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On the Elucidation of the Mechanism of *Vinca* Alkaloid Fluorination in Superacidic Medium

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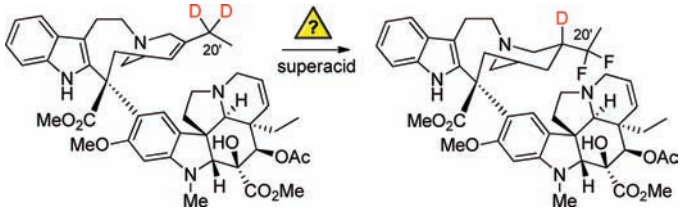
ABSTRACT



Detailed investigations on one of the key steps of the superacidic fluorination of *Vinca* alkaloids that is the origin of C20′ activation are reported. While two different pathways can be envisioned for the emergence of the transient secondary carbocationic intermediate, isotopic labeling experiments unambiguously revealed the involvement of a 1,2-hydride shift mechanism.

The dimeric *Vinca* alkaloids derived from the Madagascar periwinkle *(Catharanthus roseus (L) G. Don)* play a significant role in the treatment of cancer.[1] The first molecules in this class were the naturally occurring alkaloids vinblastine and vincristine whose total syntheses have been achieved by various groups.[2] Subsequent developments led to the approval for clinical use of the semisynthetic derivatives vinorelbine **2** (Navelbine)[3] and more recently vinflunine **4** (Javlor)[4] (Figure 1). Vinflunine differs from previously known *Vinca* alkaloids by the presence of two fluorine atoms which are incorporated in the northern portion of the dimer. The two halogens are introduced on the C20′ ethyl side chain by fluorination of vinorelbine **2** under superacidic conditions. This process works equally well starting from anhydrovinblastine **1** (to produce difluorodeoxyvinblastine **3**) and induces the concomitant

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reduction of the vicinal C3′−4′ double bond. Although harsh conditions are involved (HF/SbF_5), the reaction is remarkably efficient and regioselective.

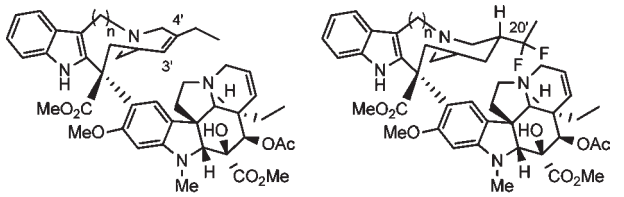
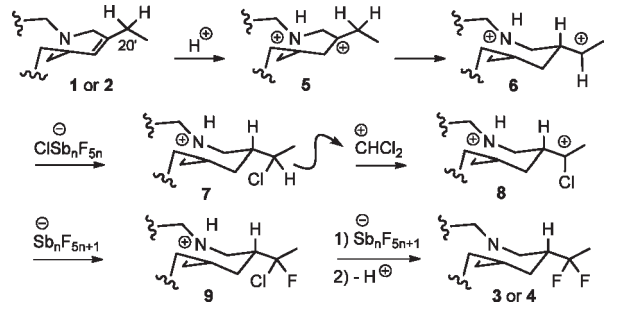


Figure 1. Structure of dimeric alkaloids.

Previous studies[4a,5] have resulted in a proposed mechanism for this transformation as illustrated in Scheme 1. The reactive species produced in situ by mixing hydrofluoric acid and antimony pentafluoride in chloroform are of general structures $Sb_nF_{5n}Cl^-$ and $Sb_nF_{5n+1}{}^-$.[6]

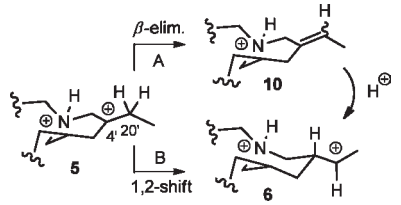
Scheme 1. Postulated Mechanism of *Vinca* Alkaloid Fluorination



The key fluorination step commences with multiple protonation of the dimeric alkaloid (e.g., **1** or **2**) including that of the C3′−C4′ double bond, giving rise to tertiary carbocation **5**. The latter evolves spontaneously to secondary carbocation **6** because of charge repulsions. Evidence for the formation of this key intermediate was provided from ionic hydrogenation studies in superacidic conditions.[7] Chlorination of **6** leads to derivative **7** whose α-hydrogen atom is withdrawn by $CHCl_2{}^+$ (a strong hydride abstracting species produced in situ from $CHCl_3$). The ensuing carbocationic intermediate **8** is then trapped by a fluoride ion from $Sb_nF_{5n+1}{}^-$ to afford **9**. The difluorinated alkaloid **3** or **4** (depending on the starting dimer **1** or **2**) is finally produced after Cl/F exchange.

