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5'-NOR ANHYDROVINBLASTINE

PROTOTYPE OF A NEW CLASS OF VINBLASTINE DERIVATIVES

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Abstract—The reaction medium obtained after applying the modified Polonovski reaction to anhydrovinblastine N_b-oxide 4 led, after hydrolysis, to 5'-nor anhydrovinblastine **10a**.

The most fruitful approach for the preparation by partial synthesis[1] of the complex antitumour alkaloids of the vinblastine 1 group found in *Catharanthus spp.*, took advantage of several stereoelectronic factors for directing functionalization of carbons $C_{20'}$ and/or $C_{15'}$ of anhydrovinblastine ($\Delta^{15'(20')}$ 20'-deoxyvinblastine) 2 or other intermediates.[2-4]

The modified Polonovski reaction[5] applied to anhydrovinblastine N_b-oxide 4 appeared to be well suited to prepare intermediates as the conjugated immonium salt 5.

In contrast with the regioselectivity generally observed in the case of tetrahydropyridines,[5] the modified Polonovski reaction applied to the N_b-oxide 4 did not lead only to the conjugated immonium 5 (path a, Scheme 1) but also to a fragmentation product of the tryptamine chain (path b, Scheme 1) giving rise to new seco C_5–C_6 derivatives 7 depending on the nucleophile being used.[6] We wish to disclose the preparation of this new class of vinblastine derivatives, the first example of which being the 5'-nor anhydrovinblastine **10a**.

Thus, the product of the reaction of anhydrovinblastine N_b-oxide 4 with trifluoroacetic anhydride was treated, after evaporation of the excess of reagent, with a mixture of water and tetrahydrofuran. In addition to a mixture of polar products, probably constituted by immonium salts and/or corresponding carbinol amines, the only product isolated was 5'-nor anhydrovinblastine **10a** (yield: 27%) (Scheme 2). Treatment of this polar mixture with sodium cyanoborohydride in acidic tetrahydrofuran led to the recovery of anhydrovinblastine 2 (35%) and the formation of 20'-deoxy leurosidine 3 (18%), these two products being formed by reduction of the conjugated immonium salt 5 (Scheme 1).

The formation of 5'-nor anhydrovinblastine **10a** can be easily rationalised: addition of water on the bisimmonium salt 6 and subsequent loss of formaldehyde followed by a nucleophilic displacement (with or without intervention of a retro-Mannich process) of the substituent on carbon $C_{6'}$ (Scheme 2).

The UV spectrum of 5'-nor anhydrovinblastine **10a** shows an indole-dihydroindole chromophore while the CD curve is analogous to those of vinblastine derivatives, indicating that there is probably no change in chirality on C atoms $C_{14'}$ and $C_{16'}$.[1,7]

In the PMR spectrum (250 MHz) of compound **10a**, the signals corresponding to the functional groups of vindoline are all present indicating that the vindoline part of the molecule has been preserved. The signal at 5.76 ppm of the ethylenic proton at $C_{15'}$ can be compared to the corresponding signals in 7 (seco C_5–C_6) which lies at ≈5.0 ppm; this striking difference disfavours structure **10b** for this compound (Scheme 2). Two doublets of one proton each appear at 4.58 and 4.41 ppm respectively; decoupling experiments allow to assign them to an A–B system (J = 14 Hz) attributed to C_6–H_2. The chemical shifts for these protons are in agreement with a gramine-type methylene[8] which are modified depending on whether the molecule is carbonated or not.

In the CMR spectrum, signals corresponding to the vindoline part are assigned in comparison with spectra of other vinblastine type derivatives.[9] These signals are generally well resolved in contrast with those corresponding to the "nor ibogane" part of the molecule. This fact could be due to either the conformational flexibility of the large membered ring[9] or to protonation of N_b during registration of the spectrum (CO_2). For this nor ibogane part of the molecule, in addition to signals attributed at high field to the Et chain and OMe, five CH_2 (53.6, 47.0, 44.1, 35.2 and 27.7) and one CH (28.2) were observed; one CH_2 is missing in comparison with anhydrovinblastine 2. In addition, a signal at 53.6 ppm, superimposed to the signal corresponding to C_7, can be attributed to a gramine-type CH_2.[10]

The mass spectrum of 5'-nor anhydrovinblastine **10a** exhibits a very small molecular ion ($M^{+\cdot}$ *m/e*: 778) and other fragments at *m/e*: 808, 794, 792, 748, 734 and 598 (100%). The presence of ions characteristic of the vindoline part at *m/e* 282, 135, 122 and 121 indicates again that this part of the molecule is indeed intact.[11] Intermolecular thermal transmethylation followed by Hofmann elimination are well known to occur during mass spectroscopy of compounds related to vinblastine 1.[12] High resolution measurements for ions at *m/e* 734 and 748 (778–CO_2 and 792–CO_2) indicate that these ions could be formed through an intra or intermolecular transmethylation process followed by the loss of a methoxy-carbonyl group.

