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Preparative access to transformation products (TPs) of furosemide: a versatile application of anodic oxidation

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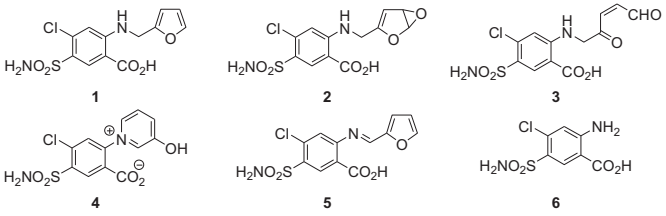
ABSTRACT

Furosemide, a pharmaceutical prescribed for the treatment of edema and hypertension, is a known contaminant of water. In this study, chemoselective anodic oxidation was implemented to assist in the identification and the preparation of furosemide transformation products (TPs), i.e., compounds deriving from furosemide and likely to appear in the environment. An aniline and a pyridinium are proposed as plausible TPs and an analytical study of the pyridinium is presented.

1. Introduction

The ubiquitous presence of drugs residues in aquatic systems has justified a large number of studies over the last decade.[1] Resulting from natural excretion (sometimes under unmetabolised form) or inappropriate disposal of unused or expired medication, these compounds contaminate surface-, ground-, and drinking water thus exposing the environment and humans to their potential effects.[2] In this context, computational studies have proposed models to assess the effects of contaminants on human health.[3] According to a prioritization methodology, lists have also been established, identifying pharmaceuticals products (PPs) that might pose a risk.[4] One of them,[4a] based on the evaluation of predicted environmental concentration (PEC), has classified forty PPs (including furosemide **1**) as highest risk compounds. However, all of these computational studies are based on available data (such as pharmacological- and environmental toxicological data), which often have to be completed. In particular, a better risk assessment would be obtained by taking into account the fate of PPs and more precisely the impact of their (a)biotic transformation products (TPs) susceptible to appear in the environment.

Furosemide (**1**), one of the forty highest risk compounds (with PEC >100 ng/L) is a sulfonamide derived from anthranilic acid. Classified as loop diuretic, it is prescribed for the treatment of edematous states and hypertension. It is rapidly eliminated by renal excretion and by biotransformation, especially conjugation.[5] It has been unambiguously detected both in sewage treatment plants and rivers.[6] Some of its TPs have been described and all of them (except conjugates) stem from an initial oxidation process (Scheme 1).



Scheme 1. Furosemide (**1**) and its TPs.

A first class of furosemide TPs highlighted the ability of the furan moiety to subject oxidation. The metabolic oxidation of furosemide by cytochrome P450 led either to the postulated epoxide (**2**) or to its γ-ketoenal equivalent (**3**).[7] Interestingly, these intermediates are suspected to induce hepatotoxicity in mice by covalently binding to biologic substrates. The chemical oxidation by dimethyldioxirane in acetone leads also to the γ-ketoenal intermediate (**3**), which rearranges in the reaction mixture to give a zwitterionic pyridinium (**4**).[8]

A second class of TPs revealed the ability of the nitrogen atom of the amino group to undergo also oxidation. The electrochemical

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oxidation at gold electrode of furosemide led to the imine (**5**).[9] Aniline (**6**) was obtained either after metabolisation by the fungus *Cuninghamella elegans*[10] or in rat.[7] Chemical oxidation of furosemide by diperiodatocuprate(III)[11] or hydrogenolysis on palladium[12] also led to the aniline (**6**).

Except in the case of the aniline (**6**), no preparative access has been described for these TPs, thus ruling out any toxicological study or the development of any protocol able to detect them in environmental matrices. In this context, the development of such preparative access appeared as a prerequisite for any attempt to study the environmental impact of furosemide. Considering previous work[9] and the well documented literature about application of anodic oxidation to generate metabolites,[13] the electrochemical approach seemed to be indicated for this purpose.

2. Results

2.1. Cyclic voltametry

The cyclic voltametry of furosemide at glassy carbon electrode in methanol revealed two oxidation peaks at 1.4 V and 1.6 V versus SCE (Fig. 1, blue curve). In agreement with the literature[14] they were, respectively, attributed to the nitrogen atom of the aniline function and to the furan ring. No peaks were observed in the return scan, indicating an irreversible process.

