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1 **Contrasting photoreactivity of β 2-adrenoceptor agonists Salbutamol**
2 **and Terbutaline in the presence of humic substances**

3

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23

24 **Abstract.**

25 The photodegradation reactions of two typical β 2-adrenoceptor agonists, salbutamol (SAL)
26 and terbutaline (TBL), alone, and in the presence of Aldrich humic acid (AHA) or Suwannee
27 River fulvic acid (SRFA) were investigated by steady-state photolysis experiments, laser flash
28 photolysis (LFP), kinetic modeling and quantum calculation. AHA and SRFA (2-20 mgC L⁻¹)
29 accelerated the phototransformation of both SAL and TBL. For SAL, an inhibiting effect of
30 oxygen on the photodegradation was observed that is fully consistent with the main
31 involvement of excited triplet states of HS (³HS*). On the contrary, oxygen drastically
32 enhanced the photodegradation of TBL showing that ³HS* were negligibly involved in the
33 reaction. The involvement of singlet oxygen was also ruled out because of the low reaction
34 rate constant measured between TBL and singlet oxygen. Quantum calculations were
35 therefore performed to explore whether oxygenated radicals could through addition reactions
36 explain the differences of reactivity of TBL and SAL in oxygen medium. Interestingly,
37 calculations showed that in the presence of oxygen, the addition of phenoxyl on TBL led to
38 the formation of adducts and to the loss of TBL while the same addition reaction on SAL
39 partly regenerated the starting compound and at the end degraded SAL less efficiently. This
40 study is of high relevance to understand the processes involved in SAL and TBL
41 phototransformation and the photoreactivity of HS. Moreover, our findings suggest that TBL
42 might be a promising probe molecule to delineate the role of oxygenated radicals.

43

44 **Keywords:** Beta2-adrenoceptor agonists; Photolysis; Humic substances; Oxygenated radicals;
45 Substituted phenols.

46

47

48

49 **1. Introduction**

50 A significant volume of pharmaceuticals are used by humans for the treatment of
51 diseases, injuries, or illnesses, and the occurrence of these chemicals in environment has been
52 widely recognized by scientists, especially in the aquatic environment (Deo, 2014). A wide
53 range of pharmaceuticals are frequently detected in surface, ground and coastal waters with a
54 concentration usually ranging from ng/L to $\mu\text{g/L}$ (Weber et al., 2016; Li, 2014). The continual
55 infusion of pharmaceuticals into surface and drinking waters may lead to chronic exposure to
56 microorganisms and even humans (Crane et al., 2006).

57 Sunlight-induced direct and indirect photolysis are the primary pathways for abiotic
58 transformation of pharmaceuticals in surface waters (Lee et al., 2014). The direct photolysis
59 of pharmaceuticals results from absorption of solar light by the compound itself, while the
60 indirect photolysis is initiated by light absorption of photo-initiators such as nitrate ions or
61 photosensitizers such as humic substances (HS). HS are brown-colored compounds and show
62 interesting properties under solar irradiation. They can generate reactive species, including
63 triplet excited state ($^3\text{HS}^*$), hydroxyl radicals ($\bullet\text{OH}$), singlet oxygen ($^1\text{O}_2$) and radicals that
64 have all been the subject to an intense research (Zepp et al., 1985; McNeill and Canonica,
65 2016; Latch and McNeill, 2006; Wang et al., 2007; Blough, 1988; Coelho et al, 2011). The
66 fate of pharmaceuticals in surface waters was shown to be affected by HS (Zhou et al., 2003;
67 Silva et al, 2016; Chen et al., 2017).

68 Two β_2 -adrenoceptor agonists, namely, salbutamol (SAL, also known as albuterol) and
69 terbutaline (TBL) were selected as the target molecules of this study due to their intense uses.
70 SAL is widely used in the treatment of respiratory diseases, such as asthma and chronic
71 obstructive airways disease (Kinali et al., 2002), it has been detected in the surface waters of
72 several European countries with concentrations ranging from 1 to 471 ng L^{-1} (Calamari et al.,
73 2003; Bound and Voulvoulis, 2006; Ternes, 1998). TBL is considered as one of the major

74 feed additive medicines to increase the proportion of lean meat of livestock (Slotkin and
75 Seidler, 2013). Several studies have been devoted to the phototransformation of SAL and
76 TBL. The quantum yield of photolysis of SAL was measured to be 0.02 for the neutral form
77 and 0.36 for the anionic form upon irradiation between 280 and 380 nm (Dodson et al., 2011;
78 Zhou et al., 2017a). In a study on 27 pharmaceutical molecules SAL and TBL were used
79 along to to establish a correlation between pharmaceuticals photodegradation and the decay of
80 effluent organic matter fluorescence peaks (Yan et al, 2017). SAL and TBL were found to
81 react with the triplet excited states of the effluent organic matter. Another study reported that
82 singlet oxygen contributed highly (19-44%) to the TBL decay when natural organic matter
83 isolates were used to photosensitize the photodegradation of TBL (Yang et al., 2013).
84 Recently, SAL and TBL were found to be both oxidized by sulfate radicals and OH radicals,
85 but interestingly they gave different patterns of reaction. Although the phenoxyl radicals were
86 generated in both cases, SAL finally yielded a benzophenone derivative while TBL gave an
87 adduct by addition of the sulfate radical on the ring (Zhou et al., 2017b).

88 Here, we compared the phototransformations of SAL and TBL in the presence of two
89 selected HS under solar-light simulated irradiation. A special attention was given to the nature
90 of reactive species involved, and to the photoproducts characterization. This study revealed
91 that oxygen affected the two molecules phototransformation very differently and quantum
92 calculations were performed to better understand the differences in the reactivity. The results
93 obtained from this study would be helpful in predicting the photochemical fate of β_2 -
94 adrenoceptor agonists, as well as the impact of HS on similar emerging contaminants.

