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Short communication

Reactivity of *Vinca* alkaloids in superacid An access to vinflunine, a novel anticancer agent

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Abstract

This is a summary of a lecture presented at the 100th Anniversary, Moissan Symposium in Paris on Friday 10th November 2006.

In HF/SbF_5, *Vinca* alkaloids react selectively at the D'ring of the molecule. In the presence of $CHCl_3$ (or CCl_4), vinorelbine yields 20′,20′-difluoro-3′,4′-dihydrovinorelbine (vinflunine), presently in phase III experimentation for treatment of bladder cancer and non small cell lung cancer.

Keywords: Superacids; Alkaloids; Fluorination; Oxidation; Ionic hydrogenation

1. Introduction

Gillespie proposed to define as a superacid any system more acidic than 100% sulfuric acid, namely $H_0 \leq -12$ [1]. One of the strongest superacids is obtained with hydrogen fluoride (HF) in combination with antimony pentafluoride (SbF_5). For example, the H_0 acidity scale for the HF/SbF_5 (1/1) has been estimated to be of the order of −28, that is 10^{16} as acidic as pure sulfuric acid [2].

Since the early 1970s, we have studied the reactions of functionalized organic compounds (terpenes, steroids, alkaloids) in the HF/SbF_5 system. Under these conditions, the substrates are mono or polyprotonated and, as a result, novel reactions can be carried out efficiently: isomerization of saturated and unsaturated ketones, functionalization of inactivated C–H bonds, dearomatization of phenols, ionic hydrogenation and fluorination (Scheme 1) [3].

Since the discovery of therapeutic efficacy of antimitotic Vinca alkaloids: vinblastine (**1a**), vincristine (**1b**), and of semi-synthetic vinorelbine (Navelbine®) (**2a**), which represent a chemical class of major interest in cancer chemotherapy, several hundreds derivatives have been synthesized and evaluated for their pharmaceutical activities (Fig. 1) [4,5].

In order to have access to new derivatives we have examined the reactivity of compounds **1a**, **2a** and of anhydrovinblastine **2b** in superacid.

2. Results

2.1. Reaction with chloromethanes

In superacids, chloromethanes, CCl_4, $CHCl_3$, CH_2Cl_2 are known to be the precursors of superelectrophiles CCl_3^+, $CHCl_2^+$, CH_2Cl^+ [6]. For example, in HF/SbF_5 in the presence of CCl_4, anilines, indoles, indolines reacting in their protonated forms yield trifluoro derivatives after halogen exchange [7].

$$ArH \rightarrow Ar{-}CCl_3 \rightarrow Ar{-}CF_3$$

Furthermore, these chloromethyl ions possess a strong hydride power and therefore act as oxidizing agents [8].

Consequently, a $CHCl_3$ solution of vinblastine **1a** or of anhydrovinblastine **2b** was added to the superacid at −50 °C. An HPLC kinetic study permitted the identification of two intermediates, 20′ diastereoisomers of 20′-chloro-4′-deoxy-vinblastine 4, which disappeared simultaneously with the formation of compound **3b** (>50% yield). Under the same conditions, vinorelbine **2a** gave the difluoro analog **3a** (vinflunine; 35% yield). The main byproduct (15%) was

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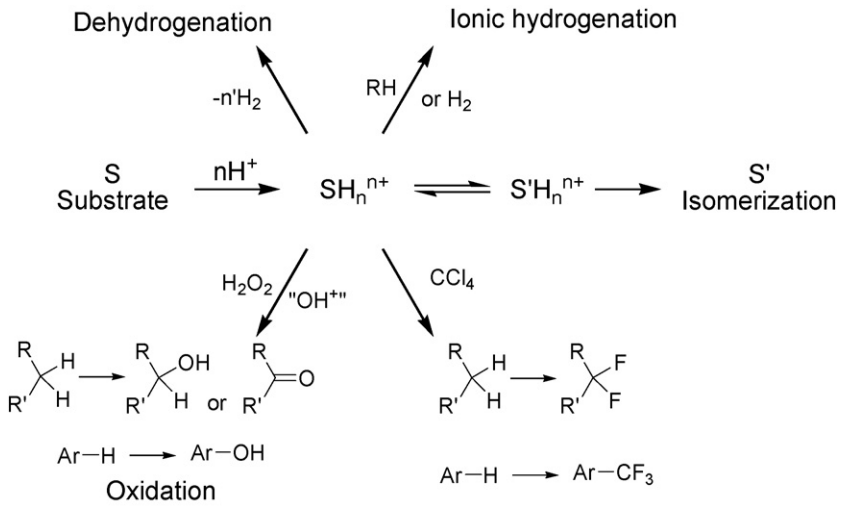


