The Electrochemical Oxidation of Aminopurines and Their Hydroxy Derivatives at the Glassy Carbon Electrode

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The electrochemical oxidation of aminopurines and their hydroxy derivatives, which gave a single voltammetric peak at the glassy carbon electrode, was investigated by means of linear-sweep voltammetry, cyclic voltammetry, coulometry, and macroscale controlled-potential electrolysis. In general, the ease of oxidation of these compounds increased in proportion to the number of hydroxyl or amino groups on the molecule. The coulometric *n*-values for monosubstituted purines were 5.86—6.08, for disubstituted purines they were 3.90—4.20, and for trisubstituted purines they were 2.07—2.33. The electrochemical oxidation of the different aminopurines appeared to follow initially the same path as the enzymatic oxidation; *i.e.*, the primary attack for the electrochemical oxidation occurred first at the C-8 position, and then the C-2 position. Thus, aminopurines were oxidized in two sequential two-electron processes to give first 8-hydroxy and then 2,8-dihydroxy(or 6,8-dihydroxy) intermediates. Then, this latter intermediate was further oxidized in a two-electron process to produce a diimine intermediate, which was unstable and which then broke down to the ultimate products, such as parabanic acid, oxaluric acid, urea, ammonia, allantoin, and guanidine.

Aminopurines, especially the two bases of adenine and guanine, are important components of nucleic acids and play an important role in many metabolic processes. Several works[1-3] have suggested a possible parallelism between the modes of electrochemical process and the biological transformation, *e.g.*, the enzymatic oxidation. The enzymatic and biological oxidation of adenine and guanine have been examined; they are covered in part in the reviews by Lister[4] and Robins.[5] The oxidation of adenine by the enzyme xanthine oxidase gives 6-amino-8-hydroxypurine as an intermediate in the formation of 6-amino-2,8-dihydroxypurine.[6] Also, guanine is slowly oxidized to uric acid in the presence of very large excesses of xanthine oxidase;[7] this process may be brought about by traces of guanine present in the xanthine oxidase.

Most of the aminopurines were not reduced easily at the dropping mercury electrode(DME) in aqueous solutions. However, some aminopurines can be voltammetrically oxidized at the pyrolytic graphite electrode[2,8,9] and the glassy carbon electrode (GCE).[10-12]

In this paper, the electrochemical oxidation of the aminopurines and their hydroxy derivatives was investigated by means of linear-sweep voltammetry, cyclic voltammetry, controlled-potential coulometry, and the polarography of the oxidation products. On the basis of the data reported here, different and plausible oxidation products were proposed; the mechanisms for the electrochemical oxidation were also proposed. In order to compare the mechanisms and products of oxidation of aminopurines with those of previously known biological oxidations, these studies were carried out in some detail.

Experimental

Chemicals. The 6-aminopurine (adenine), 2-aminopurine, and 2-amino-6-hydroxypurine (guanine) were obtained from the Wako Pure Chemical Co. The 6-amino-2-hydroxypurine (isoguanine) and 2,6-diaminopurine were obtained from the Sigma Chemical Co. The 6-amino-8-hydroxypurine, 6-amino-2,8-dihydroxypurine, 2-amino-8-hydroxypurine, 2-amino-6,8-dihydroxypurine, and 2,6-diamino-8-hydroxypurine were all synthesized according to methods previously described.[13] All the products were recrystallized three times from distilled water before drying *in vacuo* at 60 °C, and their purities were checked by both elemental analysis and linear-sweep voltammetry.

Apparatus. The polarograms and anodic voltammograms were recorded using a Yanagimoto P-8 type Polarograph. The cyclic voltammograms were obtained with a versatile solid-state instrument constructed in this laboratory, with triangular voltage sweeps supplied by a NF Circuit Design Block Co. Model FG-104T function generator. The current-voltage curves were recorded on an IWATSU Model DS-5016 dual-beam oscilloscope equipped with a camera or on a Hewlett Packard Model 7045A X-Y recorder.

A three-electrode cell was used for all the experiments. A saturated calomel electrode (SCE) was used as a reference electrode, while a platinum wire was used as a counter electrode.

The DME used had the following characteristics: mercury flow rate, $m=2.32$ mg/s and drop time, $t=3.32$ s at an open circuit with a mercury head of 70 cm in 1 M H_3PO_4.

