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CHARACTERIZATION OF SOLUTION-PHASE AND GAS-PHASE REACTIONS IN ON-LINE ELECTROCHEMISTRY–THERMOSPRAY TANDEM MASS SPECTROMETRY

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SUMMARY

Electrochemistry was used on-line with high-performance liquid chromatography–thermospray tandem mass spectrometry to provide insight into the solution-phase decomposition reactions of electrochemically generated oxidation products. Products formed during electrooxidation were monitored as the electrode potential was varied. The solution reactions which follow the initial electron transfer at the electrode are affected by the vaporizer tip temperature of the thermospray probe and the composition of the thermospray buffer. Either hydrolysis or ammonolysis reactions of the initial electrochemical oxidation products can occur with pH 7 ammonium acetate buffer. Both the electrochemically generated and the synthesized disulfide of 6-thiopurine decompose under thermospray conditions to produce 6-thiopurine and purine-6-sulfinate. Solution-phase studies indicate that nucleophilic and electrophilic substitution reactions with purine-6-sulfinate result in the formation of purine, adenine, and hypoxanthine. Products were identified and characterized by tandem mass spectrometry. This work shows the first example of high-performance liquid chromatography used on-line with electrochemistry to separate stable oxidation products prior to analysis by thermospray tandem mass spectrometry. In addition, solution-phase and gas-phase studies with methylamine show that the site of the nucleophilic and electrophilic reactions is probably inside the thermospray probe. Most importantly, these results also show that the on-line combination of electrochemistry with thermospray tandem mass spectrometry provides valuable information about redox and associated chemical reactions of biological molecules such as the structures of intermediates or products as well as providing insight into reaction pathways.

INTRODUCTION

Redox reactions play an important role in the metabolism and activation of xenobiotics and other substances. Because modern electrochemical methods have been shown to be useful in mimicking enzyme-catalyzed oxidation reactions[1–7], the combination of electrochemistry with mass spectrometry (MS) can provide important chemical information about intermediates and products formed in these redox reac-

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tions[8,9]. Most importantly, tandem mass spectrometry (MS–MS) can be used to confirm the presence of reaction intermediates as well as provide structural information. The driving force behind this work lies in the similarity between the biological oxidation and the electrochemical oxidation of many types of biologically important compounds such as purine drugs[10].

6-Thiopurine (6-TP) is a potent antineoplastic agent used in the treatment of several types of leukemia. However, its use has been restricted due to hepatotoxicity in some patients[11]. 6-TP is known to undergo extensive metabolism along several possible routes such as the transformation to its nucleotide[12], and nucleoside[13]. Hyslop and Jardine[11] have recently described a previously unknown oxidation pathway of 6-TP which involves activation by cytochrome P-450 with subsequent binding to protein via mixed disulfide bonds.

The electrochemical oxidation pathways of 6-TP and other structurally similar thiopurines such as 6-thioxanthine (6-TX), and 6-thioguanine (6-TG) have been extensively studied by off-line methods[1–3,14]. At low potentials, the first step in the oxidation of thiols is a one-electron process resulting in thiyl radicals followed by rapid dimerization to form disulfides. The S–S bonds of many disulfides have been shown to be unstable in acidic and basic media[2,15]. Cleavage of the S–S bond by a hydroxide ion will regenerate the parent thiol and give rise to a sulfinic acid[15–17]. The parent thiols are regeneratad in *ca.* 70% yield in this disproportionation process[15] (Fig. 1). The compound 6-thiopurine serves as an excellent model for studying the electrochemical oxidation of thiopurines because only the thiol group is involved in the oxidation process in the potential range from 0.0 to +1.1 V *vs.* Pd which is the normally accessible potential range in aqueous electrolytes. Other substituted thiopurines such as 6-TX and 6-TG behave differently electrochemically than 6-TP at high potentials of *ca.* 1.0 V. The difference in electrochemical behavior is due to the electronegative substituents which make the purine ring more vulnerable to oxidation. However, all three thiopurines are thought to undergo a one-electron oxidation at moderately low potentials to form a thiyl radical resulting in disulfide formation. This has been shown for 6-TX and 6-TG[2,3]. It is often difficult to verify this by coulometry because of the disproportionation reaction which regenerates the starting material in high yield[2]. The electrochemical oxidation products of 6-TP have been previously identified as bis(6-purinyl) disulfide, purine-6-sulfonic acid, and purine-6-sulfinic acid

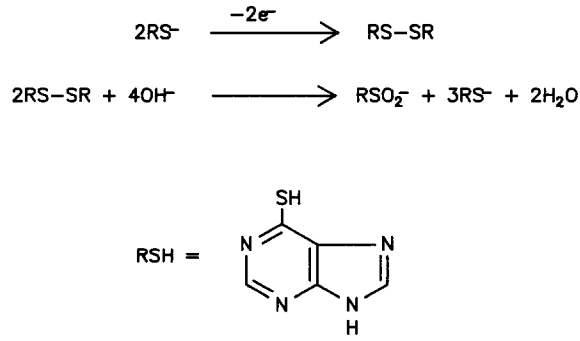

Fig. 1. Oxidation of 6-thiopurine to form 6-thiopurine disulfide, followed by disproportionation in alkaline media to form purine-6-sulfinate and the original thiol.

by comparison of the polarographic waves of electrolysis solutions with authentic standards[14].