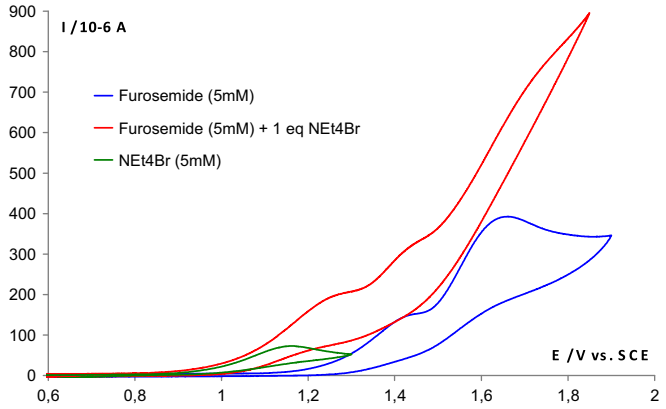
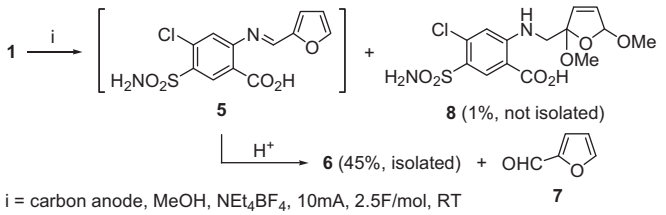


Fig. 1. Cyclic voltametry in MeOH (50 mV/s); 0.1 M NEt_4BF_4; glassy carbon electrode.

It should be noted that the possibility of a mediated oxidation of furosemide was demonstrated in the presence of 1 equiv of bromides (1.2 V vs SCE). In such conditions, the intensity of the peak attributed to the nitrogen atom was indeed decreasing (Fig. 1, red curve). At the same time, the signal of bromides was also affected, whereas the oxidation peak was increasing, the reduction peak (0.43 V vs SCE) has totally disappeared.

2.2. Preparation of TPs

Preparative oxidation was firstly carried out in methanol at room temperature in an undivided cell, using two graphite carbon electrodes. The reaction was complete after 2.5 F/mol with a constant current of 10 mA (current density: 1.25 mA/cm^2). HPLC analysis (MeOH/AcOH 0.1%, 50/50) of the reaction mixture revealed the presence of furaldehyde (**7**) and aniline (**6**). 1% of diacetal (**8**) was also detected, indicating the good chemoselectivity of the oxidation (Scheme 2).



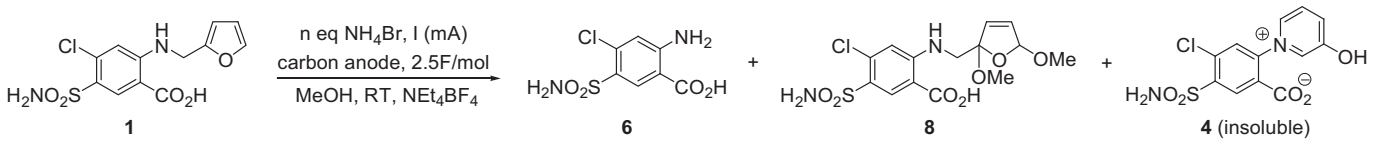
Scheme 2. Direct oxidation of furosemide (**1**) in MeOH.

In an attempt to isolate the imine (**5**), size exclusion chromatography (MeOH/CH_2Cl_2, 50/50) was conduced on the concentrated reaction mixture. Unfortunately, no trace of imine could be isolated and a pure fraction of aniline (**6**) was obtained with 45% yield.

The mediated oxidation was also tested. In the presence of ammonium bromide (20 mol %) the reaction was done at constant current of 10 mA (current density: 1.25 mA/cm^2) in an undivided cell, at room temperature. In such conditions, the reaction was complete after 2.5 F/mol and appeared to be chemoselective in favor of the diacetal (**8**), since no trace of aniline (**6**) was observed by HPLC. Concentration of the reaction mixture gave a solid, which was triturated with ethyl acetate. The mixture of dimethoxylated diastereoisomers (**8**) was obtained after evaporation of the solvent and without any further purification with 88% yield.