The mechanism of *Vinca* alkaloid fluorination in superacidic conditions has only been partially studied, as aggressiveness of the HF/SbF_5 medium restricted in depth investigations. As part of our ongoing interest in *Vinca* alkaloid chemistry,[8] we would like to give here an account of our recent findings regarding a key step of the fluorination process that is C20′ activation. As discussed above, secondary carbocation **6** is the pivotal intermediate of the superacidic fluorination. Transformation of the initial tertiary carbocation **5** into a secondary one (**6**) can follow two different pathways namely, β-elimination of H20′ (to give **10**) followed by reprotonation of the transient *exo* C4′−C20′ double bond (Scheme 2, path A) or direct 1,2-hydride shift from C20′ to C4′ (path B). In both cases, an identical secondary carbocation **6** is produced.

Scheme 2. Transformation of the Initial Tertiary Carbocation



To discriminate between the two pathways, our initial approach was to assess whether authentic **10** could provide access to the difluorinated alkaloid under superacidic conditions. To answer this question, the synthesis of anhydrovinblastine analog **10** (4′,20′-anhydrovinblastine) was undertaken. Dimeric alkaloid **10** differs from anhydrovinblastine **1** by the presence of an *exo* C4′−C20′ double bond in lieu of the classical *endo* C3′−C4′ olefin. As the upper portion of the dimeric alkaloids is classically derived from catharanthine, the synthetic scheme that was developed relied on biomimetic coupling of vindoline (lower part of *Vinca* alkaloids) with catharanthine derivative **12** (Scheme 3). The latter incorporated an *exo* C4−C20 double bond that originated from isomerization of the *endo* olefin (C3−C4) of catharanthine **11**. This unprecedented transformation was achieved using palladium over charcoal and under reduced pressure (0.3 bar) of hydrogen gas. We observed that without hydrogen, no reaction would occur and attempts to isomerize catharanthine under 1 bar of H_2 led to quantitative reduction of the double bond. The Pd/C/H_2 process thus afforded isomerized catharanthine whose structure was unambiguously assigned by X-ray crystallography (see the Supporting Information). Isocatharanthine **12**[2d] was obtained in 73% yield after recrystallization from methanol as a single isomer whose configuration of the C4−C20 double bond is *E*.

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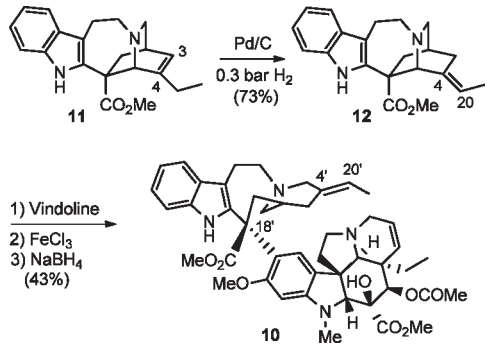
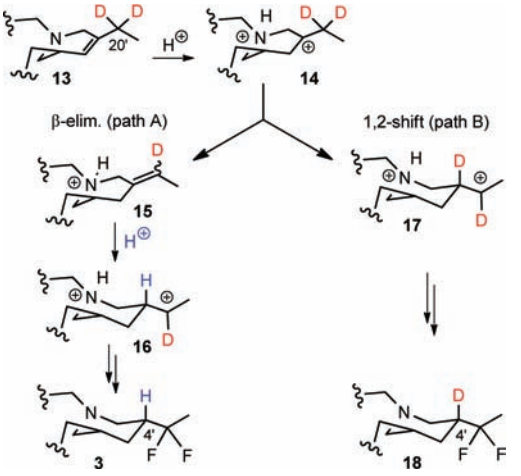
Scheme 3. Synthesis of 4′,20′-Anhydrovinblastine