Scheme 1.

the C-4′ epimeric difluorinated compound (Scheme 2) [9]. Determination of structure of compounds **3a** and **3b** was made by ^{1}H and ^{13}C NMR analysis, resonances being assigned from DEPT, COSY and HMQC data [10]. The (*R*)-C4′ configuration of compounds **3a** and **3b** was established by nuclear Overhauser effect spectroscopy (NOESY) experiments. Molecular modeling calculation favored the chair conformation of the piperidine ring, with predicted NOEs between H4′ and H7′ *endo*, and H4′ and H1′ *endo*. This result was confirmed by X-ray crystallography of compound **3a**.

Taking into account these data, the postulated mechanism is depicted in Scheme 3. Formation of cation **5** results from protonation of the 3′,4′ double bond at C3′, followed by a 1,2-hydride shift from C20′ to C4′. Reaction of the resulting ion **5**, with a chloride ion, leads to the intermediates **4**. Hydride abstraction at C20′ by the dichloromethyl ion yields ion **6**, which can trap a fluoride ion, and after halogen exchange, gives the difluorinated product (**3a** or **3b**).

Vinflunine **3a** can be prepared directly from vinorelbine **2a** but more efficiently by fluorination of anhydrovinblastine **2b** followed by C′-ring contraction [11].

2.2. Ionic hydrogenation

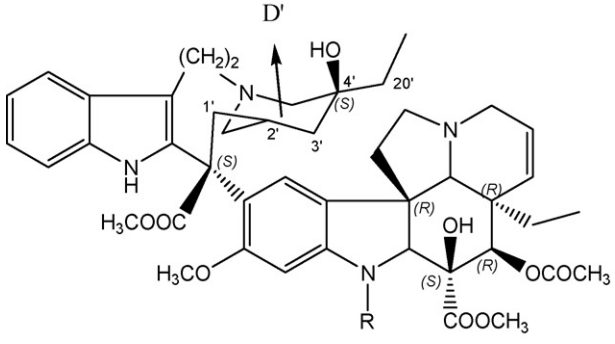
To extend the use of superacids to the synthesis of other semisynthetic derivatives, we have investigated the reactivity of compounds **1a**, **2a** and **2b** in the presence of cyclohexane or methylcyclopentane, acting as reducting agents in the media. The reduction is completely stereoselective yielding the dihydro derivative **12a** (70% yield) or **12b** (65% yield) with 4′R configuration [12].

3. Pharmacology

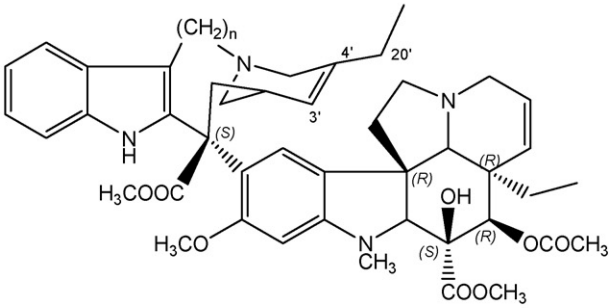
Vinca alkaloids bind to tubuline to block cell division by interfering with the function of a mitotic spindle. Evaluation of the new synthesized compounds has been based on *in vitro* and *in vivo* tests and compared to reference compounds.

3.1. In vitro tubuline interactions

The ability to inhibit tubuline polymerization *in vitro* was followed according to the method of Gaskin and Cantor [13]. Activities are determined by the IC_{50}, corresponding to the concentration of test compound inhibiting 50% of tubuline