With the aim of both scaling up the process and keeping a reasonable reaction time, the mediated oxidation was led at 30 and 50 mA. The influence of bromide concentration was studied in both cases. The composition of the solution was analyzed after 2.5 F/mol by HPLC (MeOH/AcOH 0.1%, 50/50) and quantification of aniline (**6**) and diacetal (**8**) was done using authentic standard (Table 1). Clearly, higher intensity revealed a negative effect on the chemoselectivity. In the presence of 20% of ammonium bromide,

Table 1
Mediated oxidation of furosemide (**1**) in MeOH (HPLC monitoring)



Entry[a]	*n* (equiv NH_4Br)	*I*=30 mA[b]		*I*=50 mA[c]	
		6	**8**	**6**	**8**
1	0.2	31	27	10	11[d]
2	0.4	23	27	23	11
3	0.6	29	38	15	31
4	0.8	0	91	15	42

[a] Conditions: 0.26 mmol of (**1**), 10 mL MeOH, carbon electrodes, 0.13 mmol NEt_4BF_4, rt.
[b] *J*=3.75 mA/cm^2.
[c] *J*=6.25 mA/cm^2.
[d] 3% of (**1**) observed.

significant amount of aniline (**6**) was observed at 30 mA, whereas no trace had been previously detected at 10 mA (vide supra). This is consistent with the idea of a competition between mediated and direct oxidation, the latter appearing when the capacity of the first is overtaken.

To circumvent the production of aniline, the concentration of bromide was increased. At 30 mA, 80% of bromide were necessary to obtain a complete selectivity (entries 2–4). At 50 mA, the same behavior is observed although the production of aniline could not been totally prevented (even with 100% of bromide).

Besides the problem of chemoselectivity, the increase of current intensity has also resulted in driving the formation of products other than those expected. Among them, the pyridinium (**4**) resulting from the acid decomposition of the desired diacetal (**8**) was obtained. If the quantification of pyridinium has not been possible by HPLC because of its very low solubility, it has been however observed at 30 and 50 mA. The strong acidity locally generated at the surface of the electrode and resulting from the oxidation might explain this unwanted decomposition. The presence of inorganic base in the mixture, such as cesium carbonate, did not improve the yield of (**8**), other side-products appearing during the reaction.

Pyridinium (**4**), which revealed to be insoluble in most of the organic solvents (except polar ones, such as DMSO), was easily obtained in a quantitative yield after acidic hydrolysis of (**8**) at 40 °C in a (60/40) mixture of aqueous TFA (10 mM)/MeCN and filtration.

3. Discussion

Anodic oxidation has demonstrated its ability to oxidize with an excellent selectivity the amine function as well as the furan ring of furosemide, depending on the presence or not of bromide in the reaction mixture. Surprisingly, if the anodic oxidation of furan rings in the presence of bromide ions[15] has been abundantly described and successfully applied to organic synthesis,[16] the possibility to oxidize the furan ring of furosemide remains somewhat unexpected, given the potentials measured by cyclic voltametry. In the case of furosemide, it might be suggested that a first oxidized form (only obtained in the presence of bromide, such as a bromamine) reacts in an intramolecular fashion to give the diacetal (**8**).

With this diacetal, our aim was to obtain a synthetic equivalent of epoxide (**2**) or alternatively of ketoenal (**3**). Unfortunately, this compound revealed an unexpected stability: surprisingly strong acidic conditions (pH=2.2) were indeed required to activate it. More notably, attempts to obtain adducts from this diacetal in the presence of *N*-acetyl cysteine or *N*-acetyl lysine,[7] remained unsuccessful: pyridinium (**4**) was invariably obtained.

For this reason, the preparation of more reactive species than (**8**) was attempted. Anodic oxidation of furosemide in trifluoroethanol or in a MeCN/AcOH mixture was done in order to obtain, respectively, the fluorinated diacetal or the diacetoxylated derivative.[17] None of the expected products could be obtained.

It should be also noted that in all our reactions and in accordance with the observation done on analytical scale,[8] no significant amount of γ-ketoenal (**3**) could be isolated or has even been observed. This led us to postulate that this is a species, whose presence in the environment seems very unlikely, as that of the imine (**5**).

However, it is clear from this work that aniline (**6**) and pyridinium (**4**) are two furosemide TPs whose presence (or even accumulation) in the environment is very likely. Concerning the aniline (which do not enter in the synthesis of furosemide[18]), it seems that no data refers to its toxicity, even though this compound has long been known as a derivative of furosemide.[12] Regarding the pyridinium, the synthesis presented here is the first on preparative scale: this explains the absence of data (such toxicological) relating to it.

