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Route of decomposition of thiomersal (thimerosal)

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Abstract

The route of formation and identification of the principal degradation products of thimerosal (thiomersal) has been undertaken. The initial oxidation to dithiosalicylic acid is followed by cleavage of the disulphide bond of the dithiosalicylic acid by the ethylmercuric ion to reform 1.5 mol of thimerosal with concurrent oxidation to form 0.5 mol of 2-sulfinobenzoic acid for each mole of dithiosalicylic acid. In the presence of copper ions 2-sulfobenzoic acid was also formed. A mechanism has been proposed which accounts for the stoichiometry of the cleavage reaction observed and the significance of the reaction is discussed.

Keywords: Thimerosal; Thiomersal; Degradation; 2-Sulfinobenzoic acid; 2-Sulfobenzoic acid

1. Introduction

Thimerosal (TM), an antimicrobial preservative used in immunological and opththalmic products is the sodium salt of the complex formed between thiosalicylic acid (TSA) and the ethylmercuric (EtHg) ion (Fig. 1).

Methods of analysis include atomic absorption spectroscopy (Meakin and Khammas, 1979), colorimetry (Richardson et al., 1977; Fleitman et al., 1991) and a variety of methods employing high performance liquid chromatography (HPLC)(Lam et al., 1981; Reader and Lines, 1983; Holak, 1985; Parkin, 1991a,b; Fleitman et al., 1991; Procopio et al., 1992; Pilar da Silva et al., 1993a; Caraballo et al., 1993; Pilar da Silva et al., 1993b). Most of these chromatographic methods measure intact TM and as a consequence afford significantly different results when applied to degraded samples to methods which quantitate total mercury such as AAS or colorimetry and chromatographic methods which quantitate total EtHg following derivatisation.

The preservative is reported to decompose by oxidation to 2,2′-dithiosalicylic acid (DTSA) (Fig. 1) and to TSA, EtHg and elemental mercury. This degradation is strongly influenced in a complex manner by the presence of other species in the system, for example, chloride promotes degradation (Reader and Lines, 1983; Reader, 1984; Parkin, 1991a; Caraballo et al., 1993; Rabasco et al., 1993; Faridah, et al., 1995; Perez-Barrales et al., 1997) and both tromethamine (Fleitman et al.,

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1991; Caraballo et al., 1993; Rabasco et al., 1993) and ethylenediaminetetraacetic acid (EDTA) (Faridah et al., 1995) exert a stabilising effect.

Recently Caraballo et al., (1993) have introduced an HPLC assay which enables quantitation to be made concurrently for TM and TSA and DTSA the presumed principal degradation products. Application of this assay to TM solutions demonstrated that while TSA and DTSA were originally formed, in degraded samples both DTSA and TSA were absent. The nature of the ultimate degradation products have never been elucidated or the role of EtHg in this degradation. This paper outlines an investigation to elucidate the factors which influence the formation and identify the ultimate degradation products of TM.

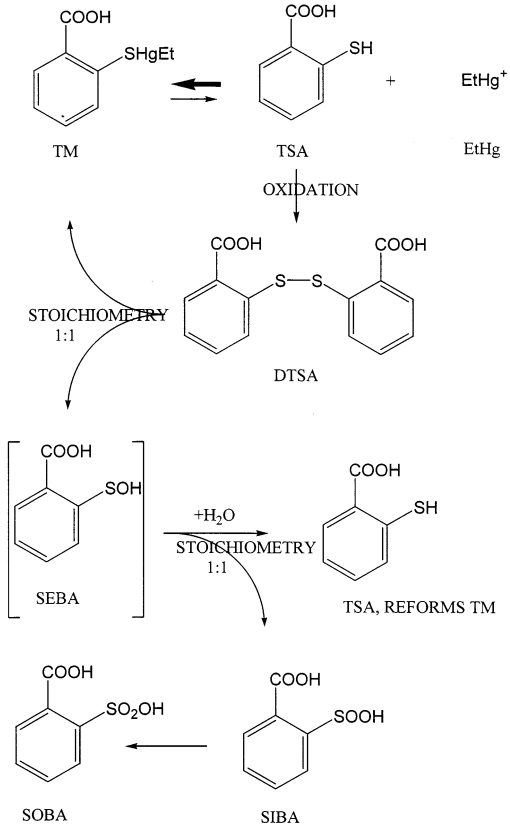

Fig. 1. Proposed route of decomposition of thimerosal. TM, thimerosal; TSA, thiosalicyclic acid; EtHg, ethylmercuric ion; DTSA, dithiosalicylic acid; SEBA, 2-sulfenobenzoic acid; SIBA, 2-sulfinobenzoic acid and SOBA, 2-sulfobenzoic acid.