The working electrode used for anodic voltammetry, GCE, was constructed as has been described before.[10] The GCE was polished for about 30 s with 1500-grade emery paper and then a paste of CeO_2 on asphalt pitch until the surface of the electrode was brought to a mirror finish. Also, in order to obtain reproducible results, the standard pretreatment procedure described previously was applied before recording each voltammogram.[10]

Controlled-potential electrolysis was done at the potentials on the crest of the anodic peaks, with a NICHIA Model NP-1 potentiostat. A glassy carbon beaker (40 ml in volume; Tokai Electrode Co.) was used as the working electrode. The colorimetric determinations of oxidation products were done using a Shimadzu Model Spectronic 20.

Colorimetric Methods for the Determination of Oxidation Products. The urea was determined by a modification of Rosenthal's method.[14,15] The ammonia was determined by the Nessler method, following King and Faulconer.[16] The allantoin was determined by a modification of the Young and Conway procedure.[17] The guanidine was determined by the procedure described by Staron and Allard.[18]

Polarographic Methods for the Determination of Parabanic and

TABLE 1. VOLTAMMETRIC DATA FOR THE OXIDATION PEAKS OF AMINOPURINES AND THEIR HYDROXY DERIVATIVES AT THE STATIONARY GCE

Compound[a)]	pH range	$E_{p/2}$ (V *vs.* SCE)	$(i_p/ACV^{1/2})$[b),c)]	$E_p-E_{p/2}$[c)] (mV)
6-Aminopurine	0—12	1.45—0.064pH	145	60
6-Amino-8-hydroxypurine	0—13	1.01—0.067pH	95	45
6-Amino-2-hydroxypurine	0—13	1.03—0.057pH	103	35
6-Amino-2,8-dihydroxypurine	0—13	0.77—0.072pH	46	52
2-Aminopurine	0—12	1.34—0.087pH	130	37
2-Amino-6-hydroxypurine	0—13	1.13—0.059pH	93	29
2-Amino-8-hydroxypurine	0—13	1.11—0.068pH	87	32
2-Amino-6,8-dihydroxypurine	0—13	0.79—0.077pH	51	27
2,6-Diaminopurine	0—13	1.03—0.054pH	93	30
2,6-Diamino-8-hydroxypurine	0—13	0.76—0.074pH	49	33

a) Concentration of each compound: 0.05 mM. b) Peak current function: $\mu A\ (cm^2 \cdot mM)^{-1}(mV/s)^{-1/2}$.
c) In 1M H_2SO_4.

Oxaluric Acids. The parabanic acid was determined on the completion of the electrolysis by transferring about 10 ml of the electrolyzed solution (1 M H_2SO_4) into a polarographic cell, deaerating it, running a polarogram between 0 and −1.1 V *vs.* SCE, and then comparing the height of the wave at −0.53 V *vs.* SCE with a calibration curve prepared from authentic parabanic acid. The oxaluric acid was determined on the completion of the electrolysis in the same way, using the height of the wave at −0.87 V *vs.* SCE.

Results and Discussion

Anodic Voltammetry. Each of the aminopurines and their derivatives studied gave a voltammetric oxidation peak at a stationary GCE over a wide pH range. The half-peak potentials ($E_{p/2}$) of their oxidation peaks shifted linearly towards negative potentials with the increase in the pH, as is summarized in Table 1. Also, the peak current function, $i_p/ACV^{1/2}$, decreased gradually with the increase in the pH, but in the electrolytes with pH values below 2.8 the peak current functions for all the aminopurines studied remained almost constant in spite of the variations in the pH values. In 1 M H_2SO_4, good linear relationships were observed between the peak current and the concentration, and the peak current functions were almost independent of the scan rate. These facts imply that, in 1 M H_2SO_4, these voltammetric oxidations are diffusion-controlled processes. Accordingly, the peak current functions for all the compounds studied were determined in 1 M H_2SO_4; these results are shown in Table 1.

