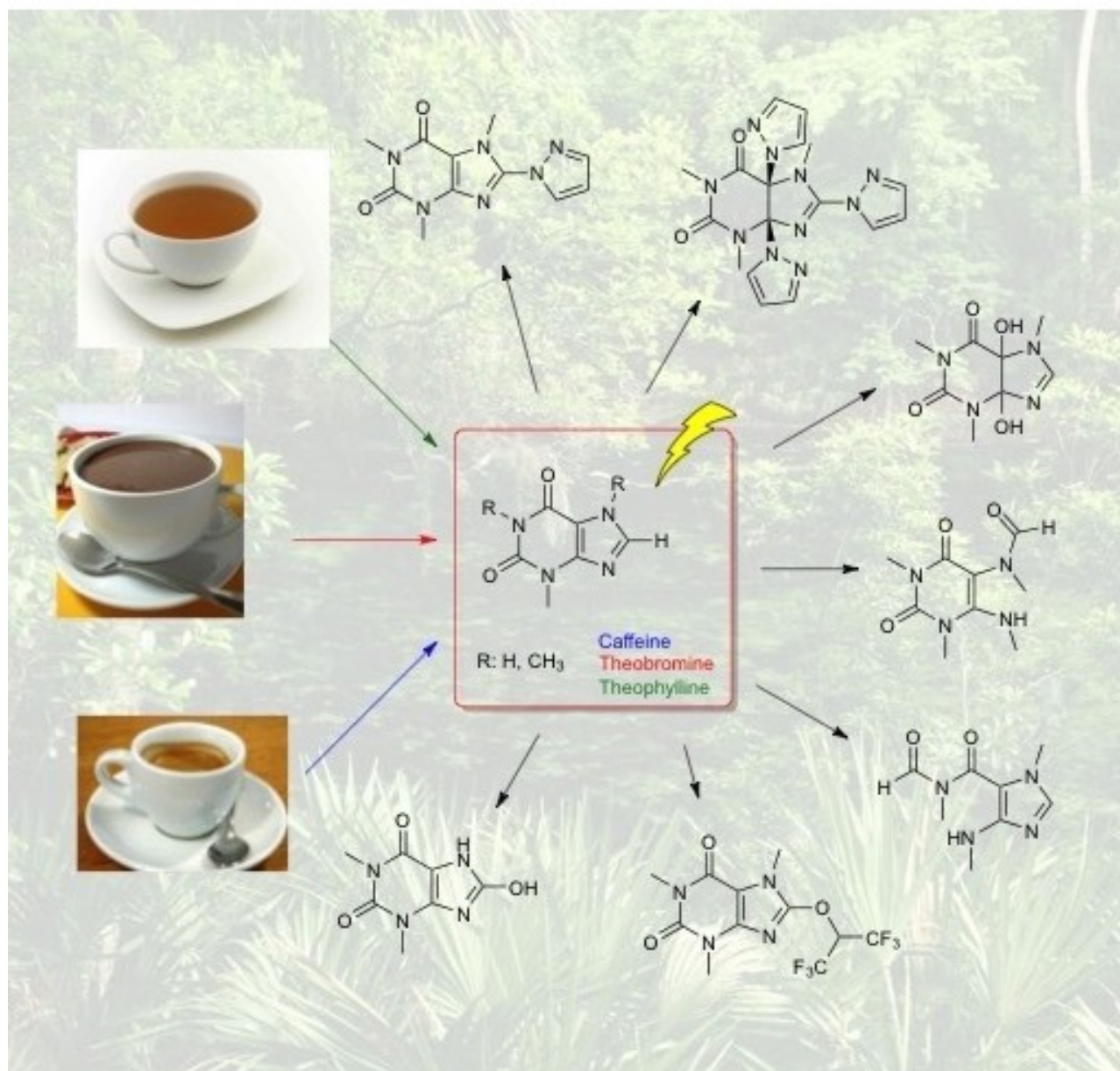


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Electrochemical Transformations of Methylxanthines in Non-Aqueous Medium

Paola Di Matteo,^[b] Martina Bortolami,^[a] Marta Feroci,^[a] Vincenzo Scarano,^[a] and Rita Petrucci*^[a]



Coffee, cocoa and tea are the main sources of the natural methylxanthines caffeine, theobromine and theophylline. They are the most consumed alkaloids worldwide, with both beneficial and harmful effects on health. In addition to more traditional therapeutic uses, beneficial effects related to the oxidative stress have been more recently ascribed to these compounds. Moreover, natural methylxanthines have recently attracted attention as bio-based and renewable starting materials for organic synthesis. By using the electron as a green and sustainable reagent, organic electrochemistry provides a precious tool to carry out redox reactions for both mechanistic and

synthetic purposes. The electrochemistry of natural methylxanthines was studied previously in an aqueous electrolytes, in which their chemistry is closely related to that of uric acid. Conversely, their electrochemistry in organic solvent is recent history: a few but successful examples of organic electro-syntheses and functionalizations have been reported, with promising applications in the pharmaceutical field. Moreover, some advances in molecular mechanisms might shed light on their potentialities as antioxidants. A prospective is given in the present minireview.

1. Introduction

Methylxanthines are heterocyclic derivatives, whose structure consists of an uracil ring fused with an imidazole one (A and B, respectively, Figure 1), and they differ for the number of the methyl groups and their position on the nitrogen atoms.

Caffeine (1,3,7-trimethylxanthine, **1**), theophylline (1,3-dimethylxanthine, **2**), and theobromine (3,7-dimethylxanthine, **3**) are the most frequently available in nature: caffeine is mainly present in coffee, theophylline is abundant in tea, and cocoa is the major source of theobromine, the reason why natural methylxanthines are widely present in the human diet.

