Selenotrisulphides from Mercaptopurines

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Products from the reaction of selenium dioxide with 2-mercapto-(2-MP) and 6-mercapto-purines (6-MP) were studied. The molar ratio of selenium dioxide to mercaptopurines was evaluated from u.v. absorption spectra obtained according to a modification of the method of continuous variations. From the results of the stoicheiometric calculations the products were shown to be seleno-trisulphides and -disulphides. From electrophoretic data equilibrium constants were computed for both reactions. In the presence of an excess of 6-MP the derived selenotrisulphide was stable in alkaline solution.

Selenium is one of the most important trace elements–nutrients in human and animal organisms.[1–4] At higher than essential levels it exhibits remarkable toxicity.[5,6] Vernie[7] described the properties of selenium as varying from carcinogenic to anticarcinogenic. On the other hand, certain illnesses can be correlated with low blood selenium levels.

In biological systems containing thiols, selenium dioxide (SeO_2 of H_2SeO_3) plays the roles of both a toxic and a nutrient factor. Compounds of type RS–Se–SR (selenotrisulphides or 2-selena-1,3-disulphides) are obtained from the reaction of SeO_2 with thiols[8–10] in agreement with equation (1) first proposed by Painter.[11] Selenotrisulphides can be considered as

$$4RSH + H_2SeO_3 \rightleftharpoons RS{-}Se{-}SR + RS{-}SR + 3H_2O \qquad (1)$$

analogues of trisulphides in which one atom of sulphur has been replaced by selenium. The earlier view that the molar ratio of SeO_2 to thiols is 1 : 4 [see reaction (1)] has not been proved for the reaction of SeO_2 with dithiols such as 2,3-dimercaptopropan-1-ol (DMP)[12] and 1,2-dimercaptoethane (DME).[13] It was found that SeO_2 and DMP or DME react in a molar ratio of 2 : 3 giving the single product $(DMP)_3Se_2(OH)_2$ or $(DME)_3Se_2(OH)_2$, respectively.[12,13] The actual importance of selenium compounds for biological systems, as well as features of the chemistry involved, stimulated our study of the reactions of SeO_2 with heterocyclic thiols.

In this paper the reactions of SeO_2 with 2-mercapto- (2-MP) and 6-mercapto-purines (6-MP) are reported.

Results

Thin-layer Chromatography of Reaction Mixtures.—The products obtained from the reactions of $Na_2{}^{75}SeO_3$ with 2-MP and 6-MP were separated after a batch process lasting 3 h over the pH range 1.8—4.3 and at 287 K. Double pairs of spots were detected on the chromatograms: the first pair can be assigned to disulphides obtained from 2-MP (R_F 0.48) and 6-MP (R_F 0.41), respectively. The second pair of ^{75}Se products (from 2-MP, R_F 0.67; from 6-MP, R_F 0.54) and unchanged selenite (R_F 0.96) were also observed as radioactive spots. As the same results were obtained after 24 h we concluded that equilibria were reached in 3 h.

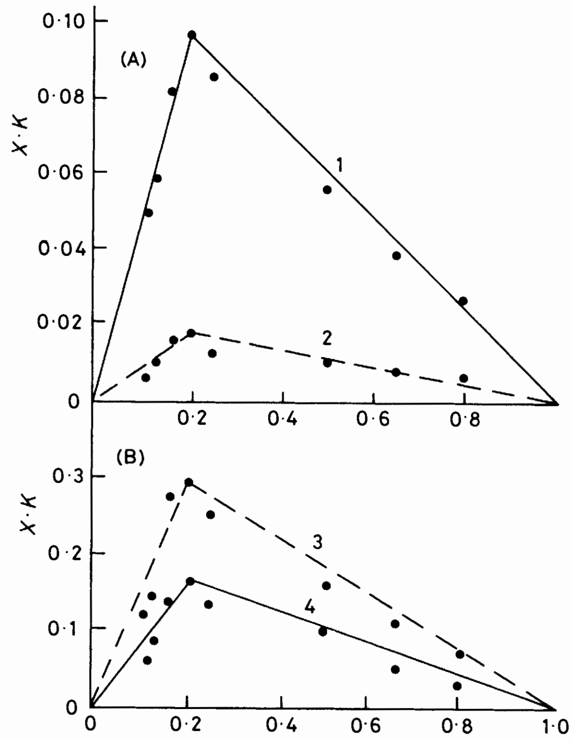

Figure 1. Variations of the mole ratio of selenite with that of 2-MP or 6-MP (the sum of molarities of reactants 11.2 μM): (A) selenite and 2-MP; optical path 1 cm; 1, absorbance at 263 nm; 2, absorbance at 350 nm, (B) selenite and 6-MP; optical path 0.33 cm; 3, absorbance at 225 nm; 4, absorbance at 327 nm

