally the structure of the focal chromogen. Work by Sinnhuber[13] pointed to this being the bispyrimidine derivative (**1**), formed by the condensation of two molecules of thiobarbituric acid and one molecule of malonaldehyde, but the absence of a molar mass estimation, supporting degradative evidence, and spectroscopic analogy, coupled with the need to propose a dihydrate to accommodate the analytical evidence, meant that the precise structure was far from established. Schmidt,[14] having shown that only one of the purported hydrating molecules could be removed by extensive drying, slightly modified Sinnhuber's suggested structure, proposing the dissociated acid structure for the compound. A recent paper by Witz and his co-workers[15] provides further support for the chromogen being the product from the condensation of two molecules of thiobarbituric acid with one molecule of malonaldehyde, but direct evidence for the skeletal structure and the tautomeric form of the pyrimidine residues in the chromogen is lacking. In this paper we report n.m.r. evidence and, in particular, an *X*-ray crystallographic study of the chromogen, which confirms the essential features of Sinnhuber and Schmidt's proposals and shows that the acidity of the compound is such that it is completely dissociated even in the solid phase. The anion responsible for the intense absorption band has the oxo-thioxo tautomeric form (**2**).


(**1**)


(**2**)

Results and Discussion

Slow generation of malonaldehyde at room temperature from tetramethoxypropane in methanol–2M-sulphuric acid (1:1) containing thiobarbituric acid gave the pigment as long lustrous purple needles which were filtered off after 2 days and dried under vacuum at 80 °C. Attempts to improve this material by recrystallisation from hot polar solvents were unsuccessful. Examination under polarised light showed the crystals to be monoclinic with a decomposition range of 200—203 °C.

The ^1H n.m.r. spectrum (250 MHz) of the product in dimethyl sulphoxide revealed exchangeable protons and a sharp A_2M spectrum (Figure 1) ascribable to three methine protons (δ_A 7.73, J_{AM} 13.8 Hz; δ_M 8.57, J_{AM} 13.8 Hz).The symmetry of this grouping was only consistent with structure (**1**) if there was very rapid proton exchange between the two pyrimidine rings or if the chromogen was fully dissociated in this solvent. The pigment was in fact found to be a very strong acid. Examination of the absorption maximum at pH values down to 0.5 showed no change in absorbance within experimental error (±1%).

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† *Supplementary data* (see Section 5.6.3 of Instructions for Authors in the January issue). H-atom co-ordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

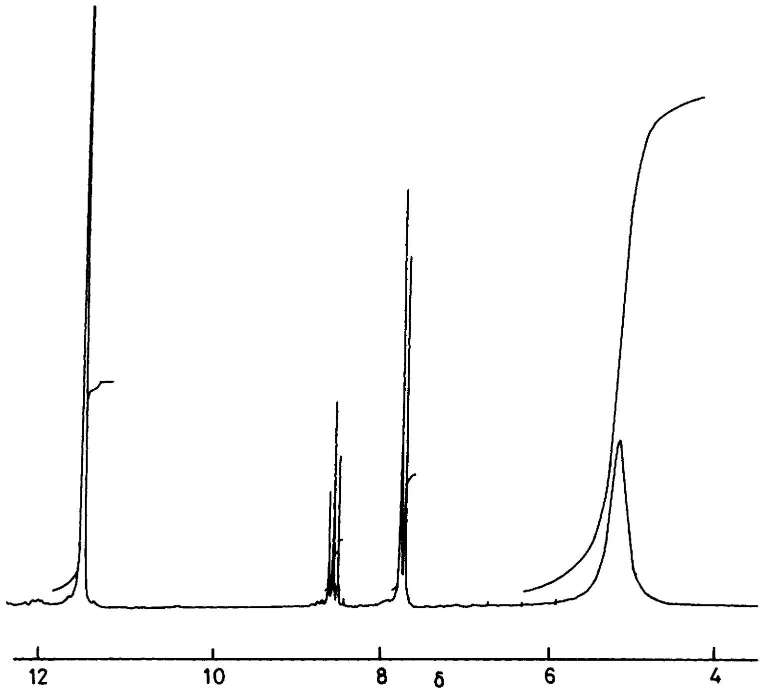

Figure 1. ^1H N.m.r. spectrum of the thiobarbituric acid–malonaldehyde chromogen (250 MHz; [$^2H_6$] DMSO; 30 °C; ref. $SiMe_4$)

