848. *Pyrimidine Polymethine Dyes and Their Formation by Ring Cleavage of Heterocycles.**

By Robert G. Shepherd.

5,5′-(Prop-1-en-1-yl-3-ylidene)bis-(2-thiobarbituric acid) (I) was prepared free of by-product dye (IIIa) and the structure and chemical and spectral properties of (I) and its derivatives were studied. It is also formed by a novel ring fission of 2-substituted pyrimidines; evidence for the reaction mechanism is presented.

The formation, in acid solution, of a red dye derived from 2-thiobarbituric acid and a brain metabolite was reported by Kohn [1] in 1944. Subsequently, the application of the colour

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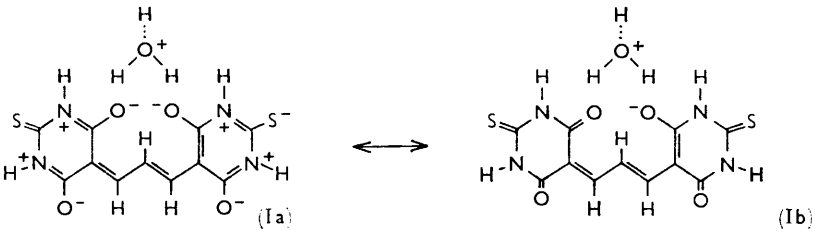
[1] Kohn and Liversedge, *J. Pharmacol.*, 1944, **82**, 292.

reaction to 2-substituted pyrimidines,[2] to fat metabolism,[1,3] and to fat oxidation [3-6] was studied in considerable detail. The reaction has also been employed in paper-chromatographic investigation [7] of metabolites of 2-sulphanilamidopyrimidine, and, more recently, in biochemical studies on a choline-containing acetal phosphatide,[8] as well as on sialic acids,[9a,10a] deoxy-sugars,[9b] and related compounds [9c] by applying it to periodate-oxidised material in solution [9] or on paper chromatograms.[10] Isolation of a crude preparation of the dye formed with thiobarbituric acid after incubation of egg lecithin was reported by Bernheim *et al.*[11] Dye solutions from these different sources had various absorption maxima in the 530—550 mμ region. We set out to investigate whether one or more dyes was involved, and to examine the structure of the dye formed from 2-substituted pyrimidines and thiobarbituric acid and the mechanism of reaction.

Preparation of the dye (I) from malondialdehyde diacetal and a large excess of 2-thiobarbituric acid avoids formation of the easily overlooked by-product which could not be conveniently removed. From comparison of analytical and spectral intensity data, it seems likely that the preparations recently reported by Sinnhuber *et al.*[12] and by Schmidt [13b] are crystalline mixtures like those we initially obtained with the same molar ratio of reagents. Since 5-substituents in the 2-thiobarbituric acid reagent prevent [2b] colour formation and *N*-substituents [6a] do not, a trimethine structure formed by reaction at the 5-position has been generally presumed for the red substance most frequently encountered



(Ia) (Ib)

($\lambda_{max.}$ 532 mμ). Linkage at the 5-positions was definitely established by reduction and acid-hydrolysis to pimelic acid. Structure (I) is supported by the synthesis from 1,3-dimethyl-2-thiobarbituric acid of the *NN'N''N'''*-tetramethyl derivative (Id) having the same very intense absorption maximum (Table). An important contribution to the resonance-hybrid by the 156 poly-ionic canonical forms [14] is suggested by its very high molar extinction coefficient, which is essentially unchanged by penta-anionisation (Table). The presence of the sulphurs would favour this contribution; cf. the shorter wavelength and

[2] (*a*) Kohn, *Proc. Soc. Exp. Biol. Med.*, 1945, **59**, 21; (*b*) Shepherd, *Analyt. Chem.*, 1948, **20**, 1150.
[3] (*a*) Oster and Oster, *J. Pharmacol.*, 1945, **85**, 332; (*b*) Abrahamson, *J. Biol. Chem.*, 1949, **178**, 179; (*c*) Wilbur, Bernheim, and Shapiro, *Arch. Biochem.*, 1949, **24**, 305; (*d*) Donnan, *J. Biol. Chem.*, 1950, **182**, 415; Zauder, *J. Pharmacol.*, 1951, **101**, 40.
[4] (*a*) Glavind and Hartmann, *Acta Chem. Scand.*, 1951, **5**, 975; (*b*) Patton and Kurtz, *J. Dairy Sci.*, 1951, **34**, 669; 1955, **38**, 901; (*c*) Sidwell, Salwin, and Mitchell, *J. Amer. Oil Chemists' Soc.*, 1955, **32**, 13.
[5] Kenaston, Wilbur, Ottolenghi, and Bernheim, *J. Amer. Oil Chemists' Soc.*, 1955, **32**, 33; Tarladgis and Watts, *ibid.*, 1960, **37**, 403.
[6] (*a*) Taufel and Zimmermann, *Fette u. Seifen*, 1961, **63**, 226; (*b*) Patton, Barnes, and Evans, *J. Amer. Oil Chemists' Soc.*, 1959, **36**, 280; Jennings, Dunkley, and Reiber, *Food Res.*, 1955, **20**, 13.
[7] Shepherd, Amer. Chem. Soc. Mtg., Chicago, Ill., U.S.A., April 1948, abstracts, p. 9K.
[8] Ohnishi, *Gann*, 1960, **51**, 1.
[9] (*a*) Aminoff, *Virology*, 1959, **7**, 355; Warren, *J. Biol. Chem.*, 1959, **234**, 1971; (*b*) Waravdekar and Saslaw, *ibid.*, 1959, **234**, 1945; Cynkin and Ashwell, *Nature*, 1960, **186**, 155; (*c*) Weissbach and Hurwitz, *J. Biol. Chem.*, 1959, **234**, 705; Ashwell, Wahba, and Hickman, *Biochim. Biophys. Acta*, 1958, **30**, 186; Levin and Racker, *Arch. Biochem. Biophys.*, 1959, **79**, 396.
[10] (*a*) Warren, *Nature*, 1960, **186**, 237; (*b*) Srinivasan and Sprinson, *J. Biol. Chem.*, 1959, **234**, 716.
[11] Bernheim, Bernheim, and Wilbur, *J. Biol. Chem.*, 1948, **174**, 261.
[12] Sinnhuber, Yu, and Yu, *Food Res.*, 1958, **23**, 626.
[13] (*a*) Schmidt, *Naturwiss.*, 1959, **46**, 379 (*Chem. Abs.*, 1960, **54**, 23336c); (*b*) *Fette u. Seifen*, 1959, **61**, 881 (*Chem. Abs.*, 1959, **53**, 14374b); (*c*) *Fette u. Seifen*, 1959, **61**, 129.
[14] There are 28 tri-, 64 penta-, 52 hepta-, and 12 nona-ionic (Ia) forms with negative charges on N, O, or S in addition to 4 monoionic forms (Ib).