These ions are shifted by three mass units in the corresponding **10d$_3$** derivative (C_{16}–CO_2CD_3) indicating that intermolecular transmethylation followed by loss of methoxy carbonyl on $C_{16'}$ is not involved. On the other hand, using a chemical ionization technique the main fragment at high molecular weights appears at *m/e* 735 (734 + 1); therefore an intramolecular transmethylation followed by a decarboxylation seems to be the most likely hypothesis.

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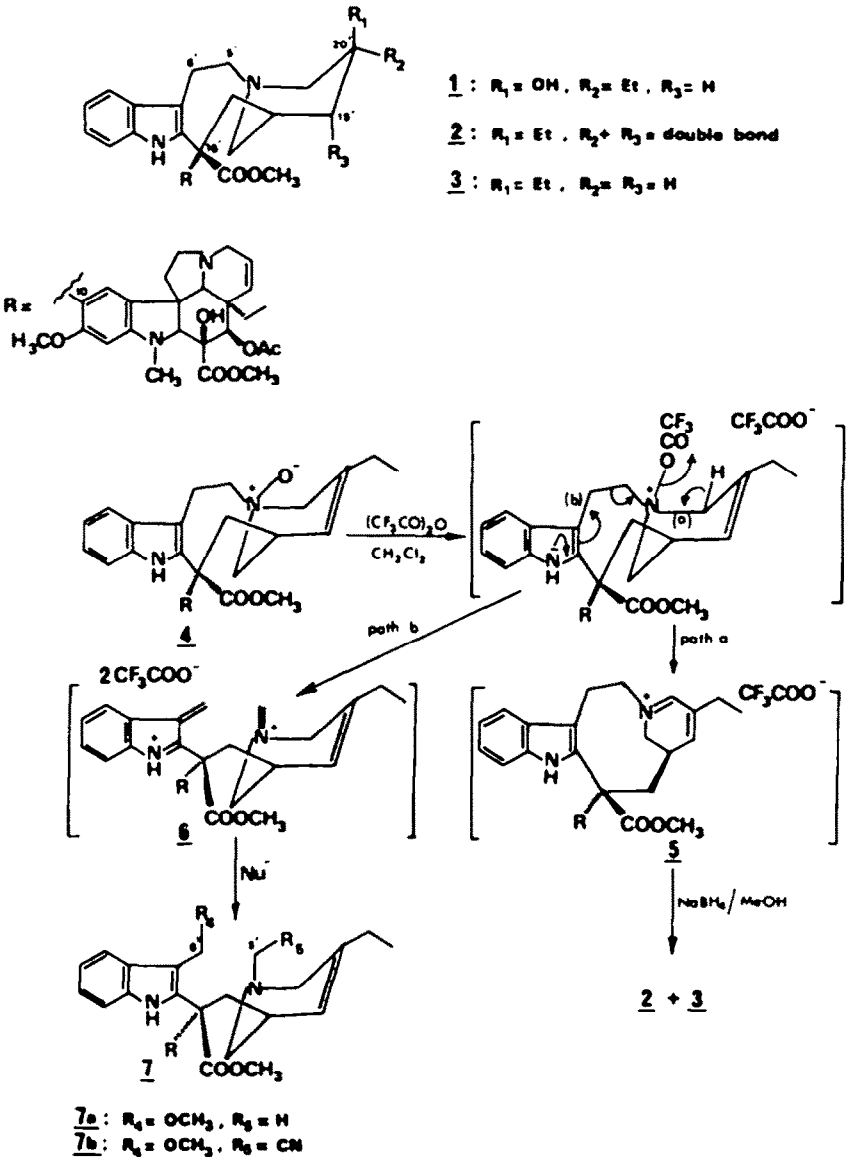


Scheme 1.

