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# Antioxidant Activity of Betanidin: Electrochemical Study in Aqueous Media

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**ABSTRACT:** The antioxidative mechanism of action of betalains is of significant interest because these pigments are recently emerging as highly bio-active natural compounds with potential benefits to human health. Betanidin, the basic betacyanin, comprises the 5,6-dihydroxyl moiety, which results in its high antioxidant activity. Oxidation of betanidin by voltammetric techniques and chromatographic identification of the oxidation products with spectrophotometric and mass spectrometric detection (LC−DAD-MS/MS) were performed. Two main oxidation peaks for betanidin are observable at pH 3−5. These peaks become merged at higher pH, suggesting a different mechanism of oxidation at higher and lower pH values. The low oxidation potential of betanidin confirms its very strong reduction properties. The presence of two prominent oxidized products, 2-decarboxy-2,3-dehydrobetanidin and 2,17-bidecarboxy-2,3-dehydrobetanidin, indicates their generation through two reaction routes with two different quinonoid intermediates: dopachrome derivative and quinone methide. Both lead to the decarboxylative dehydrogenation of betanidin. Subsequent oxidation and rearrangement of the conjugated chromophoric system results in formation of 14,15-dehydrogenated derivatives.

**KEYWORDS:** betanidin, betalains, betacyanins, cyclic voltammetry, antioxidation activity, 5, 6-dihydroxyindole, dopachrome, aminochrome, quinone methide, decarboxylation, HPLC−DAD-ESI-MS/MS

## INTRODUCTION

Betalains are water-soluble plant pigments recently emerging as very valuable health-promoting antioxidants. These compounds are immonium conjugates of betalamic acid with cyclo-DOPA (forming betanidin) or glycosylated cyclo-DOPA (forming other betacyanins), as well as amino acids or amines (forming betaxanthins).[1] They have already been used in industry as food colorants,[2] but their chemopreventive and strong antioxidative properties were only described within the past decade.[3−6] This stimulated some research on the structures of their new derivatives and especially on their potential influence on human health. Despite the huge scientific potential inherent in these pigments, the systematic research on their antioxidant activity is to date rather lacking. Isolated pure betalains were studied in few investigations.[4,7−11] Recent extensive studies on structural implications of semisynthesized natural or artificial betalains confirmed that some factors other than the presence of one or two phenolic groups are responsible for high antioxidant activity.[9,10] It is of considerable interest to investigate possible influence of other structural factors in order to better understand how these pigments may have an impact on human health.

Betanidin (Figure 1) was recently subjected[7] to oxidation mediated by enzyme tyrosinase, which plays a key role in the betalains biosynthetic scheme.[1] The products of this oxidation were tested by using high-performance liquid chromatography and spectrophotometric detection. Interestingly, the addition of ascorbic acid reverted the reaction product (apparently betanidin *o*-quinone) to the original pigment. Therefore, no further rearrangements through dopachromic or quinone methide stages were considered.[11] Very recently, spectrophotometric and mass spectrometric identification of betanidin and betanin enzymatic oxidation products shed new light on the betalain redox chemistry.[11] Within pH 4−8 range, two main oxidized derivatives of betanidin were registered, betanidin quinonoid (possibly betanidin *o*-quinone) and 2-decarboxy-2,3-dehydrobetanidin, whereas at pH 3 only dehydrogenated and decarboxylated derivatives were detected as a result of the different stability of the products at different pH values. The presence of two prominent oxidation products at pH 3: 2-decarboxy-2,3-dehydrobetanidin and 2,17-bidecarboxy-2,3-dehydrobetanidin, indicates their generation via two reaction routes with two different quinonoid intermediates: dopachrome derivative and quinone methide.[11] Both reaction paths lead to the decarboxylative dehydrogenation of betanidin. Subsequent oxidation and rearrangement of the conjugated chromophoric system results in formation of 14,15-dehydrogenated derivatives. Furthermore, in oxidized 5-*O*-glucosylated betanidin (betanin), only generation of quinone methide intermediate, which rearranges to 2,3-dehydro- or neo-derivatives, can be considered.[11] The products of enzymatic oxidation of betacyanins thus formed are therefore derivatives of 5,6-dihydroxyindole and related structures known as the key intermediates in melanogenesis.[11]

