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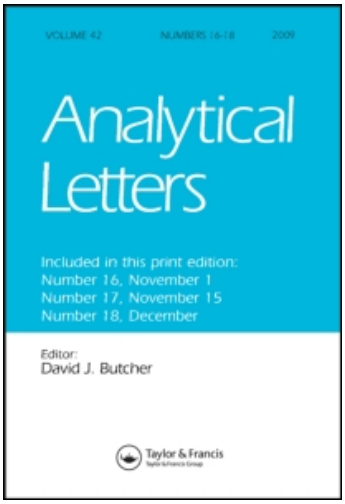

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Solid State Electrochemical Behavior of Usnic Acid at a Glassy Carbon Electrode

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Electrochemistry

SOLID STATE ELECTROCHEMICAL BEHAVIOR OF USNIC ACID AT A GLASSY CARBON ELECTRODE

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The electrochemical redox behavior of usnic acid, mainly known for its antibiotic activity, has been investigated using cyclic, differential pulse and square wave voltammetry in aqueous electrolyte. These studies were carried out by solid state voltammetry with the solid mechanically attached on the surface of a glassy carbon electrode and at different pH values. Usnic acid did not present any reduction reaction. The pH-dependent electrochemical oxidation occurs in three steps, one electron and one proton irreversible processes, assigned to each of the hydroxyl groups in the molecule. Adsorption of the non-electroactive oxidation product was also observed, blocking the electrode surface. An oxidation mechanism was proposed and electroanalytical methodology was developed to determine usnic acid.

Keywords: Electrochemistry; Oxidation; Solid state voltammetry; Usnic acid

INTRODUCTION

Lichens, more than plants, are one of the most important sources of biologically active compounds. Among known secondary lichen metabolites usnic acid (UA) [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furan-dione], Scheme 1, is the most extensively studied (Cochietto et al. 2002; Ingólfsdóttir

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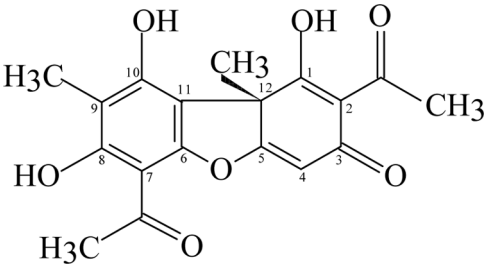

Scheme 1. Chemical structure of UA.

2002). It is an exclusive lichen product and no synthetic derivatives are known that are more effective than the natural form. Many lichens and extracts containing UA have been extensively used in the medical, pharmaceutical, cosmetic, and agricultural fields. Actually, usnic acid exhibits a wide range of pharmacological properties (Rancan et al. 2002; Dixon 2005; Francolini et al. 2004; Fernández et al. 2006): inhibition of the effects of viruses (Perry et al. 1999) as well as of tumor promotion (Yamamoto et al. 1995); analgesic and antipyretic capacity (Okuyama et al. 1995) and anti-inflammatory activity comparable to ibuprofen (Vijayakumar et al. 2000) (for (+) UA); antifungal activity against some plant pathogens (Proksa et al. 1996); antiherbivore and anti-insect effects (Romagni et al. 2000; Emmerich et al. 1993) (for (−) UA). Other characteristics of UA are ultraviolet absorption and preservation properties. The most interesting biological function of UA, however, is its antibiotic activity. Therefore, interest in this compound has grown since the 1990s, partly due to the increasing resistance to traditional antibiotics in patients developed during the last two decades, which, in turn, spurred the search for novel therapeutic alternatives (e.g., phytochemicals and other non-synthetic drugs). Both the (+) and (−) UA enantiomers are known to be effective against pathogenic bacteria (Ghione, Parrelo, and Grasso 1988).

Since usnic acid's activity is dependent on its structure (e.g., tautomeric species present), a thorough elucidation of its conformational preferences (including its H-bonding pattern and tautomeric equilibrium in physiological medium), as well as of its electrochemical behavior is of the utmost importance for understanding its mechanisms of action at a molecular level. In particular, the keto-enol tautomerism of usnic acid may be monitored by vibrational spectroscopy (both Raman and Inelastic Neutron Spectroscopy), by focusing on the deformation mode of the C–H oscillator of the chiral center of the molecule, which is exclusive to the keto tautomer.