2. Materials and methods

2.1. Materials

2-Sulphobenzoic acid (SOBA) hydrate and TSA were obtained from Aldrich, USA; EtHg chloride from TCI, Japan; TM from ICN Biochemicals Inc., USA and tetrabutylammonium hemisulfate (TBA) and DTSA from Sigma, USA. 2-Sulfinobenzoic acid (SIBA) was synthesised from TSA by the method of Kamayama et al. (1988) and isolated as the calcium salt and piperidinedithiocarbamate (PIDTC) was prepared as reported earlier (Parkin, 1991a). All the other chemicals were either analytical or HPLC grade.

2.2. Chromatographic equipment and conditions

The HPLC system consisted of a pump (501, Waters Associates, USA), 20 μl loop injector (7125, Rheodyne, USA), variable wavelength absorbance detector (486, Waters Associates) and integrating recorder (3396A, Hewlett-Packard, USA). Spectra of eluted peaks were obtained using a photodiode array absorbance detector (991, Waters Associates).

TM, TSA and DTSA and total EtHg by the method employing excess TSA were assayed using a C-18 column (250×4.6 mm I.D., Spherisorb ODS-2, 5 μ particle size) (Alltech Associates, USA) using the solvent conditions of Caraballo et al. (1993) except that eluates were monitered at 258 nm and a flow rate of 1.5 ml min^{-1} was employed. SOBA and SIBA were assayed using a C-18 column (250×4.6 mm I.D.) Econosil 10 μ particle size) (Alltech) with a solvent consisting of 0.5% (w/v) acetic acid in 35% methanol with the pH adjusted to 4.2 with sodium hydroxide and containing TBA hemisulfate (0.005 M) at a flow rate of 1.5 ml min^{-1} and monitoring at 258 nm. Total EtHg was assayed as the piperidinedithiocarbamate (PIDTC) complex by the method previously reported from these laboratories (Parkin, 1991b).

2.3. Assay of total EtHg by addition of excess TSA

Samples were diluted when necessary with water so that the resulting solution contained nominally 2×10^{-4} M EtHg or less prior to analysis. To the diluted sample for analysis (2 ml), was added a solution of TSA (6×10^{-3} M) in 25% acetonitrile in water (100 μl). The solution was allowed to stand for 1 min and submitted to analysis by HPLC.

2.4. Validation of HPLC assays

1. TM was validated by injection of freshly prepared solutions of TM: 0.04, 0.08, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8×10^{-4} M. The intra-day relative standard deviation (R.S.D.) was determined at a concentration of 0.2×10^{-4} M ($n = 6$).
2. DTSA was validated by injection of freshly prepared solution of DTSA: 0.02, 0.04, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4×10^{-4} M. The RSD was determined at a concentration of 0.1×10^{-4} M ($n = 6$).
3. Total EtHg was determined by addition of excess TSA to a freshly prepared solution of TM: 0.2, 0.4, 1.2, 2.0 and 2.8×10^{-4} M. The RSD was determined at a concentration of 0.4×10^{-4} ($n = 6$). Relative response was assessed at a concentration of 2×10^{-4} M for TM, EtHg chloride, TM plus excess TSA and TM in 0.5% (w/v) sodium chloride plus excess TSA ($n = 2$). All solutions diluted with excess TSA were corrected to give the true concentration by multiplying by the dilution factor (1.05).
4. TSA was validated by injection of freshly prepared solutions of TSA: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5 and 2.0×10^{-4} M TSA. The R.S.D. was determined at a concentration of 0.4×10^{-4} M ($n = 6$).
5. Total EtHg was assessed by the addition of PIDTC reagent to solutions of TM as outlined in the reported method (Parkin, 1991b).
6. SOBA hydrate (0.01 M) was prepared and standardised by titration with 0.01M sodium hydroxide using phenolphthalein as indicator. An appropriate volume of this solution was then diluted with water to provide a 1×10^{-3} M solution. From this 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4 and 2.0×10^{-4} M solutions were prepared and used as standards for construction of the calibration line. The R.S.D. was determined at a concentration of 0.4×10^{-4} M ($n = 6$).
7. Chromatography showed that SIBA which was synthesised by the method of Kamayama et al. (1988) contained 3.0% (w/v) of DTSA and the quantities used were adjusted accordingly. The SIBA calibration line and RSD were determined using identical concentrations as for SOBA. Solutions of SIBA were oxidised to SOBA by adding a 0.25% (w/v) solution of potassium permanganate in 0.01 M sulphuric acid (0.1 ml) to a solution of SIBA (2 ml) and allowing it to stand for 1 min. The solution was then assayed for SOBA by HPLC.