95

96 **2. Material and methods**

97 **2.1 Chemicals.**

98 Salbutamol hemisulfate (SAL, 98%), terbutaline hemisulfate (TBL, 98%), formic acid (FA,
99 96%), furfuryl alcohol (FFA, 98%), *p*-nitroanisole (PNA, 97%), pyridine (PYR, 96.5%) and
100 sorbic acid (SA, 98%) were purchased from Sigma-Aldrich. 3-Carboxybenzophenone (99%)
101 was purchased from ACROS and Suwannee River fulvic acid (SRFA, 1R101F) of reference
102 grade was obtained from the International Humic Substances Society (IHSS). Humic acid
103 (AHA) was provided by Sigma-Aldrich. HPLC or LC-MS grade acetonitrile (ACN), methanol
104 (MeOH), and 2-propanol were supplied by Fisher Chemical. Other reagents were at least of
105 analytical grade and used as received without further purification. Milli-Q water (18.2 MΩ
106 cm) was prepared from a Millipore Milli-Q system. Oxygen and helium used for oxygenation
107 and deoxygenation were of ultrapure quality (Air Products).

108 **2.2 Photochemical Experiments.**

109 Steady-state irradiation experiments were conducted in a home-made photochemical reactor
110 equipped with a 125 W high pressure mercury lamp (Cathodeon, Cambridge, UK) housed in a
111 borosilicate immersion wall ($\lambda > 290$ nm) (Figure SM-1, Supplementary Material). The lamp
112 was turned on for 20 min prior to irradiations for warming, then, 50 mL of solutions were
113 poured in a Pyrex-glass reactor (i.d. = 5 cm, height = 15 cm) and irradiated for different times.
114 The reactor placed in the photo-reactor was maintained at 24 ± 1 °C using a cooling water
115 circulation system. Typically, the solutions were maintained in contact with air. Some
116 experiments were also conducted on over-oxygenated or deoxygenated solutions. For this, we
117 bubbled a stream of O₂ in the first case, and a stream of helium in the second case during the
118 whole irradiations. The pH of the starting solutions was adjusted to 8 using phosphate buffers.
119 2-propanol (10 mM) was used as an OH radical scavenger to measure the involvement of OH
120 radicals. Furfuryl alcohol (60 μM) was used as a ¹O₂ probe and sorbic acid (SA, 100 μM) as a
121 triplet scavenger to confirm the involvement of ³HS*. Aliquots of 1 mL of the irradiated

122 solutions were withdrawn at predetermined times and analyzed directly by HPLC. The
123 detailed analytical methods are described in the Supplementary Material (Text SM-1).

124 **2.3 Laser flash photolysis experiments.** Laser flash photolysis (LFP) experiments were
125 carried out using a Quanta Ray GCR 130 Nd: YAG laser and an Applied Photophysics station
126 previously described (Bonnichon et al., 1998). The aqueous solutions were irradiated at 266
127 nm using the fourth harmonic of the laser. 3-carboxybenzophenone (CBP) was selected as a
128 model sensitizer of HS. The rate constants of reactions of SAL and TBL with $^3\text{CBP}^*$ were
129 measured by monitoring the decay of $^3\text{CBP}^*$ at 520 nm. A red filter was placed between the
130 cell and the photomultiplier to avoid parasitic signals. SAL and TBL were added in the
131 concentration range (0-400 μM).

132 **2.4 Quantum calculations.** The Gaussian09 series of programs (Frisch et al., 2010) was used
133 to perform Density Functional Theory (DFT) calculations at the B3LYP/6-31+G(d,p) level.
134 The Polarizable Continuum Model (PCM) (Tomasi et al., 2005) was used throughout to
135 model the solvent (water) implicitly. Calculations were performed to obtain Transition States,
136 minimum energy structures, Intrinsic Reaction Coordinate (IRC) paths and Minimum Energy
137 Pathways (MEP). An extensive search for conformers was pursued first in order to locate the
138 global minimum energy species (geometry given in SI section). This was achieved by a
139 systematic investigation of the variations of the Potential Energy Surface (PES) along key
140 dihedral angles.

141

142 **3. Results and discussion**

143 **3.1 Direct photodegradation**

144 In air-saturated medium and at pH 8.0, both substrates underwent photolysis. Pseudo-
145 first-order kinetics fitted the photodegradation curves of SAL and TBL until conversion
146 extents of 50 % (Eq 1):

147
$$\ln([P]_t/[P]_0) = -k_{\text{obs}} t \quad (1)$$

148 where P represents the target compound (SAL or TBL), and k_{obs} (s^{-1}) the apparent first-order
149 degradation rate constant. TBL (60 μM) was photodegraded faster ($k_{\text{obs}} = (6.9 \pm 0.2) \times 10^{-5} \text{ s}^{-1}$)
150 than SAL ($k_{\text{obs}} = (3.0 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$) (Figure 1). The photodegradations occurred due to the
151 overlapping of the absorption spectra of TBL and SAL with the emission spectrum of the
152 HPK lamp filtered by the Pyrex glass reactor ($> 290 \text{ nm}$). Laser flash photolysis experiments
153 confirmed the photoionization processes and the formation of phenoxy radicals ($\text{SAL}_{\text{-H}}^{\bullet}$ or
154 $\text{TBL}_{\text{-H}}^{\bullet}$) (Zhou et al., 2017b).

155

156 **3.2 Accelerating effect of HS in air-saturated medium**

157 Then, SRFA and AHA were added to SAL and TBL solutions at concentrations
158 comprised between 2 and 20 mgC L^{-1} . Figure 1 reveals that the photodegradations of SAL and
159 TBL in air-saturated solution were significantly enhanced by the addition of SRFA or AHA.
160 Moreover, increasing the HS concentrations led to an increase of k_{obs} values. The
161 enhancement was more pronounced for TBL than for SAL. For instance, in the presence of 20
162 mgC L^{-1} of AHA, the degradation rate constant of TBL was 3.8-fold higher compared to that
163 of the control sample (k_{obs} equal to $38 \times 10^{-5} \text{ s}^{-1}$ against $7.0 \times 10^{-5} \text{ s}^{-1}$ without AHA), while in the
164 case of SAL, k_{obs} was only 1.9 fold higher ($5.8 \times 10^{-5} \text{ s}^{-1}$ against $3.0 \times 10^{-5} \text{ s}^{-1}$ for the control
165 sample without AHA).