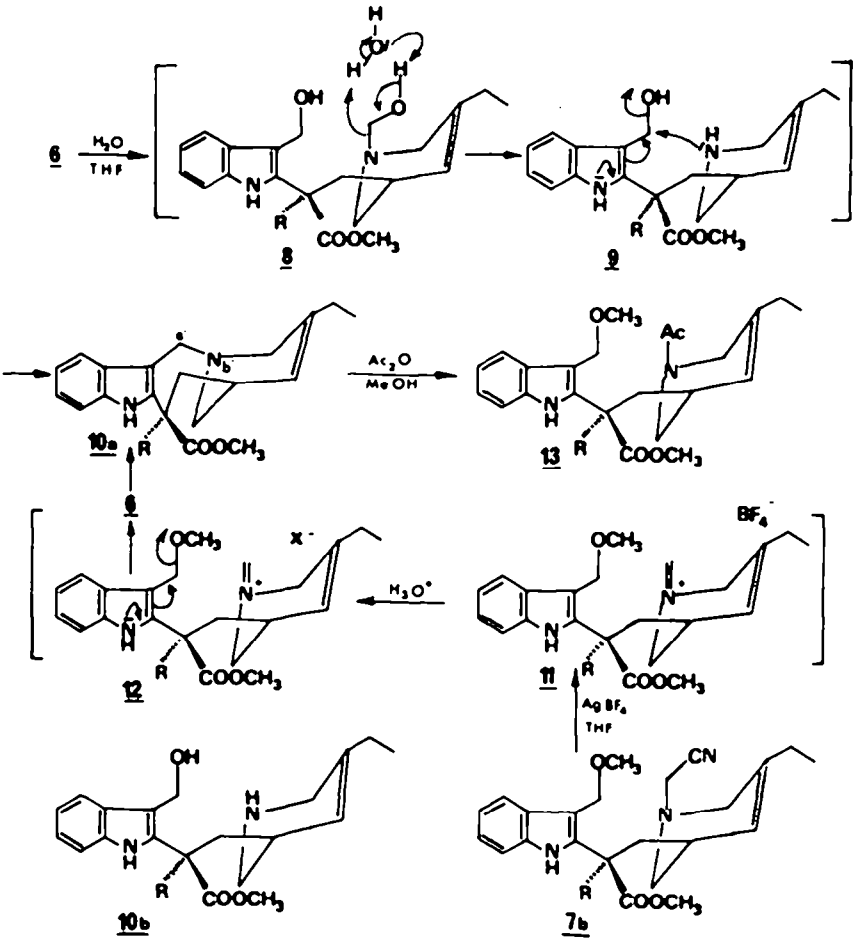
However, lack of obvious molecular ion in the mass spectrum of compound **10a**, prompt us to undertake further experiments concerning this type of compound.

Thus, compound **7b**[6] when treated with silver tetrafluoroborate in tetrahydrofuran and then with acidic water gave rise to 5'-nor anhydrovinblastine **10a**: silver salt regenerates the corresponding immonium salt **11** by decyanation and the acidic medium causes the elimination of the OMe group leading to the conjugated immonium salt intermediate **6** (or equivalent compound) (Scheme 2).

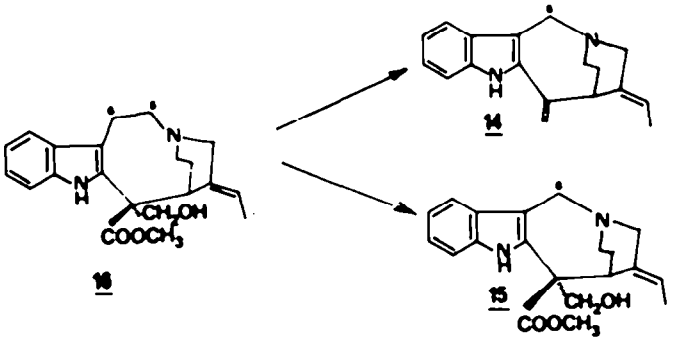
Conversely, 5'-nor anhydrovinblastine **10a** was treated by acetic anhydride in methanol[8] giving rise to the corresponding N-acetylated compound **13** by fragmentation of the N_b–C_6 bond (Scheme 2).

Structure of compound **10a** was definitely established by its clean N-oxidation with *m*-chloroperbenzoic acid a reaction which allows to exclude structure **10b** for this compound. The PMR spectrum of the N_b-oxide of 5'-nor-anhydrovinblastine **10a** was recorded at 400 MHz[18] (Fig. 1). The AB system corresponding to C_6–H_2 is shifted at 4.91 and 4.68 ppm ($J = 13$ Hz) and signals corresponding to other protons on C_{21} and/or C_3, α to N_b, also appear at lower field.