2.5. Stability and reaction studies

Stock solutions of TM (4×10^{-3} M) in water, DTSA and TSA (2×10^{-3} and 4×10^{-3} M, respectively) in pH 7.0 phosphate buffer (0.1 M) and EtHg chloride (1×10^{-2} M) in acetonitrile were freshly prepared.

TM, TSA or DTSA solutions (5 ml) were added at zero time to 100 ml volumetric flasks containing an appropriate volume (50 ml for TM or 47.5 ml for DTSA and TSA) of pH 7.0 phosphate buffer (0.2 M). Prior to this other reactants known to influence the stability of TM [10 ml of 0.1M disodium EDTA to give a final concentration of EDTA of 0.01 M, 0.5 g of sodium chloride to give a final concentration of 0.5% (w/v) or 50 μl of a 100 mg l^{-1} of Cu^{2+} as copper sulphate to give a final concentration of 50 ppb Cu^{2+}] were added. Where necessary the EtHg chloride solution was added and the flasks made to volume with water and kept in the dark at room temperature.

The flasks were submitted to analysis at appropriate time intervals for TM, DTSA and TSA and at the terminal time for these species plus SOBA, SIBA and total EtHg by both the addition of

excess TSA and by the method involving total derivatisation with PIDTC reagent (Parkin, 1991b).

3. Results and discussion

The results of Caraballo et al. (1993) in their study of the stability of TM demonstrated the transient formation of DTSA and TSA which were absent in degraded samples. DTSA, which would be expected to display reasonable stability, was, therefore, reacting with other components in the mixture. The most likely reactant is the EtHg ion. The reactivity and complexation characteristics of the EtHg ion has never been investigated but the homologous methylmercury ion has received considerable attention due to its environmental toxicity (Rabenstein, 1978; Carty and Malone, 1979). It would be expected that the EtHg ion would display similar complexation and chemical characteristics. Methylmercury is reported to not associate significantly with the disulphide group of cystine (Carty and Malone, 1979) and there are no reports of either methylmercury or EtHg reacting with disulphide compounds but initial studies demonstrated that reaction occurs rapidly between DTSA and the EtHg ion and an investigation of this reaction was undertaken. To simplify interpretation, stability and reaction studies were performed under standardised conditions [in pH 7.0 phosphate buffer (0.1 M) in the dark at room temperature] in the presence of EDTA (0.01 M), sodium chloride (0.5% w/v) or Cu^{2+} ion (50 ppb). EDTA would not be expected to complex with the EtHg ion to any extent as the ion undergoes monovalent complexation without high affinity for carboxylate and protonated amino groups (Rabenstein, 1978). The presence of EDTA would minimise metal catalysed oxidative reactions. Concurrently studies on the stability of TM, DTSA and TSA were undertaken under identical conditions to enable an assessment of the significance of the reaction between EtHg and DTSA to be made.

The concentrations chosen for the study, for DTSA (1×10^{-4} M) and TM and TSA (2×10^{-4} M) approximate those employed by TM as a preservative and enable direct comparisons of the reactions to be made as DTSA, the disulphide oxidation product, may be considered a dimer of TSA.

To enable quantitation of all the major species in the reaction mixtures the method of Caraballo et al. (1993) with minor modifications was employed for the quantitation of TM, DTSA and TSA, the retention times being TSA (3.6 min), TM (6.8 min) and DTSA (10.5 min). As previously reported the TM peak displayed forward tailing due to dissociation of the TM on the column under the chromatographic conditions (Fig. 2A–D). This did not influence the analytical precision of the method and the statistics demonstrating validity of the assay for all three species is given in Table 1.