The systemic effects of these alkaloids, especially of caffeine and theophylline, are well-known,^[1] despite the mechanisms involved are not fully understood yet. Nevertheless, these compounds found many therapeutic applications, due to their pharmacological activities.^[1] Furthermore, their potentialities as antioxidants have been recently focused on,^[2] the question remaining under discussion, however.^[3] In addition, the versatility of the xanthine scaffold makes these natural compounds very appealing as bio-based and renewable starting materials for organic synthesis, and useful precursors for molecules attractive in the pharmaceutical fields.^[4] In particular, the structural core of caffeine was evaluated in the sustainable synthesis of drugs mainly addressed to contrast damages from oxidative stress.^[5] The most extensive use of caffeine reported in the literature concerns the chemical synthesis of N-heterocyclic carbene (NHC) metal complexes, the interest in the xanthine-NHC complexes being due to their applications as catalysts, antimicrobial and anticancer agents, as well as to their photoluminescent properties, and in general to their suitability

as precursors of novel metallodrugs.^[6] Nevertheless, the high chemical stability of caffeine strongly limits *de facto* its use as starting material in organic synthesis, at least according to the limited amount of literature papers dealing with this topic.^[7]

The electrochemical reactivity of natural methylxanthines has been studied in '70s,^[8a-c] although a few previous papers are present in the literature,^[8d-f] and their electrochemical oxidation is easier when compared to the chemical route, as also evidenced by the abundance of literature papers on the electrochemical methods for caffeine detection.^[9] The majority of the reported studies is in an aqueous solvent, in which slow two-electron oxidation to the corresponding uric acid **4** followed by fast two-electron further oxidation to the corresponding 4,5-dihydroxyuric acid was proposed as an oxidative path for methylxanthines, as shown in Scheme 1 (reaction 1). A small amount of dimeric compounds as first oxidation products was evidenced only starting from theophylline.^[8c] The C⁸-N⁹ double bond (Figure 1, xanthine) was proposed as the first oxidation site.

Conversely, the electrochemistry of methylxanthines in an aprotic medium has been sparingly studied,^[3,10-15] which is unexpected taking into account the suitability of the aprotic medium to elucidate redox mechanisms (often involving radical

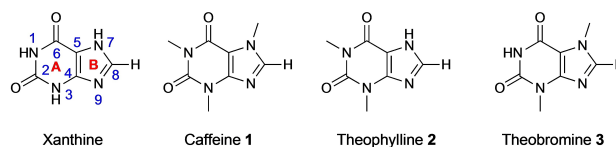
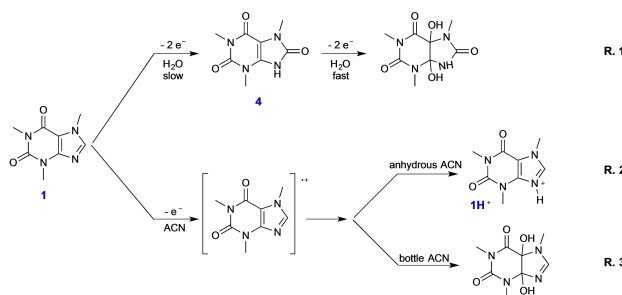



Figure 1. Methylxanthines structures.



Scheme 1. Proposed oxidation mechanisms for caffeine **1**: R. 1: in water;^[8] R. 2: in strongly dried acetonitrile;^[10] R. 3: in bottle acetonitrile.^[15]

[a] Dr. M. Bortolami, Prof. M. Feroci, V. Scarano, Prof. R. Petrucci
 Department of Basic and Applied Sciences for Engineering
 Sapienza University of Rome
 Via del Castro Laurenziano 7, 00161 Rome, Italy
 E-mail: rita.petrucci@uniroma1.it

[b] Dr. P. Di Matteo
 Department of Chemical Engineering Materials Environment
 Sapienza University of Rome
 Via Eudossiana 18, 00184 Rome, Italy

 An invited contribution to a joint Special Collection in memory of Prof. Jean-Michel Savéant.

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species more stable in organic solvent), as well as to better control electrochemical synthetic paths, avoiding chemical side-reactions often due to the presence of water. Furthermore, the aprotic medium can simulate the lipophilic environment of biological membranes, in which methylxanthines are bioavailable and exert their bioactivity.

Since the electron is the redox reagent, organic electrochemistry is considered a green technology, and a precious tool to investigate the redox reactions, for both mechanistic and synthetic purposes. In fact, organic electrochemistry allows to carry out organic reactions (not necessary redox reactions, but initiated by an electron transfer from or to the solid electrode) under mild conditions of pressure and temperature, modulating the reactivity (and thus selectivity) of the electron transfer simply by modulating the working potential. Moreover, stoichiometric amounts of reducing or oxidizing agents (and their byproducts) are avoided. In this way, functionalizations of the starting material, as well as structure transformations, can be obtained.^[16]

In the present minireview, an overview concerning the advances on the electrochemistry of natural methylxanthines in aprotic medium is provided. Caffeine and theophylline have been the most studied; the electrochemical studies were targeted to elucidate the mechanisms occurring under oxidative conditions, and to investigate the possible electrochemical synthetic routes, in both cathodic reduction and anodic oxidation. The cathodic reduction and the anodic oxidation will be discussed separately.

2. Cathodic Reduction of Caffeine

The electrochemical reduction of natural methylxanthines has been sparingly studied: up to our knowledge, only two studies have been recently reported, both dealing with caffeine 1.^[11,12] In particular, the electrochemical reduction of 1^[11] and 9-methylcaffeinium iodide^[12] has been reported, with synthetic applications.

The electrochemical reduction of 1 was studied by cyclic voltammetry (CV) and electrolysis^[11] to investigate the possibility of using 1 as starting material for cathodic organic electro-synthesis. A preliminary CV analysis was carried out in different dipolar aprotic solvents and the ionic liquid BMIM-BF₄ (1-butyl-3-methylimidazolium tetrafluoroborate), with different supporting electrolytes, and using glassy carbon (GC) and Pt as different working electrodes, with the aim to select the best experimental conditions for such cathodic reduction. DMF-NaClO₄ and GC resulted the best system to selectively reduce 1 in CV, at the cathodic peak value $E_{cp} = -2.53$ V vs SCE (Saturated Calomel Electrode). Electrolysis was then carried out, in both potentiostatic and galvanostatic conditions. The electrolyzed solutions were worked up and the reduction products were analyzed. On the basis of the collected analytical data, three possible structures were suggested for the isolated reduction product, two of which were excluded by comparison with the corresponding compounds chemically synthesized according to the literature. The third structure (compound 5 in Scheme 2) was therefore proposed as the product of the electrochemical reduction of 1 in an aprotic medium, deriving from the uracil



Paola Di Matteo, graduated in Chemical Engineering, Ph.D in Food Biotechnology (2011), is currently postDoc at Sapienza University of Rome (Italy), working in the research group of prof. Petrucci. Her research activity is focused on the production of value added molecules from natural feedstocks and on the development of new processes for the production of low-alcohol beer.