lower intensity of the sulphur-free dye (Ic) formed from barbituric acid and malondialdehyde (Table). Examination of molecular models [15] demonstrated that the *trans*-configuration (I) of 5,5′-prop-1-en-1-yl-3-ylidene)bis-(2-thiobarbituric acid) is required for a planar resonating structure. It was also clear that the molecules in the crystal can form N–H · · · O and N–H · · · S=C bonds all round their periphery and leave sufficient space for the three-dimensionally hydrogen-bonded hydronium ion indicated. The extreme stability of the monohydrate is not due to the water being chemically bound since the infrared, visible, and ultraviolet spectra were unchanged by dehydration. The highly resonance-stabilised symmetrical mono-anion renders (I) more acidic than 2-thiobarbituric acid.

Spectral properties of trimethines.

	Absorption maxima (mμ)						
	In 0·1N-NaOH [a,b]		In 0·1N-HCl [c]		In other media		
Compound	$\lambda_{max.}$	ε	$\lambda_{max.}$	ε	Soln.	$\lambda_{max.}$	ε
(I) 5,5′-Prop-1-en-1-yl-3-ylidene)bis-(2-thiobarbituric acid)	544	194,000	532 [d]	199,000	0·0002N-AcOH–MeOH	532	199,000
	311	8,700	307	11,500	1N-HCl	532	176,000
	245	20,500	245	25,500	6N-HCl	532 [d]	157,000
(Ic) 2,2′-Dioxo-analogue of (I)	498	131,000	488	124,000			
	255	16,400	241	21,000			
(Id) *N*-Me_4 derivative of (I)	533	>80,000 [b,e]	532 [d,f]	189,500			
(II) 1-Me derivative of (I)	544	dec. [g]	541 [d]	45,000	0·01N-HCl	545	86,500
			285	9,500	,,	375	61,000
1-COOH derivative [10b] of (I)			549	>72,000 [h]			
(IIIa) By-product	544	314,000	532 [d]	189,000	0·0002N-AcOH–MeOH	532	277,000
	307	18,600	245	29,600			
	245	48,000					
(IIIb) *S*-Me derivative of (I)	544	156,000	532 [d]	86,000	0·0002N-AcOH–MeOH	532	126,000
5(3-Oxo-1-butylidene)-2-thiobarbituric acid	383	31,500	372	29,000	15% AcOH	412	40,000
Indigo [32]					$CHCl_3$	604	12,300
2-Thiobarbituric acid	282	12,800	282	23,200	Conc. H_2SO_4	265	18,200
	235	17,500	235	9,300			

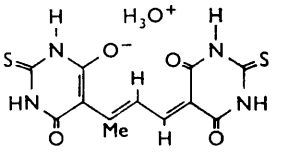
[a] All the red colours are somewhat unstable in alkali (25% decrease in 3 hr.) and absorption at 367, 265, and 245 mμ increases. [b] The colour loss is reversible on acidification to pH 1–3 and brief heating. [c] (I) had the same spectrum in 1N-sodium acetate. [d] The shoulder at 505 mμ (estimated ε 58,000) in (I) decreased in stronger acid in proportion to the peak at 532 mμ. A shoulder of various intensities was present in (Id), (II), (IIIa), and (IIIb). [e] The red colour changed to pale yellow almost immediately. [f] Maxima present also at 303 (ε 13,300) and 245 mμ (ε 19,600). The tetraethyl analogue is reported [6a] to have $\lambda_{max.}$ 540 mμ in acid. [g] Too unstable to measure, yielding peaks at 342, 282, and 236 mμ also. [h] Unstable.

Initially, erroneous spectra were obtained as a result of the instability of the dye (I) to alkali, which is very much greater (almost instantaneous decolourisation) in the *N*-tetramethyl derivative (Id). With this compound, the nucleophilic additions to the chromophore presumably responsible for decolourisation are not retarded by N–H dissociation to a polyanion, as occurs in (I). *NN*′-Dimethylindigo is also easily decomposed by alkali,[16] in contrast to indigo, but its reactivity is attributable [17] to steric inhibition of resonance which makes itself evident by a strong bathochromic shift (44 mμ) with respect to indigo. In the *N*-tetramethyl derivative (Id), the instability has a different origin, since the methyl groups do not affect its coplanarity and cause no spectral change. However, instability and a bathochromic shift of the type observed with *NN*′-dimethylindigo do result from the presence of a substituent on the methine bridge. Thus, the dye (II) with methyl on the 1-carbon of the bridge was unstable to cold dilute alkali, and to warm water. The distortion