Methods for determination of UA such as high-performance liquid chromatography (HPLC), capillary zone electrophoretic (CZE), and micellar electrokinetic chromatography (MEKC) have been developed (Demet et al. 2006; Kreft and Strukely 2001; Falk, Green, and P. Barboza 2008). All of them were carried out using acetone extracts or with a high content of methanol as UA has very low solubility in water.

Electrochemical measurements leading to the determination of physicochemical parameters, e.g., redox potential, number of electrons transferred, electrode-reaction rate constant, are relevant for understanding different redox reaction

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mechanisms. The voltammetry of microparticles (VMP), a methodology based on the mechanical immobilization of solid particles at the surface of metal electrode in the absence of binders, widens the possibilities for solid state electroanalysis of insoluble aqueous compounds due to its broad applicability (Grygar et al. 2002; Lovric and Scholz 1997). Abrasive stripping voltammetry (AbrSV) is a special case and concerns systems in which the solid compounds are dissolved during the electrochemical reactions. Virtually any solid phase containing redox centers can yield a characteristic voltammetric signal (Bond 1997). The VPM is also attractive due to the ease of preparation of the solid sample and the short time scale required for an electrochemical measurement.

Solid state electroanalysis has become an important step in the direct analysis of well defined solid powders without further pretreatment or time-consuming deposition procedures (Grygar et al. 2002), and has been used to study the electrochemistry of a variety of insoluble solid compounds (Scholz and Meyer 1994; Janeiro and Oliveira-Brett 2005; Corduneanu, Janeiro, and Oliveira-Brett 2006).

The UA is negligibly soluble in water and solid state voltammetry using UA mechanically attached onto the surface of a glassy carbon electrode (GCE) allows the investigation of UA redox reactions in aqueous medium. Therefore, the present study is concerned with the study of the electrochemical behavior of UA mechanically immobilized on a GCE over a wide pH range, using cyclic, differential pulse, and square-wave voltammetry.

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EXPERIMENTAL

Materials and Reagents

The UA was obtained from Sigma and used without further purification. All supporting electrolyte solutions (Table 1) were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μS cm^{-1}). The electrochemical experiments were done at room temperature ($25 \pm 1°C$).

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out using a Crison micropH 2001 pH-meter with an Ingold combined glass electrode.

Table 1. Supporting electrolyte buffer solutions

pH	Composition
2.1	0.2 M HCl + 0.2 M KCl
3.5	0.2 M HAcO + 0.2 M NaAcO
4.1	0.2 M HAcO + 0.2 M NaAcO
5.3	0.2 M HAcO + 0.2 M NaAcO
6.1	0.2 M NaH_2PO_4 + 0.2 M Na_2HPO_4
7.0	0.2 M NaH_2PO_4 + 0.2 M Na_2HPO_4
8.1	0.2 M NaH_2PO_4 + 0.2 M Na_2HPO_4
8.9	2 M NH_3 + 2 M NH_4Cl
10.5	2 M NH_3 + 2 M NH_4Cl
12.1	0.2 M NaOH + 0.2 M KCl

Voltammetric Parameters and Electrochemical Cells

Voltammetric experiments were carried out using an Autolab PGSTAT 30 running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands, and a three-electrode system in a 0.5 mL one-compartment electrochemical cell of capacity 2 mL (Cypress System Inc., USA). GCE ($d = 1.5$ mm) was the working electrode, Pt wire the counter electrode, and the Ag/AgCl (3 M KCl) reference electrode.

The GCE was polished using diamond spray (particle size 3 μm) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and differential pulse voltammograms were recorded until a steady state baseline voltammogram was obtained. Then, the solid UA was attached by mechanical pressing onto the GCE and electrochemical measurements were performed in different pH supporting electrolytes.

The experimental conditions for differential pulse voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}. For square wave voltammetry a frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 mV s^{-1} were used. This procedure ensured very reproducible experimental results.

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Acquisition and Presentation of Voltammetric Data

The differential pulse voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 5 mV included in GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artifact, although the peak intensity is, in some cases, reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

RESULTS AND DISCUSSION

Cyclic Voltammetry

The electrochemical behavior of UA at GCE was studied in electrolytes with different pHs by cyclic voltammetry at 100 mV s^{-1}. The cyclic voltammograms showed only one anodic peak, but no reduction peaks were observed. The oxidation is due to the hydroxyl groups attached to aromatic rings. It is irreversible and follows different oxidation pathways according to the pH of the electrolyte solution, Fig. 1. The peak P2 at $E_p^{P2} = 1.04$ V in pH 3.5 0.2 M acetate buffer was shifted to more negative values with increasing pH. In alkaline medium, pH 10.5 2 M ammonia buffer, it appeared at $E_p = 0.68$ V.