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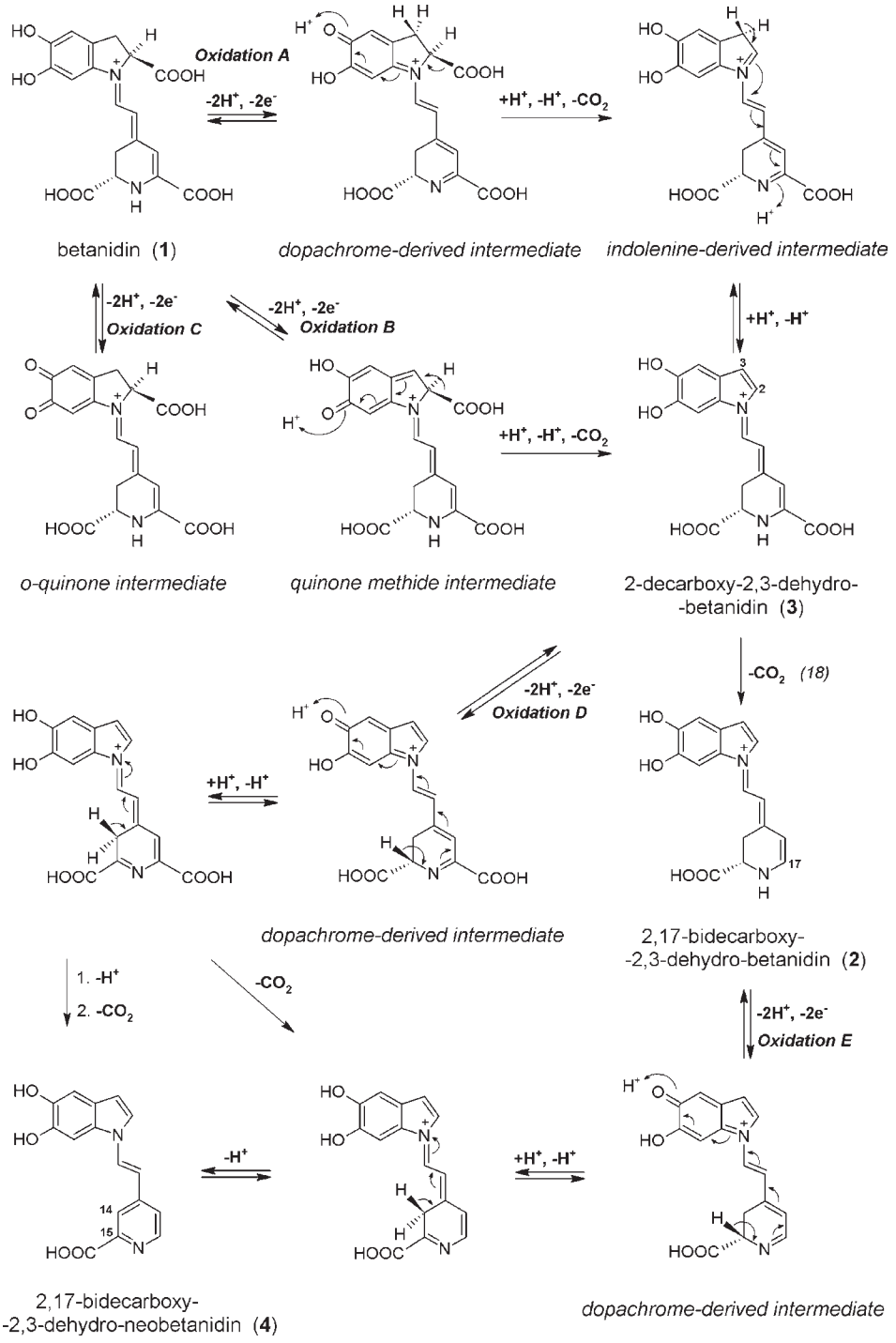


**Figure 1.** The possible pathways of betanidin oxidation and further rearrangement of two oxidized products.[11] For clarity, only the most meaningful reactions, based on the LC−MS/MS results, are depicted.

The activity of tyrosinase on betaxanthins was also demonstrated, and the reaction products were characterized directly.[12] The enzymatic hydroxylation of tyramine- and tyrosine-based betaxanthins to the dopamine- and DOPA-based betaxanthins, respectively, was documented;[12] it is equivalent to the tyrosinase-mediated oxidation of tyrosine to DOPA, considered as the first step in the biogenesis of betalamic acid and betacyanins.[1] However, despite the possible further enzymatic oxidation of dopamine and DOPA to the cyclic structures of aminochromes, the tyrosinase-catalyzed oxidation of DOPA-based betaxanthins does not result in formation of any known cyclic structure such as betanidin; other (unknown) complex isomeric structures of various intramolecular cyclization patterns are formed.[12]

To date, the electrochemical behavior of betanidin has not been studied. Betanidin (Figure 1) is the basic structure for all betacyanins, and is the only one with the 5,6-dihydroxyl moiety.[4,7,10] The mechanism of its antioxidative action has not as yet been practically revealed. In this study, oxidation of

Table 1. Chromatographic, Spectrophotometric, and Mass Spectrometric Data of Betanidin and Its Oxidation Products

| | | | | *m/z* | |
|---|---|---|---|---|---|
| no. | compound | $t_R$ (min) | $\lambda_{max}$ (nm) | $[M + H]^+$ | from MS/MS of $[M + H]^+$ |
| **1** | betanidin | 17.5 | 541 | 389 | 345 |
| **1′** | isobetanidin | 19.2 | 541 | 389 | 345 |
| **2** | 2,17-bidecarboxy-2,3-dehydro-betanidin[a] | 23.1 | 472 | 299 | 255 |
| **3** | 2-decarboxy-2,3-dehydro-betanidin[a] | 23.5 | 494 | 343 | 299; 255 |
| **4** | 2,17-bidecarboxy-2,3-dehydro-neobetanidin[a] | 27.0 | 416 | 297 | 253 |

[a] Tentatively identified.

betanidin by voltammetric techniques was investigated, and identification of the electrochemically oxidized products by mass spectrometry (LC−MS/MS) was performed.

## MATERIALS AND METHODS

**Reagents.** Formic acid, HPLC-grade acetonitrile, methanol, and HPLC-grade water were purchased from Merck (Darmstadt, Germany).