In order to initiate the study of pyridinium (**4**), a cyclic voltametry analysis was done in DMSO. The fact that no peak was observed in oxidation, led us to suppose that this compound should be a more persistent contaminant than the furosemide itself. On the other hand, the reduction peak observed at −1.98 V versus SCE (Fig. 2), highlighted its ability to behave as an electron acceptor. The comparison with ferrocene indicated monoelectronic oxidant properties (considering a charge transfer coefficient α=0.45).

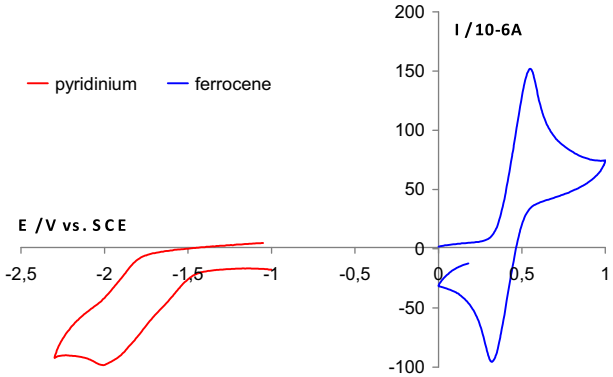


Fig. 2. Cyclic voltametry in DMSO (500 mV/s); 0.1 M NEt_4BF_4; glassy carbon electrode.

Unlike most of viologens,[19] pyridinium (**4**) showed an irreversible reduction process presumably due to the recombination of the resulting radicals. Interestingly, the reduction peak also appears at much lower potential than those usually observed for dipyridinium and pyridinium salts. This latter point seems to exclude the possibility for pyridinium (**4**) to be reduced by NADPH and NADH. However, the case of neurotoxic pyridinium salts also unable to undergo bio-reduction[20] should prompt further investigations.

4. Conclusions

It appears from this work that aniline (**6**) and pyridinium (**4**) are susceptible to be present in the environment as TPs of furosemide. Their access on preparative scale allows now to focus on them, toxicological and environmental investigations.

The access to both compounds was easily led by anodic oxidation. This reaction revealed to be highly chemoselective, the nature of the oxidized moiety depending on the presence or not of bromide in the reaction mixture. This versatility illustrated the relevance of the electrochemical tool in the elaboration of new heterocyclic structures.

5. Experimental section

5.1. Chemistry

All chemicals were purchased from either Aldrich or Acros organic. Solvents were commercially available and used as received without any further purification.

5.2. Analysis procedures

The electrochemical experiments were performed with voltalab Radiometer analytical instruments (PST006) and were carried out in a 20 mL single compartment three-electrode glass cell with a 1 mm diameter glassy carbon electrode (GCE) as working electrode, a gold wire as counter electrode, and a saturated calomel electrode as reference electrode. All experiments were carried out at ambient temperature. The GCE was polished using 0.3 μ Al_2O_3 before each experiment.

The GCE was placed in 0.1 M Et_4NBF_4 electrolyte and various voltammograms were recorded from 25 to 500 mV/s. The cyclic voltammograms of the investigated compounds were obtained in methanol.

Analysis of reaction mixtures was done by reverse phase chromatography using a VWR Hitachi Elite LaChrom HPLC chromatograph, controlled by an EZCHROM elite program. It was fitted with a quaternary pump Hitachi L-2130, a Hitachi column oven L-2300 with thermostat at 40 °C, and a Purospher RP-18 column (5 μm, 250 mm×4.6 mm) coupled with a photodiode array detector (L-2455) at 233 nm. The column was eluted at isocratic mode with a mobile phase composed of MeOH/AcOH 0.1% (50/50 v/v) at a flow rate of 0.8 mL/min and sample volumes were 20 μL.