The Influence of pH

The electrochemical oxidation of UA was studied by differential pulse voltammetry in aqueous buffer supporting electrolytes over a pH range from

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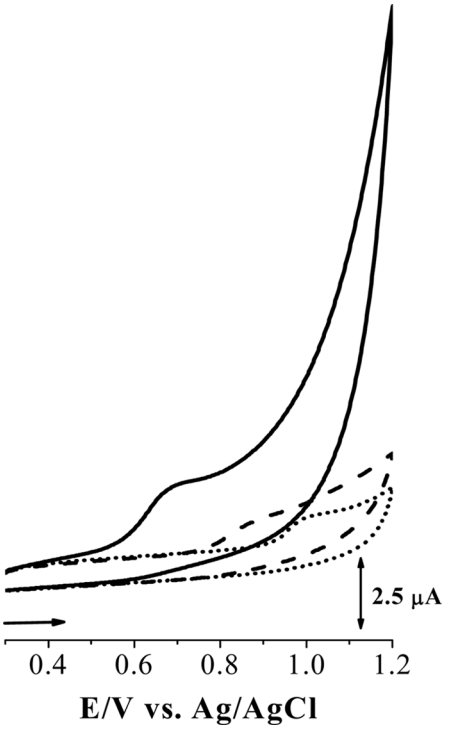

Figure 1. Cyclic voltammograms at different pH values of UA mechanically attached on the surface of GCE, $\nu = 100\,\mathrm{mV\,s^{-1}}$, (···) pH 3.5 0.2 M acetate buffer, (- - -) pH 7.0 0.2 M phosphate buffer and (—) pH 10.5 2 M ammonia buffer.

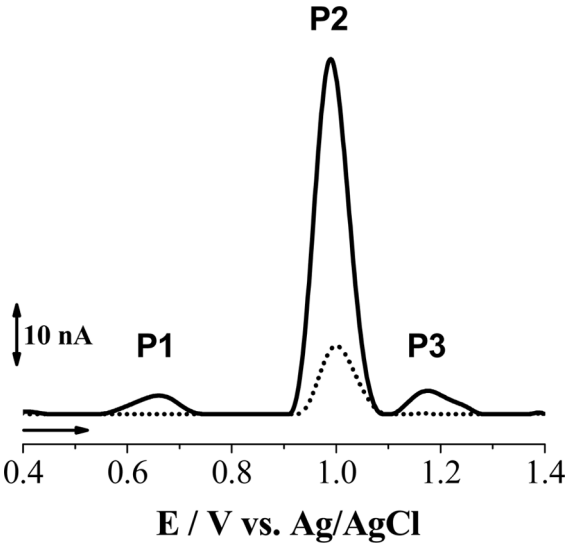

Figure 2. Differential pulse voltammograms in pH 3.5 0.2 M acetate buffer for the oxidation of UA mechanically attached on the surface of GCE, $\nu = 5\,\mathrm{mV\,s^{-1}}$: (—) first and (···) second scans.

2 to 12 (Figs. 2 and 3). The differential pulse voltammogram in pH 3.5 acetate buffer, presented another two small oxidation peaks, peak P1, at $E_p^{P1} = 0.66$ V and peak P3, $E_p^{P3} = 1.18$ V (Fig. 2). While the main peak P2, is detected for all studied pH values, peak P1 does not appear at high pH, and peak P3 cannot be seen in strong acid media at pH lower than 3. Also, in the negative potential region no peak was observed.

Successive differential pulse voltammograms in acid media, pH 3.5 0.2 M acetate buffer, showed that the peak P2 current decreased drastically in the second scan, while peaks P1 and P3 completely disappeared (Fig. 2). A similar behavior was observed in alkaline media. However, in pH 7 solution the decrease in peak P2 current was not so abrupt.

The drastic decrease of peak P2 current is due to strong adsorption of the final non-electroactive oxidation product, blocking the electrode surface. The hydroxyl groups of UA (Scheme 1) are electron donating and phenoxyl radicals are preferred targets for the oxidants and they also form polymeric products that adsorb on the electrode surface (Filipiak 2001; Jovanovic et al. 1996).

