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Reaction of reductively activated mitomycin C with aqueous bicarbonate: Isolation and characterization of an oxazolidinone derivative of *cis*-1-hydroxy-2,7-diaminomitosene

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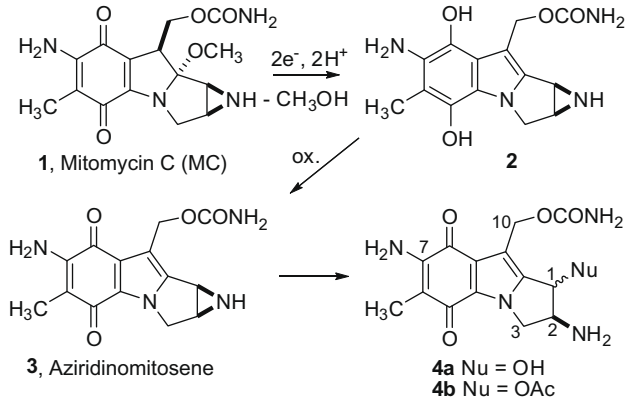
ABSTRACT

The reductive activation of mitomycin C in aqueous bicarbonate buffer resulted in the formation of a previously unknown compound, characterized as an oxazolidinone derivative of *cis*-1-hydroxy-2,7-diaminomitosene. This compound is the result of a cyclization reaction of bicarbonate with the aziridine ring of aziridinomitosene, and was observed at bicarbonate concentrations close to those present in physiological plasma.

Mitomycin C (MC, **1**) is an antitumor antibiotic used clinically in the chemotherapy of cancer[1] and in ophthalmological procedures.[2] The cytotoxic effects of the drug are believed to be originated by the formation of covalent interstrand DNA–DNA crosslinks. MC is inert towards DNA or other cellular nucleophiles in its original structure, requiring a reductive activation to expose a masked the bis-electrophile.[1]

The reductive activation of MC starts by reduction to the hydroquinone that eliminates methanol to form a reduced aziridinomitosene **2**, which can alkylate electrophiles sequentially through its 1 and 10 positions (Scheme 1).[1,3] When the reductive activation of MC is performed in vitro, an autocatalytic reduction process ensues in some cases, generating aziridinomitosene **3** that functions as a monofunctional electrophile, giving alkylation reactions only at *C*-1,[3,4] a reactivity that is also observed in the non-reductive activation of MC by acids.[5] A number of electrophiles have been reported to be alkylated monofunctionally by MC through this pathway: **3** reacts with DNA by alkylating *N*-2 of deoxyguanosine residues;[1,3] hydroxymitosenes **4a** are the major metabolites formed from 3 in water at neutral pH[4a] or by acid hydrolysis;[5] the use of phosphate buffers produced the phosphate ester of **4a**;[6] thiol adducts have been detected when MC was activated in the presence of glutathione, mercaptoethanol or *N*-acetylcysteine;[7] a sulfite adduct was observed after activation of MC with sodium dithionite as reducing agent.[8]

More relevant to the findings presented here, the solvolysis of mitomycin C with glacial acetic acid produced *trans*-acetoxy-aminomitosene *trans*-**4b** and *cis*-acetylamino-hydroxymitosene 5 (R = CH_3).[5] The latter is formed in a selective *O* to *N* migration of the acetyl group from the *cis*-acetoxyaminomitosene *cis*-**4b** (Scheme 2). A similar *O* to *N* migration of an acyl group was ob-



Scheme 1. Mechanism of the monofunctional reductive activation of mitomycin C.