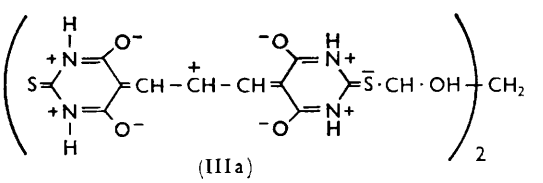
[15] Molecular models from Catalin Ltd. were used.
[16] Ettinger and Friedländer, *Ber.*, 1912, **45**, 2074.
[17] (*a*) Knott, *J. Soc. Dyers and Colourists*, 1951, **67**, 305; (*b*) Weinstein and Wyman, *J. Amer. Chem. Soc.*, 1956, **78**, 4009.

from coplanarity necessary to explain this instability and bathochromic shift (13 mμ) with respect to (I) is clearly seen in molecular models.[15] The bathochromic shift (17 mμ; Table) and instability observed [9a,c,10a] in coloured solutions formed from formylpyruvic acid and thiobarbituric acid can be similarly attributed to the steric effect of the carboxyl at the same position. The presence of an absorption maximum at the longer wavelength can be taken as presumptive evidence of a 1-substituent.

The original observations by Brunings and Corwin [18] on dipyrrylmethenes, and the further studies by Brooker and co-workers,[19] showed that a shift (up to 37 mμ) to longer wavelengths occurs in resonating symmetrical molecules as a result of substituents causing distortion from coplanarity around an element of symmetry.[20a,d] Concomitant lowering of intensity is a consequence of decreased overlap of π-electronic orbitals, and this effect is associated with steric inhibition of resonance in general.[20,21] However, the absorption maximum is commonly shifted hypsochromically when the distortion is not around an element of symmetry [21] or when the chromophore is unsymmetrical.[19c]



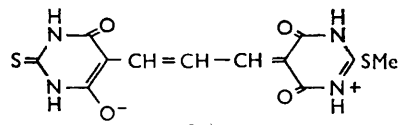
(II)



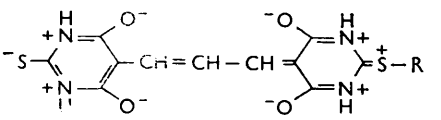
(IIIa)

In the 1-methylbis(thiobarbituryl)trimethine (II), the steric hindrance of the oxygen, the methyl, and the 3-hydrogen can be accommodated by some nonplanarity of both units attached to the central methine carbon. Since the resonating chromophore in (II) is still nearly symmetrical electronically and the distortion is around its central carbon atom, the Brunings–Corwin effect is expected to apply. Some bridge-substituted cyanines, symmetrical except for this substitution, were included in Brooker's studies [19b] of this effect. Attempts to prepare the analogue with methyl on the 2-carbon of the bridge failed as a result of even greater distortion [15] and consequently greater instability.

The crystalline dye obtained when the stoicheiometric amount, rather than a large excess, of thiobarbituric acid reacts with either malondialdehyde or a 2-substituted pyrimidine, contains about 25% of a by-product, whose presence was suggested by a high acidimetric equivalent weight, though not by elemental analyses or spectra. Results obtained with the by-product itself were consistent with a hydrated 2 : 1 adduct of dye (I) and malondialdehyde of structure (IIIa). Similar material is formed from 1,3-dimethyl-2-thiobarbituric acid. Substitution on sulphur is also supported by the similar spectral behaviour of (IIIa) and the *S*-methyl derivative (IIIb), including spectral indication of basicity (0·1N against 0·0002N acid; Table).



(IIIb)



(IIIc)

Spectral characteristics of mono- and di-*S*-alkylated derivatives of (I) suggest participation of *d*-orbital resonance. In dilute acid, the absorption maxima of (IIIa) and (IIIb) at 532 mμ have one-half the intensity, per chromophore unit, of (I). This absorption is

[18] Brunings and Corwin, *J. Amer. Chem. Soc.*, 1942, **64**, 596.
[19] Brooker, White, Sprague, Dent, jun., and van Zandt, *Chem. Rev.*, 1947, **41**, (*a*) 325, (*b*) 334, (*c*) 344.
[20] Gray, ed. "Steric Effects in Conjugated Systems," Butterworths, London, 1958; (*a*) Coulson, p. 8; (*b*) Beaven, p. 22; (*c*) Barker, p. 34; (*d*) Dewar, p. 46.
[21] Remington, *J. Amer. Chem. Soc.*, 1945, **67**, 1838.

assigned to a resonance hybrid of the 136 resonance structures involving ten-electron sulphur,[22] *e.g.*, (IIIa) and others with the positive charge on the 5- and 5′-carbons and on the other sulphur. Conjugation of the sulphurs with the bisdioxopyrimidinyltrimethine system by means of the 3*p*-orbitals of sulphur is illustrated by (IIIc) and 76 similar forms but this electron-donating resonance is generally considered [23] relatively unimportant in sulphur compounds for geometric reasons. In 0·1N-alkali, the *S*-methyl derivative (IIIb) and its analogue (IIIa) have the same absorption maximum (544 mμ) and almost the same extinction per chromophore unit as parent (I). The increased intensity associated with this shift to the same wavelength as in (I) is consistent with much greater resonance participation of methylthio *d*-orbitals in the tetra-anion since the chromophore is richer in electrons, has twice as many atoms (four nitrogen atoms in addition to the initial three carbons and one sulphur) from which to draw an electron-pair and there are now 288 canonical forms. In dilute acid, the 532 mμ maximum of the *SS*′-dimethyl derivative was also less intense than that of (I), and in alkali was likewise shifted bathochromically (to 544 mμ) and increased in intensity.