Stoicheiometry.—The reactions of SeO_2 with 2-MP and 6-MP lead to variations in the absorption spectra in the 220—350 nm range, enabling the determination of the mole ratio of SeO_2 to 2-MP and 6-MP. However, the mole ratio of SeO_2 to 2-MP and 6-MP cannot be determined by the method of continuous variations, because the u.v. absorption spectra of unchanged mercaptopurines have peaks in the range of the peaks of the products (the absorbance of SeO_2 within this range is very small). Therefore the method of continuous variations had to be modified (for details see ref. 14). For the determination of the mole ratio with which SeO_2 reacts with 2-MP and 6-MP equation (2) was applied[14] where A is the

$$A - C_0^{RSH}\cdot\varepsilon_{RSH}\cdot l = X\cdot K\cdot l \qquad (2)$$

measured absorbance, ε_{RSH} the measured molar absorption coefficient of 2-MP or 6-MP, C_0^{RSH} the initial 2-MP or 6-MP concentration, l the optical path, X the extent of the reaction, and K a constant factor.[14]

The extent of reaction X reached a maximum when the mole fractions of the reactants correspond to the stoicheiometric ratio of the reaction.[14,15] Thus, the mole fractions were obtained from intersections of straight lines, as in the method of continuous variations, corresponding to appropriate extreme.[14] Selenium dioxide and 2-MP or 6-MP were dissolved in various

Table 1. Data for the reaction of selenite with 2-MP at 286 K

pH	10^4[2-MP]/M	$10^4[Na_2{}^{75}SeO_3]$/M	$10^6[(2\text{-MP})_2]$/M	$10^6[(2\text{-MP})_2{}^{75}Se]$/M	Apparent *K*
1.6	6.621	3.421	4.1	4.1	2.6×10^5
2.2	6.612	3.280	7.3	7.3	8.4×10^5
3.1	4.552	3.721	10.5	10.5	5.3×10^6

mole ratios in 0.05M-HCl solutions (50 ml) at room temperature. The sums of the molarities of the reactants were 7.2 μM and 11.2 μM. The absorption spectra were measured with a 3 h delay. The results indicate that the value for the mole ratio of SeO_2 to 2-MP and 6-MP is 1 : 4 (Figure 1). Consequently, the reactions of SeO_2 with 2-MP and 6-MP can be written as (3) and (4).

$$4\ 2\text{-MP} + SeO_3{}^{2-} \rightleftharpoons (2\text{-MP})_2Se + (2\text{-MP})_2 + H_2O + 2\ OH^- \quad (3)$$

$$4\ 6\text{-MP} + SeO_3{}^{2-} \rightleftharpoons (6\text{-MP})_2Se + (6\text{-MP})_2 + H_2O + 2\ OH^- \quad (4)$$

Dependence of the Equilibrium State of the Reactions upon pH.—The reactants and products of reactions (3) and (4) were separated electrophoretically after 3 h reaction. The results show that pH does not affect the equilibrium state of the reaction of $^{75}SeO_2$ with 6-MP in the pH range 2.4—8.8. The equilibrium constants at 290 and 303 K were found to be 2.5×10^6 and 3.2×10^5, respectively. From van't Hoff't isobar the standard heat of this reaction was found to be 116.0 ± 2.3 kJ mol^{-1}.

The influence of the pH on the equilibrium state has been observed on reaction (3). The apparent equilibrium constants for this reaction were calculated for the concentrations specified in Table 1. The transition from the apparent to the real equilibrium constant was made by taking into account its dependence upon pH as follows: $K = 5.2 \times 10^{16}$; $K = K_{pH}[OH^-]^\alpha$; $\alpha = -0.91$.

The abundances of the reactants and products of reaction (3) were determined at 274 and 294 K at pH 3.1. The apparent equilibrium constants for this reaction were 1.2×10^7 and 3.0×10^6, respectively. So, the real equilibrium constants for 274 and 294 K were 1.0×10^{17} and 2.5×10^{16}. The standard heat of this reaction was 45.0 ± 1.4 kJ mol^{-1}. The entropy and Gibbs free-energy [16] of reactions (3) and (4) were calculated and are given in Tables 2 and 3.