Since the introduction of thermospray as a viable high-performance liquid chromatographic (LC)–MS interface, this technique has gained popularity as a soft ionization technique that gives primarily molecular weight information. Hambitzer and Heitbaum[18] were the first to successfully combine electrochemistry on-line with MS via a thermospray LC–MS interface. The experiments carried out by Hambitzer *et al.* demonstrated the potential for direct detection of electrochemically generated products by monitoring the formation of intact dimers and trimers upon electrooxidation of N,N dimethylaniline at a Pt electrode.

We have shown that the coupling of electrochemistry (EC) with tandem mass spectrometry via a thermospray interface (EC–thermospray MS–MS) can provide important chemical information about redox reactions of small biological molecules[8,10]. After the initial redox reaction has taken place, following chemical reactions can occur and produce a variety of products. Some of the products observed in on-line EC–TSP–MS result from unstable compounds undergoing electrophilic and nucleophilic substitution reactions with the mobile phase during the thermospray process. In this work, we show the first example of HPLC used on-line with electrochemistry to separate stable oxidation products prior to analysis by thermospray tandem mass spectrometry (EC–LC–thermospray MS–MS). In this paper, we report our attempts to characterize the chemical reactions which occur after the on-line electrooxidation of 6-thiopurine.

EXPERIMENTAL

Materials

Purine, hypoxanthine, adenine and 6-thiopurine were obtained from Sigma. Bis(6-purinyl) disulfide and sodium purine-6-sulfonate were prepared according to Doerr *et al.*[15].

HPLC procedure

Samples were analyzed by HPLC using a Microsorb (Rainin) C_{18} reversed-phase column (15 cm × 4.6 mm I.D.). All experiments were performed with a Rainin MacRabbit HPLC gradient system. The separation procedure for the oxidation products of 6-thiopurine required isocratic elution with 0.1 *M* ammonium acetate–methanol (pH 6.9) (98:2, v/v) for 9 min followed by a 2-min ramp from 2% methanol to 30% methanol with a flow-rate of 2.0 ml/min. The ammonium acetate and methanol solutions were filtered through a 0.45-μm filter before use. Samples were injected with a Rheodyne (Model 7125) injector fitted with a 20-μl loop. Details of the electrochemical cell have been described previously[8].

Mass spectrometry

The thermospray interface (Vestec) was mounted on a triple quadrupole mass spectrometer (Finnigan Model TSQ 45) equipped with an INCOS data system. Two temperatures were monitored in the experiments: the vaporizer exit temperature (tip temperature) and the source block temperature. In the tip temperature profile studies, the tip temperature was varied while the source temperature was held constant at

290°C. At a flow-rate of 2.0 ml/min, the typical operating temperatures were tip, 240°C, and source, 290°C.

Both positive ion and negative ion thermospray mass spectra were obtained by pulsed positive ion–negative ion chemical ionization (PPINICI). Typical conditions for thermospray MS were scan range m/z 120–300 in 0.3 s, electron multiplier voltage 1000 V, and preamplifier gain 10^8 VA^{-1}. The lower scan range limit of m/z 120 was normally used to avoid background interference from the ammonium acetate reagent ions. For MS–MS, the scan range and rates varied depending upon the m/z of the parent ion. Collisionally activated dissociation (CAD) studies were carried out using nitrogen as the collision gas (2 mTorr) with a collision energy of 30 eV.

RESULTS AND DISCUSSION

Identification of products by EC–thermospray MS–MS

Based on the electrochemical results, the known chemistry of 6-TP[14,18], EC–thermospray MS results with 6-TP were expected to indicate dimer formation by producing intact molecular-type ($[M+H]^+$ and $[M-H]^-$) ions of the disulfide. However, neither the disulfide nor its decomposition product, purine-6-sulfinate (Fig. 1), are observed in the EC–thermospray mass spectra. The results obtained by on-line EC–thermospray MS of 6-TP were initially confusing because of the unusual products which were detected and identified. Table I lists the ions identified in the EC–thermospray mass spectra of 6-TP. The formation of purine, adenine, and hypoxanthine (Table I) during the electrooxidation of 6-TP must clearly involve substitution reactions prior to mass analysis.

MS–MS was used to confirm the presence of purine, adenine, and hypoxanthine in the EC–thermospray mass spectra by comparing the daughter spectra of authentic standards with the daughter spectra of electrochemically generated products. The agreement between daughter ion abundances of standards and electrochemically generated products was generally ± 10% relative abundance (R.A.). The comparison of results is shown in Table II. The identification of intermediates and products in a mixture was based on their characteristic daughter spectra[19] which were obtained through the use of MS–MS as shown in Fig. 2.