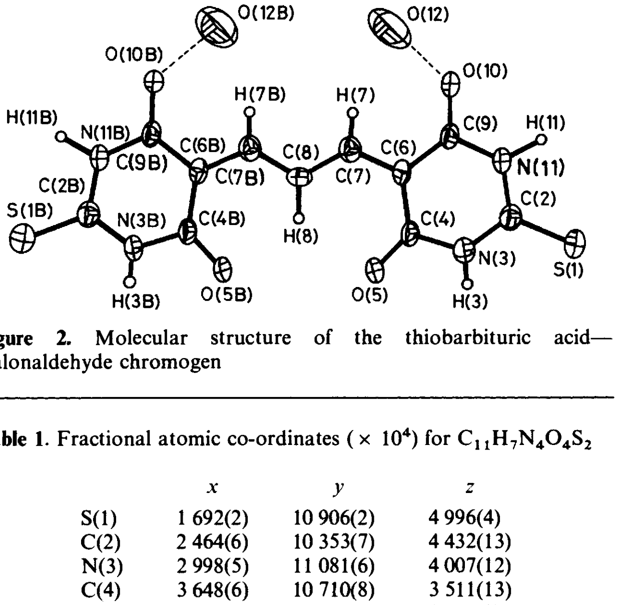

Figure 2. Molecular structure of the thiobarbituric acid—malonaldehyde chromogen

Table 1. Fractional atomic co-ordinates (× 10^4) for $C_{11}H_7N_4O_4S_2$

	x	*y*	*z*
S(1)	1 692(2)	10 906(2)	4 996(4)
C(2)	2 464(6)	10 353(7)	4 432(13)
N(3)	2 998(5)	11 081(6)	4 007(12)
C(4)	3 648(6)	10 710(8)	3 511(13)
O(5)	4 060(4)	11 520(5)	3 144(9)
C(6)	3 791(6)	9 399(7)	3 509(12)
C(7)	4 424(6)	8 887(7)	3 034(14)
C(8)	5 000	9 503(10)	2 500
C(9)	3 265(6)	8 598(7)	4 033(14)
O(10)	3 339(4)	7 449(4)	4 182(9)
N(11)	2 637(5)	9 119(6)	4 481(11)
O(12)	4 943(7)	6 265(8)	5 762(20)

Table 2. Bond lengths (Å) and angles (°) for the chromogen (**2**)

C(2)–S(1)	1.662(15)	N(3)–C(2)	1.346(17)
N(11)–C(2)	1.366(17)	C(4)–N(3)	1.377(17)
H(3)–N(3)	0.999(36)	O(5)–C(4)	1.232(16)
C(6)–C(4)	1.442(18)	C(7)–C(6)	1.393(19)
C(9)–C(6)	1.431(18)	C(8)–C(7)	1.391(17)
H(7)–C(7)	0.991(35)	O(10)–C(9)	1.253(16)
H(8)–C(8)	0.998(36)	N(11)–C(9)	1.375(16)
H(11)–N(11)	1.005(39)		

N(3)–C(2)–S(1)	122.9(11)	N(11)–C(2)–S(1)	121.7(12)
N(11)–C(2)–N(3)	115.3(13)	C(4)–N(3)–C(2)	127.1(13)
H(3)–N(3)–C(2)	118.7(*)	C(4)–N(3)–H(3)	114.1(*)
O(5)–C(4)–N(3)	117.6(14)	C(6)–C(4)–N(3)	116.1(13)
C(6)–C(4)–O(5)	126.3(14)	C(7)–C(6)–C(4)	122.7(13)
C(9)–C(6)–C(4)	118.4(13)	C(9)–C(6)–C(7)	119.0(13)
C(8)–C(7)–C(6)	127.7(14)	H(7)–C(7)–C(6)	109.6(72)
C(8)–C(7)–H(7)	121.3(73)	C(7)–C(8)–C(7)	122.6(19)
H(8)–C(8)–C(7)	118.7(10)	O(10)–C(9)–C(6)	125.9(13)
N(11)–C(9)–C(6)	118.2(14)	N(11)–C(9)–O(10)	115.9(13)
C(9)–N(11)–C(2)	124.8(13)	H(11)–N(11)–C(2)	105.5(*)
H(11)–N(11)–C(9)	128.8(*)		