Polar degradation products were formed with the loss of TM, TSA and DTSA. These eluted near the void volume and were subsequently identified as SOBA and SIBA (Fig. 1). As they eluted at or near the void volume under the chromatographic conditions of Caraballo et al. (1993) they did not interfere with the assay of the major components. Under some conditions other minor decomposition products were noted. These were identified as benzoic acid (elution time 4.9 min) and a compound tentatively identified as the corresponding thioether (2,2′-thiobisbenzoic acid, elution time 9.0 min). The identity of benzoic acid was confirmed by comparison of the retention time and ultraviolet spectrum with an authentic sample and the thioether by comparison of the ultraviolet spectrum ($\lambda_{max} = 223$, 251 and 316 nm) with the reported values for this compound (Grasselli and Ritchey, 1975). These compounds were well resolved from the TSA, TM and DTSA and did not interfere with the assay of these compounds. They were not quantitated as they constituted only minor components in reaction mixtures under some conditions.

An analytical method was developed for SOBA (a standard for which was available commercially) and SIBA [which was synthesised from TSA by the method of Kamayama et al. (1988)]. The method employed chromatography using TBA as an ion-pairing agent and this enabled quantitation of both SOBA and SIBA using a single solvent

Table 1
Statistical validation of analytical methods

Compound	Area response ($\times 10^6$) = slope concentration ($\times 10^{-4}$ M) + intercept					R.S.D.($n = 6$) (intraday)	
	Range ($\times 10^{-4}$ M)	Slope ($\times 10^{10}$)	Intercept ($\times 10^6$)	Correlation coefficient (r)	Number of data points	Concentration ($\times 10^{-4}$ M)	Value (%)
Thimerosal	0.04–2.8	5.93	−0.053	0.9985	10	0.2	2.20
Dithiosalicylic acid	0.02–1.4	4.38	0.081	0.9983	10	0.1	0.83
Thiosalicylic acid	0.1–2.0	3.78	0.41	0.9990	9	0.4	1.90
Total ethylmercury (as thiomerosal)	0.2–2.8	6.03	−0.092	0.9999	5	0.4	2.50
Total ethylmercury (as piperidinedithiocarbamate)	0.2–2.8	5.40	0.32	0.9998	5	0.4	1.40
2-Sulfobenzoic acid	0.05–2.0	0.350	0.00015	0.9983	9	0.4	2.60
2-Sulfinobenzoic acid	0.05–2.0	0.925	0.022	0.9994	9	0.4	2.93

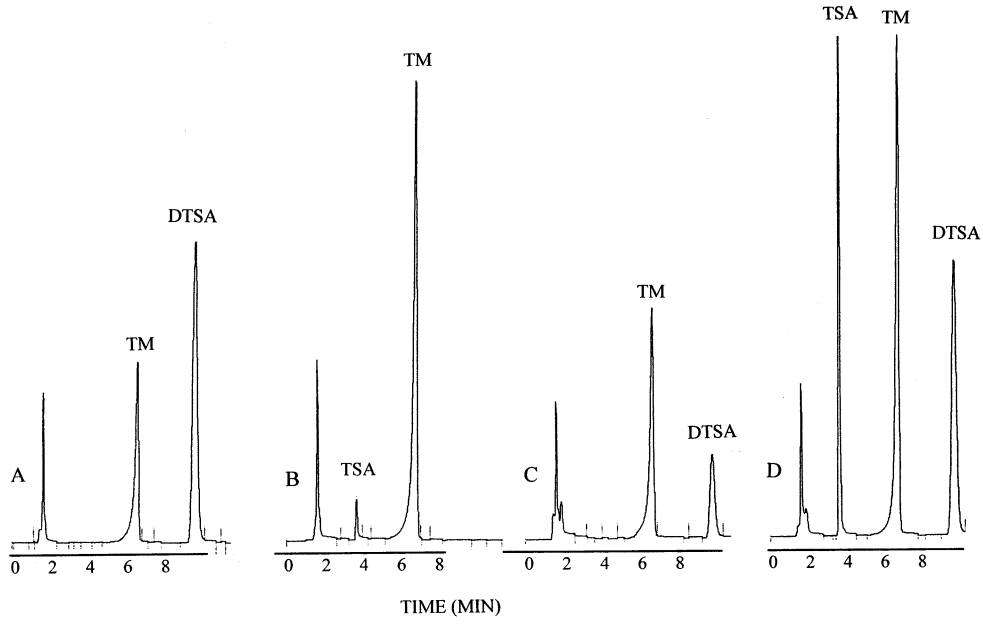

Fig. 2. Typical chromatograms for the analysis of (A), thimerosal (TM); thiosalicyclic acid (TSA) and dithiosalicylic acid (DTSA) and (B), total ethylmercury by the addition of excess TSA.

system (retention times SOBA = 3.8 min, SIBA = 5.1 min). Other species such as TM, DTSA and TSA eluted at much longer retention times and did not interfere with the analysis. See Fig. 3A and B for typical chromatograms and Table 1 for statistical details of validation of the assays.