**Betanidin Preparation.** A 100 g portion of red beet root extract containing 41% of betalains (FutureCeuticals, Inc., Momence, IL) was submitted to solid-phase extraction cleanup on C18 cartridges [13] before HPLC preparative fractionation. Purified betanin was subjected to enzymatic hydrolysis, catalyzed by $\beta$-glucosidase (Sigma, Chemical Co., St. Louis, MO), and followed by solid-phase extraction cleanup on C18 cartridges before consecutive HPLC preparative fractionation.[14] The eluates were concentrated under reduced pressure at 25 °C and submitted to HPLC preparative fractionation.

**Cyclic Voltammetry.** Cyclic voltammetry measurements were carried out using an electrochemical analyzer (model EA9/M151E) from MTM-ANKO (Cracow, Poland) with a 1.8 mm diameter glassy carbon working electrode, platinum counter electrode, and Ag/AgCl reference electrode. All measurements were carried out in a 2 mL cell at 25 °C in 0.1 M phosphate−acetic acid buffer of pH ranging from 3.0 to 8.0. All samples (1 mL) of the pigment (1.4 mM) aqueous solution were purged with argon for 5 min to remove oxygen prior to measurements. The general voltammetric parameters were as follows: initial potential, −300 mV; final potential, 1300 mV; the scan rate, varied from 25 to 1000 mV/s. Two analytical techniques were applied: cyclic voltammetry (CV) and differential pulse voltammetry (DPV). All experimental data were collected and analyzed according to the EAGRAPH EA9 program. Prior to each measurement, the working electrode was carefully polished with 1.0 $\mu$m $\alpha$-alumina paste followed by 0.05 $\mu$m $\gamma$-alumina paste.

**Bulk Electrolysis.** The controlled potential bulk electrolysis of betanidin for identification of the oxidation products was carried out using the EA9/M151E analyzer with a 10 mm glassy carbon working electrode, platinum counter electrode, and Ag/AgCl reference electrode in a 10 mL cell comprising 0.5 mM betanidin solution with buffers at pH 3−8, and anodic potential $E_a$ 350−650 mV with at least 5-fold repetition until the color of the solution turned from violet to red. The samples obtained were subjected to LC−DAD-ESI-MS/MS analysis made according to a previous report.[11] During the electrolyses, a stream of argon was bubbled through the substrate solution with rapid magnetic stirring. Because of adsorption of betanidin oxidation products [presumably generated polymers (Figure 6)] on the working electrode and a greater drop-off in the current than expected for chronoamperometry under diffusion control, the electrode was polished with 1.0 and 0.05 $\mu$m $\alpha$-alumina paste after each repetition.

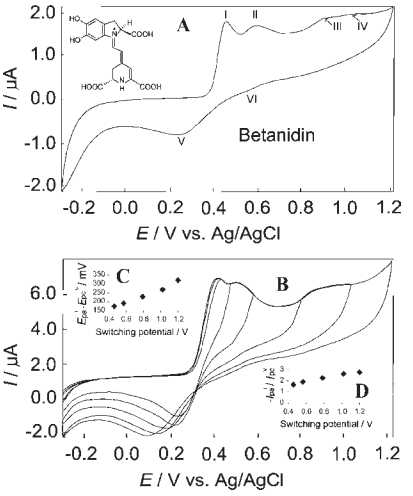


**Figure 2.** Cyclic voltammograms of 1.4 mM betanidin on a glassy-carbon electrode (1.8 mm diameter) obtained in phosphate−acetic buffer media (A) at pH 3 and scan rate 25 mV/s and (B) at pH 4 and scan rate 200 mV/s and different switching potentials, and the influence of switching potential on (C) the peak separation width $E_{pa}{}^{I} - E_{pc}{}^{V}$ and (D) the peak current ratio $-I_{pa}{}^{I}/I_{pc}{}^{V}$.

## RESULTS AND DISCUSSION

Electrochemical measurement is a very useful method for determination of physicochemical parameters of antioxidants, that enables deduction of their redox pathways. One of the frequently used techniques is cyclic voltammetry, which indicates the ability of compounds to donate electrons at a potential of an anodic wave. This technique is used for the evaluation of the scavenging activity of many frequently studied compounds such as polyphenols and also for estimation of a total antioxidant capacity of food products. The attempt to unveil the role of phenolic hydroxy groups in the antiradical activity of betalains was performed recently.[8] In this study, betanidin, the basic betacyanin comprising the 5,6-dihydroxyl moiety, was evaluated by cyclic voltammetry.

General spectrophotometric and mass spectrometric data for betanidin and its electro-oxidation products are presented in Table 1. Figure 2A shows a typical voltammogram of betanidin at pH 3.0 and a scan rate 25 mV/s. The low oxidation potential observable for peak I indicates very strong reduction properties, similar to those exhibited by phenolic antioxidants like quercetin.[15] Moreover, analogous to other polyphenolic compounds,[15] a shift of oxidation potential (from 600 to 300 mV) affected by pH change (from 3 to 8) is observed (Table 2, Figure 3A−H).