Biogenetic implications of the fragmentation of the tryptamine chain induced by the modified Polonovski reaction,[13] leading to C_3-nor-alkaloids, postulated by Potier and Janot,[14] were fully confirmed *in vivo* for apparicine **14**[15] and *in vitro* for vallesamine **15**[16] obtained from their common biogenetic precursor, i.e. stemmadenine **16** (Scheme 3). It is noteworthy that 5'-nor-anhydrovinblastine **10a** is the first example of a new class of antitumour compounds and could well be a natural product like anhydrovinblastine itself which was prepared *in vitro* in our laboratory before being isolated from *Catharanthus roseus*.[17]



Scheme 2.



Scheme 3.

EXPERIMENTAL

M.ps were taken on a Kofler apparatus, optical rotations measured ($CHCl_3$ soln, g/100 ml) on a Perkin–Elmer 141 MC, IR spectra (ν cm^{-1}, $CHCl_3$) on a Perkin–Elmer 257, UV spectra [EtOH, λ_{max}, nm (ϵ)] on a Bausch and Lomb Spectronic 505, CD curves [EtOH, λ_{max}, nm ($\Delta\epsilon$)] on a Roussel–Jouan Dichrograph II. ^1H NMR spectra were obtained ($CDCl_3$, Me_4Si, δ = 0 ppm) from Varian T 60 or IEF 240 and 400 MHz^{10} and Cameca 250 MHz spectrometers (coupling constants, J, are given in hertz; s, d, t, dd and m indicate singlet, doublet, triplet, doublet of doublet, and multiplet, respectively). Mass spectra were measured on an AEI MS 9 and MS 50. Preparative layer chromatography (preparative tlc) is performed with Kieselgel HF 254 Merck.

Anhydrovinblastine N_b*-oxide* 4. *m*-Chloroperbenzoic acid (86 mg, 0.56 mmol) in CH_2Cl_2 (5 ml), was added at 0° to a stirred soln of 2 (340 mg, 0.43 mmol) in CH_2Cl_2 (10 ml) under argon. After 10 min, the mixture was poured into a sat of Na_2CO_3 aq (3 ml) extracted by $CHCl_3$ (50 ml), washed three times with brine (5 ml). After drying with Na_2SO_4 and filtration, the $CHCl_3$ extract was evaporated under reduced pressure. Pure 4 was obtained (346 mg, 100%). UV: 268, 289, 299, 310. MS *m/e*: 822, 820, 808 ($M^{+\cdot}$), 806, 792, 733, 669, 631, 610, 282, 136, 135, 122, 121 (100%). NMR (240 MHz): 9.80 (s, 1H, C_{16}–OH, 8.19 (s, 1H, N_a–H), 7.70

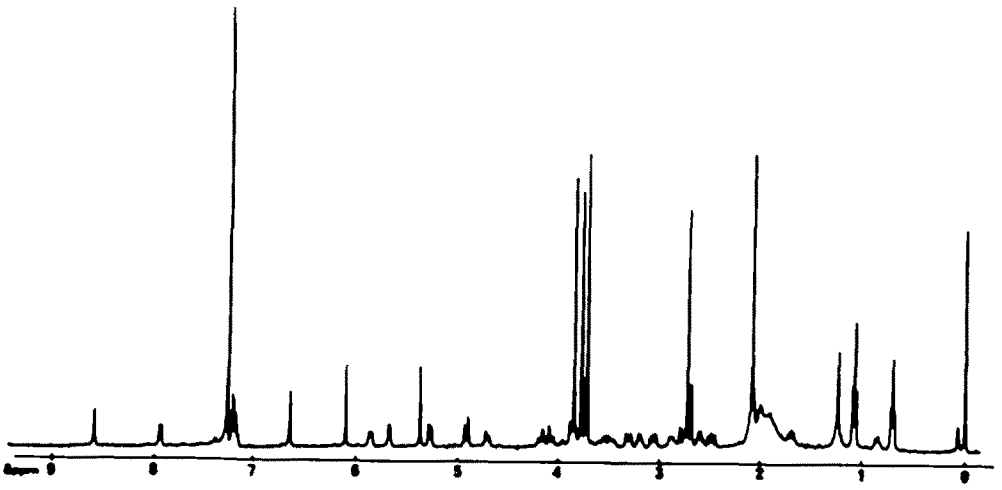


Fig. 1.