TABLE I

OXIDATION PRODUCTS OF 6-THIOPURINE

Positive ions (m/z)	*Negative ions (m/z)*	*Structure correlation*
121	–	Purine $[M+H]^+$
136	134	Adenine $[M+H]^+$, $[M-H]^-$
–	194	Adenine $[M+CH_3COO]^-$
137	135	Hypoxanthine $[M-H]^-$
154	195	Hypoxanthine $[M+NH_4]^+$, $[M+CH_3COO]^-$
153	151	6-Thiopurine $[M+H]^+$, $[M-H]^-$
170	–	6-Thiopurine $[M+NH_4]^+$

TABLE II

COMPARISON OF DAUGHTER SPECTRA OF THE $[M+H]^+$ IONS OF AUTHENTIC STANDARDS WITH THOSE OF ELECTROCHEMICALLY GENERATED PRODUCTS

Compound	$[M+H]^+$ *ion*	*Authentic standard ion (% R.A.)*	*EC generated product ion (% R.A.)*
Purine	121^+	121(100), 94(35) 67(10), 52(2)	121(100), 94(36) 67(7), 52(2)
Adenine	136^+	136(100), 119(73) 94(5), 92(5)	136(100), 119(63) 94(1), 92(5)
Hypoxanthine	137^+	137(100), 119(21) 110(23), 94(7) 82(3), 55(2)	137(100), 120(5) 119(20), 110(26) 94(10), 82(3), 55(2)

Insights into the reaction pathway provided by EC–LC–thermospray MS

Although MS-MS allowed identification of purine, adenine, and hypoxanthine in the EC–thermospray mass spectra, MS–MS did not provide any information concerning the location of the reactions responsible for these products. In order to help determine whether the reactions occurred in solution prior to analysis by thermospray or during the thermospray vaporization process, HPLC was used on-line with an electrochemical cell to separate electrochemically generated products prior to analysis by thermospray MS–MS (Fig. 2). It was postulated that if the products listed in Table I were formed in solution immediately after electrooxidation, then it should be possible to correlate the retention times of these products with the retention times of the authentic standards. If, on the other hand, these products were formed later, in the thermospray probe or in the source, their retention times should instead be the same as that of the electrochemically generated disulfide which eventually leads to their formation.

Fig. 3 illustrates the LC–thermospray positive ion $[M+H]^+$ mass chromatograms of a four-component mixture of authentic purine [molecular weight (MW)

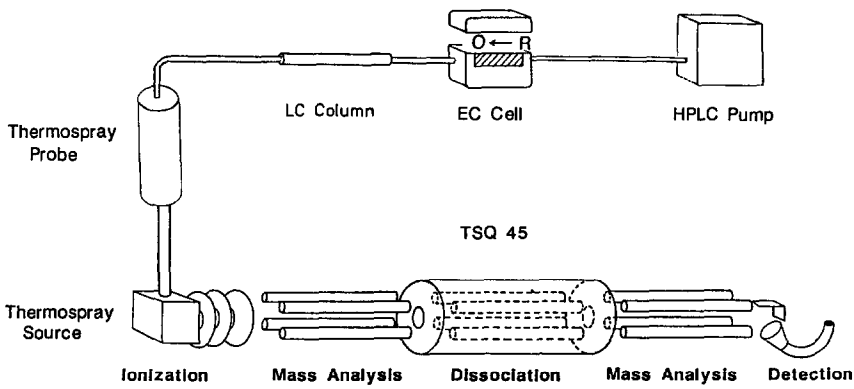

Fig. 2. On-line EC–LC–thermospray MS–MS system.

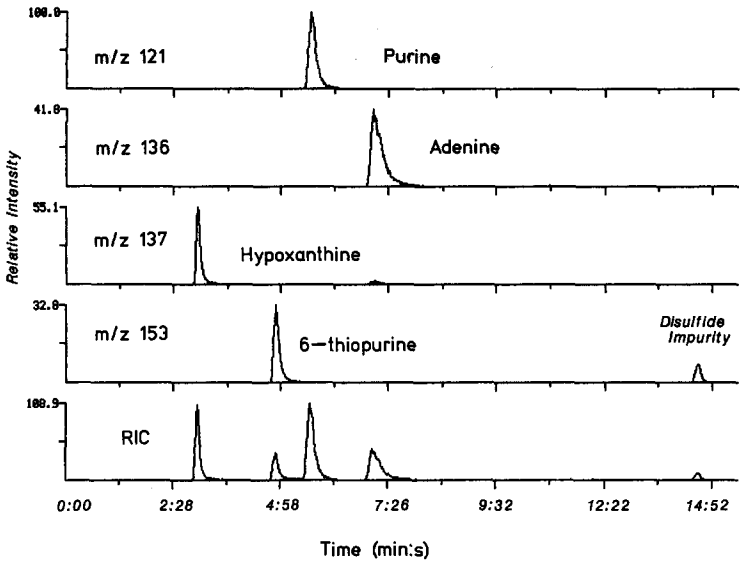

Fig. 3. HPLC–thermospray MS positive ion mass chromatograms of 4-component purine mixture. Flow-rate of 0.1 *M* ammonium acetate mobile phase 2.0 ml/min. Tip temperature 240°C, source temperature 300°C.