Total EtHg in the reaction mixture was determined by two methods. Derivatisation on as the PIDTC complex by the method previously reported from these laboratories (Parkin, 1991b) and by an adaption of the method of Caraballo et al. (1993) involving the addition of excess TSA to the sample prior to chromatography. The latter technique has been reported from these laboratories but employing the chromatographic conditions of Lam et al. (1981). The adaptation is equally suitable for the method of Caraballo et al., provided that there is excess TSA in the system, the free EtHg ion and TM chromatograph as TM enabling quantitation of the total EtHg ion to be made. The statistical results validating these two assays are provided in Table 1 and a typical chromatogram for the assay of total EtHg ion as TM is provided in Fig. 2D. To confirm that this assay quantitates total TM it was applied to solutions of 2×10^{-4} M TM, 2×10^{-4} M EtHg chloride and 2×10^{-4} M TM containing 0.5% (w/v) sodium chloride. The results confirmed the suitability of the approach (TM = nominal 100.0%, EtHg chloride = 101.8% and TM plus sodium chloride = 98.4% response, all $n = 2$).

When DTSA (1×10^{-4} M) was made to reacted with varying concentrations of EtHg ion (2×10^{-4}–10×10^{-4} M) in the presence of

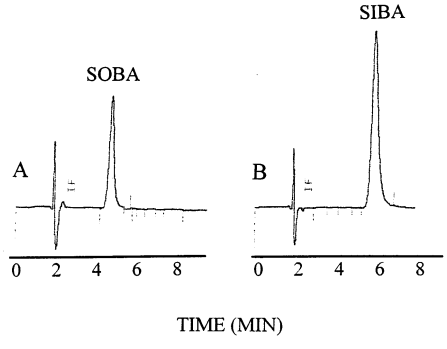

Fig. 3. Typical chronatogram for the analysis of 2-sulfinobenzoic acid (SIBA) and 2-sulfobenzoic acid (SOBA).

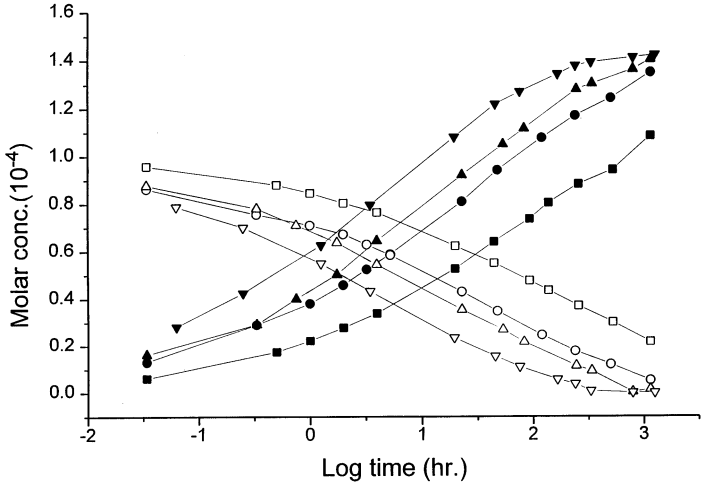

Fig. 4. Plot of the reactant and product concentrations versus log time for the reaction of ethylmercury ($2–10 \times 10^{-4}$ M) with dithiosalicylic acid (1×10^{-4} M) in the presence of EDTA. Open symbols, DTSA, closed symbols, TM in the presence of ethylmercury ($\times 10^{-4}$ M) 2.0 = squares; 4.0 = circles; 6.0 = triangles; 10.0 = inverted triangles.

EDTA there was a rapid reaction resulting in the formation of TM with loss of DTSA demonstrating that the EtHg ion was able to readily cleave the disulphide bond of DTSA. The results are displayed in Fig. 4 and the species present at the terminal time are shown in Table 2. The kinetics of the reaction is complex and the data is best displayed using a logarithmic time scale. A typical chromatogram obtained during partial reaction is shown in Fig. 2C.