Martina Bortolami, graduated in Pharmacy (2016), Ph.D. in Pharmaceutical Sciences (2019), is currently postDoc at Sapienza University of Rome (Italy), working in the research group of prof. Feroci and Petrucci. Her research activity is focused on the design, synthesis and characterization of new molecules in the field of Pharmaceutical Chemistry, using also organic electrochemistry as synthetic tool.



Marta Feroci, graduated in Organic Chemistry, Ph.D. in Organic Chemistry, now associate professor of Chemistry for Engineering at Sapienza University. Her research interests are electroorganic and classical organic synthesis. In particular, the generation of electrogenerated species by cathodic reduction in both organic solvents and ionic liquids (NHC generation) and their use in synthesis and organocatalysis.



Vincenzo Scarano graduated in Organic Chemistry in 2020 (Sapienza University), under the supervision of prof. Marta Feroci. He is now a Ph.D. student (Engineering and Applied Science for Energy and Industry) in the same research group. His research interest is in the fields of electroorganic and classical organic chemistry.



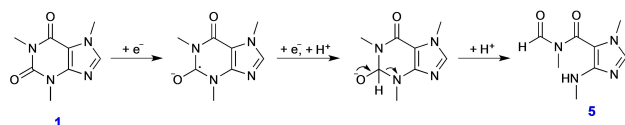
Rita Petrucci graduated in Organic Chemistry in 1988 (Sapienza University of Rome). She is an assistant professor of Chemistry for Engineering at Sapienza University. Her research interests are in the fields of molecular electrochemistry, spectroelectrochemistry, and mass spectrometry, applied to natural antioxidants, in particular to polyphenols in food and beverages.

ring opening *via* a two-electron two-proton process, shown in the scheme.

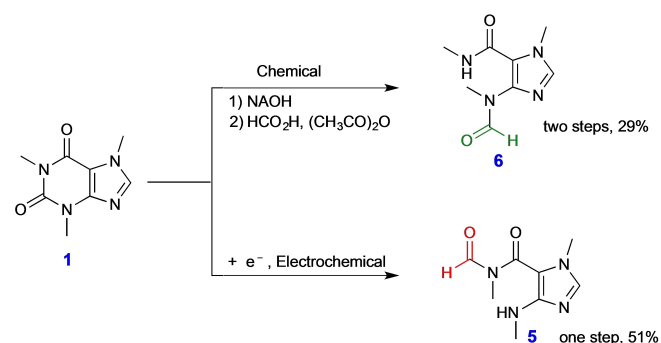
Further experiments were carried out to optimize the electrochemical synthesis of **5** in terms of yields, and the best electrochemical performance was obtained in DMF-Et₄NBF₄ at a Pt working electrode, under galvanostatic conditions, in the absence (47% yield) and presence (51% yield) of added water. A 50% conversion can be considered a good result, taking into account that the highly negative E_{cp} value of **1** is close to the solvent/electrolyte discharge potential, which cannot be completely avoided, with a consequent partial current consumption. Noteworthy, compound **5** was not obtained under classical chemical conditions: in fact, in the electrochemical route the formylation occurred at the exocyclic amide nitrogen atom, while in the non-electrochemical route at the exocyclic amine nitrogen atom (Scheme 3).^[17] Thus, the cathodic reduction of the bio-based **1** in aprotic medium provided the highly amino-functionalized imidazole **5**, which can find valuable applications as starting material in the synthesis of enamides and related compounds.

A different reactivity was observed for the 9-methylcaffeinium ion, whose cathodic reduction led to the opening of the imidazole ring instead of the uracil one.^[12]

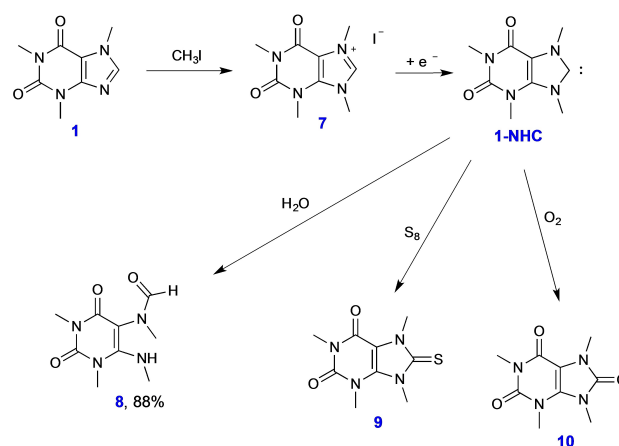
Caffeinium salts have been used in organic synthesis as precursors of NHC in metal complexes, some of which are biologically active.^[4,5c-d,18] Nevertheless, free NHC derivatives have never been evidenced. The electrochemical behavior of 9-methylcaffeinium iodide **7** (Scheme 4) was studied by CV, differential pulse voltammetry (DPV) and electrolysis in an aprotic medium, to explore the possible electrochemical production of the corresponding NHC.^[12]



Scheme 2. Cathodic reduction of caffeine **1**: proposed mechanism for the amino-functionalized imidazole product **5**.^[11] Reprinted with permission from Ref. [11]. Copyright © 2019, Rights Managed by Georg Thieme Verlag KG Stuttgart • New York.



Scheme 3. Fate of caffeine **1** by chemical^[16] and cathodic reduction.^[11] Adapted with permission from Ref. [11]. Copyright © 2019, Rights Managed by Georg Thieme Verlag KG Stuttgart • New York.