**Table 2. Basic Voltammetric Data of 1.4 mM Betanidin (acetic−phosphate buffer) Measured on a Glassy Carbon Electrode (1.8 mm diameter) at Different pH**

| | scan rate $\nu$ = 50 mV/s | | | | | | | scan rate $\nu$ = 1000 mV/s | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | anodic peaks | | | | cathodic peaks | | | anodic peaks | | | | cathodic peaks | | |
| | I | II | III | IV | V | VI | $E_{pa}^{I} - E_{pc}^{V}$ $I_{pa}^{I}/I_{pc}^{V}$ | I | II | III | IV | V | VI | $E_{pa}^{I} - E_{pc}^{V}$ $I_{pa}^{I}/I_{pc}^{V}$ |
| | | | | | | | pH 3 | | | | | | | |
| $E$ (mV) | 428 | 580 | 872 | 996 | 268 | 532 | 160 | 468 | 620 | 892 | 1044 | 292 | 556 | 176 |
| $I$ ($\mu$A) | 2.2 | 1.4 | 0.6 | 0.3 | −1.1 | −0.1 | −2.0 | 10 | 7.6 | 0.1 | 0.6 | −5.5 | −0.8 | −1.8 |
| | | | | | | | pH 4 | | | | | | | |
| $E$ (mV) | 396 | 484 | 824 | nd[a] | 136 | 460 | 260 | 444 | 516 | 852 | 972 | 100 | 468 | 344 |
| $I$ ($\mu$A) | 3.0 | 2.3 | 0.7 | nd | −1.4 | −0.1 | −2.1 | 11 | 10 | 1.0 | 1.2 | −5.5 | −0.4 | −2.0 |
| | | | | | | | pH 5 | | | | | | | |
| $E$ (mV) | 385 | 436 | 836 | nd | 56 | 384 | 329 | 434 | 492 | 892 | nd | 12 | 396 | 422 |
| $I$ ($\mu$A) | 3.5 | 3.5 | 0.9 | nd | −1.6 | −0.1 | −2.2 | 16 | 15 | 2.5 | nd | −6.7 | −0.7 | −2.4 |
| | | | | | | | pH 6 | | | | | | | |
| $E$ (mV) | 376 | 822 | nd | −28 | 352 | 404 | 420 | nd | nd | −28 | 340 | 448 | | |
| $I$ ($\mu$A) | 3.2 | 0.2 | nd | −1.3 | −0.2 | −2.5 | 16 | nd | nd | −6.2 | −0.9 | −2.6 | | |
| | | | | | | | pH 7 | | | | | | | |
| $E$ (mV) | 332 | 828 | nd | −44 | 284 | 376 | 372 | 844 | nd | −60 | 276 | 432 | | |
| $I$ ($\mu$A) | 3.4 | 0.2 | nd | −0.8 | −0.3 | −4.2 | 17 | 0.9 | nd | −3.8 | −2.2 | −4.5 | | |
| | | | | | | | pH 8 | | | | | | | |
| $E$ (mV) | 308 | 868 | nd | −56 | 248 | 364 | 332 | 836 | nd | −68 | 244 | 400 | | |
| $I$ ($\mu$A) | 2.8 | 0.2 | nd | −0.8 | −0.2 | −3.5 | 15 | 1.1 | nd | −2.8 | −2.2 | −5.4 | | |

[a] Not detected.

Initial oxidation of betanidin generates the transient radical cation, which rapidly loses a proton, forming a neutral phenoxy radical.[11] This process is equivalent to donation of a hydrogen atom by betanidin or to the loss of a proton accompanied by electron transfer. Further oxidation with a loss of a proton and electron results most probably in formation of a two-electron oxidized form, namely, the *o*-quinone (oxidation C) (Figure 1).[11] Therefore, it can be stated that protons participate in the electro-oxidative mechanism and that their concentration produces significant changes in the voltammetric profiles of betanidin. The question arises regarding how easily the *o*-quinone can tautomerize to other quinonoid forms (aminochrome or quinone methide) [11,16] as well as whether the other forms are generated according to the oxidations A or B (Figure 1) besides the typical *o*-quinone.

Interestingly, in more acidic media (pH 3−5), two main oxidation peaks for betanidin are observable. These peaks become merged at higher pH (Figure 3), indicating different mechanism of oxidation at higher and lower pH values. Additionally, it can be deduced from the pH dependence of the first anodic peak potential ($E_{pa}^{I}$) that there are two pH ranges of different line slopes (Figure 3G) which change at pH 5−6. At lower pH,[3−5] the slope d$E$/d(pH) is low (21 mV) and changes to 52 mV in the pH interval 6−8. Such a dependence of the potential on pH for $E_{pa}^{I}$ indicates a participation of a duplicate number of electrons with respect to that of protons (pH 3−5) and a change in the mechanism of the reaction (pH 6−8) with participation of an equal number of electrons and protons.

