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Total synthesis of Crotogossamide using an on-resin concomitant cyclization/cleavage reaction

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ABSTRACT

Crotogossamide, a cyclic peptide isolated from the latex of *Croton gossypifolius*, has been synthesized by a rapid and efficient Boc solid-phase peptide synthesis. The strategy takes advantage of the oxime resin nucleophile susceptibility and comprises the synthesis of a linear precursor followed by on-resin head-to-tail concomitant cyclization/cleavage. In addition, we report the first antimicrobial and antibiofilm investigations on Crotogossamide.

1. Introduction

Crotogossamide **1** is a cyclic nonapeptide isolated from the latex of *Croton gossypifolius* (Quintyne-Walcott et al., 2007). To our knowledge, only one report has so far described the total synthesis of Crotogossamide **1** using free-radical desulfurization as the key step (Wan and Danishefsky, 2007). Though several natural products have been isolated from the genus *Jatropha* of the *Euphorbiaceae* family, Crotogossamide is the first cyclic peptide isolated from a Croton species. Since the first report of a natural cyclic peptide isolation, Gramicidin S, in 1944 (Gause and Brazhnikova, 1944), macrocycles constitute a privileged scaffold for the scientific community (Marsault and Peterson, 2011; Nielsen et al., 2017; Peña et al., 2015; Russo et al., 2016; Tapeinou et al., 2015; Tsomaia, 2015; White and Yudin, 2011; Yudin, 2015). Over the years, a rich diversity of homodetic and heterodetic natural cyclic peptides have been isolated from different living organisms (Wessjohann et al., 2005). The first natural cyclic peptide drug, cyclosporine, (Borel et al., 1976) was launched on the market in 1983. The unique properties of cyclosporine prove that peptide cyclization could improve pharmaceutical properties by reducing polarity and conformational flexibility and increasing proteolytic stability (Marsault and Peterson, 2011).

Though peptide macrocycles are attractive for their unique properties, macrocyclization is always challenging (White and Yudin, 2011). Oligomerization and racemization often occur during macrocyclization processes, which require highly diluted conditions to counterbalance entropic loss by cyclization. Among the available synthetic strategies to cyclize peptides, our research group focused on the use of oxime resin (DeGrado and Kaiser, 1980; DeGrado and Kaiser, 1982). Previously, we reported parameters affecting the cyclization-oligomerization reactions favouring cyclic monomers of large peptide macrocycles (Blanchette et al., 2007). Cyclic hexa- to octa-peptides were prepared efficiently with amino acid sequence L-Pro-Gly-L-Pro-Gly to facilitate peptide folding (Fig. 1) (Blanchette et al., 2007). To investigate the unknown pharmacological potential of Crotogossamide **1**, we report here the total synthesis, exploiting the key step developed in our lab (Bérubé et al., 2017; Bérubé et al., 2015; Bérubé and Voyer, 2016; Blanchette et al., 2007).

2. Results and discussion

Ring size is one of the most important factors in peptide cyclization processes. It is well documented (White and Yudin, 2011; Yudin, 2015) that cyclization of large peptides (more than seven amino acids) is easier than cyclization of shorter ones, due to their higher flexibility. In fact, natural peptide macrocycles with 14- to 18-member rings are the most common scaffold (Yudin, 2015). However, the primary sequence of Crotogossamide **1** contains nine amino acids, of which seven are hydrophobic. Consequently, the on-resin linear precursor could form β-sheets, decreasing the probability of cyclization occurring.

However, based on precedents in the literature about peptide macrocyclization, we anticipated that the linear precursor would promote the cyclization of Crotogossamide **1** in a head-to-tail fashion with minimal oligomerization/racemization side reactions. Indeed, examples of sequence-dependent cyclization/cleavages that favour the intramolecular process have been described on oxime resin (Blanchette

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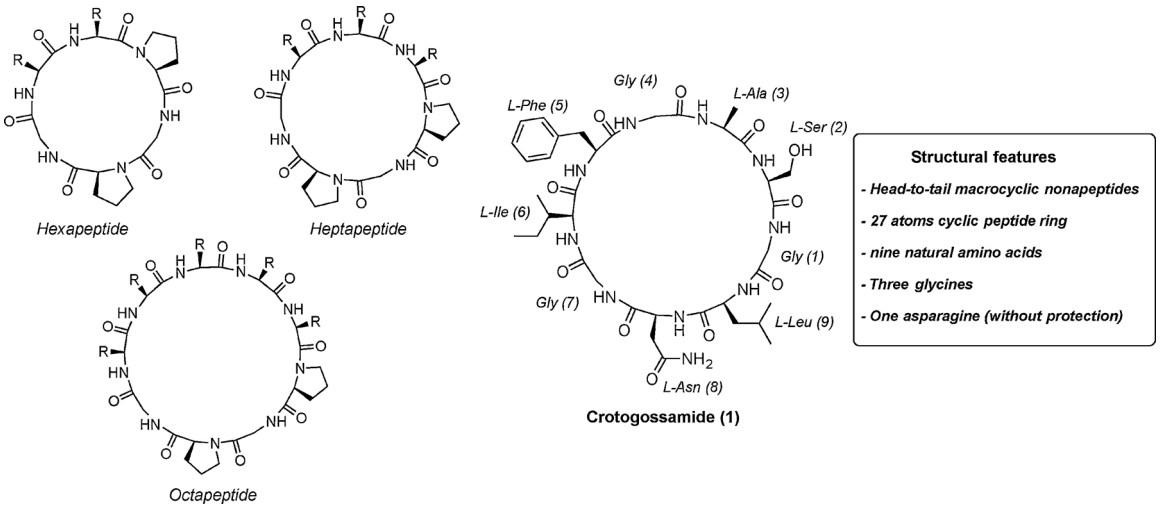

Fig. 1. Peptide macrocycles prepared on oxime resin and Crotogossamide **1** with its structural features.

et al., 2007; Nishino et al., 1992a; Nishino et al., 1992b; Smith et al., 1998).