There was an initial rapid reaction resulting in the formation of TM with loss of DTSA. The precise mechanism is difficult to explain but must involve complex equilibria. At all concentrations of EtHg ion 2 mol of DTSA formed 3 mol of TM accompanied by the formation of 1 mol of SIBA. The reaction reached completion after 1000 h at higher concentrations of EtHg, the overall rate being concentration dependent.

The identity was confirmed as being SIBA and SOBA by comparison of their retention times with authentic samples under two sets of chromatographic conditions (those employed for their assay and 30% methanol in 0.1% w/v phosphoric acid) and comparisons of their ultraviolet spectra using a photodiode HPLC detector. SIBA could also be readily oxidised by permanganate to an equivalent molar concentration of SOBA.

In the presence of 50 ppb Cu^{2+} the reaction proceeded with different kinetics, the rapid formation of TM being accompanied by the complete loss of DTSA (Figs. 5 and 6 and Table 2). At higher concentrations of EtHg ($> 2 \times 10^{-4}$ M) the reaction was so rapid that it could not be followed in detail but at 2×10^{-4} M EtHg there was a equimolar conversion of DTSA to TM with complete loss of DTSA after 20 h (Fig. 5). With 1×10^{-4} M EtHg the reaction was slower and biphasic. Up to 100 min there was an equimolar conversion of DTSA to TM but subsequently there was a loss of reactant and product such that after 20 h, following the complete loss of DTSA, there was a conversion to 0.75 mol of TM (utilising 75% of the available EtHg ion). Assay for EtHg ion at this point in these reactions by the addition of excess TSA demonstrated the expected amount of EtHg ion in the system (2×10^{-4} M EtHg = 2.05×10^{-4} M at 1×10^{-4} M EtHg = 1.03×10^{-4} M). These reaction mixtures when allowed to stand for extended periods (500 h) showed a continued loss of TM, no DTSA and the conversion of reactant and initial reaction product to SOBA and SIBA. No loss of EtHg ion was observed in any system. The explanation for these results is difficult and it is not obvious why, at the lower concentration, the DTSA does not

Table 2
Reaction of ethylmercury with dithiosalicylic acid (1×10^{-4} M)

EtHg initial concentration ($\times 10^{-4}$ M)	Concentration at terminal time ($\times 10^{-4}$ M)				Total TSA equivalents[a]	Final EtHg present	
	DTSA	TM	SIBA	SOBA		EtHg by PIDTC derivatisation	EtHg by use of excess TSA
In the presence of EDTA (1×10^{-2} m) after 16:00 h							
1.0	0.415	0.738	0.467	0	2.135	1.03	1.03
2.0	0.170	1.068	0.666	0	2.074	2.07	1.97
4.0	0.027	1.375	0.633	0	2.062	4.05	4.00
6.0	0.004	1.422	0.547	0	1.977	6.20	6.10
10.0	0	1.440	0.539	0	1.979	10.20	10.30
In the presence of CU^{2+} (50 ppb) after 05:00 h							
1.0	0	0.388	1.465	0.184	2.037	–[b]	0.99
2.0	0	0.607	1.302	0.186	2.095	–[b]	2.05
4.0	0	0.692	1.071	0.200	1.963	–[b]	4.00
6.0	0	0.788	0.920	0.243	1.951	–[b]	5.90

[a] Total TSA equivalents defined as the total molar concentrations of TM + SIBA + SOBA + (2 × DTSA).
[b] Not measured.

react rapidly up to the total available EtHg ion except that for TM to form there must be a 25% loss to form SIBA. Arylsulfinic acids generally are readily oxidised to the corresponding arylsulfonic acids (Finar, 1976) and the presence of SOBA may arise from subsequent oxidation of SIBA under the influence of Cu^{2+}.

The stoichiometry of the equation may be explained by a sequence of reactions involving ethylmercuration of the disulphide sulphur followed by attack by water to give TM and the arylsulfenic acid (SEBA) (Figs. 6 and 7). Arylsulfenic acids are known to be in an unstable oxidation state and undergo disproportionation