Based on the possibility of a formation of a two-electron oxidized form of betanidin *o*-quinone (oxidation C) (Figure 1) or the two tautomeric forms[11] it can be deduced, therefore, that a two-electron, one-proton process can be attributed to the oxidation peak I at pH 3−5, and a two-electron, two-proton process is indicated at pH 6−8. The two-electron, one-proton process is possibly an effect of the lack of the second deprotonation step in the oxidized molecule, which is caused by the low pH. In contrast, at pH 6−8, the general pathways of the initial betanidin oxidation can be presented as in Figure 1.[11]

The pH dependence with a slope d$E$/d(pH) = 52 mV of the second anodic peak ($E_{pa}^{II}$) is linear throughout the whole pH range (Figure 3G). In this case, the participation of an equal number of electrons and protons is apparent; therefore, based also on a previous study,[11] a two-electron, two-proton process can be attributed to the oxidation peak II (oxidations D or E) (Figure 1) in the whole tested pH range. Therefore, at pH 6−8 the anodic potentials $E_{pa}^{I}$ and $E_{pa}^{II}$ are very close to each other, possibly because of a similarity of the oxidized compounds and the reaction mechanisms (oxidations A or B versus D or E). The reaction path of the second oxidation step based on previous results on enzymatic oxidation of betanidin[11] was confirmed here by additional bulk electrolysis experiments and analysis of the generated products by mass spectrometry.

One distinct cathodic peak V, at $E_{pc}^{V}$ in the range from ca. −50 to 300 mV, and a smaller peak VI, $E_{pc}^{VI}$ ca. 250−550 mV, are visible for betanidin during the reversed scan (Figure 2), and their position is also dependent on pH (Table 2, Figure 3H).

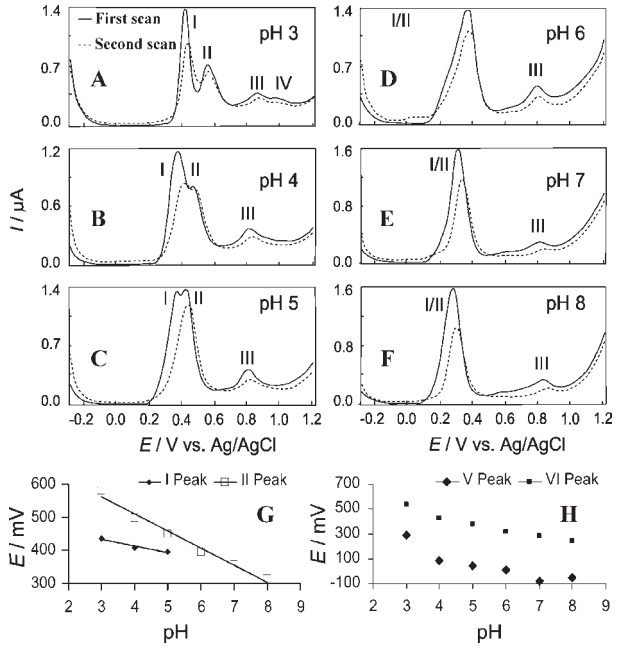


**Figure 3.** Differential pulse voltammograms of 1.4 mM betanidin with consecutive second scan runs on a glassy-carbon electrode (1.8 mm diameter) at a scan rate 25 mV/s and different pH (A−F) and pH dependence of the cyclic voltammetric oxidation potentials $E_{pa}^{I}$ and $E_{pa}^{II}$ (G) as well as the reduction potentials $E_{pc}^{V}$ and $E_{pc}^{VI}$ (H) at a scan rate 74 mV/s.

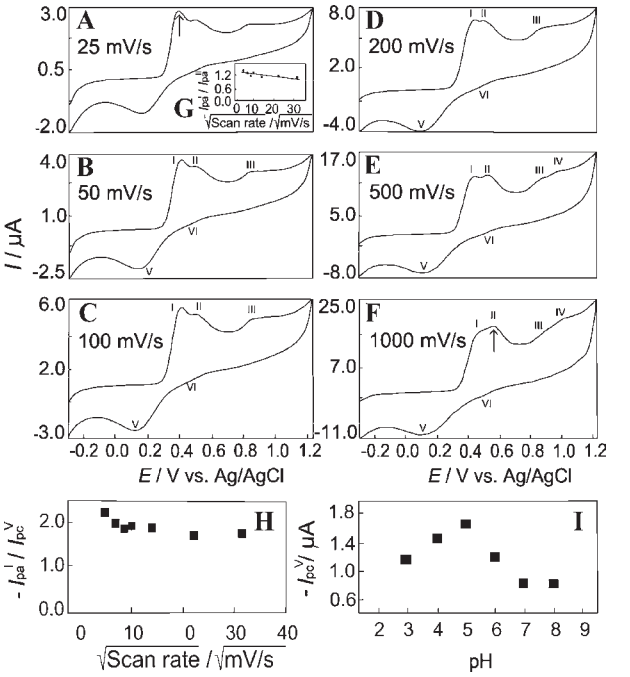


**Figure 4.** Cyclic voltammograms for 1.4 mM betanidin solutions obtained on glassy-carbon electrode at pH 4 and different scan rates (A−F). (G) The peak current ratio for the two anodic peaks $-I_{pa}^{I}/I_{pa}^{II}$. (H) Scan rate dependence of the current ratio $-I_{pa}^{I}/I_{pc}^{V}$ at pH 3. (I) pH dependence of the reduction current $I_{pc}^{V}$ at a scan rate 50 mV/s.