Reaction of 2-sulphanilamidopyrimidine with 2-thiobarbituric acid in hot acetic acid (pH 2) gave the same dye (I), embodying the 4-, 5-, and 6-pyrimidinyl carbons in the trimethine bridge and producing sulphanilylguanidine as the only other ring fragment. Degradation of the ring under these rather mild conditions could involve either (1) an unfavourable equilibrium (hydrolytic ring-opening) which is shifted by consumption of ring-opened aldehyde by thiobarbituric acid, or (2) a ring-opening which requires specific attack by the latter. Evidence against the first possibility is the fact that displacement of the supposed equilibrium does not occur with other " aldehyde reagents " (various hydrazine derivatives) or with oxidising agents. Further, 2-benzenesulphonamidopyrimidines are stable to 5-chlorination [24a,b] or bromination [24b] in acid and to 5-iodination [24c] in hot acetic acid and mercuric acetate. Evidence for the second possibility is the fact that 2-sulphanilamidopyrimidine was unchanged after exposure to boiling acetic acid and gave no trace of distillable malondialdehyde. Also 2-imino-1-methylpyrimidine is stable to hot acetic acid, but if thiobarbituric acid is present, (I) is rapidly formed. Brown [25] has shown that, when the ring in this imine opens, it isomerises by reclosure to 2-methylaminopyrimidine. The observation that various 4- or 5-substituents prevent ring-opening as well as colour formation [2b] indicates that a bulky reactant is involved, whereas attack by hydroxide ion at another ring-carbon should not be hindered. Therefore, we conclude that degradation of pyrimidines under these mild conditions requires attack by the 2-thiobarbituric acid reagent.

The mechanism is outlined in the sequence (IV)—(VIII). The unfavourable effect of higher and lower pH on the reaction rate arises from consequent decreases in concentrations of the reactants: protonated heterocycle (V) and of thiobarbiturate anion, respectively. 2-Thiobarbituric acid is approximately half-ionised [26] at pH 2 and the slower reaction of barbituric acid would follow from the 40-fold lower concentration [27] of its anion. Reaction between thiobarbituric acid enol (a minor component [28] compared to the oxo-form of the

[22] Cf. Ten-electron sulphur resonance in various systems: Knott, *J.*, 1955, 916, 937; Duffin and Kendall, *J.*, 1956, 361; Craig and Magnusson, *J.*, 1956, 4895; Cilento, *Chem. Rev.*, 1960, **60**, 152, 154.

[23] Ingold, " Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, Ithaca, N.Y., U.S.A., 1953, ch. 2; Bordwell and Bouton, *J. Amer. Chem. Soc.*, 1956, **78**, 854; Baker and Harris, *J. Amer. Chem. Soc.*, 1960, **82**, 1923.

[24] (*a*) English, Clark, Clapp, Seeger, and Ebel, *J. Amer. Chem. Soc.*, 1946, **68**, 455; (*b*) English, Clark, Shepherd, Marson, Krapcho, and Roblin, *ibid.*, 1946, **68**, 1048; (*c*) Shepherd and Fellows, *ibid.*, 1948, **70**, 157.

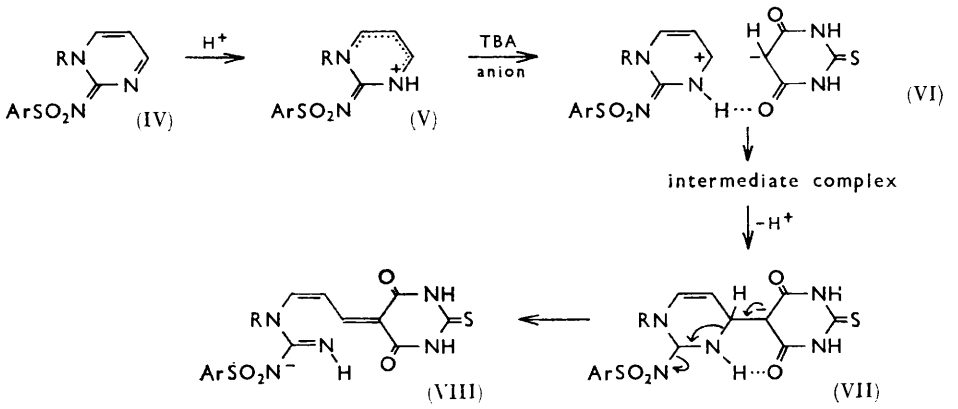
[25] Brown, Hoerger, and Mason, *J.*, 1955, 211, 4035.

[26] Measured pK_a 2·25, in agreement with Sato, *Nippon Kagaku Zasshi*, 1957, **78**, 921 (*Chem. Abs.*, 1960, **54**, 4598b), but not with Mautner and Clayton, *J. Amer. Chem. Soc.*, 1959, **81**, 6271.

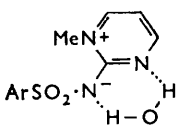
[27] Based on measured pK_a 3·82, approximately in agreement with Mautner and Clayton (*loc. cit.*) and Biggs (*J.*, 1956, 2485) with values of 4·12 and 4·04, respectively.