166

166 **Figure 1**

167

168 **3.3 Photoproducts.**

169 We investigated the effect of AHA and SRFA on the SAL and TBL photoproducts. In
170 general, the same photoproducts were detected in the absence and in the presence of 10 mgC
171 L^{-1} of HS (details in Table SM-2 and SM-3, SM). In the case of SAL, we identified by HPLC-

172 MS analysis 2-(*tert*-butylamino)-1-(4-hydroxyl-3-hydroxymethylphenyl) ethanone (SAL-_{2H}),
173 2-(*tert*-butylamino)-1-(4-hydroxyl-3-hydroxymethylphenyl) ethylene (SAL-_{H₂O}) and the
174 hydroxylated product of SAL-_{2H} (SAL-_{2H+O}) as the main photoproducts (Figure 2). For TBL,
175 2-(*tert*-butylamino)-1-(2,3,5-trihydroxyphenyl) ethanol (TBL-_{H+OH}) and 5-[2-(*tert*-
176 butylamino)-1-hydroxyethyl]-2-hydroxyl-1,4-benzoquinone (TBL-_{+O-2H}) were found (Figure 2).
177 Some of these photoproducts were reported in the literature, in particular the
178 hydroxybenzoquinone derivative of TBL (TBL-_{+O-2H}) (Yang et al, 2013; Yan et al, 2017).
179 However, from the quantitative point of view, we got some differences. After a conversion
180 extent of 10%, the area of the SAL photoproduct detected in HPLC-UV was of 1100 ± 50 at
181 224 nm, both in the absence as in the presence of AHA, while the area of the TBL
182 photoproduct showed a peak area of 2500 ± 53 at 230 nm in the absence of AHA, but of only
183 700 ± 20 in the presence of AHA. These results suggest that a significant change of reaction
184 occurred for TBL but not for SAL.

185 **Figure 2**

186

187 **3.4 Reactivity of SAL and TBL toward singlet oxygen and ³CBP*.**

188 When irradiated in the presence of HS, SAL and TBL might react with ¹O₂ and ³HS*.
189 To further evaluate the contribution of these species in the HS-mediated photoreactions, we
190 first measured their reactivities with our substrates. The reaction rate constants of SAL and
191 TBL with ¹O₂ were obtained using Rose Bengal as a sensitizer, and FFA as a reference
192 compound, as described in Text SM-2 of SM. The reactivities of SAL and TBL with ³HS*
193 were estimated using CBP as a proxy HS sensitizer (Avetta et al, 2016).

194 In the case of TBL, a rate constant of $(8.0 \pm 0.3) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ at pH 8.0 was determined
195 for the reaction with ¹O₂. This is comparable to the value of $(7.1 \pm 0.3) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ reported in
196 the literature at pH 7.0 by Yang et al., 2013. For SAL a reaction rate constant of (1.1 ± 0.1)

197 $\times 10^8 \text{ M}^{-1}\text{s}^{-1}$, i.e. ~14 fold faster than that of TBL was determined. The reaction rate constants
198 of $^3\text{CBP}^*$ with TBL and SAL were directly measured by laser flash photolysis. We got $(2.7 \pm$
199 $0.3) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ and $(6.0 \pm 0.6) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, respectively.

200

201 **3.5 Reactive species involved in the HS-mediated photoreactions.**

202 In this section, we investigated the effect of AHA and SRFA (5 mgC L^{-1}) on the rate
203 of SAL and TBL photodegradation. In these conditions, k_{obs} is the sum of 2 terms, one
204 corresponding to the direct photolysis and the other to the sensitized or indirect reaction. At
205 the level of 5 mgC L^{-1} AHA and SRFA reduced the absorbances of SAL and TBL by 60%
206 and 35%, respectively, through screen effect filter (Table SM-4, SM). Therefore, the direct
207 photodegradation rate constants of SAL and TBL (k_{direct}) have to be corrected for this screen
208 effect. The corrected k_{direct} are finally equal to $1.2 \times 10^{-5} \text{ s}^{-1}$ and $2.0 \times 10^{-5} \text{ s}^{-1}$ for SAL in the
209 presence of AHA and SRFA and to $2.6 \times 10^{-5} \text{ s}^{-1}$ and $4.2 \times 10^{-5} \text{ s}^{-1}$ for TBL in the presence of
210 AHA and SRFA (Table 1). Hereinafter, the indirect degradation rate constants k_{indirect} will be
211 calculated according to:

$$212 \quad k_{\text{indirect}} = k_{\text{obs}} - \text{corrected } k_{\text{direct}} \quad (2)$$

213 *i) Hydroxyl radical.* In order to test the involvement of $\text{HO}\cdot$, 10 mM of 2-propanol, used as a
214 $\text{HO}\cdot$ scavenger ($k = 1.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$), were spiked into the reaction solutions. The addition of
215 2-propanol in excess had no effect on the SAL photodegradation rate and decreased that of
216 TBL by less than 10% (Figure SM-2, SM). Thus, the involvement of $\text{HO}\cdot$ in the reactions was
217 very limited despite the high reactivity of SAL and TBL with $\text{OH}\cdot$ ($k_{\cdot\text{OH}, \text{SAL}} = (5.3 \pm 0.7) \times 10^9$
218 $\text{M}^{-1} \text{ s}^{-1}$ and $k_{\cdot\text{OH}, \text{TBL}} = (5.9 \pm 0.8) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) (Zhou et al., 2017b).