Scheme 4. From caffeine **1** to NHC derivatives: hypothesis on the reactivity of the electrochemically reduced 9-methylcaffeinium iodide **7**.^[12]

CV of **7**, easily synthesized from **1**, was carried out in DMF-Et₄NBF₄ on a GC working electrode: an irreversible cathodic peak at E_{cp} = -1.76 V vs Ag/AgCl was observed, with an anodic peak in the reverse scan in the region of NHCs oxidation.

The observed electrochemical behavior was similar to one of other imidazolium salts,^[19] suggesting that the NHC might have been generated by cathodic reduction of the 9-methylcaffeinium ion, and likely furtherly reacting. Controlled potential electrolysis (CPE) of **7** was then carried out in DMF-Et₄NBF₄ at a Pt working electrode, at the cathodic potential of -2.0 V vs Ag/AgCl. Hymeniacidin **8** (Scheme 4) was found as the main oxidation product, isolated in high yield (88%) from the electrolyzed solution after workup. The collected data supported the hypothesis of a cathodic reduction of **7** to the corresponding NHC, followed by the opening of the imidazole ring to the final hymeniacidin **8**. Moreover, the NHC electrogeneration was also confirmed by the isolation of products **9** and **10**, obtained when the catholyte was treated with elemental sulfur and oxygen (Scheme 4).^[20] From a mechanistic point of view, the NHC electrogeneration appeared well supported and the hypothesis on the reactivity of the electrochemically reduced **7** appeared reasonable.

In conclusion, two different electrochemical synthetic routes were found starting from the bio-based **1**: in the first one, the opening of the uracil ring could be obtained by direct cathodic reduction of **1**, leading to a highly amino-functionalized imidazole **5**; in the second one, the opening of the imidazole ring could be obtained by cathodic reduction of **7**, chemically obtained from **1**, *via* the electrogenerated NHC, highly reactive. The last route led to the first electrosynthesis of the natural product hymeniacidin **8**.

3. Anodic Oxidation of Methylxanthines

3.1. Mechanistic Studies

The electrochemical oxidation of methylxanthines in an aprotic medium has been studied, too.^[3,10,13–15]

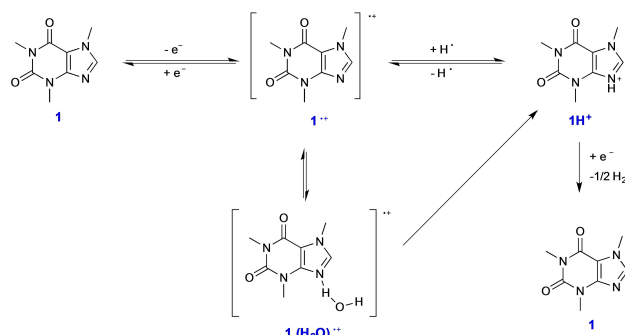
The beneficial effects of methylxanthines have been ascribed, in part, to antioxidant properties: protection from the oxidative damage was evidenced by *in vivo* and *in vitro* studies, mainly as OH[•] radical scavenger.^[2,21] On the other hand, pro-oxidant properties have been also reported,^[21b] and lastly, **1** is inactive towards moderate hydrogen/electron scavengers such as DPPH (1,1-diphenyl-2-picrylhydrazyl, free radical) and ABTS^{•+} (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, radical cation).^[2c] The potentiality of methylxanthines as antioxidants is a topic of high interest, and an open discussion, up to now.

To elucidate the behavior of the lipid-soluble **1** under metabolic oxidation, the first electrochemical study was carried out by CV and CPE in MeCN,^[10] that is a relatively unreactive solvent with a wide potential window, and in which radical intermediates can be stabilized and characterized, because reactions with water can be avoided or limited. Bu₄NPF₆ was used as a supporting electrolyte; Pt and GC were used as working electrodes in the preliminary CV study; Pt was chosen as working electrode for CPE. The effect of water on the oxidation of **1** was evaluated by using both undried and strongly dried MeCN.

On the basis of CV experiments, a reversible one-electron process, E_{ap} = +1.2 V vs Fc/Fc⁺, was proposed for the oxidation of **1** to the corresponding radical cation **1^{•+}**, whose lifetime was strongly dependent on the water content. In fact, the corresponding cathodic peak, E_{cp} ≈ +1.1 V in the reverse scan, was much more intense in dried MeCN than in undried MeCN, in which the peak was hardly detected. CPE confirmed that one-electron per molecule was consumed in the full oxidation of **1**. Further, CV evidenced that **1** was regenerated after reducing its oxidation product. The mono-electronic oxidation of **1** was then chemically carried out^[22] and the oxidation product was studied by CV, for a comparison. Cyclic voltammograms proved that the same species was obtained both by non-electrochemical and by electrochemical oxidation of **1**. NMR and X-ray analysis supported the formation of the diamagnetic cation **1H⁺** as oxidation product of **1** in MeCN, as shown in Scheme 1 (reaction 2).

The radical cation **1^{•+}**, electrogenerated in dried MeCN, was proposed to react with a hydrogen atom donor to form the cation **1H⁺**, which can be easily converted back to **1** by cathodic reduction.

Despite the origin of the H atom was not clarified, the possibility that trace water might aid the transfer of a H atom from the solvent to the radical cation **1^{•+}** was suggested (stabilizing **1^{•+}** by H-bonding). The hypothesis of mechanism for the oxidation of **1** in MeCN, the reduction of its oxidized product, and the H atom transfer are resumed in Scheme 5. The electrochemical potentials vs Fc/Fc⁺ and kinetic parameters, evaluated by digital simulation experiments, were also reported by the authors.