Stability of Selenotrisulphides derived from 2-MP and 6-MP.—The u.v. absorption spectra of solutions of selenite with 2-MP proved that colloidal selenium was formed at pH >5 and for a mole ratio of 2-MP to selenite of at least 5. The colloidal selenium is due to the decomposition of selenotrisulphide [reaction (5)]. Moreover, with time and a higher temperature the amount of the colloidal selenium increased. To examine in detail the influence of proportions of the reactants and the pH on the stability of selenotrisulphides in solution a paper-chromatographic procedure was applied to the separation of unchanged substrates and products of reactions (3) and (4) at 288 K. There were no spots corresponding to a ^{75}Se peak. It was found that only in mixtures of $^{75}SeO_2$ with 2-MP was the formation of free selenium observed at pH 5.4. The yield of selenium formation (R_F 0) increase with increasing the mole ratio of 2-MP to SeO_2 and the duration of the reaction (Figure 2).

$$C_5H_3N_4S\text{-}Se\text{-}SN_4H_3C_5 \longrightarrow C_5H_3N_4S\text{-}SN_4H_3C_5 + Se^\circ\downarrow \quad (5)$$

Table 2. Thermodynamic data for the reaction of SeO_2 with 2-MP

T/K	$\Delta S^\ddagger$/J mol^{-1} K^{-1}	$\Delta G^\ddagger$/kJ mol^{-1}
274	490 ± 3	−89.2 ± 2.1
286	477 ± 3	−91.5 ± 2.2
294	468 ± 3	−92.7 ± 2.2

Table 3. Thermodynamical data for the reaction of SeO_2 with 6-MP

T/K	$\Delta S^\ddagger$/J mol^{-1} K^{-1}	$\Delta G^\ddagger$/kJ mol^{-1}
290	523 ± 4	−35.6 ± 1.3
303	489 ± 3	−32.0 ± 1.2

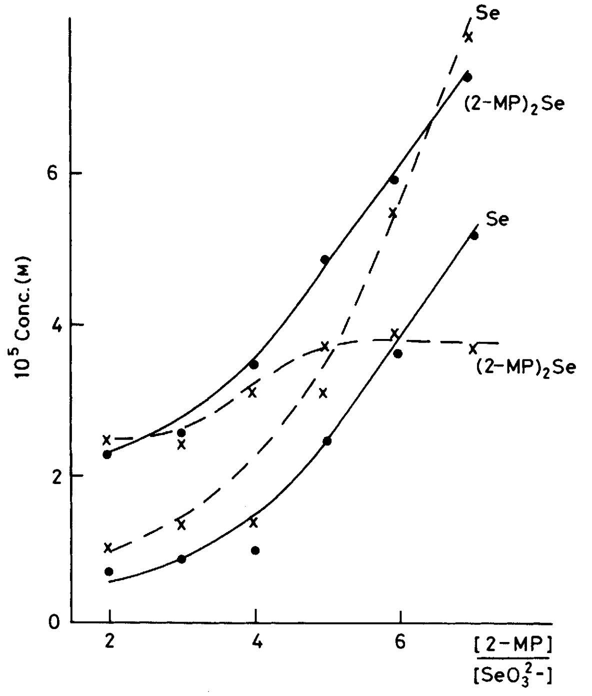

Figure 2. Influence of 2-MP : selenite mole ratio on the yield of selenotrisulphide and colloidal selenium in the dark: after 1 day, ——; after 6 days, — — —

Against expectation, for the reaction of SeO_2 with 6-MP no colloidal selenium was observed even after 5 weeks at pH 5.4. The selenotrisulphide formed in this reaction is stable in the 6-MP : SeO_2 mole ratio range 1—8. A similar stability of the selenotrisulphides was found when the yield of products of reactions (3) and (4) was examined over the pH range 4.0—11.0 and for a mole ratio of mercaptopurines : selenite of 3. For this pH range the selenotrisulphide from 6-MP was stable and even after 6 weeks no formation of selenium was observed. However, decomposition of this selenotrisulphide was observed when reaction mixtures were not protected from light. The systematic study of the influence of pH on the yield of the products of reaction (3) has shown that the yield of free selenium within the pH range 4.0—6.7 is increasing (Figure 3).

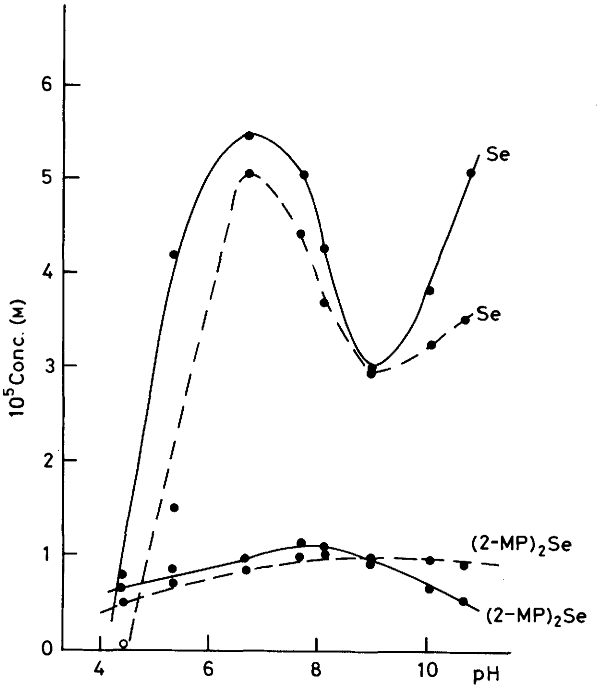

Figure 3. Yield of selenotrisulphide from 2-MP and colloidal selenium in the pH range 4.0—10.7 in the dark: after 1 day, ——; after 6 days, — — — —