120], adenine (MW 135), hypoxanthine (MW 136), and 6-TP (MW 152) which are chromatographically separated in *ca.* 8 min. The small peak at *ca.* 14 min (Fig. 3) is due to trace amounts of the 6-TP disulfide, an impurity in the 6-TP standard. The retention time of this small peak at *ca.* 14 min is identical to the retention time of the synthesized disulfide (Fig. 4).

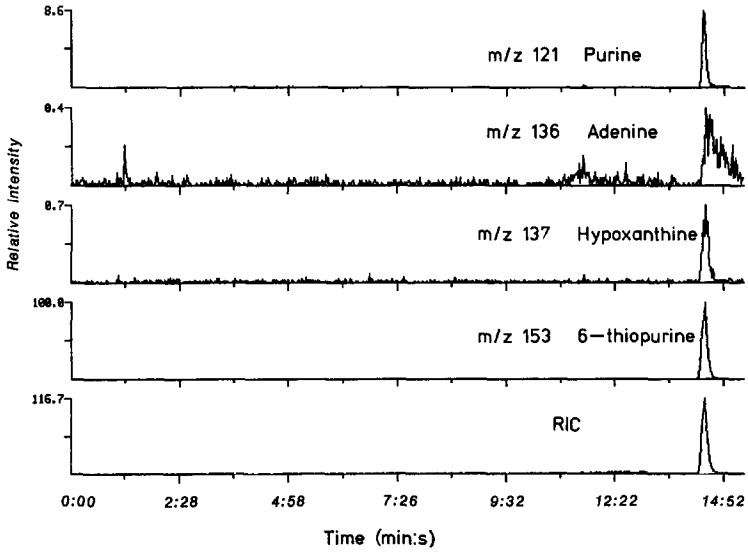

Fig. 4. HPLC–thermospray MS positive ion mass chromatograms of synthesized 6-thiopurine disulfide. Flow-rate 2.0 ml/min, tip temperature 240 °C, and source temperature 300°C.

When a sample of synthesized 6-TP disulfide is analyzed by LC–thermospray MS, decomposition reactions occur during the thermospray vaporization process which result in the formation of 6-thiopurine, adenine, hypoxanthine, and purine (Fig. 4). The LC–MS mass chromatograms of the $[M+H]^+$ ions formed during the decomposition of the authentic disulfide standard can be seen in Fig. 4. The fact that all four products originate from a single peak with a retention time of *ca.* 14 min reveals that decomposition of the disulfide occurs somewhere during the thermospray ionization process. This is clear from the comparison of the retention times of the authentic purine standards (Fig. 3) with the retention times of the decomposition products arising from the disulfide (Fig. 4).

The on-line EC–LC–thermospray MS results obtained for the electrochemical oxidation products of 6-thiopurine can be seen in Fig. 5. The positive ion mass chromatograms obtained in an EC–LC–thermospray MS experiment (Fig. 5) are in agreement, when both the retention time and relative intensity are compared, with the positive ion mass chromatograms obtained for the authentic 6-TP disulfide standard (Fig. 4). In addition to the peak corresponding to the disulfide at *ca.* 14 min in EC–LC–thermospray mass chromatograms in Fig. 5, a few smaller peaks with much shorter retention times (2 and 4 min) are also observed. Although these ions correspond to oxidation products of 6-TP, they could not be positively identified because their retention times did not match those of the available standards and their low intensities precluded the use of MS–MS for structural elucidation. The only conclusion which can be drawn is that the product at *ca.* 2 min forms only a positive ion at *m/z* 121 and the product at *ca.* 4 min forms only positive ions at *m/z* 121 and *m/z* 136.

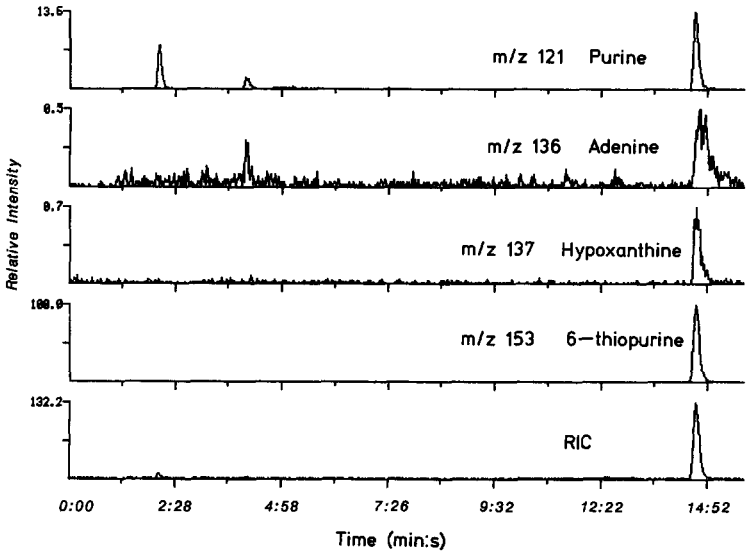

Fig. 5. EC–LC–thermospray MS positive ion mass chromatograms of the oxidation products of 6-thiopurine. Flow-rate 2.0 ml/min, tip temperature 240°C, and source temperature 300°C. Potential +0.60 V *vs.* Pd.