219 *ii) Humic triplet state.* To investigate the contribution of $^3\text{HS}^*$ in the HS-mediated
220 photodegradations, experiments were conducted at different oxygen concentrations because
221 oxygen is a well-known triplet scavenger. Removal of oxygen by continuous helium (He)

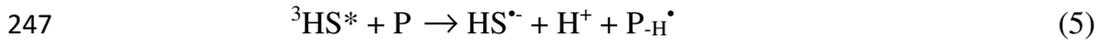
222 purging should led to an increase of the steady-state concentration of $^3\text{HS}^*$, $[\text{}^3\text{HS}^*]_{\text{ss}}$ and thus
 223 to an increase of k_{indirect} if $^3\text{HS}^*$ is involved in the reactions. As seen in Figure 3, the rate of
 224 SAL phototransformation in the presence of AHA and SRFA was much higher in helium-
 225 purged solution than that in air-saturated medium. The indirect rate constants were in the
 226 range $(17-18) \times 10^{-5} \text{ s}^{-1}$ in He-saturated medium against $(2.8-3.1) \times 10^{-5} \text{ s}^{-1}$ in air-saturated
 227 medium (Table 1). This result demonstrates the involvement of $^3\text{HS}^*$ in the reactions. The
 228 involvement of $^3\text{HS}^*$ was confirmed by adding the triplet quencher, sorbic acid (SA, 100 μM)
 229 in the presence of which the SAL indirect photodegradation rate constant was reduced by a
 230 factor of 2 in air-saturated solution. In contrast, for TBL, the solution deoxygenation through
 231 He purging reduced the indirect rate to $(3.2-3.3) \times 10^{-5} \text{ s}^{-1}$ against $(12.1-18.9) \times 10^{-5} \text{ s}^{-1}$ (Table
 232 1). This result is opposite to the one found for SAL. Afterwards, the addition of SA (100 μM)
 233 did not show a significant effect on k_{obs} , confirming that $^3\text{HS}^*$ contributed in a minor way to
 234 the TBL photodegradation.

235 At last, the solutions of SAL and TBL containing SRFA or AHA were irradiated after
 236 having been saturated by oxygen. For SAL, k_{indirect} became very low $(0.2-0.8) \times 10^{-5} \text{ s}^{-1}$ while
 237 in the case of TBL, k_{indirect} was close to the one measured in air-saturated medium (Figure 3
 238 and Table 1).

239 **Figure 3**

240 To rationalize these results, mechanistic analysis is necessary. In a simplified
 241 mechanism, we considered that $^3\text{HS}^*$ produced in (3) undergoes desactivation (4), oxidizes
 242 SAL and TBL, labeled as P, by electron transfer or H atom abstraction (5), or reacts with
 243 oxygen to yield $^1\text{O}_2$ (6). We make the hypothesis that the yield of $^1\text{O}_2$ formation in process (6)
 244 is equal to 1:





251 Photoproducts of P are expected to arise from further reactions of $\text{P}\text{-H}^{\cdot}$ and from reaction (7).

252 The lowest triplet (E_{T1}) excitation energy of SAL was calculated as 3.68 eV in our previous
 253 work (Zhou et al., 2017a), which is larger than that of most kinds of HS (2.23 – 2.60 eV, for
 254 SRFA, E_{T1} was 2.37 eV) (Zhang et al., 2010). Thus, energy transfer from ${}^3\text{HS}^*$ to SAL is
 255 not possible from a thermodynamical point of view, and we excluded this reaction in the
 256 proposed mechanism.

257 In He-saturated solution, the rate of HS-mediated phototransformation of P is equal
 258 to:

259
$$r^{\text{He}}_{\text{indirect}} = k_5[{}^3\text{HS}^*]_{\text{SS}}[\text{P}]$$
 (9)

260 where $[{}^3\text{HS}^*]_{\text{SS}}$ the steady-state concentrations of ${}^3\text{HS}^*$ is equal to:

261
$$[{}^3\text{HS}^*]_{\text{SS}} = I_a^{\text{HS}} \Phi_T^{\text{HS}} / (k_4 + k_5[\text{P}])$$
 (10)

262 with I_a^{HS} represents the rate of photon absorption by HS and Φ_T^{HS} is the quantum yield
 263 of ${}^3\text{HS}^*$ generation.

264 In air-saturated solution, the rate of indirect P phototransformation can be written as :

265
$$r^{\text{Air}}_{\text{indirect}} = k_5[{}^3\text{HS}^*]_{\text{SS}}[\text{P}] + k_7[{}^1\text{O}_2]_{\text{SS}}[\text{P}]$$
 (11)

266 with

267
$$[{}^3\text{HS}^*]_{\text{SS}} = \frac{I_a^{\text{HS}} \Phi_T^{\text{HS}}}{k_4 + k_6[\text{O}_2] + k_5[\text{P}]}$$
 (12)

268

269 As the rate constants of reaction with $^1\text{O}_2$ are respectively 55 and 32-fold lower than
270 the rate constants of reaction with $^3\text{CBP}^*$ for SAL and TBL, Eq 11 can be simplified, in a
271 first approximation, by neglecting the term corresponding to $^1\text{O}_2$. This gives:

$$272 \quad r^{\text{P, He}}_{\text{indirect}} / r^{\text{P, Air}}_{\text{indirect}} = (k_4 + k_6[\text{O}_2] + k_5[\text{P}]) / (k_4 + k_5[\text{P}]) \quad (13)$$

273 If the scheme and assumptions are correct, the experimental ratios must be higher than 1. It
274 is the case for SAL with 6.3 in the case of SRFA and 5.8 in the case of AHA. Taking $k_4 \approx$
275 $5 \times 10^4 \text{ s}^{-1}$ (Huber et al., 2003), $k_6[\text{O}_2] \approx 5 \times 10^5 \text{ s}^{-1}$ (data provided by Canonica et al., 1995 in
276 the case of natural waters), we finally get $k_5 \approx 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This value is consistent with
277 data obtained with other electron-rich phenols (McNeill and Canonica, 2016; Canonica et
278 al., 1995), but it is 6-fold lower than that measured using 3-carboxybenzophenone as a
279 sensitizer.