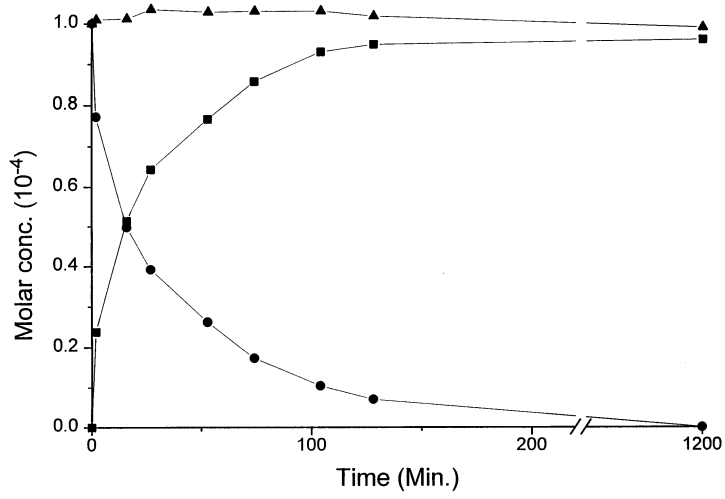

Fig. 5. Plot of the reactant concentrations versus time for the reaction of ethylmercury (2×10^{-4} M) with dithiosalicylic acid (1×10^{-4} M) in the presence of 50 ppb Cu^{2+}. ● = DTSA; ■ thimerosal and ▲ total concentration of reactant and product.

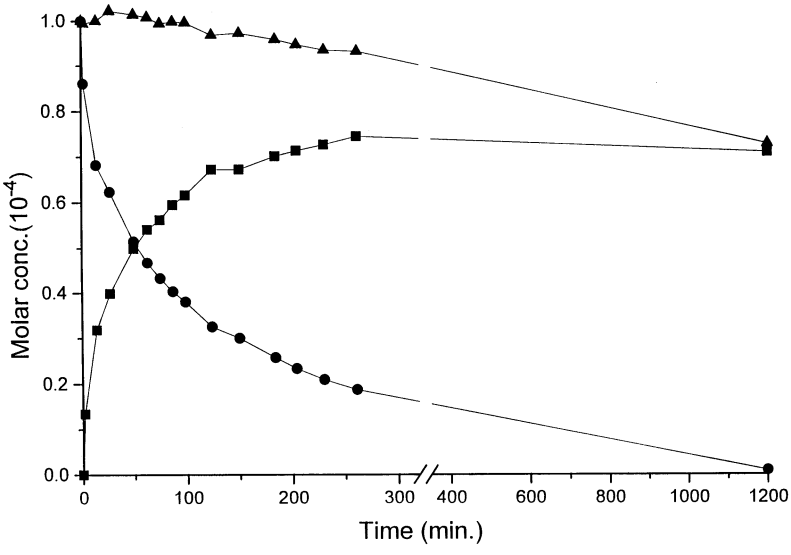

Fig. 6. Plot of the reactant concentrations versus time for the reaction of ethylmercury (1×10^{-4} M) with dithiosalicylic acid (1×10^{-4} M) in the presence of 50 ppb Cu^{2+}. ● = DTSA; ■ thimerosal and ▲ total concentration of reactant and products.

Table 3
Decompositon of TM (2×10^{-4} M), TSA (2×10^{-4} M) and DTSA (1×10^{-4} M) in the presence of EDTA and Cu^{2+}

Reactants	Duration of study (days; number of data points in parenthesis)	Rate of decomposition (approximate percentage per day)	Composition at terminal time ($\times10^{-4}$ M)					Total TSA Equivalents[a]	EtHg by PIDTC	EtHg by excess TSA	Comments
			TM	DTSA	TSA	SOBA	SIBA				
TM+EDTA	78 (15)	<0.01	1.990	0.012	0	0	0	2.05	1.94	2.05	Stable
TM+Cu^{2+}	50 (10)	1.5	1.115	0	0	0.125	0.758	2.00	2.01	2.02	–
TM +NaCl +EDTA	78 (15)	0.1	1.848	0.016	0.046	0	0.044	1.97	2.02	2.07	–
TM+NaCl +Cu^{2+}	50 (10)	4.3	0.138	0	0	0.402	1.553	2.09	1.90	2.04	–
TM+EDTA +EtHg (2×10^{-4} M)	78 (12)	<0.01	2.020	0	0	0	0	2.02	4.07	4.04	Stable
TM+Cu^{2+} EtHg (2×10^{-4} M)	42 (10)	0.06	1.952	0	0	0.020	0.050	2.02	[b]	4.08	TM stabilised by EtHg
TSA+EDTA	42 (10)	1.8	–	0.756	0.443	0	0	1.95	[b]	[b]	–
TSA+Cu^{2+}	3h (2)	>20% h^{-1}	–	1.860	0	0.022	0.48	1.93	[b]	[b]	Benzoic acid and thioether formed
DTSA +EDTA	78 (15)	<0.01	–	0.999	0	0	0	2.00	[b]	[b]	Stable
DTSA+Cu^{2+}	42 (10)	0.95	–	0.602	0	0.100	0.432	1.73	[b]	[b]	Benzoic acid and thioether formed
DTSA+TM (0.8×10^{-4} M) +EDTA	42 (10)	<0.01	0.780	1.02	0	0	0	2.82	[b]	0.76	Stable