Scheme 5. Hypothesis of mechanism for the oxidation of caffeine **1** and reduction back to **1** of the oxidized product in acetonitrile.^[10] Adapted with permission from Ref. [14]. © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

A deeper insight into the oxidative mechanisms methylxanthines might undergo was given in a comprehensive study carried out in anhydrous MeCN, in which the combined tools of electrochemistry, spectroelectrochemistry, computational analysis and chemical reactivity towards reactive oxygen species (ROS) were used to investigate on the behavior of **1**, **2** and **3** under oxidative conditions.^[3]

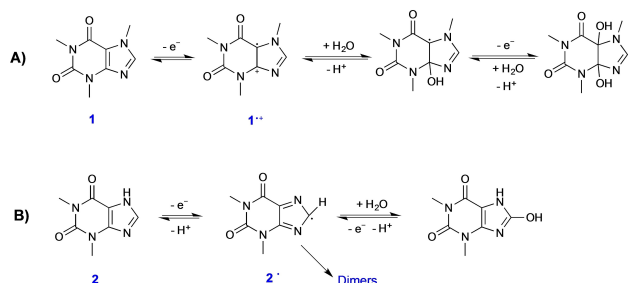
The comparative electrochemical study was carried out in anhydrous MeCN, using Et₄NClO₄ as supporting electrolyte and a GC as working electrode, in the absence and presence of a deprotonating agent as well as of water as proton donor.

An almost identical anodic behavior was evidenced for the two N⁷-methyl substituted xanthines, **1** and **3**, with a quasi-reversible anodic peak at the same potential value of +1.26 V vs Ag/AgClO₄, suggesting that the N¹ nitrogen atom should not be involved in the oxidation process. Conversely, a different behavior for the N⁷-H **2** was observed, with an irreversible anodic peak at +1.22 V. A different oxidative route was therefore suggested, depending on the presence or not of a H-atom on the N⁷ position. Some differences between **1** and **2** were also evidenced by the Density Functional Theory computational analysis. Different spectral changes were also observed along the CPE carried out on **1** and **2**, in turn, in an UV-vis cell modified for spectroelectrochemistry.

Based on all the experimental and theoretical data, the primary oxidation of natural methylxanthines was proposed starting from the C⁴-C⁵ olefinic site, unlike what was previously reported. A mono-electronic electron transfer (ET) process was proposed for the oxidation of **1** (and likely for **3**) to give a quite stable radical cation **1^{•+}** (Scheme 6, A), while a mono-electronic ET coupled with a proton transfer process was proposed for the oxidation of **2** to give the neutral radical **2[•]** (Scheme 6, B).

A different chemical reactivity was then expected for the radical cation **1^{•+}** and the neutral radical **2[•]**: 4,5-saturated derivatives were proposed as final oxidation products of **1**, while 8-substituted derivatives were proposed as final oxidation products of **2**.

The possibility of dimerization was also proposed for **2**. The hypothesis of reactivity of **1^{•+}** and **2[•]** with water as a nucleophile is shown in Scheme 6, (A) and (B), respectively.



Scheme 6. Anodic oxidation of caffeine (1, A) and theophylline (2, B) and hypothesis of reactivity of the radical cation 1^{•+} (A) and the neutral radical 2[•] with water as a nucleophile.^[3] Reprinted with permission from Ref. [3]. © 2018 Elsevier B.V. All rights reserved.

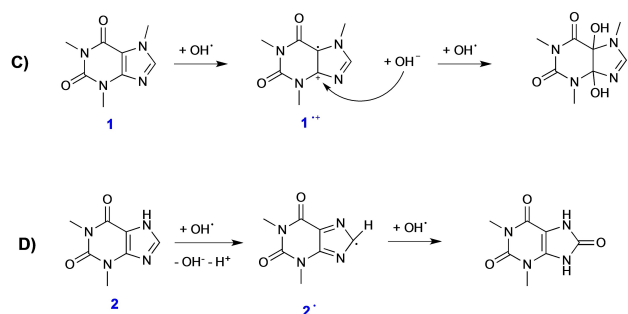
The antioxidant properties of methylxanthines were re-discussed, considering the reaction with PbO₂, the radical anion superoxide O₂^{•-} and the galvinoxyl radical. No UV-vis spectral changes were observed in all the cases. The antiradical properties *via* a H-atom transfer mechanism were therefore excluded. Furthermore, because of the high oxidation potential values of methylxanthines, the antioxidant capacity *via* an ET mechanism was reported possible only towards strong oxidants. The previously reported reactivity of 1 towards the highly oxidant HO[•] was therefore explained with an ET mechanism, as reported in Scheme 7.

3.2. Synthetic Routes

The interest in 2 ranges from biomedical applications and antioxidant potentiality, to environmental contamination and the use as starting material in organic synthesis, just to name a few. Nevertheless, as highlighted above, a very small number of papers on the electrochemistry of 2 are available in the literature, even less than those for 1.

A systematic study of the oxidation products of 2 in organic solvents, and water for comparison, has been recently reported.^[13]

CPEs of 2 were carried out in MeCN and DMF, in turn, with Et₄NBF₄ as supporting electrolyte and Pt electrode as working electrode, in both cases in the absence and presence of



Scheme 7. Hypothesis of reactivity of caffeine (1, C) and theophylline (2, D) towards the hydroxyl free radical HO[•] via an ET mechanism.^[3] Reprinted with permission from Ref. [3]. © 2018 Elsevier B.V. All rights reserved.

stoichiometric amounts of water. For comparison, CPEs were also carried out in water/KCl. The anodic peak potential values in the range +1.3/+2.0 V vs SCE, in MeCN/DMF and water, respectively, were in good agreement with the literature.^[3,8c]

The anodic oxidation was monitored during the electrolysis by CV and UV-vis spectral analysis *ex-situ*, after 1 Faraday (1F) and 2 Faradays (2F) current consumption. While weak spectral changes were observed during the electrolysis in water, a λ_{\max} /274 nm absorbance decrease and new absorption bands in the range 310–370 nm were observed in organic solvents, suggesting a high current efficiency and oxidation products with extended conjugation, mainly in organic solvents.

The electrolyzed solutions were analyzed by liquid chromatography coupled with a photodiode array detector and a mass spectrometry detector (HPLC-PDA-ESI-MS/MS system). Five main chromatographic peaks were studied, and the structures were tentatively assigned on the base of chromatographic parameters, UV-vis and mass spectral data, and mass fragmentation patterns (Figure 2).