It was proved in the previous report[12] that uric acid (2,6,8-trihydroxypurine) gives a well-defined oxidation peak when in acidic solutions, but the peak tends to be ill-defined because of the adsorption of a depolarizer on the electrode surface when in neutral or alkaline solutions, and that the oxidation peak obtained in acid solutions, such as 1M H_3PO_4 and H_2SO_4, is attributable to a two-electron, diffusion-controlled process. The value of the peak current function for uric acid was 47.1 $\mu A(cm^2 \cdot mM)^{-1}(mV/s)^{-1/2}$ in 1 M H_2SO_4. This value was compared with the values for all the compounds studied by assuming that their diffusion-constant values are equal to that of uric acid. The results indicated that the values of the peak current function for the oxidation peaks of monosubstituted purines were *ca.* 130—145 $\mu A(cm^2 \cdot mM)^{-1}(mV/s)^{-1/2}$, corresponding to a six-electron process. Similarly, it is feasible that disubstituted purines are oxidized in a four-electron process, and that trisubstituted purines are oxidized in a two-electron process.

Coulometry. The coulometric measurements at the controlled potentials were performed in order to determine the number of electrons involved in the overall electron-transfer reactions. In 1 M H_2SO_4, the time taken to oxidize completely all of the depolarizer was from one to two hours. However, in the Britton-Robinson buffers with pH values above 4, exhaustive electrolysis required a much longer time and the electrolytic current remained at a low value throughout the electrolysis; this is indicative of a very slow intermediate step in the oxidation, the deposition of an insoluble reaction product, film formation on the electrode surface, or a combination of these phenomena.

Consequently, the coulometric *n*-values were determined in 1 M H_2SO_4. The results are presented in Table 2; the *n*-values for monosubstituted purines were 5.86—6.08, for disubstituted purines they were 3.90—4.20, and for trisubstituted purines they were 2.07—2.33. These results were consistent with the *n*-values determined from the peak current functions of the voltammetric peaks.

TABLE 2. COULOMETRIC *n*-VALUES FOR THE CONTROLLED-POTENTIAL ELECTROLYSIS OF AMINOPURINES AND THEIR HYDROXY DERIVATIVES IN 1M H_2SO_4

Compound	Applied potential (V *vs.* SCE)	n[a)]
6-Aminopurine	1.55	5.86
6-Amino-8-hydroxypurine	1.05	3.90
6-Amino-2-hydroxypurine	1.03	4.20
6-Amino-2,8-dihydroxypurine	0.75	2.28
2-Aminopurine	1.30	6.08
2-Amino-6-hydroxypurine	1.15	4.11
2-Amino-8-hydroxypurine	1.15	4.18
2-Amino-6,8-dihydroxypurine	0.75	2.33
2,6-Diaminopurine	1.05	3.92
2,6-Diamino-8-hydroxypurine	0.75	2.07

a) Average value of three determinations.

Table 3. Effect of pH on the peak potential[a] of anodic peaks observed on the cyclic voltammograms of aminopurines and their hydroxy derivatives

A, Original peak; B, anodic peaks of intermediates.

Compound	1M H_2SO_4		Britton-Robinson buffer					
			pH 2.1		pH 3.0		pH 4.1	
	A	B	A	B	A	B	A	B
6-Aminopurine	1.45	0.99	1.37	0.92	1.31	0.86	1.24	0.78
		0.76		0.66		0.61		0.54
6-Amino-8-hydroxypurine	0.99	0.76	0.91	0.68	0.85	0.61	0.78	0.53
6-Amino-2-hydroxypurine	1.01	0.76	0.95	0.68	0.89	0.61	0.83	0.54
6-Amino-2,8-dihydroxypurine	0.76		0.67		0.61		0.53	
2-Aminopurine	1.30	1.08	1.19	1.00	1.12	0.94	1.02	0.88
		0.75		0.79		0.59		0.52
2-Amino-6-hydroxypurine	1.10	0.76	1.03	0.79	0.98	0.59	0.91	0.52
2-Amino-8-hydroxypurine	1.08	0.75	0.99	0.79	0.94	0.58	0.88	0.51
2-Amino-6,8-dihydroxypurine	0.75		0.79		0.59		0.51	
2,6-Diaminopurine	1.01	0.73	0.95	0.64	0.90	0.58	0.84	0.50
2,6-Diamino-8-hydroxypurine	0.73		0.63		0.57		0.50	

a) in V *vs.* SCE.