(d, 1H, J = 7.5, H aromatic indolic), 7.14 (m, 2H, H aromatic), 6.43 (s, 1H, C_9–H), 6.11 (s, 1H, C_{17}–H), 5.84 (dd, $J_{14,15}$ = 10 and $J_{3,14}$ = 4.5, C_{14}–H), 5.44 (1H, C_{15}–H), 5.41 (s, 1H, C_{17}–H), 5.27 (d, 1H, $J_{14,15}$ = 10, C_{15}–H), 4.51 (m, 2H), 4.32 (d, 1H) and 3.98 (d, 1H) N_b–CH, 3.84, 3.78 and 3.69 (3s, 9H, C_{11}–OCH_3, $C_{16}CO_2CH_3$ and $C_{16}CO_2CH_3$), 2.62 (s, 3H, N_a–CH_3), 2.09 (s, 3H, $OCOCH_3$), 1.06 and 0.64 (2t, $J_{18,19}$ = 7, 6H, C_{19}–H_3 and C_{18}–H_3).

Preparation of 5'-nor-anhydrovinblastine **10a**. Compound 4 (345 mg, 0.42 mmol) in soln in dry CH_2Cl_2 (2 ml) was treated at 0° under argon for 2 hr with trifluoroacetic anhydride (0.267 ml, 1.8 mmol). The medium was evaporated *in vacuo* without heating and dissolved in dry THF (8 ml). Water (0.03 ml) was introduced and the resulting soln was stirred for an additional 1 hr under argon at room temp, poured into brine, extracted with $CHCl_3$, washed with brine until neutral. The resulting organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude residue was purified by preparative tlc (eluent: $CHCl_3$–MeOH, 90–10) and **10a** (90 mg, 27%) and polar products (257 mg) were isolated.

The polar products were dissolved in a tetrahydrofuran-trifluoroacetic acid mixture (10 ml/0.08 ml) and reduced with an excess of sodium cyanoborohydride. After neutralisation with $NaHCO_3$ and extraction with $CHCl_3$, the crude mixture was purified by preparative tlc ($CHCl_3$–MeOH, 90–10) and 2^{1b} (120 mg, 35%) and 3^{1b} (60 mg, 18%) were isolated.

5'-Nor-anhydrovinblastine **10a**. $[\alpha]_D^{20}$: + 52.4° ($CHCl_3$, C = 0.3); IR: 3460, 2965, 1745, 1620; UV: 215(3700), 268(11000), 282(9500), 293(7600), 310(4400); DC: 312 (+ 3.4), 305 (+ 3.2), 258 (+ 13.3), 230 (+ 31.6), 260 (− 44.3); MS: 808.4420 (calc.: 808.4411, $C_{47}H_{60}N_4O_8$, 2%), 794.4235 (794.4254, $C_{46}H_{58}N_4O_8$, 4%), 792.4897 (792.4098, $C_{46}H_{56}N_4O_8$, 11.5%), 748.4155 (748.4200, $C_{45}H_{56}N_4O_6$, 100%), 734.4001 (734.4043, $C_{44}H_{54}N_4O_6$, 23%), 598.2909 (598.2917), $C_{35}H_{40}N_3O_6$, 70%), 282.1335 (282.1341, $C_{14}H_{20}NO_3$, 8.2%), 135.1048 (135.1048, $C_9H_{13}N$, 8%), 122.0976 (122.0970, $C_8H_{12}N$, 3%). NMR ^1H (250 MHz): (s, 1H, N_a–H), 7.17 (m, 2H, H aromatic indolic), 6.34 and 6.08 (2s, 2H, C_9–H and C_{17}–H), 5.83 (dd, 1H, $J_{14,15}$ = 9.5 and $J_{3,14}$ = 3.5, 1H, C_{14}–H), 5.76 (m, 1H, C_{15}–H), 5.37 (s, 1H, C_{17}–H), 5.25 (d, $J_{14,15}$ = 9.5, 1H, C_{15}–H), 4.58 and 4.41 (d not well resolved and d, $J_{6'-6'b}$ = 14, 2H, C_6–H_2), 3.82, 3.13 and 3.68 (3s, 9H, C_{11}–OCH_3, C_{16}–CO_2CH_3 and C_{16}–CO_2CH_3), 2.69 (s, 3H, N_a–CH_3), 2.57 (s, 1H, C_{21}–H), 2.06 (s, 3H, $OCOCH_3$), 1.07 and 0.20 (2t, $J_{18,19}$ = 7, 6H, C_{19}–H_3 and C_{18}–H_3). NMR ^{13}C:[19] 173.9 (C_{16}–COO), 171.3 and 170.6 (–$OCOCH_3$ and C_{16}COO), 157.9 (C_{11}), 152.7 (C_{13}), 134.2 (C_{17} and C_{20}), 132.5 (C_7), 129.6 (C_{14}), 128.3 (C_8), 124.8 (C_{14}), 122.7 and 120.6 (C_{16} and C_{11}), 118.7 (C_7?), 118.4 (C_9), 110.4 (C_{12}), 93.8 (C_{12}), 83.1 (C_2), 79.8 (C_{16}), 64.7 (C_{21}), 55.8 (C_{11}–OCH_3), 54.7 (C_{16}), 53.6 and 53.4 (C_6 and C_7), 52.9 and 52.1 (C_{16}–CO_2CH_3 and C_{16}–CO_2CH_3), 50.3 and 49.8 (C_5 and C_3), 47.0 and 44.1 (C_{21} and C_7), 44.6 (C_4) (attributed with respect to the resolution for this signal), 42.8 (C_{20}), 38.3 (N_a–CH_3), 35.2 and 27.7 (C_{17} and C_{19}), 30.8 (C_{19}), 28.2 (C_{14}), 21.3 ($COCH_3$), 12.2 (C_{18}), 8.3 (C_{18}).