Strongly supported by experimental data, compounds 11–15 were proposed as the main products obtained by anodic oxidation of 2 in organic solvents and water.

The dimeric products 13–15 were prevalent in organic solvents; 13 and 15 were not previously reported. Monomeric compounds 11 and 12 were prevalent in water, with 12 never experimentally evidenced before. These results were in good agreement with the literature^[3] discussed in the previous section. In fact, the electrogenerated radical cation of 2 can react with water, giving the 4,5-diol 12, while the deprotonated neutral radical can give dimers 13–15 by coupling reaction, in different ratio according to the medium. Compound 11 resulted from the opening of the imidazole ring, likely starting from 12. Hypotheses of mechanisms for the formation of compounds 11–15 were also given.^[13]

A similar systematic study of the oxidation products of 1 in organic solvent has been recently reported.^[15]

CVs of 1, carried out in MeCN-Et₄NBF₄ at GC and Pt working electrode, evidenced an irreversible anodic peak at E_{ap} = +

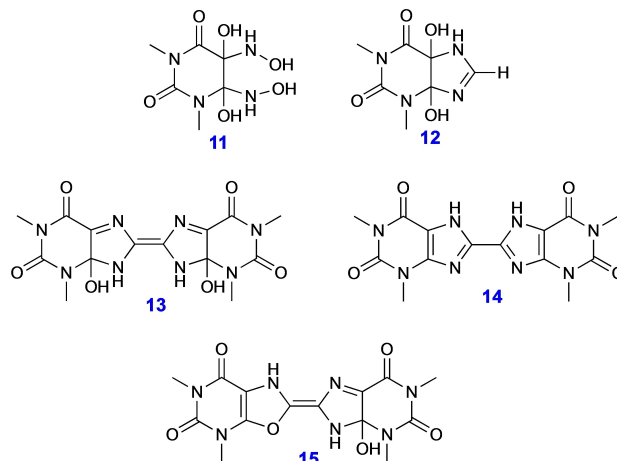


Figure 2. Theophylline 2 oxidation main products tentatively assigned on the basis of HPLC-PDA-ESI-MS/MS data.^[13]

1.87 V vs SCE. A slight shift to +1.92 V and an increase of the anodic peak current density, till doubling, was observed when 1 equivalent of water was added using a GC working electrode. These data were in good agreement with data previously reported^[3,9b-c,10] and discussed above. The absence of the cathodic peak in the reverse scan was likely due to the trace water.

Consequently, CPE of **1** was carried out in MeCN at a Pt working electrode, in the absence and presence of water, and the anodic oxidation was monitored by CV. After 2F the electrolyzed solutions were analyzed by HPLC-PDA-ESI-MS/MS.

Similar chromatographic profiles were obtained for the solutions electrolyzed in MeCN and MeCN-H₂O. Four chromatographic peaks were evidenced, two of which suggested higher polarity and the other two lower polarity than **1**. The oxidation products, assigned on the base of chromatographic parameters, UV-vis and mass spectral data, and mass fragmentation patterns, are shown in Figure 3.

The presence of dimeric forms of **1** was excluded by the mass spectrum analysis, in agreement with the anodic generation of the quite stable radical cation **1**^{•+}, unable to undergo coupling reactions because of the methylated N⁷ position, as previously hypothesized.^[3]

Moreover, the presence of the diamagnetic cation **1H**⁺ previously reported^[10] as an oxidation product could be excluded, at least under the applied experimental conditions, in which undried MeCN was used.

Last, a higher current efficiency was found in MeCN-H₂O, in which only 14% of the starting material was found at the end of electrolysis vs a 42% in MeCN.

Experimental data well supported all the proposed structures **16–19** (Figure 3), which suggested deriving from chemical reactions between the electrogenerated radical cation **1**^{•+} and nucleophilic species available in solution, as water.

Moreover, it was also possible to identify 4,5-diol **16** as the primary oxidation product of **1** in MeCN in the presence of controlled amount of water. All the other compounds were suggested to derive from **16**.

Noteworthy, this was the first experimental evidence for compound **16**, which strongly supported the olefinic site oxidation previously proposed.^[3] A 4,5-diol was also proposed as an oxidation product of **2**.^[13]

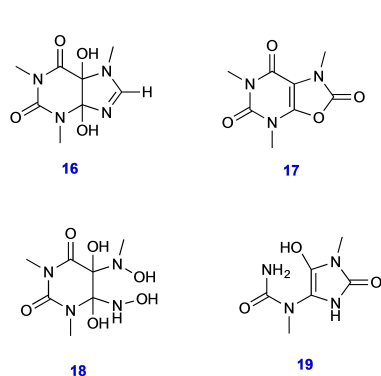


Figure 3. Caffeine **1** oxidation main products tentatively assigned on the basis of HPLC-PDA-ESI-MS/MS data.^[15]

In conclusion, the anodic oxidation products of **1** and **2** in an aprotic medium provided experimental evidence for the different reactivity of the electrogenerated radical cation **1**^{•+} and neutral radical **2**[•] previously proposed, as discussed in the section above, with a meaningful impact on the discussion concerning the behavior of methylxanthines under oxidative stress in a lipophilic environment.

Moreover, most of the oxidation products of both **1** and **2** had never been reported before.

Another example of electrochemical oxidative functionalization of **1** has been recently reported,^[14] with interesting synthetic applications.