A peak separation between I and V ($E_{pa}^{I} - E_{pc}^{V} \gg 56$ mV) (Table 2) and a peak current ratio $-I_{pa}^{I}/I_{pc}^{V}$ ($\gg 1$) (Table 2, Figure 4H) are clear indications of the chemical instability of the initial oxidation products.[17] The separation width $E_{pa}^{I} - E_{pc}^{V}$ reaches its maximum at pH 6 (Table 2). The irreversible nature of the reactions does not permit the direct calculation of any thermodynamic parameters (e.g., formal oxidation potential $E^{o\prime}$ or electron stoichiometry $n$) from the voltammograms.

The signal $|I_{pc}^{V}|$ is maximal at pH 5 (ca. 1.6 $\mu$A) (Table 2, Figure 4I). This is likely due to a higher stability of the oxidized form/forms of betanidin at pH 5, which undergo slower chemical transformations. Betanidin, as well as other betacyanins and their derivatives, are known to be most stable at pH 5−5.5.[1,2,14] Under these conditions, the oxidized forms (the betanidin derivatives) should be present at higher concentration and, therefore, should be ready for subsequent reduction.

Increasing the scan rate results in a slight diminution of the peak current ratio $-I_{pa}^{I}/I_{pc}^{V}$ (Figure 4H), indicating a small increase in accessability of the oxidation products for subsequent reduction. However, $-I_{pa}^{I}/I_{pc}^{V}$ as well as the width of peak separation $E_{pa}^{I} - E_{pc}^{V}$ depend significantly on the switching potential; $-I_{pa}^{I}/I_{pc}^{V}$ ca. 1.7 and $E_{pa}^{I} - E_{pc}^{V}$ ca. 175 mV at pH 3 are observed, after setting the switching potential just after peak I (Figure 2C,D). This indicates that the system tends towards semi-reversibility which can be attributed to the transformations between betanidin and its three possible quinonoid derivatives in the course of the oxidations A, B, or C (Figure 1).[11,16]

Taking into consideration the probable reaction paths,[11,16] the two quinonoid intermediates (dopachromic derivative and quinone methide intermediates originated through the oxidations A and B) should rather be considered (Figure 1). In addition, the presence of the second prominent anodic peak (II) close to the peak I at pH 3−5 or merged with it at pH 6−8 suggests that these two reaction routes (A and B) might be possible and would take place at slightly different $E_a$. However, it is also possible that a further oxidative conversion (D or E) of one of the generated quinones is represented by the peak II. The possibilities of parallel oxidation, decarboxylation, dehydrogenation, and rearrangement reactions still render the whole process of betanidin oxidation obscure. For clarity, in Figure 1, only the most meaningful reactions based on the LC−MS/MS results are depicted (Figure 5, Table 1). For example, the tautomerization between the three quinonoid forms,[11] as well as the dehydrogenation of the quinonoids at C-2 (instead of decarboxylation at C-2), are not shown.

A second oxidative scan performed immediately after the first scan without polishing the electrode results in formation of voltammograms with diminished anodic peaks. These results are conveniently presented by the DP technique at different pH values (Figure 3A−F). This indicates an adsorption of the oxidized derivatives or their chemically transformed products. Interestingly, the anodic peak I is diminished at greater extent than the peak II.

The peak current ratio $-I_{pa}^{I}/I_{pa}^{II}$ diminishes with the scan rate $v$, as a result of a significant $I_{pa}^{II}$ increase, which is very well observable at pH 3 and 4 (Figure 4A−G), indicating that at low scan rates (25 mV/s), newly formed quinonoid/quinonoids (oxidations A or B) have time for other undefined reactions that diminish their concentration prior to the next oxidation step (D or E). Therefore, the $E(C_{irr})EC_{irr}$ mechanism for the initial two oxidation steps can be taken into account. It may be adjusted with next $EC_{irr}$ stages, because the presence of a small anodic peak III (and a peak IV at higher scan rates), without corresponding cathodic peaks, indicates further irreversible oxidation/degradation steps of the structures **2**−**4** (Figure 1) thus formed.