Comparing the oxidation potentials at different pHs provides information on the mechanism of UA oxidation. For buffer solutions $2 < pH < 9$, the potential of peak P2 is shifted to more negative values with increasing pH (Fig. 3a). The slope of the E_p^{P2} vs. pH curve (Fig. 3b), -59 mV per pH unit, shows that the mechanism of this oxidation process involves the same number of electrons and protons. The peak current for the peak P2 decreases until pH 6 (Fig. 3b) afterwards increasing and reaching a maximum value for pH 10 above which it decreases again.

The number of electrons transferred, n, was determined from the peak width at half height, $W_{1/2} \sim 87$ mV, and is close to the theoretical value of 90 mV, corresponding to an electrochemical reaction involving the transfer of one electron (Brett and Oliveira-Brett 1993). Consequently, it can be concluded that the oxidation process below $pH < 9$ occurs with the transfer of one electron and one proton. The second

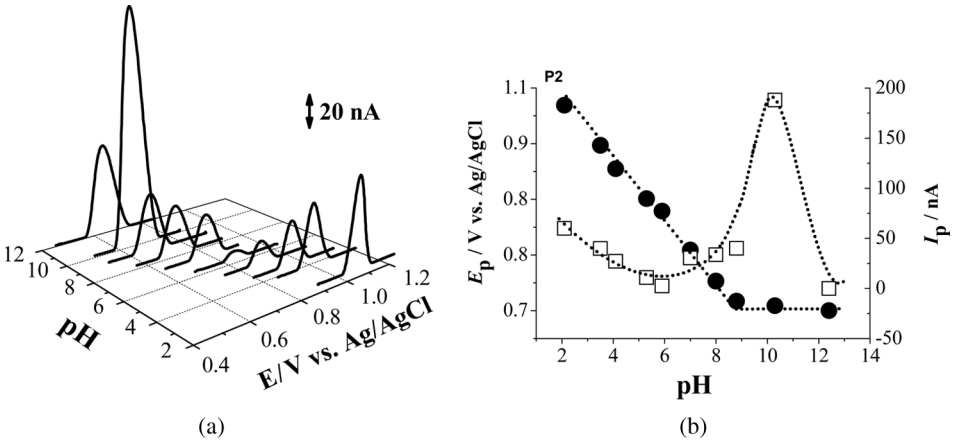

Figure 3. (a) 3D plot of background-subtracted differential pulse voltammograms obtained for UA mechanically attached on the surface of GCE as a function of pH; (b) Plot of E_p (●) and I_p (□) of peak P2 vs. pH.

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oxidation peak of UA corresponds to the hydroxyl group at position 10 (Scheme 1). For $pH > 8.8$, the oxidation process was pH independent. The value found for $pK_a \sim 8.8$ is in agreement with the literature (Cochietto et al. 2002).

The oxidation peaks P1 and P3 correspond to the oxidation of the hydroxyl groups in positions 1 and 8 (Scheme 1). The plot of E_p vs. pH for peak P1 showed a linear pH dependence for $3.5 < pH < 6$. For peak P3, this dependence was linear in the interval $3.5 < pH < 12$. Slopes of $-59\,mV$ per pH unit were found for both electrode reactions; therefore, it can be also concluded that the same number of protons and electrons was involved in two electrode processes. Other parameters such as $W_{1/2}$, were not determined since these peaks presented very low currents.

Square Wave Voltammetry

Experiments by square wave voltammetry were carried out over the same pH range, and showed similar results to differential pulse voltammetry. The advantages of square wave voltammetry are greater speed of analysis, lower consumption of the electroactive species in relation to differential pulse voltammetry, and reduced problems regarding the blocking of the electrode surface (Brett and Oliveira-Brett 1993). Furthermore, it is possible to see during only one scan if the electron transfer reaction is reversible or not. Since the current is sampled in both positive and negative-going pulses, peaks corresponding to the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment.