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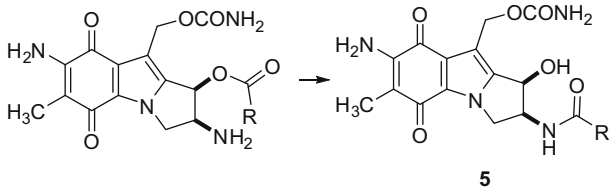


Scheme 2. Acyl migration on *cis*-acyloxymitosenes to form *N*-acylmitosenes **5** (R = CH_3,[5] CF_3[9]).

served in the reaction of MC with trifluoroacetic acid, that gave **5** (R = CF_3) as major product.[9]

We report here the isolation and characterization of a novel mitosene containing an 1,3-oxazolidin-2-one ring, formed after reductive activation of MC in the presence of bicarbonate, the quintessential biological buffer.

During our recent research on the reductive activation of MC by thiols[4a] we used carbonate–bicarbonate as a buffer in a number of experiments. LC–MS analysis of these reaction mixtures revealed, in some cases, the formation of a previously unknown mitosene in addition to the expected known hydroxymitosenes **4a** (Fig. 1a).

The UV spectrum of the novel compound showed the maximum absorbance at 252 and 310 nm characteristic of the mitosene chromophore. The peaks observed in the EI-MS indicated a molecular weight of 346 g/mol (Fig. 1b), that we attributed to the oxazolidinone derivative **6** (Scheme 3). Differential IR spectroscopy using the IR spectrum of *cis*-**4a** as subtrahend showed the presence of an additional band in **6** at 1716 cm^{-1}, that we assigned to the oxazolidinone carbonyl group.

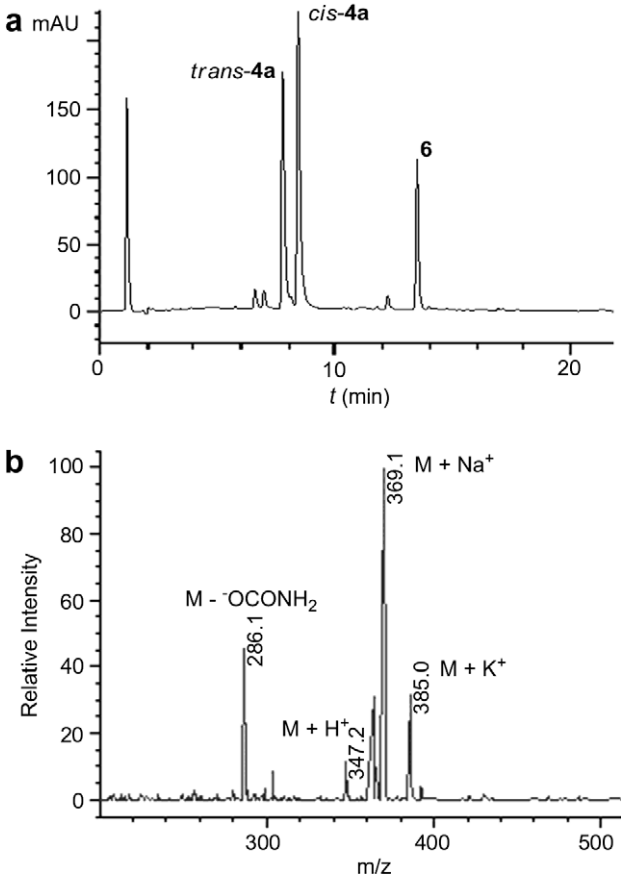
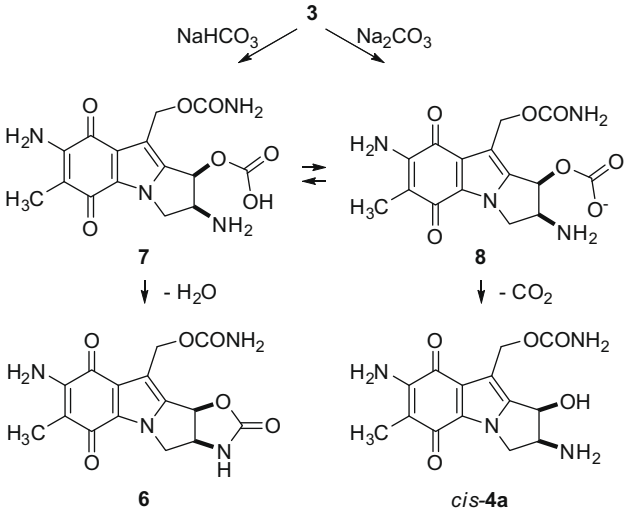


Figure 1. (a) HPLC trace of the reaction of activated MC with 1.0 M bicarbonate in water. (b) EI-MS of **6**.