Against expectations, over the pH range 6.7—8.9, the yield of colloidal selenium dropped to a minimum at pH 8.9. Finally, as shown in Figure 3 at pH >8.9 the yield of selenium increased. Moreover, the yield of the decomposition product of the selenotrisulphide from 2-MP (R_F 0.52) increased with time.

Discussion

The results of the present work disclosed the different behaviour of selenotrisulphide derived from 2-MP, compared with that from 6-MP. While the selenotrisulphide from 6-MP was stable in the dark at pH 2.4—11.0 in the presence of the excess of 6-MP, the stability of the selenotrisulphide from 2-MP was found to vary with pH and the mole ratio of 2-MP to SeO_2 (Figures 2 and 3). This variation is due to shifts in the thiol–thione equilibrium, which is affected by both H^+ and OH^- ions.[17-19] Apart from the thiols, the concentration of OH^- is another factor affecting the decomposition rates of the selenotrisulphides,[8,9] *e.g.* the decomposition of selenodicysteine is first-order in cysteine and 0.5-order in OH^- ion.[20] Accordingly an increase of the latter was observed in the pH interval 4.0—6.7. The decrease of this rate in the interval 6.7—8.9 can be explained by the gradually vanishing thiol form of the sulphur derivative of the purine. The minimum of the decomposition rate of selenotrisulphide from 2-MP at pH 8.9 can be attributed to the maximum of the shift of the tautomeric equilibrium involving the formation of thione. The increase in the decomposition rate starting from pH 8.9 seems to be due to the shift of the equilibrium towards the formation of the thiol form, accompanied by the rise of $[OH^-]$.

The unexpected stability of the selenotrisulphide from 6-MP presumably results from the lack of a free thiol group required for the decomposition of selenotrisulphides. There may be intramolecular hydrogen bonding between the sulphur atom at position 6 and the nitrogen atom at position 7.[21] Hydrogen bonding of this type was found for 6-amino- and 6-hydroxypurine.[17] The experimental data suggest that intermolecular hydrogen bonds in the solutions of 2-MP [19] are less stable than the intramolecular ones preferred in 6-MP solutions.

Experimental

Selenium dioxide was obtained from POCh. 2-Mercaptopurine was purchased from Sigma and 6-mercaptopurine from Fluka. The remaining chemicals, (standard reagent purity) were used as received. Labelled $Na_2{}^{75}SO_3$ was obtained from the Radioisotope Production and Distribution Centre, Świerk, Poland, the specific activity being 424 MBq per mg Se. The purity of 2-MP, 6-MP, and $Na_2{}^{75}SeO_3$ was tested by high voltage paper electrophoresis and by ascending paper chromatography. The reactions of Na_2SeO_3 with 2-MP and 6-MP were studied in 0.1M-sodium acetate solutions, in darkness. The pH was adjusted with 1M-HCl. The post-reaction mixtures were analysed by ascending paper chromatography, silica-gel t.l.c., and high voltage paper electrophoresis (70 V^{-1} cm). The u.v.-absorption spectra were obtained with a Specord spectrophotometer. Whatman 3MM paper was used for the chromatographic and electrophoretic procedures. The components of mixtures of Na_2SeO_3 with 2-MP were separated using ethyl acetate–acetone–concentrated hydrochloric acid (50 : 50 : 5 v/v) for elution of chromatographic strips and plates. For the reaction of Na_2SeO_3 with 6-MP the solvent system was ethyl acetate–acetone–concentrated hydrochloric acid–water (50 : 50 : 8 : 20 v/v). Strips and plates were run for 24 h and 6 h, respectively. Nitroprusside-sprayed strips at 40—60 °C for 1—2 h were developed. The distribution of the labelled selenium compounds along chromatographic and electrophoretic strips was scanned with the 4πG-M radiochromatograph (Packard model 7201) or measured as the activity of 5 mm sections of plates with a well shaped NaI–Tl scintillation counter. Because the reaction products of SeO_2 with 2-MP and 6-MP and 2-MP and 6-MP all give peaks in the 250—350 nm range a modified method of continuous variations was used.[14]

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