280 For TBL however, the ratio is much less than 1 (0.26 and 0.17 for SRFA and AHA,
281 respectively) showing that assumptions/scheme are not working in this particular case. In
282 air-saturated solution, the percentage of $^3\text{HS}^*$ trapped by oxygen is equal to
283 $100 \times k_6[\text{O}_2] / (k_6[\text{O}_2] + k_4 + k_5[\text{P}])$ and thus to 80%. On the other hand, the percentage of $^1\text{O}_2$
284 trapped by TBL is equal to $100 \times k_7[\text{TBL}] / (k_7[\text{TBL}] + k_8[\text{H}_2\text{O}])$. Given the value of $k_7 = (8.0$
285 $\pm 0.3) \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, and $k_8 \approx 2.3 \times 10^5 \text{ s}^{-1}$ in water (Rodgers and Snowden, 1982), TBL at the
286 concentration of 60 μM only trapped 0.2% of $^1\text{O}_2$. If we assume that SAL trapped all the
287 $^3\text{HS}^*$ in the absence of oxygen, it gives a rate of $^3\text{HS}^*$ formation of $1.1 \times 10^{-8} \text{ M s}^{-1}$ and a
288 rate of formation of $^1\text{O}_2$ of $0.88 \times 10^{-8} \text{ M s}^{-1}$ in air-saturated medium. The percentage of $^1\text{O}_2$
289 trapped by TBL being of 0.2%, the rate of reaction of TBL with $^1\text{O}_2$ was equal to 1.8×10^{-11}
290 M s^{-1} fully negligible behind the rate of TBL decay $1.1 \times 10^{-8} \text{ M s}^{-1}$ for AHA and $7.2 \times 10^{-9} \text{ M}$
291 s^{-1} for SRFA, ruling out a significant importance of this pathway in the HS-mediated
292 phototransformation.

293 The low rate of TBL photodegradation in the absence of oxygen is not consistent
294 with the high reactivity of TBL with $^3\text{HS}^*$ deduced from the high rate constant measured
295 between $^3\text{CBP}^*$ and TBL. The explanation of this apparent discrepancy could be that TBL
296 is actually oxidized by $^3\text{HS}^*$ at a significant rate (process 5) but regenerated through
297 process (5'). Such reactions have been shown to take place (Vione et al, 2018).



299 If reaction (5') also occurs for SAL, the rate constant k_5 deduced from Eq 13 may be
300 minimized. This is in accordance with the low value of calculated k_5 from Eq 13 compared to
301 that obtained in the experiment with $^3\text{CBP}^*$.

302 The possible existence of reaction 5' does not explain however why in air and oxygen
303 saturated media the photodegradation of TBL was so fast. Apart commonly mentioned
304 reactive species, radicals that are surely formed in significant amounts in irradiated HS
305 solutions (Blough, 1988) might be responsible for these our results and this option needs to be
306 explored. Due to the difficult to generate oxy and peroxy radicals in water by selective
307 excitation, we performed quantum calculations to determine in which conditions and for
308 which reasons oxygenated radicals in the presence of oxygen would induce degradation of
309 TBL but not of SAL.

310

311 **3.6 Reactivity of TBL and SAL with phenoxyl radicals**

312 These calculations were performed using the phenoxyl radical as reacting radical
313 because phenols are a class of oxygenated radicals well represented in humic materials.
314 Moreover, humic substances produces long-lived phenoxyl radicals (Golanoski et al, 2012;
315 Chen et al, 2018). In these calculations, we first investigated the reactivity of TBL and SAL
316 with $^3\text{O}_2$ and phenoxyl radicals Two different pathways were investigated: one where $^3\text{O}_2$ or

317 PhO reacted first in H radical abstraction reactions, the other where PhO reacted through an
318 addition reaction on TBL, followed by oxygen addition (see Scheme 1).

319 Both reactions are endothermic overall, but the reaction energy is significantly more
320 favorable in reaction 1b. A reaction energy of +31.2 kcal/mol was found for 1a when reactive
321 species was PhO (+61.2 kcal/mol for $^3\text{O}_2$). In comparison, the reaction energy was +18.3
322 kcal/mol for the phenoxyl radical addition, and +12.0 overall for 1b. Thus, reaction 1b, being
323 thermodynamically more favorable, was investigated more thoroughly. A transition state (see
324 SM) was found for the addition reaction of PhO. Secondly, the addition reaction of $^3\text{O}_2$ was
325 barrierless. It was also found that the potential energy surfaces did not exhibit significant
326 differences between TBL and SAL (activation energies were respectively 21.9 and 22.8
327 kcal/mol; see Figure 4). Thus, it was decided to investigate the fate of peroxy radical **I** for
328 TBL and SAL in order to understand the differences of reactivities that were observed
329 experimentally.

330 The peroxy radical **I** in Figure 4 can abstract a hydrogen atom on the cycle (H_a in
331 Figure 4) or on the chain (H_b). For TBL, both pathways require similarly activation energies.
332 Abstraction of H_b requires lower energy and is slightly more favorable (activation energy is
333 36.8 kcal/mol, while it is 37.7 kcal/mol for H_a). MEP calculations were performed to obtain
334 the PES for HO_2 dissociation from species **II** (see Figure 5). This was achieved by computing
335 partial optimizations for fixed C-OOH bond lengths. It was found that 2.2 kcal/mol was
336 required to dissociate HO_2 from **II**. Further keto-enol rearrangement gives the more stable
337 degradation product **III** (see Figure 5). Note that abstraction of H_a or H_b leads indistinctly to
338 the same degradation product **III**.

339 Similarly, for SAL, H_a abstraction was slightly less favorable than that of H_b
340 (activation energy is 36.7 kcal/mol whereas it was 32.9 kcal/mol for H_b). However, for TBL
341 the position of the hydroperoxyl group in **II** (adjacent to the radical site) leads readily to a

342 stable closed-shell species, for SAL, the positions of the hydroperoxyl and the radical site in
343 **II** are in meta position and the closed-shell species cannot be reached easily. Spin density in **II**
344 is larger on the ring (0.70) than on the chain (0.45; see SM). In that case, dissociation of HO₂
345 (obtained from MEP calculations) lead to the spontaneous dissociation of radical PhO, a
346 reaction that yields the reactant species.