Scheme 3. Proposed mechanism for the formation of **6**.

Due to the limited availability of MC we devoted some time to maximize the yield of the novel adduct. It was found that the optimum yield of **6** was reached in a two step approach: an initial generation of aziridinomitosene **3**, that in a subsequent step was reacted in situ with bicarbonate. A solution of **3** was prepared by treating MC with a substoichiometric amount of 1,3-propanedithiol at pH around 10.[4a] This resulted in a quick autocatalytic activation, observed by the naked eye as a sudden change in the color of the solution. Subsequent treatment with a large excess of bicarbonate gave **6** in yields around 20%, based on the HPLC trace. Desalting by solid phase extraction followed by preparative TLC gave pure **6**. We found later a more convenient protocol to isolate **6**, based on its solubility properties and the selective chemical derivatization of undesired by-products: the crude reaction mixture (containing mostly **4a**, decarbamoyl-**4a**, **6**, and some unreacted MC) was treated with Boc_2O, a reagent that converted all 2-aminomitosenes to the corresponding hydrophobic *N*-Boc-derivatives, while **6** remained intact; liquid–liquid extraction selectively removed the Boc-substituted mitosenes from the aqueous phase; desalting by solid phase extraction gave an aqueous solution of **6**, from which pure **6** precipitated after cooling. This later protocol provided enough material (5–6 mg) to perform NMR experiments.

The ^1H NMR spectrum of the new mitosene showed the expected peaks for all *C*- and *N*-bound hydrogens, and a COSY spectrum showed connectivity from *C*-1 to *C*-3. The ^{13}C NMR spectrum showed peaks for all 15 carbons that were identified by DEPT, HSQC and HMBC experiments. The HMBC spectrum showed a peak at 158 ppm coupled to *H*-1, *H*-2 and the *NH* of the oxazolidinone, corresponding to the new carbonyl group inserted by bicarbonate.

Two observations provide evidence for the mechanistically predicted *cis* stereochemistry of **6**: firstly, the coupling constant $J_{1\text{-}2}$ in the ^1H NMR spectrum was 7.8 Hz, a value characteristic of a *cis*-fused 1,3-oxazolidin-2-one;[10] secondly, a strong NOE on *H*-2 was observed upon irradiation of *H*-1 (Fig. 2), denoting close proximity between these hydrogens. The relative stereochemistry in many 1-substituted 2-aminomitosenes has also been assigned using circular dichroism, using the sign of the Cotton effect at 520–550 nm as diagnosis.[11] The CD spectrum of **6** did not follow this general rule, as **6** was not optically active between 450 and 600 nm, likely due to distortions induced by the strained oxazolidinone ring. However, two active bands at 390 and 350 nm showed a Cotton effect with the expected sign for a *cis* configuration (Fig. 3).

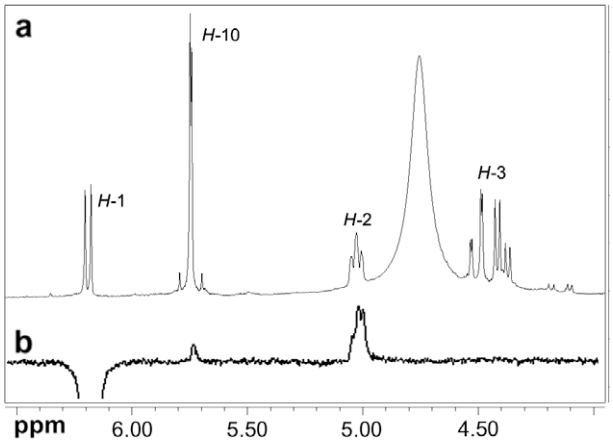


Figure 2. (a) Fragment of the ^{1}H NMR spectrum of **6** (pyridine-d_5). See Scheme 1 for numbering. (b) NOE spectrum obtained upon irradiation of *H*-1.