Cyclic Voltammetry. Cyclic voltammograms at fast scan rates were recorded in 1 M H_2SO_4 for each of the four 6-aminopurines. The results are shown in Fig. 1. When scanning was done from 0.0 V at a clean electrode toward a positive potential, only a single anodic peak was observed for 6-aminopurine (Curve A). The principal point of interest in this voltammogram is that two new, small anodic peaks (Peaks I and II) were observed on the second sweep toward the positive potential at a fast scan rate of more than 0.25 V/s. Peak I appeared at almost the same peak potential as the original anodic peak of 6-amino-8- or 6-amino-2-hydroxypurine. Also, the peak potential of Peak II was the same as that of the anodic peak of 6-amino-2,8-dihydroxypurine. Each of 6-amino-8- and 6-amino-2-hydroxypurine gave a new anodic peak on the second sweep toward the positive potential; the peak potential of this new peak was consistent with that of the original peak of 6-amino-2,8-dihydroxypurine.

However, since the peak potentials of these new peaks were dependent on the pH of the electrolyte solutions, cyclic voltammograms were recorded in electrolyte solutions of various pH values. The results are shown in Table 3. At various pH values, Peak I and Peak II of 6-aminopurine coincided in potential with the original peak of 6-amino-8-hydroxypurine and 6-amino-2,8-dihy-

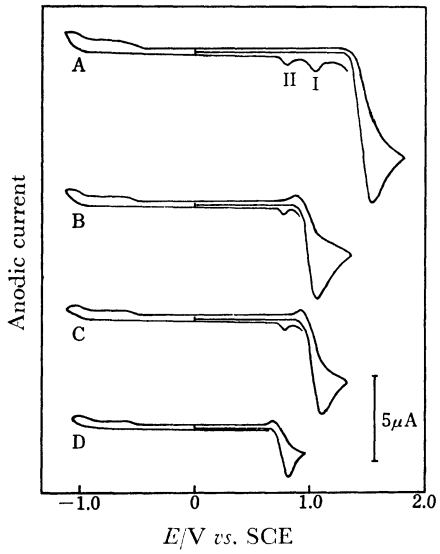

Fig. 1. Cyclic voltammograms of 6-aminopurines in 1 M H_2SO_4.
(A), 6-Aminopurine; (B), 6-amino-8-hydroxypurine; (C), 6-amino-2-hydroxypurine; (D), 6-amino-2,8-dihydroxypurine. Concn of all the compounds: 0.5 mM. GCE geometric area: 0.071 cm^2. Scan rate: (A), 0.64 V/s; (B), (C), (D), 0.56 V/s.

Table 4. Comparison of polarographic behavior of parabanic and oxaluric acids with products of the electrolytic oxidation of 6-aminopurine

pH	Oxidation products[a]				Parabanic acid[b]		Oxaluric acid[b]	
	1st wave		2nd wave					
	$E_{1/2}$ (V *vs.* SCE)	i_d (μA)	$E_{1/2}$ (V *vs.* SCE)	i_d (μA)	$E_{1/2}$ (V *vs.* SCE)	i_d (μA)	$E_{1/2}$ (V *vs.* SCE)	i_d (μA)
1M H_2SO_4	−0.566	0.56	−0.957	0.32	−0.569	1.25	−0.955	1.06
Britton-Robinson buffer								
2.1	−0.652	0.55	−1.077	0.35	−0.655	1.26	−1.080	1.12
3.0	−0.715	0.49	−1.165	0.30	−0.717	1.32	−1.171	1.00
4.1	−0.795	0.51	−1.287	0.26	−0.793	1.28	−1.283	0.95

a) Exhaustive electrolysis of 1.0 mM 6-aminopurine. b) Concentration: 0.2 mM.

TABLE 5. DETERMINATION OF OXIDATION PRODUCTS AFTER EXHAUSTIVE ELECTROLYSIS

Compound[a]	Polarographic method		Colorimetric method			
	Parabanic acid (mM)	Oxaluric acid (mM)	Urea (mM)	Ammonia (mM)	Allantoin (mM)	Guanidine (mM)
6-Aminopurine	0.09	0.06	0.25	2.36	0.24	—
6-Amino-2-hydroxypurine	0.04	0.05	0.21	2.98	0.30	—
6-Amino-8-hydroxypurine	0.05	0.03	0.16	3.36	0.34	—
6-Amino-2,8-dihydroxypurine	0.05	0.03	0.32	3.48	0.29	—
2-Aminopurine	0.02	0.01	0.29	0.93	0.55	0.60
2-Amino-6-hydroxypurine	0.01	0.01	0.20	0.52	0.78	0.63
2-Amino-8-hydroxypurine	0.01	0.01	0.30	0.82	0.60	0.61
2-Amino-6,8-dihydroxypurine	0.01	0.01	0.32	1.00	0.48	0.64
2,6-Diaminopurine	0.04	0.03	0.25	2.50	0.52	0.55
2,6-Diamino-8-hydroxypurine	0.03	0.03	0.21	2.75	0.60	0.51