Only in > 1.0M-hydrochloric acid did the absorbance begin to decrease, implying that the pK_a is significantly lower than 0 and placing the acidity of this product amongst the highest for known carbon acids [*cf.* trifluoroacetic acid, pK_a (25 °C) + 0.23].[16]

Unequivocal confirmation that the pigment is the fully dissociated acid was obtained by an *X*-ray structure analysis. The crystal structure comprises an organic anion, sited on a two-fold axis, coupled with one oxonium cation and one water molecule (Figure 2). The positioning of the anion on the two-fold axis implies that the two halves are equivalent. Such a result could be achieved by disordering of a species with two different halves, but the displacement ellipsoids for all atoms are well shaped and we believe the symmetry equivalence to be real with the electron density fully delocalised over the sp^2-hybridised system. The crystallographic symmetry also implies that the H_3O^+–H_2O pair are also disordered over the two equivalent sites.

Atomic co-ordinates are listed in Table 1. Bond lengths and angles (Table 2) are generally consistent with the model proposed, although the C(6)–C(9) bond is slightly longer than might be expected.

Experimental

Thiobarbituric acid (Aldrich) was recrystallised from hot water before use. 1,1,3,3-Tetramethoxypropane (Aldrich) was used without further purification. ^{1}H N.m.r. spectra were measured with $(CD_3)_2SO$ as solvent, using a JEOL PS/PFT instrument.

Preparation of Pigment.—2M-Sulphuric acid (20 cm^3) was added at room temperature to a filtered and stirred solution of thiobarbituric acid (0.1 g) in methanol (10 cm^3), followed by 1,1,3,3-tetramethoxypropane (60 µl). A purple colouration developed within 4 min. The solution was kept at 22 °C for 48 h, during which time the pigment slowly crystallised as long lustrous needles showing a purple reflectance (yield 78%). On drying (80 °C and 0.5 mmHg for 6 h) the pigment showed a decomposition range of 200—203 °C when examined under polarised light on a Mettler hot-stage (lit.,[13] decomp. > 300 °C).

X-Ray Crystallography.—The crystal used for the *X*-ray study was a small, blade-like fragment, cut from a larger piece and mounted on a glass fibre. Following preliminary photography, unit-cell and intensity data were obtained by using a CAD4 diffractometer and Ni-filtered Cu-K_α radiation (λ 1.5418 Å), following previously detailed procedures.[17] The diffraction peaks were broad and variable in width, the poor crystal quality probably arising out of the strong drying of the sample. The structure was solved by direct methods and refined by full-matrix least-squares techniques.

Crystal data. $[C_{11}H_7N_4O_4S_2^-]\cdot[H_3O^+]\cdot H_2O$, *M* 360.37, monoclinic, $a = 16.183(9)$, $b = 10.841(s)$, $c = 8.683(10)$ Å, $\beta = 112.05(7)°$, $V = 1\,466$ Å^3, space group $C2/c$, $Z = 4$, $D_c = 1.632$ g cm^{-3}, $\mu(\text{Cu}–K_\alpha) = 33.4$ cm^{-1}, $F(000) = 744$. A total of 1 532 data were measured, of which 1 356 were unique and only 677 were observed. The data were corrected for absorption empirically, $R = 0.084$, $R_w = 0.064$ for 128 parameters. Non-hydrogen atoms were refined anisotropically. The hydrogens attached to C and N in the anion were located in a difference map and were refined freely with individual U_{iso} values. Those on the H_3O^+/H_2O oxygen were omitted. The weighting scheme $w = 1/[\sigma^2(F) + 0.000\,05F_0{}^2]$ gave satisfactory agreement analysis.

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