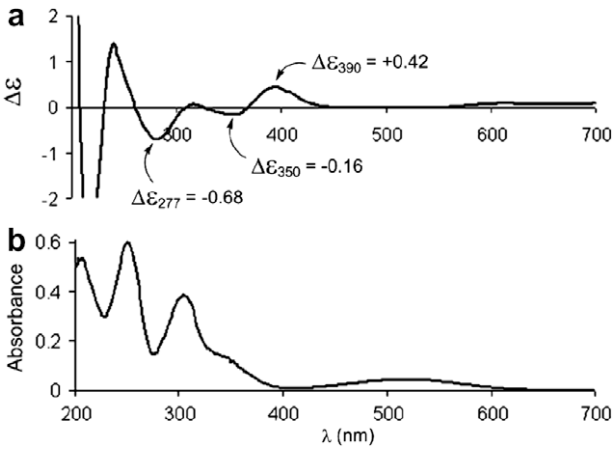


Figure 3. (a) CD spectrum of **6** in MeOH. (b) UV–vis spectrum of **6** in MeOH.

The ratio of **6** relative to hydroxymitosenes (**4a** and decarbamoyl-**4a**) obtained in the reaction of **3** with aqueous bicarbonate was markedly affected by the pH of the bicarbonate–carbonate solution (Table 1, entries 1–4), with a maximum yield of **6** obtained with pure bicarbonate (pH around 8.0). From these observations we propose the mechanism shown in Scheme 3: the first step is favored by a large concentration of anion, and it probably occurs both with bicarbonate or carbonate as nucleophiles. On the contrary, the second step (**7**→**6**) requires the presence of protonated alkylbicarbonate **7**. Higher pH values will favor the formation of alkylcarbonate anion **8** that does not cyclize, but decarboxylates to give *cis*-**4a**, while lower pH values will favor the presence of **7**, that we propose is the species competent to undergo cyclization to form the oxazolidinone **6**. The *trans* isomers of **7** and **8** are likely also formed, but stereoelectronic factors impede its conversion to an oxazolidinone and they are converted to *trans*-**4a**. The global outcome of the reaction with bicarbonate resembles the previously observed reactivity of 1-acyloxy mitosenes, where a selective *O*- to *N*-acyl migration occurs only in the *cis* isomer, resulting in the formation of *N*-acyl *cis* isomer 5 (Scheme 2) and an *O*-acyl *trans* isomer.[5a,9]

When the influence of bicarbonate concentration was studied, a proportional relationship between the yield of **6** and the concentration of bicarbonate was found (Table 1, entries 4–7), with the yield of **6** peaking at 18% when 0.9 M bicarbonate was used. The use of 30 mM bicarbonate, a concentration close to that present in biological plasma, resulted in a yield of **6** around 3–4%.

Table 1
Influence of pH, concentration of carbonate/bicarbonate buffer and presence of chloride anion in the yield of **6**

Entry	$[HCO_3^-] + [CO_3^{2-}]$	$[Cl^-]$ (mM)	pH	**6** (% yield)[a]
1	0.9 M	–	11.0	2
2	0.9 M	–	10.0	6
3	0.9 M	–	9.0	15
4	0.9 M	–	8.0	18
5	300 mM	–	8.0	14
6	100 mM	–	8.0	8
7	30 mM	–	8.0	3.5
8	300 mM	500	8.0	15
9	30 mM	150	8.0	3.3
10	30 mM	150	7.5	3.7
11	30 mM	150	7.0	4.2
12	KHS solution[b]		7.6	4

[a] The yield of **6** was calculated from the area of the peaks in the HPLC trace as 100 × [**6**/(Σ all mitosenes)].
[b] The ionic composition of KHS solution was (mM): HCO_3^- 25, Cl^- 130, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, Ca^{2+} 2.5, K^+ 6, Mg^{2+} 1.2.