a) Exhaustive electrolysis of 1 mM aminopurines in 1M H_2SO_4.

droxypurine respectively. Also, the new anodic peaks of 6-amino-8- and 6-amino-2-hydroxypurine were observed at almost the same potentials at various pH values, and their peak potentials were in very close agreement with that of the original peak of 6-amino-2,8-dihydroxypurine.

Similarly, 2-aminopurine gave two new, small anodic peaks on the second sweep toward the positive potential. At various pH values, the peak potentials for these two peaks were the same as those of the anodic peaks for the oxidation of 2-amino-8-hydroxypurine and 2-amino-6,8-dihydroxypurine, as is shown in Table 3. Also, 2,6-diaminopurine gave a new anodic peak corresponding to an oxidation peak of 2,6-diamino-8-hydroxypurine on the second sweep toward the positive potential.

Controlled-potential Electrolysis. After the exhaustive electrolysis of 1 mM aminopurines, linear-sweep voltammetry at the GCE showed no anodic peak, but polarography at the DME showed two reduction waves. These waves were probably due to the reduction of the ultimate products produced by the oxidation; their half-wave potentials shifted toward negative potentials with the increase in the pH values of the electrolyte solutions. The polarographic behavior of the products in the electrolyzed solutions was compared with those of parabanic and oxaluric acids. In the case of 6-aminopurine,

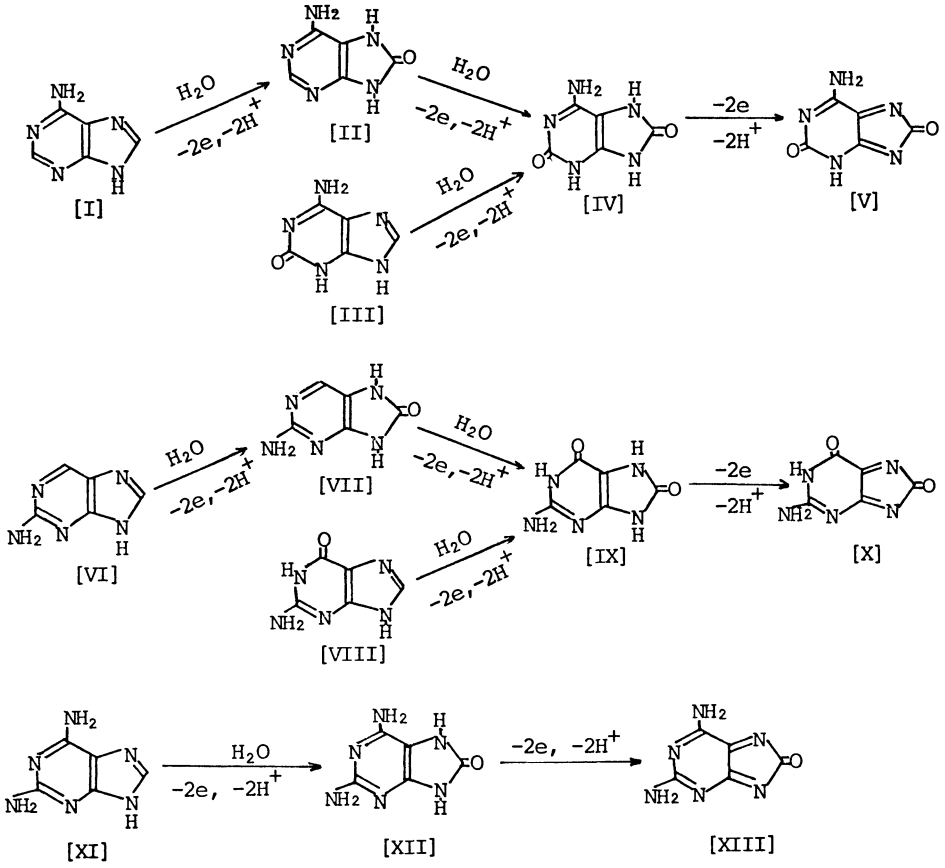

Fig. 2. Proposed primary electrochemical pathways for electrooxidation of different aminopurines.

the experimental results are summarized in Table 4. The $E_{1/2}$ values of the first and second waves agreed very closely with those of parabanic and oxaluric acids respectively.