The synthesis of 4′,20′-anhydrovinblastine **10** was thereafter continued by biomimetic coupling of **12** with vindoline, under Fe(III) activation. This process, which was originally developed for catharanthine by Kutney,[9] generates the isocatharanthine amine radical cation that undergoes oxidative fragmentation and coupling with vindoline. Subsequent reduction of the immonium intermediate by $NaBH_4$ afforded isoanhydrovinblastine **10** (4′,20′-anhydrovinblastine, 43% yield) with the same C18′-(*S*) configuration as that of naturally occurring *Vinca* alkaloids. With isoanhydrovinblastine **10** in hand, the fluorination was attempted through the reaction of the dimeric alkaloid with HF/SbF_5 and $CHCl_3$ at −35 °C. The reaction was stopped after 25 min, and we were pleased to observe that, similar to anhydrovinblastine **1**, the reaction of isoanhydrovinblastine **10** furnished difluorodeoxyvinblastine **3** in comparable yield (40%) and byproduct profile, as determined by LC/MS. This result suggests that the intermediacy of a species analogous to **10** in the course of anhydrovinblastine's **1** fluorination cannot be excluded (Scheme 2, path A). However, this hypothesis neither implies an exclusive β-elimination route (path A) nor rules out a parallel 1,2-hydride shift (path B). To establish whether the latter mechanism is also operating in the superacidic fluorination of *Vinca* alkaloids, we undertook to prepare isotopically labeled anhydrovinblastine. Specifically we sought to replace the two protons originally borne by C20′ with deuterium atoms (compound **13**). If following fluorination of the labeled dimeric alkaloid no deuterium atoms were detected on C4′ in the final fluorinated product, this would imply that β-elimination and C4′ reprotonation by the superacidic mixture is the main pathway (Scheme 4, path A). On the contrary, if deuterium were detected on C4′, this would indicate that the C20′ to C4′ 1,2-deuteride shift mechanism is active (Scheme 4, path B). Both mechanisms may not be exclusive and a mixture of labeled **18** and unlabeled **3** could be obtained after fluorination. In this case, the respective contribution of each path to the fluorination process will be given by the **18**/**3** ratio.

Scheme 4. β-Elimination vs 1,2-Deuteride Shift Pathway



C20′-deuterated anhydrovinblastine **13** was synthesized starting from catharanthine **11** (Scheme 5). The indole and tertiary nitrogen atoms, which may be readily oxidized, were protected as a carbamate and lactam, respectively.[10] The protected catharanthine **19** was subsequently submitted to regioselective allylic oxidation (SeO_2) which provided the expected alcohol as a single epimer of unknown configuration. The latter was oxidized to ketone **20** using MnO_2. Introduction of the first deuterium atom on C20 was achieved using sodium borodeuteride in the presence of $CeCl_3$ to favor 1,2- over 1,4-addition.[11] The monodeuterated alcohol **21** was then activated as the corresponding acetate before the second atom of deuterium was introduced using the same deuteride source ($NaBD_4$) but in the presence of $Pd(PPh_3)_4$.[12] The masked catharanthine derivative **22** was finally deprotected by removal of the carbamate group and selective reduction of the lactame.[13] C20-bis-deuterated catharanthine **23** (91% isotopic enrichment) was obtained in nine steps with an overall yield of 9%. Labeled catharanthine **23** was thereafter coupled to vindoline using the previously mentioned $FeCl_3/NaBH_4$ conditions to give 20′,20′-dideuteroanhydrovinblastine **13**. The latter exhibited a nearly identical ^1H NMR spectrum to that of anhydrovinblastine **1** except for the disappearance of both H20′ signals and a change in the multiplicity of the H21′ protons (triplet → singlet). The biomimetic coupling of **23** with vindoline provided efficient access to **13**, thus setting the stage for the key fluorination step.

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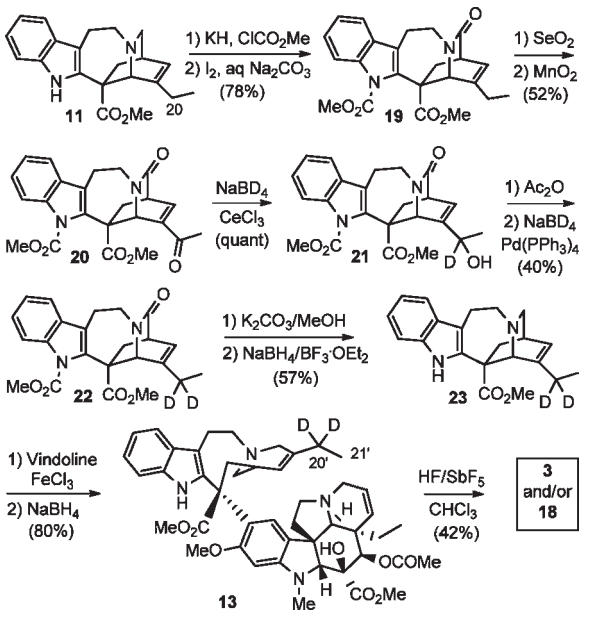
Scheme 5. Synthesis of 20′,20′-Dideuteroanhydrovinblastine and Further Reaction under Superacidic Conditions