[28] Katritzky and Lagowski in " Advances in Heterocyclic Chemistry," Vol. 1, p. 375, Academic Press, New York, N.Y., 1963.

acid) and (IV) is a less likely and only slightly modified form of the first stage of the reaction. The intermediate complex partly ionises to (VII) and the resulting lone-pair then provides the driving force for ring-opening [29] as shown in (VII). The benefit of more electron-attracting character at the 2-position (arylsulphonamido > acylamido > amino in reaction rate [25]) is seen in this ring-opening process. Completion of the reaction results from similar



nucleophilic attack at the electron-deficient carbon adjacent to the other ring-nitrogen after protonation of (VIII). Activation of the pyrimidine ring for nucleophilic attack by the reagent anion is a consequence of the resonance-stabilised cationisation in (V). Participation of structure (V) is supported by the predominant sulphonimido-structure of the sulphonamido-heterocycles so far investigated [30] and by the unique behaviour of the *N*-alkyl isomers. An alkyl group on the sulphonamide-nitrogen prevents the reaction entirely while one on a ring-nitrogen (IV; R = Me or $HO{\cdot}C_2H_4$) has only a slight rate-decreasing effect.[25] In contrast to the sulphonamide (IV; R = H), the methyl derivative (IV; R = Me) is still reactive in neutral or alkaline solution due to its partly cationic nature (IX).



(IX)

Quaternised pyridine or glutacondialdehyde gave a blue colour with 2-thiobarbituric acid which on mild heating changed to the spectrum of (I). Synthesis of the latter is akin to the unusual formation of a trimethine unit from a pyridinium compound recently reported [31] except that, in the present instance, the pentamethine is formed and then decomposes.

Experimental

Spectra (Table) were obtained with a Cary recording spectrophotometer. Instabilty errors were avoided by dissolving samples in cold 100 : 1 (v/v) dimethylformamide : acetic acid and diluting immediately before recording.

5,5′-(Prop-1-en-1-yl-3-ylidene)bis-(2-thiobarbituric Acid) (I).—To 2-thiobarbituric acid monohydrate (64·8 g., 0·4 mole) in refluxing 15% (v/v) acetic acid (pH 2·1; 1100 ml. used to keep excess of reagent dissolved on subsequent cooling) 1,1,3,3-tetraethoxypropane (22·0 g., 0·1 mole) was added during 5 min. and heating was continued for 45 min. The silky, blue crystals were filtered at 20° and washed with dilute acetic acid (30·6 g.), air-dried (85% of theoretical as dihydrate). The dye was insoluble in most organic solvents but was highly soluble (>16 g./100

[29] Shepherd, Symposium on Heterocyclic Chemistry, Amer. Chem. Soc. Mtg., Washington, D.C., U.S.A., March 1962, abstract, p. 16N. Heterocyclic rings are not opened by strongly nucleophilic alkyl anions whose intermediate complexes do not tend to form anions.

[30] (*a*) Shepherd, Bratton, and Blanchard, *J. Amer. Chem. Soc.*, 1942, **64**, 2532; (*b*) Angyal and Warburton, *Austral. J. Sci. Res.*, 1951, **4**, 93; (*c*) Jones and Katritzky, *J.*, 1961, 378.

[31] Ficken and Kendall, *J.*, 1959, 3988.

ml.) in liquid amides, dimethylsulphoxide, and concentrated sulphuric acid. Most attempts at purification from organic solvents gave voluminous, solvated brown material and in various aqueous media gave colloidal solutions. However, dissolving in boiling 15% acetic acid (1 g./200 ml.) followed by filtration, addition of 1/200 vol. of 2N-hydrochloric acid and immediate rapid cooling gave dense, blue crystalline *trimethine* (93% recovery). Air-drying gave the *dihydrate* (Found: C, 37·0; H, 3·4; N, 15·8; S, 17·5%; Equiv., 366. $C_{11}H_8N_4O_4S_2,2H_2O$ requires C, 36·7; H, 3·3; N, 15·6; S, 17·8%; Equiv., 360). After being dried at 20° (20 mm.) over sulphuric acid or at 80°/0·1 mm. over phosphoric anhydride, the monohydrate (m. p. >370°) was obtained (Found: C, 38·6; H, 2·8; N, 16·5; S, 18·5. $C_{11}H_8N_4O_4S_2,H_2O$ requires C, 38·6; H, 2·9; N, 16·4; S, 18·7%). Only after rigorous drying (10^{-5} mm. over P_2O_5 for 20 hr. at 150°) was the remaining water removed (Found: N, 17·2. $C_{11}H_8N_4O_4S_2$ requires N, 17·3%). In air, fast absorption of 1 mole of water occurred (Found: H_2O, 5·2 by weight increase. Monohydrate requires H_2O, 5·3%).

The τ-value for n.m.r. of the methine hydrogens was 5·32 (5 mm. spinning sample in formamide at 40 Mc. sec.$^{-1}$); pK_a in 75% dimethylformamide (2 mg./ml.; 1N-potassium hydroxide; potentiometric) 2·5 for the first equivalent, compared to 3·7 for 2-thiobarbituric acid. In stronger alkali, two more hydrogens dissociated (pK_a ~12—12·5) (Found: Equiv., 174, 185. Dihydrate requires Equiv., 180); additional inflection corresponding to two additional hydrogens occurred at pH ~13·6.

The dye crystallises as ammonium, diethylammonium, or sodium salt monohydrates from 4N-ammonia solution, liquid diethylamine, or dilute alkali even when dilute solutions are added to aqueous acetic acid. The dye prepared in citrate buffer[32] reaction medium contained appreciable sodium. Salts were converted into (I) by precipitation from 20 equivalents of 2N-hydrochloric acid and crystallisation from hot 15% acetic acid. *Ammonium salt* was obtained quantitatively from aqueous or colloidal solutions by adding ammonium acetate (2 g./100 ml.); the latter was removed from the solid at 40°/1 mm. (Found: C, 36·9; H, 3·9; N, 19·4; volatile basic N, 3·6. $C_{11}H_8N_4O_4S_2,NH_3,H_2O$ requires C, 36·8; H, 3·7; N, 19·5; volatile basic N, 3·9%). No basic properties were detected: it was precipitated chloride-free from concentrated hydrochloric acid and as sulphate-free *monohydrate* from concentrated sulphuric acid and ether; potentiometric titration in glacial acetic acid or in nitromethane with perchloric acid.