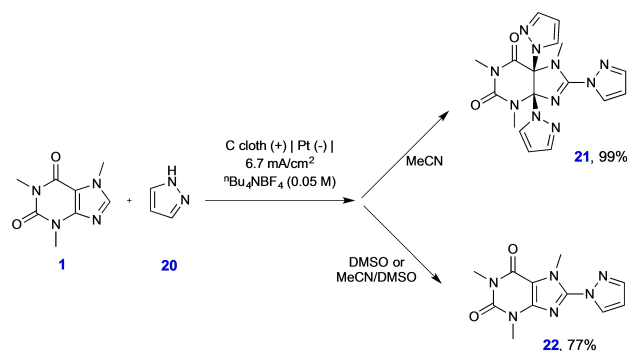
The transition metal-catalyzed functionalization of heterocyclic compounds, including **1**, is not recommended because of the N atoms that would poison the catalysts,^[23] and strategies for the dearomatizative functionalization of indole with stoichiometric amount of chemical oxidants has been reported.^[24]

Due to the advances of the electrochemical functionalization in the last decades,^[25] providing the advantage of the green and sustainable electron, the possibility to selectively functionalize **1** was studied by anodic oxidation in organic solvent.^[14]

Solutions containing **1** and pyrazole **20** as a nucleophile in MeCN, DMSO or MeCN/DMSO, with nBu₄NBF₄ as supporting electrolyte, a carbon cloth as anode and a Pt sheet as cathode, were electrolyzed under galvanostatic conditions (Scheme 8).

By changing the electrolysis solvent, it was possible to obtain in a selective way the dearomatized amination product **21** (in MeCN) and the C⁸ amination product **22** (in DMSO or in MeCN/DMSO mixture). The structures of compounds **21** and **22** were confirmed by X-ray analysis, and they were also supported by DFT calculation. The dearomatized amination product **21** was obtained in quantitative yield with high diastereoselectivity by anodic oxidation in MeCN after 10 h reaction (Scheme 8). The amination product **22** was proposed as the precursor of **21**, as confirmed by control experiments.

The optimized synthetic routes were further investigated by using various azoles, differing for steric hindrance and substituent nature: all of them resulted from good to excellent substrates, leading to **1** dearomatization amination products in 66–99% yields, and to **1** amino-functionalized products in 31–



Scheme 8. Reaction between caffeine **1** and pyrazole **20** under anodic oxidation conditions in organic solvents.^[14] Adapted with permission from Ref. [14]. © 2020 Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

77% yields. A poor 12% yield was obtained only when 4-iodopyrazole was used for the dearomatization amination of **1**. Noteworthy, the use of substituted pyrazole provides other reactive sites for further functionalization.

Other nucleophiles were also tested, such as alcohols, azides and sodium trifluoromethane-sulfonate, with good yield ranging between 40% and 62%. Also, gram-scale experiments were carried out to evaluate the scalability of the optimized process, obtaining **21** in 88% yield.

A CV investigation on the system **1**/pyrazole provided information on the possible mechanism for the C⁸ amination of **1** to **22** and the subsequent dearomatization to **21**.

In MeCN, **1** was easier to be oxidized than pyrazole, with a first anodic peak at +2.0 V vs Ag/AgCl, in good agreement with literature,^[3,10] versus a value of +2.3 V for pyrazole. The anodic oxidation of **1** to **1**^{•+}, followed by chemical reaction with the nucleophilic pyrazole and a further oxidation to the C⁸ substituted **22** was proposed as mechanistic path.

On the other hand, the low first oxidation potential of +1.2 V measured for **22**, easier to oxidize than **1**, pyrazole and **21**, explained the low yield of **22**. In fact, **22** is easily oxidized to the corresponding radical cation, that after reaction with pyrazole leads to the final **21**. Conversely, no anodic peak was observed for **22** in MeCN/DMSO, explaining the selective amino-functionalization in a mixture of solvents.

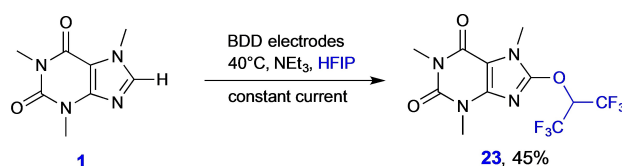
The electrochemical oxidative functionalization of methylxanthines was reported in a recent comprehensive study.^[26] Due to some **1** and **2** C⁸ functionalized pharmaceuticals, which are approved drugs for the treatment of type II diabetes and Parkinson's disease,^[27] there is a great interest in the functionalization strategies aimed to develop new xanthine-derived pharmaceuticals.

The oxidative activation and alkoxylation of C⁸ in **1** structure were obtained by constant current electrolysis of **1** carried out in hexafluoroisopropanol (HFIP) as a solvent working also as a reagent, at a boron-doped diamond (BDD) electrode, and in the presence of triethylamine (NEt₃) as a base. No additional supporting electrolyte was added due to the highly conductive system HFIP/NEt₃. The reaction conditions were optimized using Design of Experiment (DoE) techniques: five parameters, namely the stirrer speed, the concentration of **1** and NEt₃, the amount of the applied charge and the current density, were followed in a first experimental design using 5 mL HFIP, BDD electrodes and 40 °C as static parameters; a fixed concentration of NEt₃ was used in a second experimental design, in which the four parameters left were followed.^[26]

The HFIP caffeyl ether, 8-((1,1,1,3,3,3-hexafluoropropan-2-yl)oxy)caffeine (**23**, Scheme 9), was obtained both in 1 mmol and 10 mmol scale, in 42% and 45% yield, respectively. The scalability of the electrolysis was demonstrated and results suggested that the yield could be further improved.

The oxidative activation of C⁸ in **2** structure was similarly obtained and the corresponding 8-((1,1,1,3,3,3-hexafluoropropan-2-yl)oxy)theophylline was isolated, 31% yield.

The reaction mechanism was also studied by CV and theoretical calculations. CV of **1** in HFIP/NEt₃ evidenced an irreversible anodic peak at +1.80 V (vs Ag/AgCl in saturated



Scheme 9. Anodic oxidation of caffeine **1** in the presence of HFIP by constant current electrolysis.^[26] Reprinted with permission from Ref. [26]. © 2020 Published by Wiley-VCH Verlag GmbH & Co. KGaA.

LiCl in EtOH), shown in Figure 4 (left). An ECEC mechanism (electrochemical-chemical-electrochemical-chemical four step mechanism) was proposed, in which the first monoelectronic oxidative step coupled with a fast chemical reaction was followed by a second oxidation at lower potential. A different voltammetric behavior was observed when tributylmethylammonium sulfate (MTBS) was used instead of NEt₃ in HFIP, with two different oxidative steps (Figure 4, right), coupled with a fast irreversible chemical reaction also in this case.