The reaction of MC with bicarbonate is, to the best of our knowledge, the first report of the direct reaction of an aziridine with bicarbonate to form an oxazolidinone. However, this transformation is not completely unprecedented. A synthesis of 2-oxazolidinones from the reaction of aziridines and bicarbonate was reported in the mid 70s, but required an initial conversion of the aziridine to the 2-chloroethylamine by treatment with anhydrous HCl.[12] In a closely related reaction, the formation of 2-oxazolidinones in cell culture media and blood plasma from 2-chloroethylamines and bicarbonate has been reported.[13] Additionally, the synthesis of 2-oxazolidinones by reaction of CO_2 and aziridines has been extensively investigated in the last decade. Reaction conditions for this conversion reported recently include: LiI in THF,[14] quaternary ammonium salts and halides,[15] $ZrOCl_2$ in solvent-free conditions,[16] polyethylene glycol functionalized with a quaternary alkylammonium bromide,[17] and (salen)chromium complexes,[18] among others. A common feature in most of the reported conversions of aziridines to oxazolidinones is the involvement of halide ions.[12–16] This observation raised the question if chloride ion, present physiologically at concentrations of up to 150 mM, could increase the formation of **6**, probably by generating an intermediate β-chloroethylamine that then alkylates bicarbonate. To test this hypothesis we added 0.5 M NaCl to the reaction of **3** with 0.3 M bicarbonate, but no appreciable differences were observed (Table 1, entries 5 and 8). The outcome of the reaction employing concentrations of bicarbonate and chloride close to those biologically relevant was also studied (Table 1, entries 9–11). No significant changes were observed with respect to the reaction in absence of chloride (Table 1, entry 7). We also performed the reaction of **3** with the classic Krebs–Henseleit solution (KHS), a saline solution designed to emulate the ionic composition of rat plasma, containing 25 mM HCO_3^- and 130 mM Cl^-, among other salts.[19] Again, this reaction resulted in yield of **6** comparable to other reactions that contained similar bicarbonate concentrations, but without chloride (Table 1, entries 7 and 12).

From these results we hypothesize that **6** may be formed in vivo as a MC metabolite, although in minor quantities relative to hydroxymitosenes **4a** or 2,7-diaminomitosene.[6] The low water solubility of **6** may have precluded its detection in vivo, while the infrequent use of bicarbonate-containing buffers ruled out its formation during in vitro experiments.

In conclusion, we have discovered a novel MC derivative, resulting from the reaction of reductively activated MC with bicarbonate. This compound is formed in low yields with concentrations of bicarbonate resembling those present physiologically, therefore

we hypothesize that the biological relevance of this novel MC derivative is unlikely to be significant.[20]

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Supplementary data

Supplementary data (experimental details for the synthesis of **6** and its spectroscopic characterization. HPLC traces for the reactions of activated MC with bicarbonate) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.046.

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20. We consider that the results presented here are also interesting from a synthetic organic chemistry perspective, as they open the door to the development of an alternative to existing methods to synthesize oxazolidinones from aziridines.[12–18] While the ratio of oxazolidinone to aminoalcohols obtained here is rather low, the potential optimization of this reaction would provide an environmentally attractive method for the synthesis of oxazolidinones from aziridines: it uses water as solvent, the reaction may be performed at standard pressure and temperature, and utilizes bicarbonate (easier to handle than gas) as CO_2 source.