In concentrated or 100% sulphuric acid, the 532 mμ peak of (I) was only briefly visible, new absorptions at 505 and 480 mμ also rapidly disappeared; absorption after a few hours was characteristic of thiobarbituric acid. On dilution with water to 0·4N, reversal to the spectrum of (I) occurred at once. When a 17% solution in concentrated sulphuric acid was added after 5 hr. at 20° to 10 times its weight of ice, an 88% recovery was obtained of material having the characteristics of (I).

Extraction from aqueous acid with n-butanol can be used to increase the sensitivity of the colour reaction to the nanogram range (cf. ref. 9*a*) and to obtain micro-amounts for paper-chromatographic comparisons. n-Butanol : 15N-ammonia (4 : 10) gave R_F 0·15 for (I) and R_F 0·66 for the tetra-*N*-methyl analogue. Other chromatographic behaviour has been reported.[12,13]

Structure Proof of (I) *by Reduction and Hydrolysis to Pimelic Acid.*—Irreversible catalytic (PtO_2) reduction of the barbituric acid analogue of (I) failed. In the following procedure, decolorisation (presumably by reduction of one C=C bond, reversible in air) proceeded at moderate speed but irreversible reduction much more slowly.

To dye (I) monohydrate (684 mg.) in 5N-sodium hydroxide (15 ml.) was added Raney nickel–aluminium alloy (0·6—1·2 g.) until external heating regenerated only faint colour. The precipitate (220 mg.) collected after acidification was refluxed in 80% sulphuric acid (4 ml.) for 5 hr. After addition of water (14 ml.) and extraction with ether (10 × 5 ml.), the extract was washed 3 times with a small volume of 30% sulphuric acid and once with water. Evaporation and drying (H_2SO_4) yielded long, white needles, m. p. 103° (8·9 mg.; 3% of theoretical C_7 acid), lit.,[33] m. p. 104°, mixed m. p. 103°. Infrared absorption curves of the unknown and of authentic pimelic acid were superimposable.

By-product (IIIa) *of Synthesis of* (I).—When the preparation of (I) above was carried out with a 2 : 1 mole-ratio of 2-thiobarbituric acid to malondialdehyde diacetal, the same yield

[32] Brode, Pearson, and Wyman, *J. Amer. Chem. Soc.*, 1954, **76**, 1035.
[33] Müller, *Monatsh.*, 1934, **65**, 18.

(85%) was obtained of "analytically pure" (C,H,N,S) intensely-coloured material whose elemental analysis, and infrared, visible, and ultraviolet spectra, were the same as for (I) and were unchanged after crystallisation from boiling 15% acetic acid. Potentiometric titration showed a high equivalent weight (400—410) for the first dissociation but almost the same equivalent weight (175) as (I) for the second dissociation of two hydrogens. These data suggested an impurity with the same chromophore but lacking the strongest acidic group, *e.g.*, the disulphide or a thioacetal of (I). Although extraction with 0·01N-sodium hydroxide or boiling 15% acetic acid demonstrated a contaminant, pure (I) could not thereby be prepared. (IIIa) was obtained by extraction of the product mixture with boiling 15% acetic acid [1·5 times the vol. necessary to dissolve all the (I) present]. Only a little of the insoluble portion was dissolved on re-extraction with acetic acid (600 ml./g.), and it was then dissolved in 0·05N-sodium hydroxide and the dark red *by-product* precipitated by addition at once to 20 equivalents of 2N-hydrochloric acid (Found: C, 40·9; H, 3·3; N, 15·1; S, 17·7%; Equiv., 175. $C_{25}H_{20}N_8O_{10}S_4,H_2O$ requires C, 40·7; H, 3·0; N, 15·2; S, 17·4%; Equiv., 184).

The infrared absorption curve (KBr disc) had the same principal peaks as pure (I). On titration in dimethylformamide the single, most acidic hydrogen was absent and at pH 12·5 there was an inflection corresponding to an equivalent weight of 175. The molecular weight (700 for 4 dissociable H's) calculated therefrom, the analyses, and the spectra are consistent with the postulated structure (IIIa). An alkaline solution of (I) with air or hydrogen peroxide gave no disulphide formation. The analytical data are not consistent with a product formed from one mole each of (I), thiobarbituric acid, and malondialdehyde.

This by-product was also formed during synthesis of (I) by ring-degradation of 2-substituted pyrimidines.

1,3-*Dimethyl*-2-*thiobarbituric Acid*.—A mixture of alcohol (110 ml.), sodium ethoxide (13·6 g., 0·2 mole), diethyl malonate (16·0 g., 0·1 mole), and 1,3-dimethylthiourea (10·4 g., 0·1 mole) was refluxed for 7 hr. After evaporation to 50 ml. and addition of water (50 ml.), the solution was added to 6N-hydrochloric acid (40 ml.). The precipitate was crystallised from hot water to give the white *product* (30% yield, dried over H_2SO_4), m. p. 183° (Found: C, 41·8; H, 4·8; N, 15·9; S, 18·6. $C_6H_8N_2O_2S$ requires C, 41·9; H, 4·7; N, 16·3; S, 18·6%).