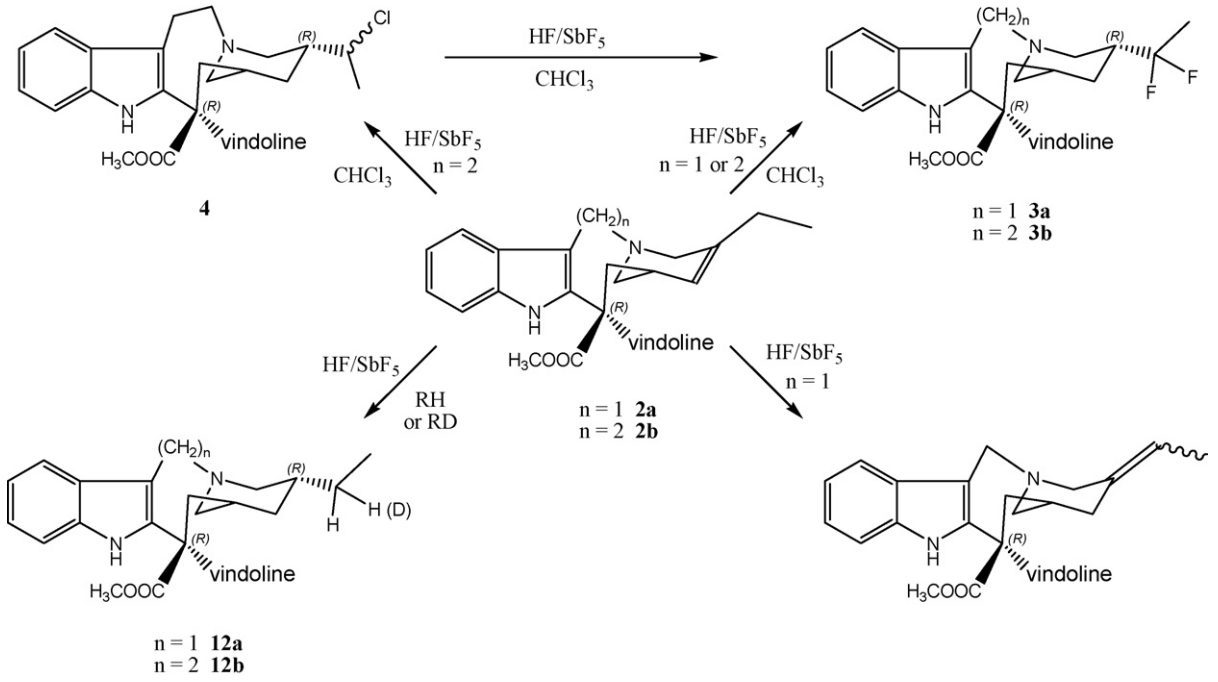


1a R=CH_3
1b R= CHO

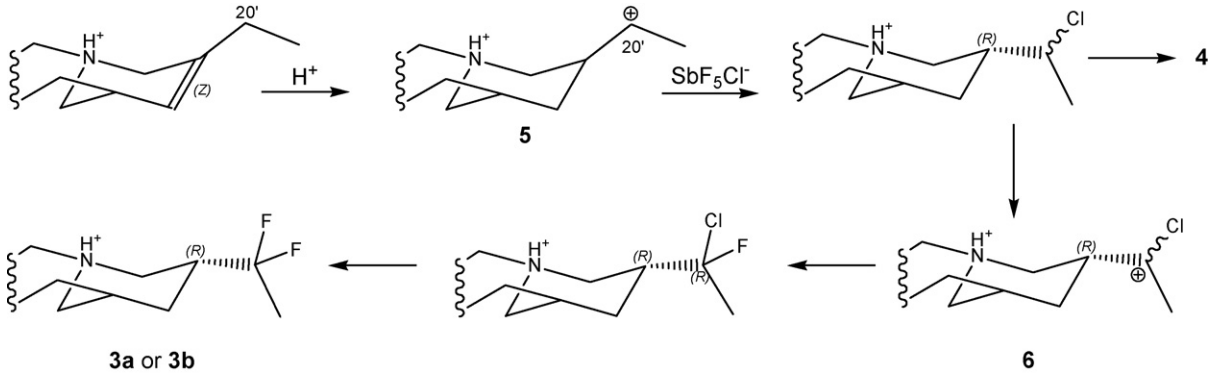


n = 1 **2a**
n = 2 **2b**

Fig. 1. Natural Vinca alkaloids and semisynthetic derivatives.



Scheme 2.



Scheme 3.

polymerization. The observed IC_{50} values for **1a**, **1b**, **2a**, **3a** and **3b** were all within a too narrow micromolar range (1.7–3.1 μM) to permit any clear classification [14].

3.2. Cytotoxicity

The new derivative **3a** has been evaluated for its cytotoxic properties against a panel 11 human and murine cancer cell lines. Vinflunine **3a** exhibited a relatively low cytotoxicity compared to the other *Vinca* alkaloids **1a**, **1b** and **2a**. On the basis of these *in vitro* results, vinflunine could be rejected in a classic drug screen [14].

3.3. In vivo antitumor properties

Activity of test compound is reflected by an increase of life span define as (the median survival of treated mice/median survival of control mice) × 100 (% T/C). Antitumor activity against the P388 murine leukemia (IV/IP) cell line was evaluated in single or multiple doses according various schedules of administration. The T/C ratios for vinflunine are ranging from 200% (single dose) to 457% (weekly injection over 4 weeks), superior to those of 129–186% obtained with the other *Vinca* alkaloids [15]. Vinflunine was also evaluated against 11 human tumor xenograft models and exhibited high or moderate antitumor efficacy in 64% (7/11) of the models, versus moderate activity recorded for vinorelbine in 27% (3/11) [16].