347 Finally, the experimental photodegradation rate constants ($1\text{-}20\times 10^{-5}\text{ s}^{-1}$) found in this
348 work were coherent with the activation energies found from our theoretical calculations. For
349 example, unimolecular rate constants that range between 10^{-5} s^{-1} and $20\times 10^{-5}\text{ s}^{-1}$ require
350 activation energies of 24.3 and 22.5 kcal/mol, respectively (see Eq 14). The activation
351 energies calculated for the limitant unimolecular reactions depicted in Figure 5 have
352 activation energies of 24.8 and 20.5 kcal/mol, in agreement with the experimental results.

$$353 \quad k_{uni} = \frac{k_B T}{h} e^{-\Delta G/k_B T} \quad (14)$$

354 Moreover, if one considers the bimolecular association reaction of PhO and TBL (or
355 SAL) in Figure 4 to be the limitant step, and provided the concentration of PhO is $\sim 10^{-6}\text{ M}$, an
356 activation energy between 16.2 and 18.0 kcal/mol is required to obtain apparent first order
357 rate constants of $20\times 10^{-5}\text{ s}^{-1}$ and 10^{-5} s^{-1} , respectively (See Eq 15). This is slightly smaller than
358 the values found in this work (21.9 ad 22.8 kcal/mol for TBL and SAL, respectively).
359 However, the overall concentration of alkoxy radicals in HS is not well known and may be
360 larger than 10^{-6} M . In conclusion, our experimental data show that the limiting step in the
361 degradation process requires an activation energy of $\sim 20\text{ kcal/mol}$, which is in agreement with
362 our theoretical results. Note also that the energies calculated in our work are obtained for a
363 standard state of 1 atm, and it was necessary to multiply our rate constant by 0.08206T in
364 order to have the proper reference standard state, i.e. 1 mol/L (de Sainte Claire et al., 1996;
365 Bryantsev et al, 2008).

366
$$k_{\text{apparent uni}} = [\text{PhO}] \times 0.08206T \times \frac{k_B T}{h} e^{-\Delta G/k_B T} \quad (15)$$

367 Thus, the different reactivities observed experimentally between TBL and SAL may be
368 explained mainly by the differences of the positions of the alcohol groups on the phenyl
369 ring. For SAL, the photodegradation rate constant is smaller because the reaction with
370 oxygen yields the reactant back.

371

372 **4. Conclusion**

373 To conclude, the present study focused on the photo-transformation of two β 2-
374 adrenoceptor agonists. In pure water the direct photolyses were relatively slow, but the
375 presence of HS significantly enhanced reactions. Therefore, β 2-adrenoceptor agonists are
376 expected to be photolyzed in surface waters enriched in natural organic matter and terrestrial
377 inputs. Although SAL and TBL share common side chain, their major photooxidation
378 pathways in the presence of HS were very different. For SAL, the enhancement by HS was
379 essentially due to $^3\text{HS}^*$ and $^1\text{O}_2$. In the case of TBL that underwent faster photodegradation in
380 air-saturated medium than SAL, none of these species was significantly involved. Quantum
381 calculations suggest that oxygenated such as phenoxy radicals could significantly enhance the
382 photodegradation of TBL through addition reactions. This study and in particular the
383 unexpected behavior of TBL stresses several points. First, the different behaviors of SAL and
384 TBL in HS remind us that different substituted phenols would undergo different
385 photochemical fates in natural waters. Second, the influence of quenchers like HS should be
386 considered carefully and comprehensively, depending on the target substances. Third, TBL
387 could be a potential probe molecule for oxygenated radicals.

388

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393

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CAPTIONS FOR FIGURES

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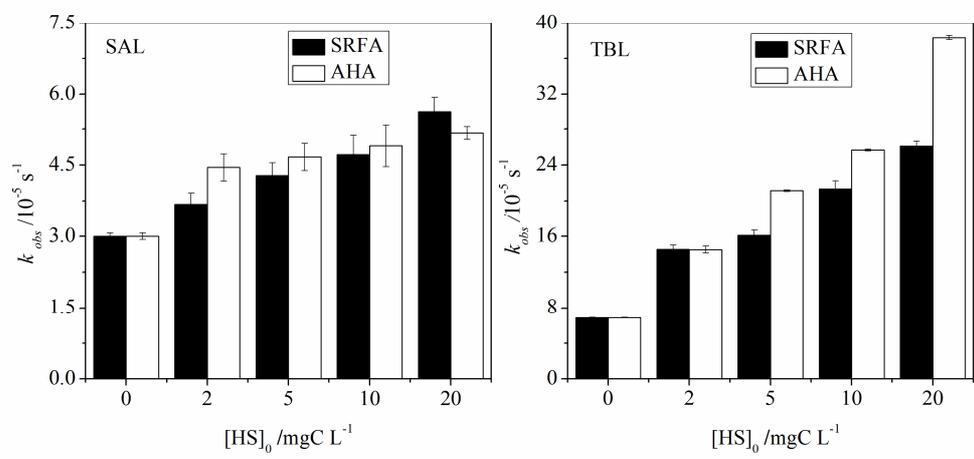
Figure 1. Photodegradation rate constants of SAL (60 μM) and TBL (60 μM), k_{obs} , in the presence of different concentrations of Suwannee River Fulvic Acid Standard II (SRFA) and Aldrich Humic Acid (AHA) under HPK irradiation, at pH = 8.0 and in air-saturated medium. Error bars represent the standard deviation.

Figure 2. Photoproducts of SAL and TBL as identified by HPLC/MS.

Figure 3. Photodegradation rate constants of SAL and TBL (60 μM), k_{obs} , under different conditions in the presence of 5 mg C L⁻¹ HS under HPK irradiation, pH = 8.0, [SA]₀ = 100 μM . Error bars represent the standard deviation.

Figure 4: The consecutive addition reactions of PhO and ³O₂ on TBL. Electronic energies (kcal/mol) were obtained at the B3LYP/6-31+G(d,p)+PCM level. The respective energies for SAL are indicated in parentheses. The transition state is indicated with a dashed level.