5,5′-(*Prop*-1-*en*-1-*yl*-3-*ylidene*)*bis*-(1,3-*dimethyl*-2-*thiobarbituric Acid* (Id).—To 1,3-dimethyl-2-thiobarbituric acid (6·9 g., 0·04 mole) in refluxing 15% acetic acid (210 ml.) was added 1,1,3,3-tetraethoxypropane (2·2 g., 0·01 mole). After being boiled for 45 min., the mixture was cooled in ice and the purple crystals were washed with dilute acetic acid, water, and acetone (wt. 0·96 g., 23%). After crystallisation from boiling n-butanol (110 ml./g.), the purple-red *trimethine* was dried (H_2SO_4) *in vacuo*, m. p. 260° (decomp.) (Found: C, 43·4; H, 4·8; N, 13·7; S, 15·2; H_2O, 8·5%; Equiv., 423. $C_{15}H_{16}N_4O_4S_2,2H_2O$ requires C, 43·3; H, 4·8; N, 13·5; S, 15·4; H_2O, 8·6%; Equiv., 416).

A neutral by-product comparable to (IIIa) was formed in about 20% yield when 1,3-dimethylbarbituric acid was not present in excess. On removing the desired product with hot butanol, the by-product was obtained (Found: C, 46·0; H, 4·3; N, 13·5; S, 15·4. $C_{33}H_{36}N_8O_{10}S_4,H_2O$ requires C, 46·5; H, 4·5; N, 13·2; S, 15·1%).

5-(3-*Oxo*-1-*butylidene*)-2-*thiobarbituric Acid*.—Thiobarbituric acid hydrate (1·95 g., 0·12 mole) and 3-oxobutyraldehyde dimethylacetal (0·66 g., 0·05 mole) in refluxing 15% acetic acid (40 ml.) were heated for 20 min. and the red-brown *alkylidene derivative* was filtered off, washed with dilute acetic acid, and dried (P_2O_5); it (220 mg.) had m. p. 270° (decomp.) (Found: C, 44·9; H, 4·0; N, 12·9; S, 14·9. $C_8H_8N_2O_3S$ requires C, 45·3; H, 3·8; N, 13·2; S, 15·1%). The 1670 cm.$^{-1}$ absorption (ketone) is not present in the methine analogues. Absorption at 532 mμ (shifted to 544 mμ in alkali) was only about 0·5% (ε *ca.* 900) of the intensity produced by malondialdehyde under the same reaction conditions.

5,5′-(1-*Methylprop*-1-*en*-1-*yl*-3-*ylidene*)*bis*-(2-*thiobarbituric Acid*) (II).—2-Thiobarbituric acid (170 mg., 1·05 mmole) and 5-(3-oxo-1-butylidene)-2-thiobarbituric acid (212 mg., 1 mmole) were suspended in warm 2-ethoxyethanol (4 ml.) and treated with sodium (23 mg., 1 mmole) dissolved in 2-ethoxyethanol (1 ml.). On boiling, a clear red solution quickly formed and in 2 min. heavy crystallisation occurred. The crystals were centrifuged, washed well with 2-ethoxyethanol, and dissolved in cold water to give a neutral solution. Addition of acetic acid and hydrochloric acid [see above for (I)] caused crystallisation of the C-*methyltrimethine* (100 mg.), m. p. >370° (Found: C, 37·8; H, 3·9; N, 14·6. $C_{12}H_{10}N_4O_4S_2,2·5H_2O$ requires C, 37·6; H, 3·9; N, 14·6%).

5-[3-(3,4-*Dihydro*-6-*hydroxy*-2-*methylthio*-4-*oxo*-5-*pyrimidinyl*)*allylidene*]-2-*thiobarbituric Acid*

(IIIb) *by* S-*Monomethylation of* (I) *under Acidic Conditions*.—To dye (I) (324 mg., 0·95 mmole) in dimethylformamide (2 ml.) was added dimethyl sulphate (0·166 g., 1·32 mmole in 0·5 ml. of dimethylformamide). After 30 min. at 60°, the crystals were centrifuged and washed twice with dimethylformamide and propanol. On extraction with hot 15% acetic acid (15 ml.), very little solid or colour was removed; the product (200 mg.) was dried at 40° for 18 hr. (over KOH and P_2O_5) to remove volatile material from this *S*-methyl derivative, m. p. >370° (Found: C, 41·2; H, 3·6; N, 15·7. Calc. for $C_{12}H_{10}N_4O_4S_2,0{\cdot}83H_2O$: C, 40·9; H, 3·3; N, 15·9%). The product (46 mg.) was identified through the dianilino-analogue by boiling it with aniline (1 ml.) for ½ hr., at which time the colour change (red–blue–pale yellow) was complete and evolution of methanethiol ceased. White dianilino-analogue (30 mg.) obtained on addition to 1 : 1 benzene–petroleum (12 ml.) was washed with hot benzene and then ether (Found: C, 58·8; H, 4·8; N, 18·3. Calc. for $C_{23}H_{18}N_6O_4,1{\cdot}4H_2O$: C, 59·0; H, 4·5; N, 18·0%). Loss of one carbon and two sulphurs confirms monosubstitution on sulphur.

5-[3-(3,4-*Dihydro*-6-*hydroxy*-2-*methylthio*-4-*oxo*-5-*pyrimidinyl*)*allylidene*]-2-*methylthio*-*pyrimidine*-4,6-*dione by* SS′-*Dimethylation of* (I) *in Alkali*.—The dye (324 mg., 0·95 mmole) in 2·75N-sodium hydroxide (20 ml., 55 mmoles) was treated dropwise with dimethyl sulphate (2·52 g., 20 mmole). After stirring for ½ hr., the product was filtered off, redissolved in water (40 ml.), and added to 2N-hydrochloric acid (20 ml.). Blue SS′-*dimethyl derivative* was washed by centrifugation (three times each) in dilute acetic acid, water, acetone, and ether, and then vacuum-dried over sulphuric acid (yield, 85 mg.) (Found: C, 40·3; H, 4·3; N, 14·1. $C_{13}H_{12}N_4O_4S_2,2H_2O$ requires C, 40·2; H, 4·2; N, 14·4%). Its colour was unstable in 0·1N-sodium hydroxide (peaks at 544 and 525 mμ soon disappear) and after 1 hr. in 0·1N-hydrochloric acid (peaks at 485 and 243 mμ, but not at 532 mμ).