*5.2.1. 2-Amino-4-chloro-5-sulfamoyl-benzoic acid (**6**).* Furosemide (0.5 mmol) was dissolved in methanol (10 mL) and tetraethylammonium tetrafluoroborate (0.4 mmol) was added and employed as the supporting electrolyte. The furosemide was oxidized at room temperature at a plate of carbon (I=10 mA) in undivided cell under magnetic agitation. After consumption of 2.5 electrons per molecule, the electrolysis was stopped. The methanol was removed under reduced pressure and the residue was purified by size exclusion chromatography on Sephadex LH-20 (61 mg, 45%). The expected product was obtained as a white solid. t_R(HPLC)= 2.83 min; mp 278 °C (decomp.); ^{1}H NMR (400 MHz, acetone-d_6): δ=8.54 (s, 1H), 7.16 (br s, 2H), 7.02 (s, 1H), 6.50 (br s, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ=168.7, 155.4, 137.0, 134.9, 128.0, 118.7, 108.0; IR: 3498, 3370, 3248, 1662, 1616, 1536, 1419, 1326, 1241, 1159, 1127, 968, 882, 842, 715, 686, 628, 597 cm^{-1}; HRMS m/z calcd for $C_7H_8ClN_2O_4S$ $[M+H]^+$: 250.9888. Found: 250.9831.

*5.2.2. 4-Chloro-2-[(2,5-dimethoxy-2,5-dihydro-furan-2-ylmethyl)-amino]-5-sulfamoyl-benzoic acid (**8**).* Into an undivided glass cell equipped with two plates of carbon electrodes were dissolved furosemide (250 mg, 0.75 mmol), ammonium bromide (61 mg, 0.6 mmol), and tetraethylammonium tetrafluoroborate (81 mg, 0.4 mmol) in methanol (10 mL). Magnetic stirrer was used to get an agitation of the reaction solution. A constant current of 30 mA passed through reaction until 2.5 F/mol of charge was consumed. The solution was transferred to a round bottom flask and methanol was removed under reduced pressure. The residue was extracted with ethyl acetate and the inorganic salt was eliminated by filtration. Ethyl acetate was evaporated and a beige solid was obtained. Two diastereomers were present in a 50/50 ratio (268 mg, 91%). t_R(HPLC)= 4.78 min (dia 1)–5.52 min (dia 2); mp 136–140 °C; ^{1}H NMR (400 MHz, acetone-d_6): δ=8.55 (s, 2H), 7.07 (s, 1H), 7.05 (s, 1H), 6.22–6.15 (m, 2H), 6.05 (d, J=13.4 Hz, 1H), 6.03 (d, J=13.5 Hz, 1H), 5.79 (br s, 1H), 5.52 (br s, 1H), 3.72–3.61 (m, 2H); 3.57–3.47 (m, 2H); 3.45 (s, 3H), 3.31 (s, 3H), 3.20 (s, 3H), 3.12 (s, 3H); ^{13}C NMR (acetone-d_6): δ=169.3, 169.1, 154.8, 154.5, 138.0, 137.8, 135.0, 135.0, 134.2, 134.2, 132.4, 132.1, 127.4, 127.2, 114.9, 114.7, 114.6, 114.0, 109.9, 108.6, 108.5, 56.4, 56.0, 50.7, 50.4, 50.1, 49.9; IR: 3257, 1674, 1595, 1503, 1455, 1331, 1229, 1162, 1043, 1012, 957, 899, 832, 799, 686, 626, 579 cm^{-1}; HRMS m/z calcd for $C_{14}H_{18}ClN_2O_7S$ $[M+H]^+$: 393.0523. Found: 393.0445.

*5.2.3. 1-(2-Carboxylate-5-chloro-4-sulfamoyl-phenyl)-3-hydroxy-pyridinium (**4**).* This compound was obtained by dissolving (**8**) (136 mg, 0.3 mmol) in 2 mL of a 60/40 mixture of TFA (10 mM)/MeCN at 40 °C. The product **4** was obtained after filtration as a white solid (126 mg, 100%). Mp 258–260 °C; ^{1}H NMR (100 MHz, DMSO-d_6): δ=8.78 (br s, 1H), 8.59 (br s, 1H), 8.50 (s, 1H), 8.09 (s, 1H), 8.00–7.87 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ=164.8, 158.0, 143.2, 142.7, 134.9, 134.4, 133.1, 133.1, 131.5, 131.4, 129.8, 127.4; IR: 3096, 1613, 1573, 1494, 1379, 1321, 1252, 1155, 1026, 963, 927, 865, 805, 694, 654, 603, 584 cm^{-1}; HRMS m/z calcd for $C_{12}H_{10}ClN_2O_5S$ $[M+H]^+$: 328.9999. Found: 328.9995. Anal. Calcd for $C_{12}H_9ClN_2O_5S\cdot 2H_2O$: C 39.51%, H 3.59%, N 7.68%. Found: C 39.47%, H 3.61%, N 7.46%.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2011.10.006.

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