The ultimate electroinactive products (urea, ammonia, allantoin, and guanidine) were determined by the colorimetric methods given in the Experimental section. The results are shown in Table 5.

Mechanisms. The characteristic ultraviolet absorption spectra of the aminopurines (λ_{max}=260—270 nm) are attributable mainly to the –C(4)=C(5)–C(6)=N(1)– chromophoric group.[19,20] The disappearance of these peaks after exhaustive electrolysis indicates that the –C(4)=C(5)– bond is ultimately oxidized and cleaved. In addition, an analysis of the product solutions after exhaustive electrolysis suggested the presence of parabanic acid, oxaluric acid, urea, ammonia, allantoin, and guanidine. The cyclic voltammetric data also suggested the presence of various intermediates which were more easily oxidized than their parent compounds. The presumed mechanisms of the primary oxidations of the different aminopurines studied at the GCE are summarized in Fig. 2.

The coulometric *n*-value for the oxidation of 6-aminopurine was *ca.* 5.86. Also, cyclic voltammetric experiments revealed the presence of both 6-amino-8-hydroxypurine(II) and 6-amino-2,8-dihydroxypurine(IV) as the electrochemical intermediates, both of which were more easily oxidized than 6-aminopurine(I). The coulometric *n*-values for the oxidation of Compounds II and IV were *ca.* 3.90 and 2.28 respectively. These facts supported the idea that the electrochemical oxidation of 6-aminopurine proceeds initially by two sequential two-electron steps to give first Compound II and then Compound IV. The latter compound was similar to uric acid except for the presence of an amino group in place of an hydroxyl group at the 6-position; then it was probably oxidized in a further two-electron step to produce a diimine intermediate(V) of the same type as that proposed for the electrochemical oxidation of uric acid.[1] The continuous oxidation to the diimine intermediate, as soon as the initial two-electron oxidation had occurred, was caused by the fact that the ease of oxidation generally increased with the number of hydroxyl groups on the molecule (see Table 1). Compounds II and IV were easily oxidized electrochemically than was 6-aminopurine itself and, consequently, were unstable with respect to the oxidation at the potential at which 6-aminopurine was oxidized. Also, an intermediate for the two-electron oxidation of 6-aminopurine was 6-amino-8-hydroxypurine, and no evidence for the presence of 6-amino-2-hydroxypurine(III) was given by cyclic voltammetry. In addition, both Compounds II and III were oxidized in a two-electron step to produce the same intermediate, Compound IV. Thus, under electrochemical conditions oxidation occurred first at the C-8 position and then at the C-2 position.

Similarly, 2-aminopurine(VI) was electrochemically oxidized to 2-amino-6,8-dihydroxypurine(IX) *via* 2-amino-8-hydroxypurine(VII). Also, 2,6-diaminopurine(XI) was oxidized in a two-electron step to produce 2,6-diamino-8-hydroxypurine(XII), as was shown by cyclic voltammetric experiments. Compounds IV, IX, and XII were oxidized in a two-electron step to produce the corresponding unstable diimine intermediates (V, X, and XIII), which were then hydrolyzed in water to the ultimate compounds, such as parabanic acid, oxaluric acid, urea, ammonia, allantoin, and guanidine.

Conclusions

The different aminopurines studied were electrochemically oxidized by a mechanism very similar to that observed for other naturally occurring purines, such as uric acid and xanthine. The evidence put forward supported the view that the locus of the initial electron removal was the same for enzymatic and electrochemical processes, *i. e.*, in both the enzymatic and electrochemical oxidations the attack was first at the C-8 position and then at the C-2 position. Unlike most enzyme reactions, where the 2-amino group of guanine is removed, this group remained intact under electrochemical oxidations, and further electrochemical reaction occurred at the –C(4)=C(5)– bond.

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