Tip temperature studies

Optimum thermospray interface parameters such as tip temperature are traditionally determined by maximizing the solvent-buffer ion intensities for a given flow-rate[20–22]. Using the solvent-buffer ion intensity method, a signal maximum is obtained at a tip temperature of *ca.* 240°C for a flow-rate of 2.0 ml/min. As expected, this tip temperature also produces the most intense reconstructed ion current for samples in EC–thermospray MS. However, some ions produced during the vaporization process of 6-TP oxidation products do not follow the tip temperature profile of the authentic standards. In this study, we have identified the ions which show different responses. These ions have been identified as ammonolysis products and are believed to result from purine-6-sulfinate. Purine-6-sulfinate is one of the 6-TP disulfide disproportionation products (Fig. 1). Because hydrolysis reactions of intermediates following electrochemical oxidation are well known[1], the formation of ammonolysis products under EC–thermospray MS conditions is not surprising but has only recently been reported[23–25].

We hypothesize that ammonia and water react with purine-6-sulfinate by nucleophilic substitution to form adenine and hypoxanthine (Fig. 6). Electrophilic substitution by a proton followed by elimination of SO_2 gas is responsible for the formation of purine (Fig. 6). Decomposition of purine-6-sulfinate in 98–100% formic acid to yield purine has been previously reported[15]. It is important to note that no ions corresponding to either H_2SO_2, the leaving group produced by nucleophilic substitution, or SO_2, the leaving group produced by electrophilic substitution, are observed in the thermospray mass spectra when the lower m/z scan limit is reduced from m/z 120 to m/z 25.

Fig. 7 compares the tip temperature profiles of authentic adenine and hypoxanthine with the tip temperature profiles of electrochemically generated adenine and hypoxanthine. As can be seen in Fig. 7, the profiles differ dramatically at tip temperatures lower than 240°C due to the decreased efficiency with which the product of electrochemical oxidation, postulated to purine-6-sulfinate, undergoes ammonolysis and hydrolysis reactions. The decreased intensity of products at lower tip temperatures is indicative that the reactions of the electrochemical product forming adenine and hypoxanthine are occurring inside the vaporizer probe.

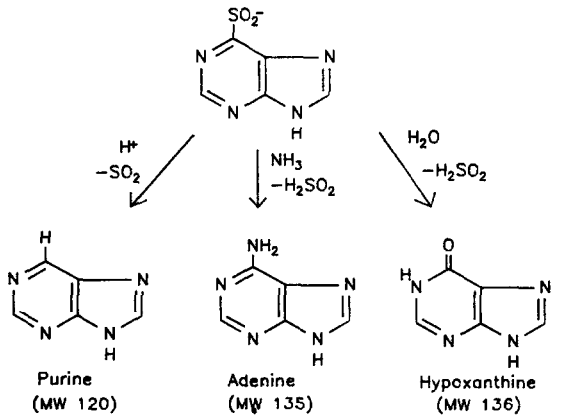

Fig. 6. Reaction pathways of purine-6-sulfinate under thermospray conditions.

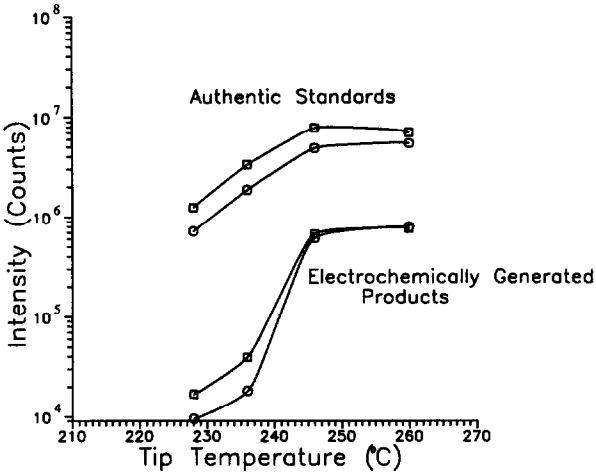

Fig. 7. EC–thermospray MS tip temperature study of hydrolysis and ammonolysis reactions with purine-6-sulfinate under thermospray conditions. (○) Hypoxanthine, (□) adenine.