5,5′-(*Prop*-1-*en*-1-*yl*-3-*ylidene*)*dibarbituric Acid* (Ic).—After 1,1,3,3-tetraethoxypropane (22·0 g., 0·1 mole) and barbituric acid (51·2 g., 0·4 mole) had been heated in boiling 15% acetic acid (2500 ml.) for 40 min., the mixture was filtered hot, treated with 2N-hydrochloric acid (25 ml.), and cooled rapidly to 20°. The fine, red crystals (dihydrate) were washed with dilute acetic acid, water, and acetone and dried *in vacuo* at 90° (19·6 g., 60%); crystallisation from hot 15% acetic acid (230 ml./g.) (90% recovery) gave the bright red *trimethine monohydrate*, m. p. 330° (decomp., dried (H_2SO_4) *in vacuo* (Found: C, 42·3; H, 3·5; N, 17·9. $C_{11}H_8N_4O_6,H_2O$ requires C, 42·6; H, 3·3; N, 18·1%). This trimethine forms insoluble salts as does (I); pK_a 3·4 the first equivalent in 75% dimethylformamide.

5,5′-(*Prop*-1-*en*-1-*yl*-3-*ylidene*)*bis*-(2-*thiobarbituric Acid*) *from* 2-*Substituted Pyrimidines*.—2-Sulphanilamidopyrimidine (0·50 g., 2 mmole) and 2-thiobarbituric acid hydrate (1·30 g., 8 mmole) in 15% acetic acid were heated for 1·5 hr. The product was the red monohydrated *sulphanilylguanidine salt* of the dye (yield 65%) (Found: C, 39·2; H, 3·8; N, 19·8; S, 17·5%; Equiv., 563. $C_{11}H_8N_4O_4S_2,C_7H_{10}N_4O_2S,H_2O$ requires C, 38·9; H, 3·6; N, 20·1; S, 17·2%; Equiv., 557) ($\lambda_{max.}$ 532 mμ, ε 198,000 requires $\lambda_{max.}$ 532 mμ, ε 199,000 in 0·1N-hydrochloric acid). Addition of a strong dimethyl sulphoxide solution of this material to 20 equivalents of 2N-hydrochloric acid followed by crystallisation from dilute acetic acid gave material identical to (I) in R_F in infrared, visible, and ultraviolet spectra, and elemental analyses.

Paper chromatography in 1 : 1 butanol–3% aqueous ammonia with butyl nitrite in acetic acid followed by *N*-1-naphthylethylenediamine showed 1 mole of sulphanilylguanidine (R_F 0·48) in the reaction mixture and in the salt above. Less than 1% of sulphanilylurea (R_F 0·04), sulphanilylcyanamide (R_F 0·05), sulphanilamide (R_F 0·62), or sulphanilic acid (R_F 0·07) was formed, none being detected.

An intermediate formed by reaction at 25° had $\lambda_{max.}$ about 460 mμ which disappeared as the peak at 532 mμ increased. Paper chromatography of a partially reacted mixture revealed material with R_F 0·12 (distinctly separated from unchanged 2-sulphanilamidopyrimidine, R_F 0·17) whose spot become red when heated with thiobarbituric acid; the mixture also contained primary arylamine.

The absence of an equilibrium producing malondialdehyde by hydrolytic ring-opening without 2-thiobarbituric acid was demonstrated by slow distillation (900 ml. in 3 hr.) of 15% acetic acid (1300 ml.) containing 2-sulphanilamidopyrimidine (1·25 g., 5 mmoles). The distillates gave no colour with 2-thiobarbituric acid; a solution of 2-sulphanilamidopyrimidine and sulphanilylguanidine 10^{-5}M in malondialdehyde acetal gave a clearly positive distillate. Schmidt's [13c] positive colour test could have resulted from the strong acid (1N-H_2SO_4) used or from the extreme sensitivity of the test in detecting minute amounts of entrained spray. When

our experiment was carried out without a 6 in. Vigreux column, a faint colour was formed with thiobarbituric acid but diazotisable arylamine was also present in the distillate.

The reaction of 2-imino-1-methylpyrimidine [25] (55 mg.) with thiobarbituric acid (325 mg.) in boiling 15% acetic acid was about 50% complete in 1 hr. Under the same conditions, but in the absence of thiobarbituric acid, 2-imino-1-methylpyrimidine was unchanged (90% recovery; m. p. and mixed m. p. 103—105°). An artificial mixture (1 : 10) of the lower-melting rearrangement product and the imine melted at 65—95°.

2-Aminopyrimidine formed (I) more slowly than the imine above. Pyrimidine and its 2-mercapto-derivative gave unstable and stable red colours, respectively.

No colour was formed from pyrazole, 1-phenylpyrazole, 3-amino-1,2,4-triazine, pyridazine, imidazole, 1-methylimidazole, pyridine, or pyridine N-oxide. Cleavage to glyoxal would produce a red colour ($<0{\cdot}3\%$ of malondialdehyde intensity) and to formic acid would form a yellow colour.[13] Pyridine produced a blue colour after quaternisation with 1-chloro-2,4-dinitrobenzene or dimethyl sulphate. Unsuccessful application of the test to certain other heterocycles was previously reported.[2] Glutacondialdehyde and its dianil produced blue colours ($\lambda_{max.}$ 625 mμ, $\varepsilon > 50{,}000$) whose intensity decreased as absorption at 532 mμ ($\varepsilon > 80{,}000$) increased.

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