Labeled anhydrovinblastine was accordingly reacted with HF/SbF_5 in the presence of $CHCl_3$ for 25 min at −35 °C, and after workup, the major product was isolated by column chromatography. The first observation is that the superacidic fluorination worked equally well with both labeled and unlabeled anhydrovinblastine, with no impact arising from the isotopic substitution. The difluorinated alkaloid was isolated in 42% yield, but neither ^{1}H NMR nor ^{19}F NMR permitted to unambiguously detect the presence of deuterium on C4′. Indeed, the signal of the putative residual H4′ proton is masked in the dense 2.1−2.9 ppm region and accurate integration of the peak of interest was not feasible. On the other hand, ^{19}F NMR (H-decoupled) showed a well-defined AB system for the two C20′-fluorine atoms but no coupling constant with the adjacent deuterium. This may not be detrimental as the reported ^3J (H4′/F20′) value for analogous systems (e.g., vinflunine **4**) is only of 6 Hz.[14] Taking into account the deuteron gyromagnetic ratio and the small H/F coupling value of vinflunine, the D4′/F20′ coupling in **18** is thus anticipated ≤1 Hz. Deuteration of the fluorinated alkaloid was finally revealed by mass spectrometry analysis which detected, together with the $[M+H]^+$ peak at 832 Da, an increased peak at 833 Da which corresponds to deuterated alkaloid **18** and natural isotopic ^{13}C abundance contribution. The relative intensities of these peaks, combined to MS analysis of the unlabeled alkaloid **3**, were used in the calculation of the isotopic enrichment which was found to be 20% (see the Supporting Information for details). These results thus unambiguously showed that the difluorinated alkaloid incorporated some deuterium; without being able to ascertain definitively if this was in the C4′-position. The site of deuterium incorporation was eventually elucidated by ^{2}H NMR spectroscopy. Indeed, the deuteron spectrum of the difluorinated alkaloid showed the appearance of a unique singlet centered at 2.6 ppm whose chemical shift matches that of H4′. Taken together, these results indicate that the fluorinated alkaloid incorporates 20% of deuterium at the expected C4′ position which suggests that the process is, to some extent, going through 1,2-hydride shift (Scheme 4, path B). This would also imply that the other mechanism (i.e., elimination/reprotonation, path A) is preponderant with a contribution of 80%. However, we were intrigued by the peculiar position of the deuterium atom which is adjacent to the CF_2 group. Although electron-withdrawing groups classically decrease the σ-basicity of adjacent protons under superacidic conditions, some D/H exchange might still occur. If isotopic dilution is taking place, the deuterium incorporation value obtained for **18** would be altered. To find out whether isotopic dilution intervened, the partly labeled dimer **18** was simply reprocessed under classical superacidic conditions. After the usual workup, the difluorinated alkaloid was recovered by flash chromatography and analyzed to measure its isotopic enrichment. While **18** initially incorporated 20% of deuterium at C4′, mass spectrometry indicated that the deuterium content dropped to 4% in the reacted sample. This result clearly shows that D/H exchange at C4′ is active under superacidic conditions and that the initially recorded 20% deuterium incorporation did not reflect respective contributions of paths A and B (Scheme 4). Isotopic exchange proceeded likely by σ-protonation[15] of the C4′−D bond which might be anchimerically assisted by the neighboring fluorine atoms.

In conclusion, although the elimination−reprotonation pathway cannot be excluded in the formation of the key C20′-carbocation, its occurrence is not likely to be the major pathway as long-lived carbocations are generally produced under superacidic conditions. If any elimination were still to occur, the concentration of the exocylic alkene **15** would be limited as equilibrium with carbocation **14** would be favored in the presence of the superacid. We believe that, in the case of dimeric *Vinca* alkaloids, the main pathway is thus governed by the 1,2-hydride (deuteride) shift mechanism. Even though involvement of the latter could not be precisely measured because of the inexorable D/H exchange, its actual contribution is most likely superior to the detected 20% value.

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Supporting Information Available. Experimental procedures, spectroscopic data, copies of ^{1}H and ^{13}C NMR spectra, and crystallographic data for compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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