Because the synthesis of sodium purine-6-sulfinate ($RSO_2^-Na^+$) using the method described by Doerr *et al.*[15] was not successful, it was not possible to analyze it under thermospray conditions to prove our hypothesis. However, a very similar compound, sodium purine-6-sulfonate ($RSO_3^-Na^+$), was prepared instead[15] and analyzed by LC–thermospray MS. Fig. 8 illustrates the LC–thermospray positive ion mass chromatograms of sodium purine-6-sulfonate. As can be seen in Fig. 8, pu-

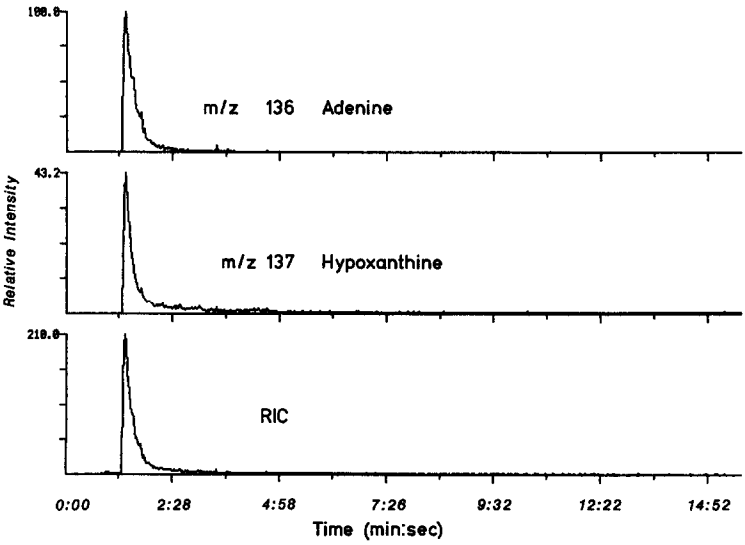

Fig. 8. LC–thermospray MS positive ion mass chromatograms of the thermospray-induced decomposition products of purine-6-sulfonate. Flow-rate 2.0 ml/min, tip temperature 240°C, and source temperature 300°C.

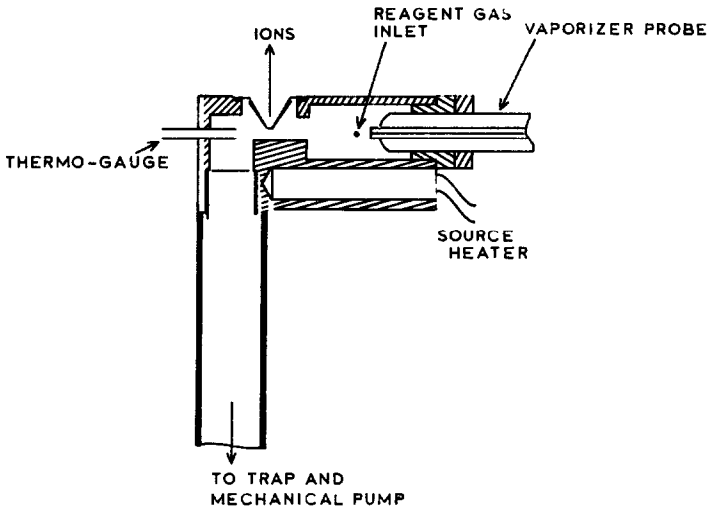

Fig. 9. Modified Vestec thermospray source. Reagent gases introduced in the orifice normally holding the discharge electrode. Source pressure monitored by replacing vapor temperature sensor with thermogauge.

rine-6-sulfonate completely decomposes during the thermospray process to produce adenine and hypoxanthine. Hydrolysis studies have shown that both purine-6-sulfinate and purine-6-sulfonate are very labile to acid resulting in the formation of hypoxanthine[15]. No ions indicative of intact purine-6-sulfonate are observed in the LC–thermospray mass spectra. It is important to point out that purine is not produced during the decomposition of sodium purine-6-sulfonate under thermospray conditions; however, purine is produced during the thermospray-induced decomposition of 6-TP disulfide which may occur via electrophilic substitution reactions with purine-6-sulfinate.

Solution-phase and gas-phase studies

These studies were performed to determine if substitution reactions such as ammonolysis and hydrolysis reactions shown for purine-6-sulfinate were occurring

TABLE III

PRODUCTS FORMED IN SOLUTION-PHASE AND GAS-PHASE STUDIES OF ELECTROOXIDATION PRODUCTS OF 6-THIOPURINE

Reagent gas added to source	*Buffer composition*	*Products*
None	0.1 *M* NH_4OOCCH_3	Purine, hypoxanthine, 6-thiopurine and adenine
None	0.1 *M* NH_4OOCCH_3–CH_3NH_2	Purine, hypoxanthine, 6-thiopurine, adenine and N6-methyladenine
CH_3NH_2	0.1 *M* NH_4OOCCH_3	Purine, hypoxanthine, 6-thiopurine and adenine
NH_3	0.1 *M* CH_3COOH	Purine, hypoxanthine, 6-thiopurine and 6-TP disulfide

inside the thermospray vaporizer probe or in the thermospray source. By modifying a standard Vestec thermospray source, we were able to introduce various reagent gases capable of acting as nucleophiles into the thermospray source (Fig. 9). The electrochemical oxidation products of 6-TP were monitored as the mobile phase composition and reagent gas were varied.