In Fig. 4 the forward and backward components of the total current for UA at pH 3.5 are depicted and confirm the irreversibility of the main oxidation peak P2. Based on the results obtained, a mechanism for the oxidation of UA is

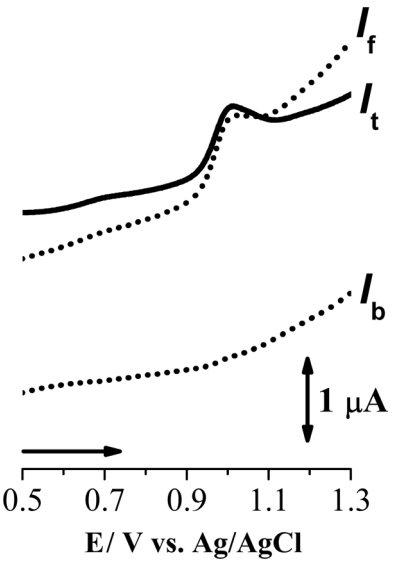

Figure 4. Square wave voltammograms in pH 3.5 0.2 M acetate buffer of UA mechanically attached on the surface of GCE, $\nu_{effective} = 100\,mV\,s^{-1}$, I_t – total current, I_f – forward current and I_b – backward current.

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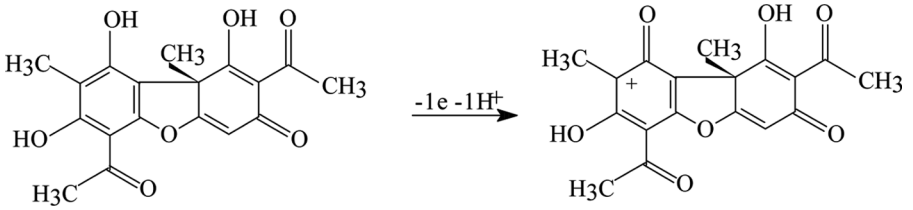

Scheme 2. Proposed oxidation mechanism of UA.

proposed, (Scheme 2). The oxidation peak P2 is related with the hydroxyl group in the position 10.

However, the electrochemical oxidation of UA is pH dependent in a one electron one proton irreversible process and occurs in the three hydroxyl groups in the molecule, corresponding to position 1, peak P1, position 10, peak P2, and position 8, peak P3.

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Analytical Determination

Square wave voltammograms were recorded for standard additions of UA in ethanol/pH 7.0 0.2 M phosphate buffer. This supporting electrolyte was preferred since in this medium a less strong adsorption was observed. A good linearity was found for peak P2 current for UA concentrations in the range of 1.00×10^{-6} M to 6.00×10^{-6} M, according to the equation: I_p (A) $= -8.12 \times 10^{-7} + 2.92 \times 10^{-7}$ [UA] (M), where R = 0.99. These data confirm that is possible the development of an electroanalytical methodology for UA determination.

CONCLUSIONS

The solid state voltammetry methodology enabled the investigation of the electrochemical oxidation mechanism of UA at a glassy carbon electrode which is pH dependent and occurs in three consecutive, one electron one proton irreversible processes, assigned to each of the hydroxyl groups in the molecule. An adsorption process of the non-electroactive oxidation products was also observed, blocking the electrode surface. The data obtained suggest that voltammetric studies using mechanically attached insoluble solids provides a feasible and quick method for studying the electrochemical redox mechanism of UA in aqueous medium. The electroanalytical determination of UA in biological fluids is foreseen and would provide very important and useful data for clinicians.

REFERENCES

Bond, A. M. 1997. Voltammetric techniques for studying the redox chemistry of solids. *J. Solid State Electr.* 1(3): 185–186.

Brett, C. M. A., and A. M. Oliveira-Brett. 1993. *Electrochemistry: Principles, Methods and Aplications*. Oxford: Oxford Science Publications.

Cochietto, M., N. Skert, P. L. Nimis, and G. Sava. 2002. A review on usnic acid, an interesting natural compound. *Naturwissenschaften.* 89(4): 137–146.

Corduneanu, O., P. Janeiro, and A. M. Oliveira-Brett. 2006. On the electrochemical oxidation of resveratrol. *Electroanal.* 18(8): 757–762.

Demet, C., C. Demet, H. M. Gokhan, and A. Orhan. 2006. Determination of usnic acid in some rhizoplaca species from middle anatolia and their antimicrobial activities. *Z. Naturforsch. C* 61(1–2): 47–51.

Dixon, B. 2005. Liking Lichens. *Lancet Infectious Diseases* 5(9): 534–534.

Emmerich, R., I. Giez, O. L. Lange, and P. Proksch. 1993. Toxicity and antifeedant activity of lichen compounds against the polyphagous herbivorous insect spodoptera-littoralis. *Phytochemistry* 33(6): 1389–1394.