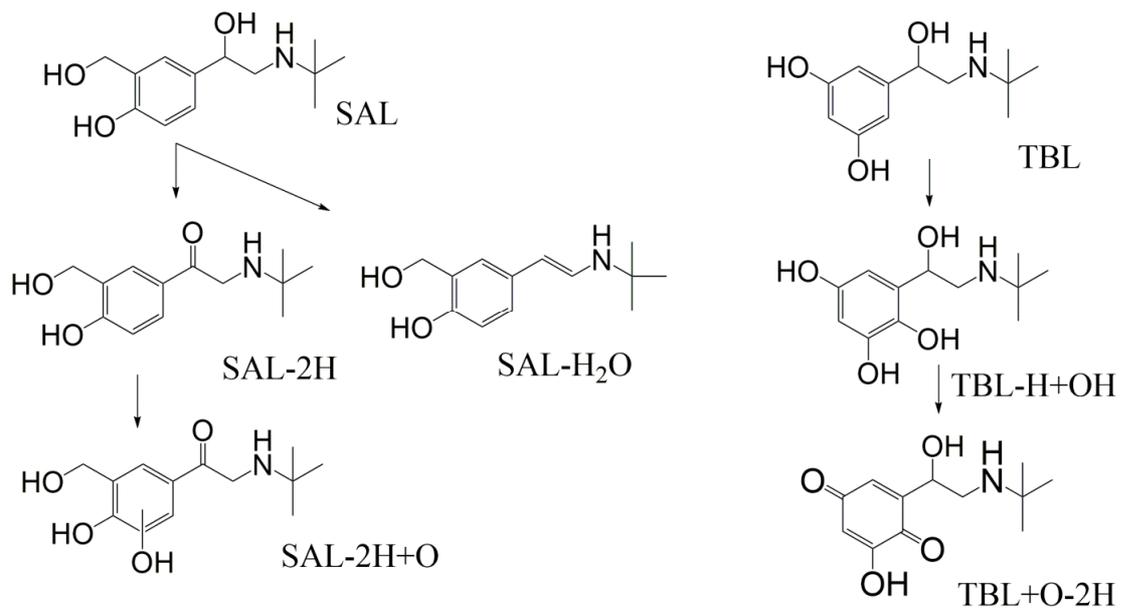
Figure 5: The intramolecular H abstraction reaction (**I**→**II**) and HO₂ dissociation pathway (**II**→**III**) for TBL and SAL. Energies (kcal/mol) were obtained at the B3LYP/6-31+G(d,p)+PCM level. Transition states and MEP maximum are indicated with dashed levels.



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568 Figure 1.

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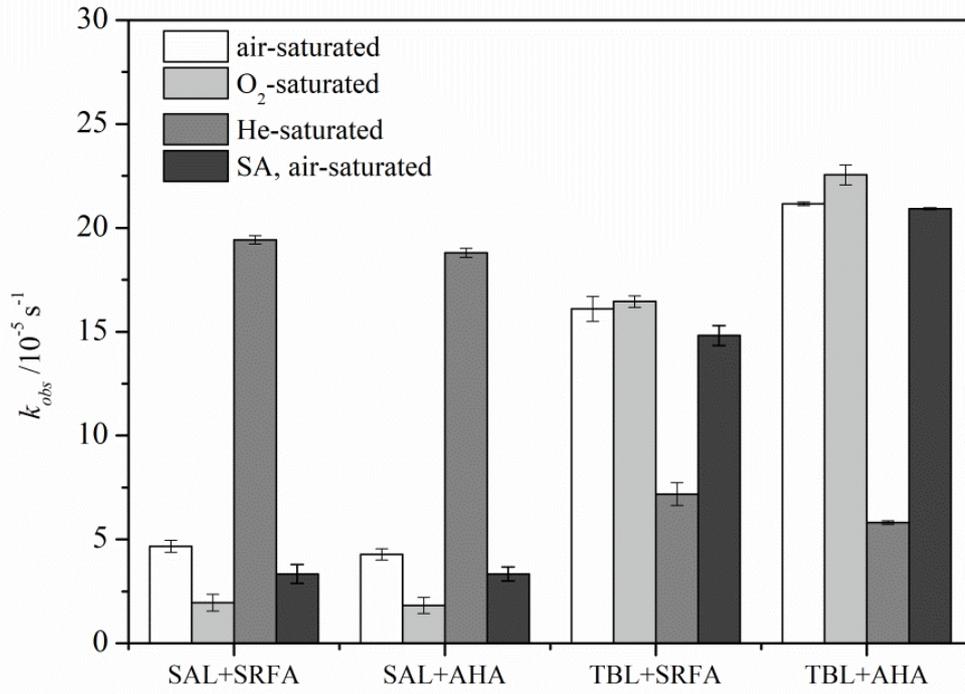
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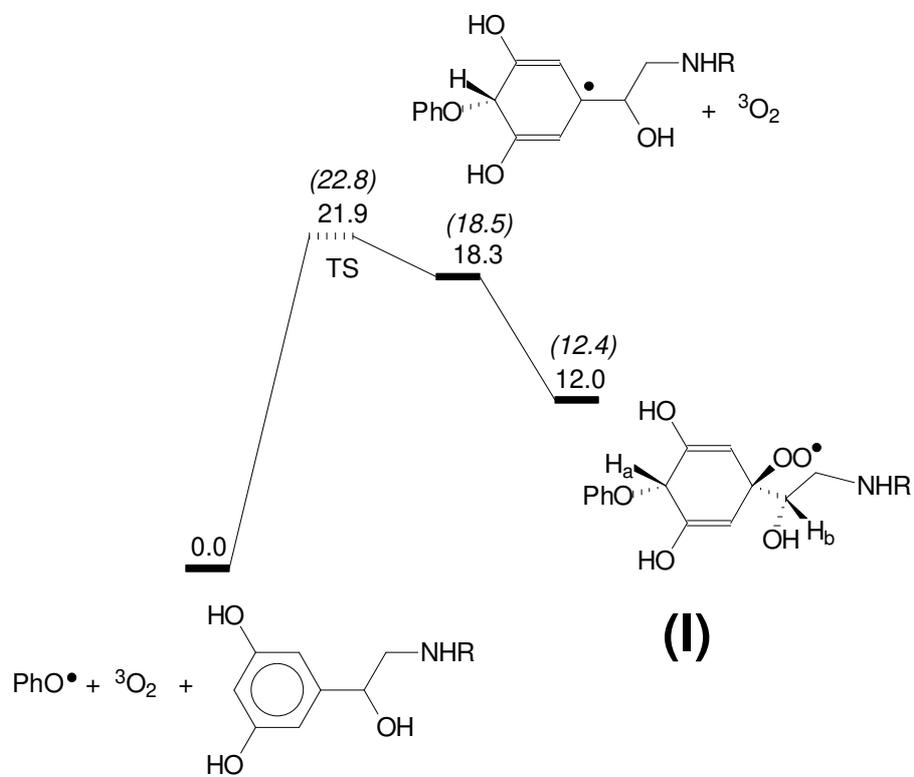
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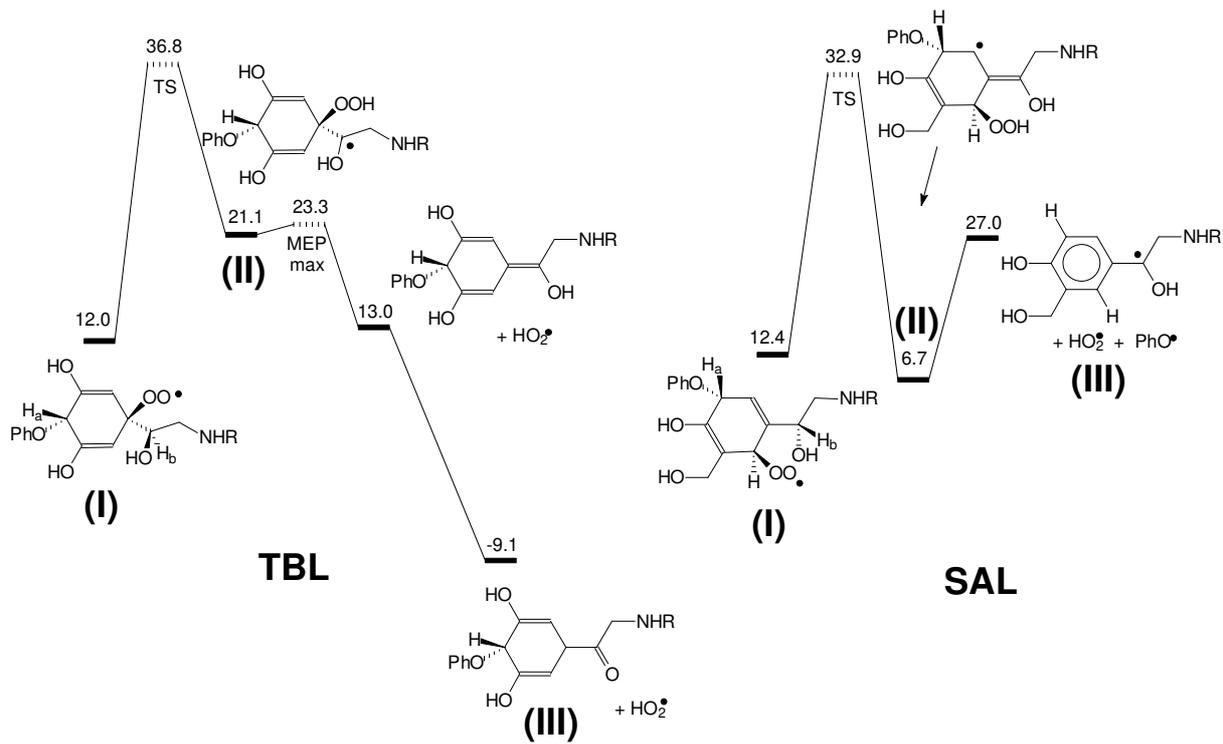