Methylamine was chosen as a model nucleophile in these experiments because its chemical properties and reactivity are similar to those of ammonia and it is not present in the normal thermospray buffer. When a mixture of 0.1 *M* methylamine (CH_3NH_2) and 0.1 *M* ammonium acetate pH 7 is used as a buffer during the electrooxidation of 6-TP, a new product, N6-methyladenine, is observed in the EC–thermospray mass spectra (Table III). N6-methyladenine is formed as a result of the electrochemically formed product undergoing a nucleophilic substitution with methylamine in analogy to the reaction with NH_3 which forms adenine (Fig. 6).

To determine if the nucleophilic reaction with methylamine can occur inside the thermospray source, methyl amine (0.4 Torr) was introduced into the thermospray source while 0.1*M* ammonium acetate was used as the buffer. Under these conditions, no gas-phase nucleophilic reactions between methylamine and the electrochemical oxidation products of 6-TP are observed. Specifically, N6-methyladenine was not detected. Hence, it appears that methylamine must be present in the buffer solution to react and form N6-methyladenine. Therefore, this reaction and other nucleophilic substitution reactions (hydrolysis, ammonolysis) probably occur inside the vaporizer probe during the thermospray vaporization process, rather than in the thermospray ion source.

Additional results supporting this conclusion were obtained using 0.1 *M* acetic acid (pH *ca.* 2.8) as the buffer while introducing ammonia (0.4 Torr) into the thermospray source. The use of acetic acid as the mobile phase was designed to control conditions so that ammonia was present only in the source and was not part of the solution phase. Under these conditions, the normal ammonolysis product, adenine, is not observed following electrooxidation of 6-thiopurine. However, hypoxanthine and purine are still observed in addition to small amounts of the intact disulfide. The inability to detect the disulfide by EC–thermospray MS at pH *ca.* 7 with the traditional ammonium acetate buffer is consistent with the disproportionation process which is favored at high pH[15]. Detection of the disulfide shows that under acidic conditions, the disproportionation process (Fig. 1) will not be favored. This supports the results obtained with methylamine and indicates that the ammonolysis reaction occurs inside the vaporizer probe.

Mass spectrometric hydrodynamic voltammograms

Typical electrochemical techniques such as cyclic voltammetry and chronocoulometry rely on monitoring current or charge as a function of potential or time. These techniques provide little chemical information about the actual molecular structures of the species which form as a result of electron transfer at the electrode surface. One of the most important uses of on-line EC–thermospray MS or EC–thermospray MS–MS is the monitoring of reactants, intermediates, and products as a function of electrode potential[8].

Fig. 10 illustrates the mass spectrometric hydrodynamic voltammograms of 6-thiopurine obtained by EC–thermospray MS. Oxidation of 6-TP begins at poten-

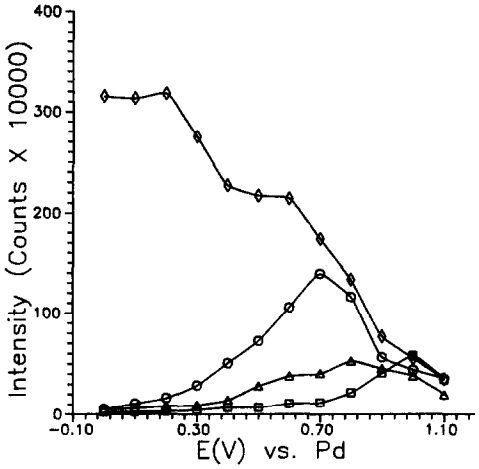

Fig. 10. MS hydrodynamic voltammograms of 6-thiopurine. Flow-rate of 0.1 *M* ammonium acetate mobile phase 2.0 ml/min. Tip temperature 240°C and source temperature 300°C. (○) Purine, *m/z* 121; (□) adenine, *m/z* 136; (△) hypoxanthine, *m/z* 137; (◇) 6-thiopurine, *m/z* 153.

tials > +0.20 V and reaches a steady-state level at potentials < +0.40 V. Steady-state behavior is expected for both reactants and products when operating in the limiting current region due to the hydrodynamic flow of reactant in the electrolysis cell. This is what is observed in HPLC with detection by electrochemistry[25]. At *ca.* +0.65 V, a second oxidation process appears to occur. This process can be followed by noting the decrease in the intensity of the $[M+H]^+$ ion of 6-TP at *m/z* 153. This second oxidation process does not favor the formation of purine. In addition, the intensities of the ions corresponding to hypoxanthine and adenine also change at potentials higher than +0.65 V. This behavior is expected if further electrochemical oxidation of 6-TP results in the formation of an intermediate which does not readily undergo the same substitution reactions or react at the same rate as the intermediate formed at lower potentials.