[a] Total TSA equivalents defined as the total molar concentration of TM+TSA+SOBA+SIBA+(2×DTSA).
[b] Not measured.

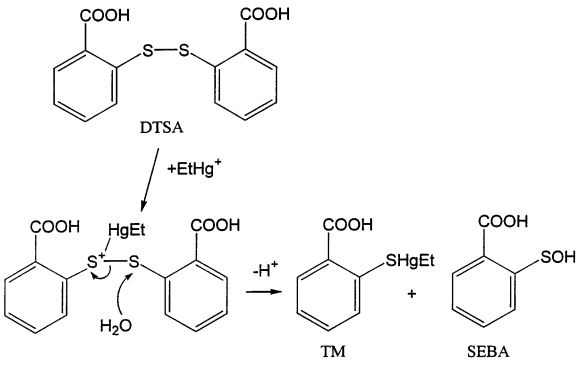

Fig. 7. Proposed mechanism for the cleavage of dithiosalicylic acid (DTSA) by ethylmercuric ion ($EtHg^+$) to form thimerosal (TM) and sulfenobenzoic acid (SEBA).

to form arylsulfinic acids with reduction of a second molecule to the thiol (Fig. 1) (Kice, 1980; Davis and Billmers, 1985). This TSA may then react to reform TM the overall result being the stoichiometry observed.

The stability of TM, TSA and DTSA were assessed under the conditions under which the reaction of EtHg and DTSA was investigated (Table 3). In the presence of EDTA, TM and DTSA are stable ($< 0.01\%$ per day degradation) and TSA undergoes oxidation at approximately 1.8% per day to form DTSA. In the presence of Cu^{2+} ions all species degrade (TM at 1.5% per day and TSA at 20% h^{-1}). These values are averages over the times specified and the rates of decomposition varied significantly with time as the proportions of the reactant species changed. Assessment of the reaction mixtures at terminal times show that the ultimate degradation products for TM, DTSA and TSA are SIBA and SOBA with small amounts of benzoic acid and a compound tentatively identified as the corresponding thioether as minor products following degradation in the presence of Cu^{2+}.

Excess EtHg has a stabilising influence on TM and sodium chloride promotes the degradation. This must arise by the presence of chloride ion complexing with the EtHg ion displacing free TSA which is prone to oxidation. The association constant of alkylmercury for chloride ($\log K_a = 5.25$ for methylmercury) is sufficiently high that, in combination with the high concentration of chloride, displacement of the EtHg ion from the thiol group of the TSA occurs with the formation of EtHg chloride. Initial chromatography of these reaction mixtures shows the presence of 6.5% free TSA, and consequently an equivalent percentage of free EtHg (Fig. 2B). As degradation proceeds the stoichiometric excess of EtHg results in the suppression of dissociation of the TM, which may explain the stabilisation of the TM following its initial rapid loss. In all the studies there was no loss of EtHg ion when assayed by either of the chromatographic methods employed (Table 3).

On the basis of this evidence a route for the degradation of TM can be deduced (Fig. 1). The rate determining step is that of oxidation of the TSA to DTSA which is metal catalysed. This reaction is controlled by the amount of free TSA, and the oxidation product (DTSA) reacts rapidly with the free EtHg ion to reform TM with the loss of 25% of reactant to form SIBA and ultimately SOBA. Continuous recycling of the reaction ultimately results in the complete loss of TM. The overall reaction kinetics are complex as during the reaction there develops a stoichiometric excess of EtHg which both promotes degradation of the DTSA and suppresses oxidation of TSA to DTSA by suppressing dissociation of the TM.

Finally, it is of significance that under all of the conditions studied there is no loss of total EtHg ion. It is probable that this is the antibacterial species and this would explain the observation that degraded solutions of TM display greater antibacterial activity than freshly prepared solutions (Davies et al., 1983).

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