Since the amino acid sequence influences the oligomerization/cyclization process, we examined the primary sequence of potential linear precursors of Crotogossamide **1**. As shown in Fig. 1, the cyclic nonapeptide (**1**) contains six L-amino acids and three glycines with a primary sequence of *cyclo*(Gly-L-*S*er-L-Ala-Gly-L-Leu-L-Asn-Gly-L-Ile-L-Phe). Absence of a β-turn-inducing proline in the sequence makes it more challenging to avoid oligomerization side reactions. Nevertheless, Crotogossamide **1** contains two amino acids between each glycine, which has more flexibility than branched amino acids, and less steric hindrance at the carbonyl group.

Hence, we chose to incorporate a glycine at the first position. Placing this achiral glycine at the linking position also avoids racemization during the formation of *O*-acetyl urea in the presence of diisopropylcarbodiimide (DIC) during the coupling of the first amino acid. On the other hand, Crotogossamide **1** contains one asparagine in its primary sequence. By the *N*-t-Boc strategy, asparagine can be incorporated without side-chain protection. Though side reactions have been reported to occur with a variety of coupling agents, the addition of HOBt minimizes nitrile formation by carboxyamide dehydration. More importantly, weak acids catalyze intramolecular cyclization of glutamine into pyroglutamate, which can be avoided using a high TFA concentration for *N*-t-Boc removal (Dimarchi et al., 1982). Using 75% TFA/DCM deprotection conditions allows the deprotection to proceed without side reactions.

With the above considerations in mind, we synthesized Crotogossamide **1** on oxime resin using classical protocols with 0.3 mmol/g loading (Fig. 2). The first amino acid was coupled for three hours using DIC as coupling reagent. The *N*-t-Boc protecting group was removed using a mixture of 1:1 trifluoroacetic acid (TFA)/dichloromethane, while the second amino acid was activated with hydroxybenzotriazole (6-Cl-HOBt) and 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HCTU). After appropriate coupling/deprotection steps, the linear peptide was simultaneously cyclized and cleaved from the resin in the presence of diisopropylethylamine (DIEA; 2.5 equiv) and acetic acid (AcOH; 5 equiv) in dichloromethane at a 10^{-2} M precursor concentration, leading to a good 41% macrocyclization yield. The remaining of the linear precursor left on the resin could not be macrocyclized, probably due to its oligomerization on the resin.

HPLC-TOF-MS mass spectrometry was used to distinguish between the desired protected Crotogossamide and its cyclic dimer. Results revealed only two peaks, one at 7.47 min with 99% of the total surface area, and the second at 11.38 min, both with identical masses (Fig. 3). However, the peak at 7.47 min possesses an isotopic *m/z* ratio equal to one, while the second peak at 11.38 has an isotopic *m/z* ratio equal to 0.5. The isotopic ratio distribution analysis allows us to assign the desired protected Crotogossamide **1** at 7.47 min. The peak at 11.38 min has been assigned to the Crotogossamide cyclic dimer (see ESI for details). Then, very low side-product (1%) arising from oligomerization were identified in the cyclization process. In addition, the cyclization of the linear precursor provided the desired protected Crotogossamide in high purity up to 99% after a simple trituration with cold diethylether. Having confirm that the cyclization product is the right one, the final step was the serine benzyl deprotection. Even if this deprotection seems to be an easy task, the hydrogenolysis was a hard one. Indeed, performing hydrogenolysis in EtOH, MeOH and in THF provided Crotogossamide **1** in poor yields. However, Crotogossamide **1** was obtained by hydrogenolysis using 50 psi of H_2 in AcOH in 83% crude yield and 41% pure isolated yield after preparative HPLC purification. Spectroscopic data of synthetic Crotogossamide **1** matched entirely those reported for the isolated natural product (see ESI for details).

As mentionned, peptide macrocycles exhibit a large diversity of biological properties and macrocyclic head-to-tail peptides are well-known powerful antimicrobial agents. In order to investigate the unknown pharmaceutical potential of Crotogossamide **1**, we evaluated antimicrobial properties towards *Escherischa coli* ATCC 15939, *Streptococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 10145 and *Candida albicans* ATCC 28366. In addition, its anti-biofilm activity was evaluated on both prokaryotic (*Streptococcus mutans* ATCC 25175) and eukaryotic (*Candida albicans* ATCC 28366) models. Unfortunately, no significant antimicrobial or antibiofilm activity was observed for concentrations of Crotogossamide **1** up to 96 ug/mL.

3. Conclusion

We achieved the total synthesis of Crotogossamide **1** on solid support by concomitant cyclization/cleavage with almost no oligomerization side reactions, and an overall yield of 34%. The cyclization of the linear precursor proceeded in an excellent yield and provided almost exclusively the desired protected Crotogossamide monomer, as confirmed by the isotopic ratio distribution in mass spectrometry. Access to sufficient amount of Crotogossamide **1** allowed us to perform the first antimicrobial investigation on that natural macrocyclic peptide, though no activity was observed. Work is currently underway to prepare a library of Crotogossamide analogs, as well as other natural macrocyclic peptides by head-to-tail, on-resin concomitant cyclization/cleavage using the oxime resin.