CONCLUSIONS

Electrochemistry has been used on-line with LC–thermospray MS–MS to provide insight into redox reactions of the purine drug 6-thiopurine. After the initial oxidation reaction of 6-TP has taken place, the disulfide which forms disproportionates in solution inside the thermospray vaporizer probe to regenerate 6-thiopurine and to form small amounts of an intermediate proposed to be purine-6-sulfinate. Neither the disulfide nor purine-6-sulfinate are detected under normal thermospray conditions. The disulfide can be detected if acetic acid is used as a mobile phase. Formation of purine-6-sulfinate was tested by synthesis of a structurally similar derivative, purine-6-sulfonate, which reacted under thermospray conditions to form the products found in EC–thermospray MS–MS. The observed products, adenine, hypoxanthine, and purine were shown to form inside the vaporizer probe by nucleophilic and electrophilic substitution reactions of purine-6-sulfinate.

In addition to the solution-phase studies, tip temperature profiles also support the disulfide disproportionation process. The LC–thermospray mass chromatograms of authentic 6-TP disulfide and the EC–LC–thermospray mass chromatograms of the electrochemically generated disulfide indicate that all decomposition products arise from a single LC peak corresponding to the retention time of 6-TP disulfide thereby supporting the hypothesis of decomposition occurring inside the vaporizer probe. This is further supported by the formation of the nucleophilic substitution product, N6-methyladenine, when methylamine is added to the thermospray buffer. Because N6-methyladenine is not detected when methylamine is added to the thermospray source, these results indicate that the site of nucleophilic and presumably electrophilic attack is within the thermospray probe.

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REFERENCES

1 G. Dryhurst, *Electrochemistry of Biological Molecules*, Academic Press, New York, 1977.
2 K. McKenna and A. Brajter-Toth, *J. Electroanal. Chem.*, 233 (1987) 49.
3 P. J. Kraske and A. Brajter-Toth, *J. Electroanal. Chem.*, 207 (1986) 101.
4 R. N. Goyal, A. Brajter-Toth and G. Dryhurst, *J. Electroanal. Chem.*, 131 (1982) 181.
5 D. Astwood, T. Lippincott, M. Deysher, C. D'Amico, E. Szurley and A. Brajter-Toth, *J. Electroanal. Chem.*, 159 (1983) 295.
6 D. J. Miner, J. R. Rice, R. M. Riggin and P. T. Kissinger, *Anal. Chem.*, 53 (1981) 2258.
7 J. R. Rice and P. T. Kissinger, *Biochem. Biophys. Res. Commun.*, 104 (1982) 1312.
8 K. J. Volk, M. S. Lee, R. A. Yost and A. Brajter-Toth, *Anal. Chem.*, 60 (1988) 270.
9 E. G. Rogan, E. L. Cavalieri, S. R. Tibbels, P. Cremonesi, C. D. Warner, D. L. Nagel, K. B. Tomer, R. L. Cerny and M. L. Gross, *J. Am. Chem. Soc.*, 110 (1988) 4023.
10 A. Brajter-Toth, T. Peterson, K. McKenna, P. J. Kraske and K. J. Volk, in G. Dryhurst and K. Niki (Editors), *Redox Chemistry and Interficial Behavior of Biological Molecules*, Plenum Press, 1988.
11 R. M. Hyslop and I. Jardine, *J. Pharmacol. Exp. Ther.*, 218 (1981) 621.
12 R. W. Brockman, *Cancer Res.*, 23 (1963) 1191.
13 S. Zimm, G. E. Johnson, B. A. Chabner and D. G. Poplack, *Cancer Res.*, 45 (1985) 4156.
14 G. Dryhurst, *J. Electrochem. Soc.*, 116 (1969) 1097.
15 I. L. Doerr, I. Wempen, D. A. Clarke and J. J. Fox, *J. Org. Chem.*, 26 (1961) 3401.
16 E. L. Carr, G. P. Smith and G. J. Alliger, *J. Org. Chem.*, 14 (1949) 921.
17 A. J. Parker and N. Kharasch, *Chem. Rev.*, 59 (1959) 583.
18 G. Hambitzer and J. Heitbaum, *Anal. Chem.*, 58 (1986) 1067.
19 R. J. Perchalski, R. A. Yost and B. J. Wilder, *Anal. Chem.*, 54 (1982) 1466.
20 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750.
21 C. Linberg and J. J. Paulson, *J. Chromatogr.*, 394 (1987) 117.
22 R. D. Voyksner and C. A. Haney, *Anal. Chem.*, 57 (1985) 991.
23 K. J. Volk, R. A. Yost and A. Brajter-Toth, *Anal. Chem.*, (1989) in press.
24 R. A. Yost, K. J. Volk, M. S. Lee and A. Brajter-Toth, *Adv. Mass Spectrom.*, (1988) in press.
25 P. T. Kissinger and W. R. Heineman, *Laboratory Techniques in Electroanalytical Chemistry*, Marcel Dekker, New York, 1984.