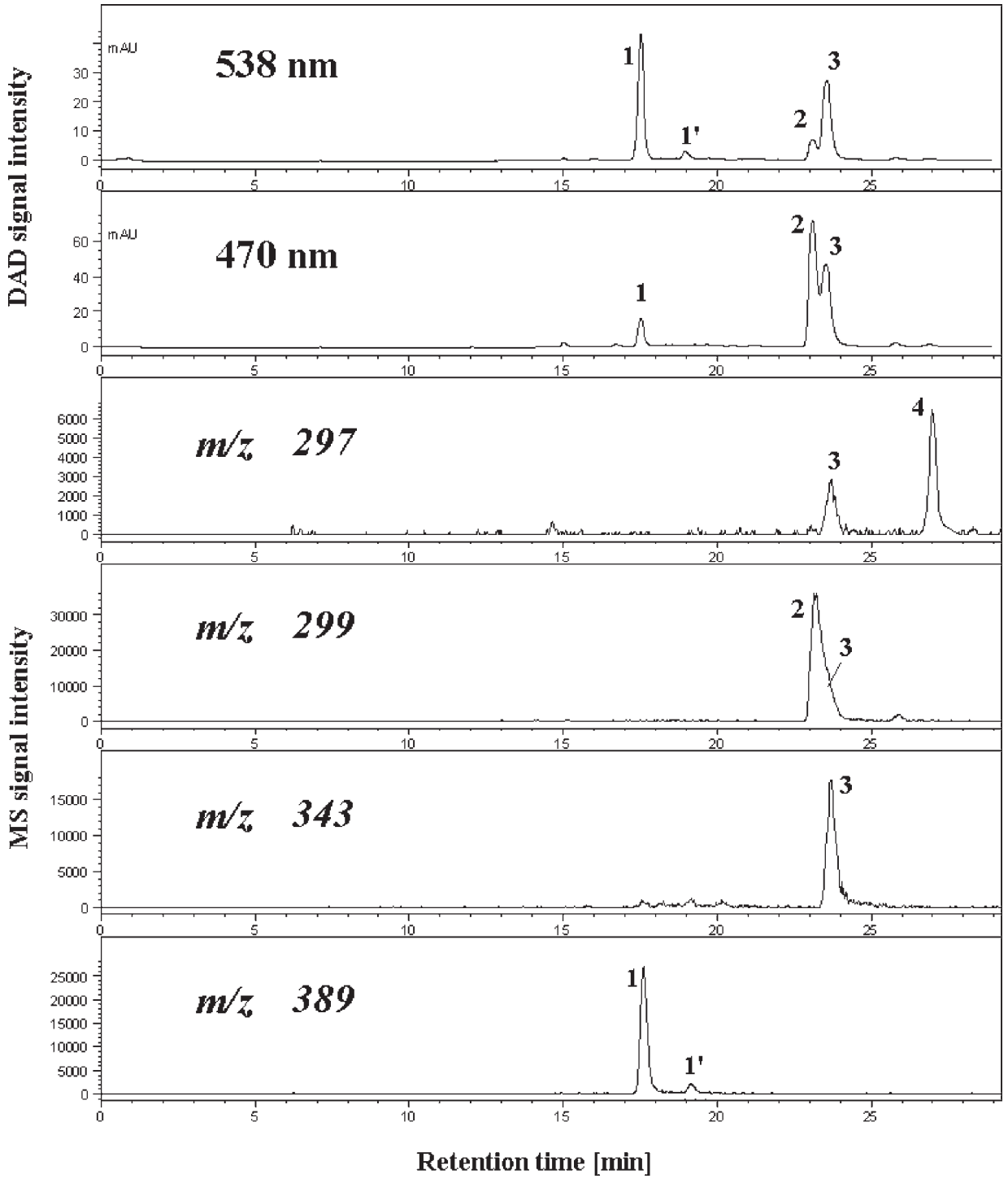


**Figure 5.** Chromatographic traces of betanidin (**1**) and its oxidation products (**2**−**4**) (Figure 1) monitored by DAD optical detector (at 470 and 538 nm) and MS detector (at *m*/*z* 297, 299, 343, and 389) (see Table 1).

One of the irreversible transformations can be hydrolytic decomposition of any reaction product splitting of the aldimine bond[19,20] and subsequent polymerization of the derivatives of betalamic acid or cyclo-DOPA,[16] which is represented for **3** as an example in Figure 6A. Further oxidation pathways of **4** that might be responsible for the peak III appearance are presented in Figure 6B. Note that for the oxidations F and G only one proton and two electrons are exchanged. In any case, the generation of adsorbed polymers is possible, leading to diminished signals after the second scans (Figure 3A−F).

Identification of many betanidin intermediates in the reaction pathways was difficult to accomplish due to the great reactivity of the short-lived pigments thus generated; however, three of them were sufficiently to survive the chromatographic separation conditions and the LC−ESI-MS/MS identification. A set of electrolytic experiments, performed accordingly at potential *E*