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588 Figure 5

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Conditions	SAL		TBL	
	AHA	SRFA	AHA	SRFA
	$k/10^{-5} \text{ s}^{-1}$	$k/10^{-5} \text{ s}^{-1}$	$k/10^{-5} \text{ s}^{-1}$	$k/10^{-5} \text{ s}^{-1}$
corrected k_{direct}	1.2	2.0	2.6	4.2
k_{indirect} under air	3.1	2.8	18.9	12.1
k_{indirect} under He	18.0	17.6	3.3	3.2
k_{indirect} under pure oxygen	0.8	0.2	20.4	12.3

599 Table 1: Degradation rate constants of SAL and TBL in the presence of SRFA and AHA at 5

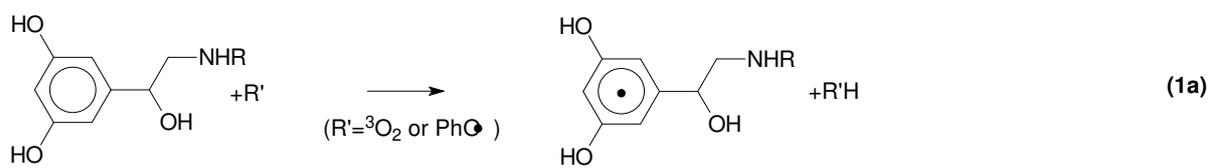
600 mgC L^{-1} . The uncertainties are of 10%.

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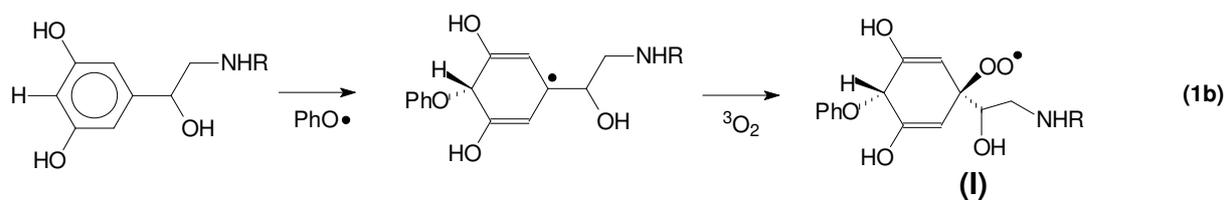
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607 Scheme 1 : Comparison of two initiation reactions that lead to degradation products for TBL.

608 The peroxyl radical in 1b is labeled **I** in the following.

609