Theoretical data were in good agreement with the experimental voltammetric data. On the basis of all the experimental and theoretical data, the electro-synthesis of HFIP caffeyl ether **23** was proposed to start from the mono-electronic oxidation of **1** leading to the radical cation (Scheme 10), whose spin density is delocalized across C⁸, N⁷, C⁴ and C⁵, in good agreement with data previously reported.^[3] NEt₃ deprotonates HFIP, whose anion may either deprotonate the radical cation, unlikely, or

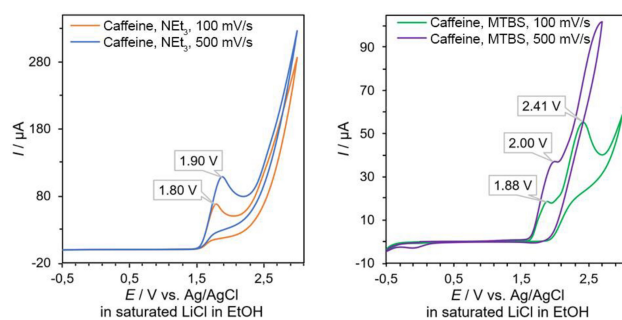
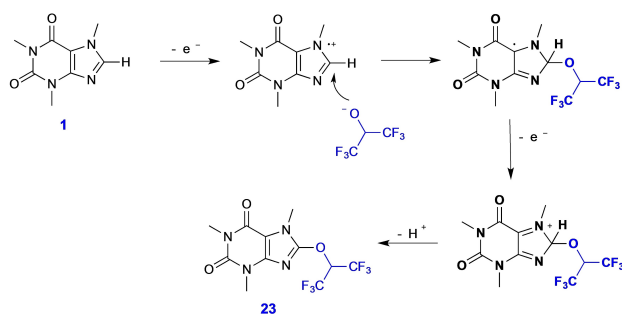
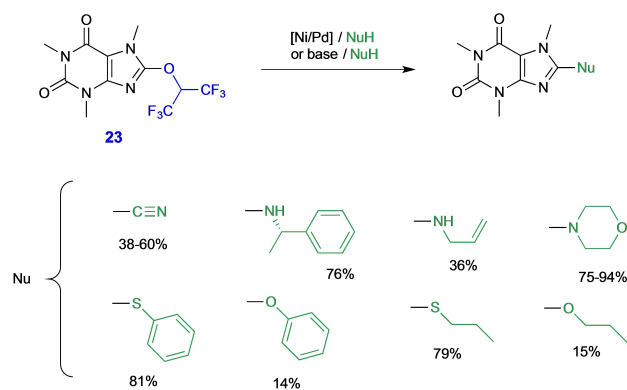


Figure 4. CVs of **1** in HFIP/NEt₃ (left) and in HFIP/MTBS (right).^[26] Reprinted with permission from Ref. [26]. © 2020 Published by Wiley-VCH Verlag GmbH & Co. KGaA.



Scheme 10. Hypothesis of mechanism for the anodic alkoxylation of caffeine with HFIP.^[26] Adapted with permission from Ref. [26]. © 2020 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA.



Scheme 11. Reactions of the HFIP caffeine ether electrochemically obtained **23**, and yields of the isolated products.^[26] Adapted with permission from Ref. [26]. © 2020 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA.

likely react by a second-order nucleophilic attack on C⁸. The addition product is further oxidized and after deprotonation yields the final product **23**, with a global ECEC mechanism.

The versatility of the HFIP moiety as leaving group was then shown by different functionalization reactions, carried out by transition metal-catalysis with nickel or palladium, or by direct metal-free reaction, as shown in Scheme 11, evidencing the values of **1** intermediates electrochemically obtained for further synthetic applications.

4. Conclusions and Perspectives

A comprehensive overview on the electrochemistry of the widespread natural methylxanthines caffeine, theophylline and theobromine in aprotic organic solvent was herein given. The mild conditions of the electrochemical methods allowed to study electrogenerated radical species, and to evidence intermediates and oxidation products that cannot be evidenced under the harsh condition usually necessary for chemical oxidation. Thus, some lights were shed on the reaction mechanisms of methylxanthines under oxidative stress conditions in lipophilic environment. Moreover, different oxidation products were found, and corresponding mechanisms were proposed, depending on the starting methylxanthine and on the amount of water present in the solvent, as the diamagnetic 1H⁺, dimers from theophylline, and dearomatized diols from caffeine and theophylline. Different degradation products were also found, deriving from the opening of the imidazole ring or the uracil ring. Apart from mechanistic studies, natural methylxanthines are renewable starting materials for the obtainment of highly functionalized organic molecules, via both cathodic reduction and anodic oxidation. As regards the cathodic reduction, very different electrolysis products were obtained starting from caffeine or its methylated salt. In particular, the opening of the uracil ring or of the imidazole one, respectively, was obtained. As regards the anodic functionalization, a successful highly diastereoselective dearomatization functionalization of caffeine by direct electrochemical oxidation of

caffeine in the presence of pyrazole, pyrazole derivatives and other nucleophiles, under metal-free and external oxidant-free conditions, was also reported, with both synthetic applications and mechanistic discussion. Finally, a successful C⁸ functionalization of caffeine and theophylline was obtained by simple and quite selective anodic oxidation in HFIP/NEt₃, leading to the corresponding HFIP ethers, useful intermediates for further synthetic applications.

Electrochemistry was confirmed as a simple, selective, sustainable, green technology whose application in organic synthesis has a wide margin of growth in the upcoming years. Nonetheless, only few papers report the use of natural products as starting material for further electrochemical transformations. The main advantage of natural compounds (apart from their renewable nature) is due to the chemical complexity of their structures, which allows to obtain highly functionalized molecules in a few steps, avoiding the multistep, expensive and yield-limiting way of the total synthesis.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: natural methylxanthines · electro-organic synthesis · oxidative mechanisms · mass spectrometry · antioxidant capacity

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