Falk, A., T. K. Green, and P. Barboza. 2008. Quantitative determination of secondary metabolites in cladina stellaris and other lichens by micellar electrokinetic chromatography. *J. Chromatogr. A* 1182(1): 141–144.

Fernández, E., W. Quihot, C. Rubio, M. E. Hidalgo, R. Diaz, and J. Ojeda. 2006. Effects of UV radiation on usnic acid in *Xanthoparmelia microspora*. *Photochem. Photobiol.* 82(4): 1065–1068.

Filipiak, M. 2001. Electrochemical analysis of polyphenolic compounds. *Anal. Sci.* 17: i1667–1670.

Francolini, I., P. Norris, A. Piozzi, G. Donelli, and P. Stoodley. 2004. Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. *Antimicrob. Agents Chemother.* 48(11): 4360–4365.

Ghione, M., D. Parrelo, and L. Grasso. 1988. Usnic acid revisited, its activity on oral flora. *Chemioterapia* 7(5): 302–305.

Grygar, T., F. Marken, U. Schroder, and F. Scholz. 2002. Electrochemical analysis of solids. A review. *Collec. Czech. Chem. C.* 67(2): 163–208.

Ingólfsdóttir, K. 2002. Usnic acid. *Phytochemistry* 61(7): 729–736.

Janeiro, P., and A. M. Oliveira-Brett. 2005. Solid state electrochemical oxidation mechanisms of morin in aqueous media. *Electroanal.* 17(9): 733–738.

Jovanovic, S. V., S. Steenken, Y. Hara, and M. G. Simic. 1996. Reduction potentials of flavonoid and model phenoxyl radicals. Which ring in flavonoids is responsible for antioxidant activity. *J. Chem. Soc. Perkin Trans.* 2: 2497–2504.

Kreft, S., and B. Strukely. 2001. Reversed-polarity capillary zone electrophoretic analysis of usnic acid. *Electrophoresis* 22(13): 2755–2757.

Lovric, M., and F. Scholz. 1997. A model for the propagation of a redox reaction through microcrystals. *J. Solid State Electr.* 1(1): 108–113.

Okuyama, E., K. Umeyama, M. Yamazaki, Y. Kinoshita, and Y. Yamamoto. 1995. Usnic acid and diffractaic acid as analgesic and antipyretic components of usnea-diffracta. *Planta Med.* 61(2): 113–115.

Perry, N. B., M. H. Benn, N. J. Brennan, E. J. Burgess, G. Ellis, D. J. Galoway, S. D. Lorimer, and R. S. Tangney. 1999. Antimicrobial, antiviral and cytotoxic activity of New Zealand lichens. *Lichenologist* 31: 627–636.

Proksa, B., M. Sturdíková, N. Prónayová, and T. Liptaj. 1996. (−)-Usnic acid and its derivatives. Their inhibition of fungal growth and enzyme activity. *Pharmazie* 51(3): 195–196.

Rancan, F., S. Rosan, K. Boehm, E. Fernández, M. E. Hidalgo, W. Quihot, C. Rubio, F. Boehm, H. Piazena, and U. Oltmanns. 2002. Protection against UVB irradiation by natural filters extracted from lichens. *J. Photochem. Photobio. B* 68(2–3): 133–139.

Romagni, J. G., G. Meazza, D. Nanayakkara, and F. E. Dayan. 2000. The phytotoxic lichen metabolite, usnic acid, is a potent inhibitor of plant p-hydroxyphenylpyruvate dioxygenase. *FEBS Lett.* 480(2–3): 301–305.

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Scholz, F., and B. Meyer. 1994. Electrochemical solid-state analysis-state of the art. *Chem. Soc. Rev.* 23(5): 341–347.

Vijayakumar, C. S., S. Viswanathan, M. Kannappa-Reddy, S. Parvathavarthini, A. B. Kundu, and E. Sukumar. 2000. Anti-inflammatory activity of (+)-usnic acid. *Fitoterapia* 71(5): 564–566.

Yamamoto, Y., Y. Miura, Y. Kinoshita, M. Higuchi, Y. Yamada, A. Murakami, H. Ohigashi, and K. Koshimizu. 1995. Screening of tissue cultures and thalli of lichens and some of their active constituents for inhibition of tumor promoter-induced Epstein-Barr virus activation. *Chem. Pharm. Bull.* 43(8): 1388–1390.

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