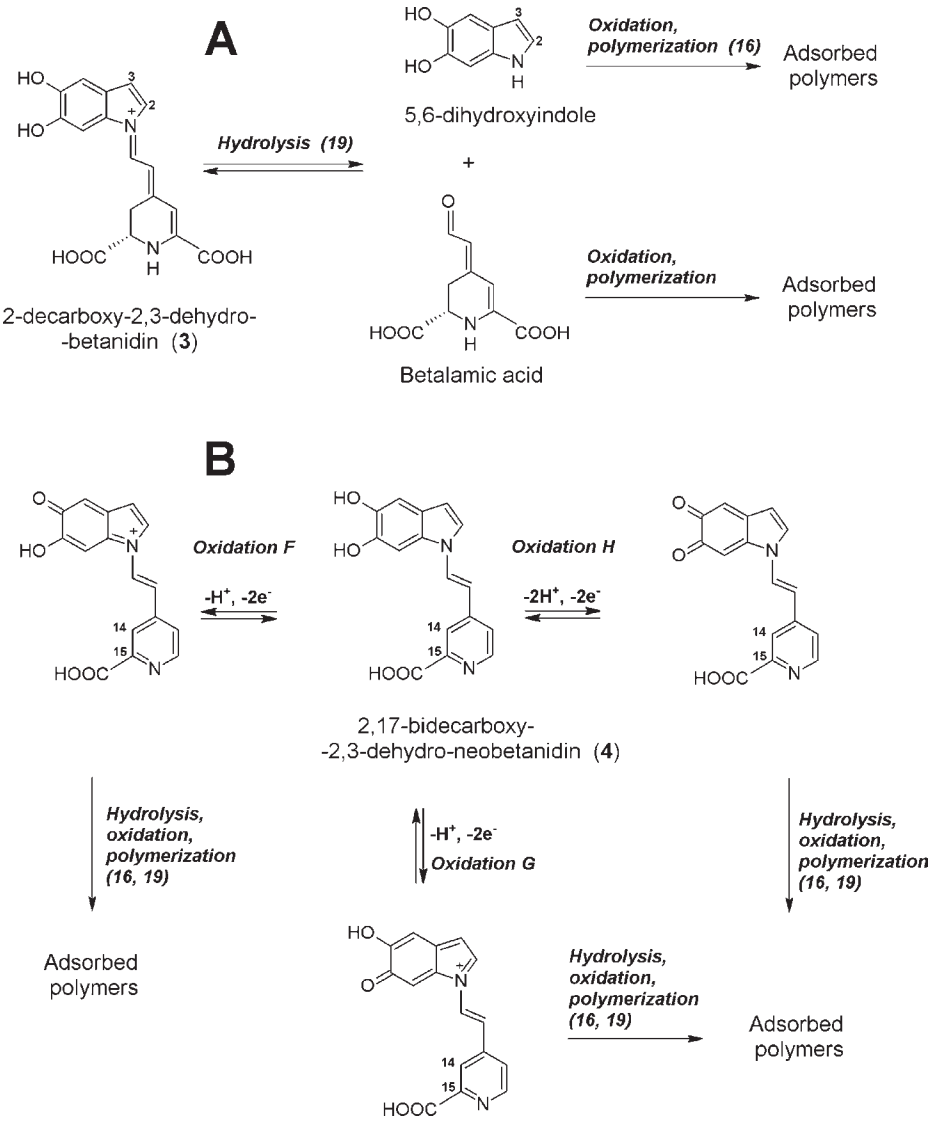


**Figure 6.** Hydrolytic decomposition of **3** leading to subsequent oxidation and polymerization [16]of the derivatives of betalamic acid and cyclo-DOPA (A). Further oxidation pathways of **4** and possible subsequent hydrolysis, oxidation, and polymerization of the derivatives (B). Note that for the oxidations F and G only one proton and two electrons are exchanged.

values that were more positive than the potential of the peaks I, II, III, or IV, respectively, was accomplished. In any case, an interesting pattern of betanidin-derived products, the presence of which was mostly independent of the electrolysis potential and pH, was detected (Figure 5, Table 1). The presence of two compounds of tentatively identified structures was especially noticed, namely, 2,17-bidecarboxy-2,3-dehydrobetanidin **2** ($m/z$ 299 for $[M + H]^+$) and 2-decarboxy-2,3-dehydrobetanidin **3** ($m/z$ 343 for $[M + H]^+$).

The decarboxylation at positions C-2 and C-17 is the most often detected reaction pattern during thermal degradation of betacyanins.[14] The mechanism of the most frequent betanidin decarboxylation, leading to a formation of 17-decarboxybetanidin, was studied by Dunkelblum et al.,[18] in ethanolic solutions. However, the decarboxylation at C-15 is not excluded, as well. The MS/MS patterns confirmed the typical fragmentation pathways of betalainic pigments. The structures of these pigments fall very well into the reaction scheme specified in Figure 1[11] and involve the first fairly stable reaction products.

The possible structures **2** and **3** correspond to the decarboxylative rearrangements after the oxidations A or B that, however, can be oxidized in next successive steps, likely at higher potential related to the peak II (oxidations D or E). The possible anodic reactions D or E can result in formation of further dehydrogenated derivatives via oxidation of the indolic moiety and subsequent rearrangements in the conjugated system of quinonoid structures into 14,15-dehydrogenated derivatives ("neobetacyanins")[1] (Figure 1).

Inspection of mass spectra of chromatographic effluents revealed the presence of a doubly dehydrogenated pigment, possibly 2,17-bidecarboxy-2,3-dehydroneobetanidin **4** ($m/z$ 297 for $[M + H]^+$); it seems, therefore, that this is another relatively stable betanidin derivative. No traces of the three quinonoid forms, nor of neobetanidin,[1] characterized by $m/z$ 387, were found in the chromatograms, which confirms that these structures are highly unstable under these conditions.

This investigation is the first evaluation of the electrochemical properties of betanidin. The knowledge regarding the nature of

the oxidation products of betanidin gained from previous studies is still scarce and limited to enzymatic reactions.[11] Application of mass spectrometry enabled the tentative determination of stable decarboxylated and dehydrogenated oxidation products of betanidin. The pH effect on the oxidation potential for betanidin was demonstrated. In addition, the presence of the carboxyl moieties in the conjugated chromophore system may influence the redox properties of the pigments. This aspect is currently under investigation.

The present study aimed to characterize the electrochemical properties of betanidin. This topic is of great interest mainly because of its high antioxidation activity and its presence in many red beetroot preparations containing 5-*O*-glucosylated betanidin (betanin). Revealing the possible paths of betanidin oxidation by different chemical and electrochemical methods at different physicochemical conditions will help to understand the mechanisms of antioxidative action of this beneficial pigment as well as other betalains present in any daily diet containing red beetroot. This will be useful in the evaluation of food quality and creation of beneficial features of betalain-based products.

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