In vivo antitumor activity of vinflunine has been compared with that of the nonfluorinated analog **12a**, experiments being conducted on the P388 (IV/IP) model. Reduced compound **12a** is less active (T/C 140) than vinflunine, showing the importance of the two fluorine atoms [17].

4. Conclusion

On account of its promising antitumor activity, vinflunine (Javlor®) is synthesized on a kilogram scale and presently in phase III experimentation for treatment of bladder cancer and of non small cell lung cancer.

References

[1] R.J. Gillespie, T.E. Peel, Adv. Phys. Org. Chem. 9 (1971) 1–24.

[2] G.A. Olah, G.K.S. Prakash, J. Sommer, Superacids, Wiley–Interscience, 1985.

[3] (a) J.C. Jacquesy, G.K.S. Prakash, P.v.R. Schleyer (Eds.), Stable Carbocation Chemistry, John Wiley and Sons Inc., New York, 1997,, pp. 549–574, Chapter 17;
(b) J.C. Jacquesy, G.A. Olah, G.K.S. Prakash (Eds.), Carbocation Chemistry, John Wiley and sons, Inc., New York, 2004,, pp. 359–375, Chapter 14.

[4] G. Blasko, G.A. Cordell, Antitumor Bisindole Alkaloids from *Catharanthus roseus* (L), in: G. Blasko, A. Brossi, M. Suffness (Eds.), The Alkaloids, 37, Academic Press, San Diego, 1990, pp. 1–76.

[5] R.L. Noble, Biochem. Cell. Biol. 68 (1990) 1344–1351.

[6] G.A. Olah, Angew. Chem. Int. Ed. Eng. 32 (1993) 757–788.

[7] (a) S. Debarge, B. Violeau, B. Bendaoud, M.P. Jouannetaud, J.C. Jacquesy, Tetrahedron Lett. 44 (2003) 1747–1750;
(b) S. Debarge, K. kassou, H. Carreyre, B. Violeau, M.P. Jouannetaud, J.C. Jacquesy, Tetrahedron Lett. 45 (2004) 21–23.

[8] S. Thibaudeau, A. Martin-Mingot, M.P. Jouannetaud, J.C. Jacquesy, Tetrahedron 58 (2002) 6643–6649.

[9] J. Fahy, A. Duflos, J. Ribet, J.C. Jacquesy, C. Berrier, M.P. Jouannetaud, F. Zunino, J. Am. Chem. Soc. 119 (1997) 8576–8577.

[10] Characteristic NMR signals for compound 3a (δ ppm). 1H NMR: δ 1.60 (3H, t, ^{3}J = 18.9 Hz, H21′), δ 2.8 (1H, m, H4′), loss of the ethylenic H3′. 13C NMR: δ 125.4 (dd, ^{1}J = 240.0 Hz, C20′), δ 21.5 (dd, ^{2}J = 31.5 Hz, C21′), δ 31.2 (dd, ^{2}J = 31.5 Hz, C4′). 19F NMR: AB spin system (proton noise decoupling), δ = −20.1 and −21.3 Hz, J = 242 Hz.

[11] P. Mangeney, R.Z. Andramialisoa, J.Y. Lallemand, N. Langlois, Y. Langlois, P. Potier, Tetrahedron 35 (1979) 2175–2179.

[12] C. Lafitte, M.P. Jouannetaud, J.C. Jacquesy, J. Fahy, A. Duflos, Tetrahedron Lett. 39 (1998) 8281–8282.

[13] F. Gaskin, C.R. Cantor, J. Mol. Biol. 89 (1974) 737–758.

[14] A. Kruczynski, J.M. Barret, C. Etievant, F. Colpaert, J. Fahy, B.T. Hill, Biochem. Pharmacol. 55 (1998) 635–648.

[15] A. Kruczynski, F. Colpaert, J.P. Tarayre, P. Mouillard, J. Fahy, B.T. Hill, Cancer Chemother. Pharmacol. 41 (1998) 437–477.

[16] A. Kruczynski, B.T. Hill, Crit. Rev. Oncol. Hematol. 40 (2001) 159–173.

[17] J. Fahy, A. Duflos, P. Schambel, A. Kruczynski, C. Etievant, J.M. Barret, et al. Proc. Am. Assoc. Cancer Res. 39 (1998) 1136.