Preparation of 5'-nor-anhydrovinblastine **10a** *from compound* **7b**. Compound **7b** (80 mg, 0.095 mmol) in dry THF (3 ml) was treated in the dark under argon at room temp. by silver tetrafluoroborate (38 mg, 0.2 mmol). The medium was stirred for 3 hr and then diluted with trifluoroacetic acid (0.12 ml) and water (0.12 ml). The resulting soln was stirred for an additional 1 hr, poured into brine and extracted with $CHCl_3$. The organic layers were washed with water until neutral. The crude mixture obtained after usual treatment was purified by preparative tlc ($CHCl_3$–MeOH: 92–8), and a product identical $[\alpha]_D^{20}$, IR, UV, SM, ^1H NMR and HPLC with **10a** was obtained (50 mg, 68%).

Preparation of compound **13** *from 15'-nor-anhydrovinblastine* **10a**. A soln of **10a** (5 mg, 0.006 mmol) in dry MeOH (0.5 ml) was treated, at room temp. under argon with Ac_2O (0.02 ml) in the presence of KOAc (5 mg). The medium was stirred for 30 min, poured into brine and extracted with $CHCl_3$. After the usual treatment, the crude product was purified by preparative tlc (AcOEt–MeOH: 90–10) and compound **13** was isolated (3 mg, 55%).

Compound **13**. IR: 3420, 2920, 1740, 1640; UV: 220, 226, 284, 292, 314; SM: 852($M^{+\cdot}$), 821, 692, 661(100%), 553, 497, 496, 380, 282, 273, 223, 135, 122, 121; ^1H NMR (240 MHz): 6.0 and 5.5 (2s, 2H, C_9–H and C_{17}–H), 3.80 (s), 3.72 and 3.70 (2s), 3.44 and 3.40 (2s) (9H, $C_{16}CO_2CH_3$, C_{16}–CO_2CH_3 and C_{11}–OCH_3), 3.14 and 3.06 (2s) (3H, C_3–OCH_3), 2.70 (s, 3H, N_a–CH_3), 2.10 (s, 3H, $OCOCH_3$), 1.90 (s, 3H, N_b–$COCH_3$).

Preparation of 5'-nor-anhydrovinblastine N_b*-oxide*. Compound **10a** (10 mg, 0.013 mmol) in soln in CH_2Cl_2 (5 ml) was treated at 0° for 10 min with 3-chloroperbenzoic acid (3 mg, 0.017 mmol). The medium was poured into $CHCl_3$ washed with Na_2CO_3 aq (40 g/l) and 5'-nor anhydrovinblastine N_b-oxide was obtained (quantitative) after the usual treatment.

SM: 808, 794, 792, 776, 748, 656, 589(100%), 538, 282, 152, 135, 122, 121; ^1H RMN (400 MHz): 8.56 (s, 1H, N_a–H), 7.91 (1H, C_9–H), 6.65 and 6.1 (2s, 2H, C_9–H and C_{17}–H), 5.93 (dd, 1H, C_{14}–H), 5.67 (m, 1H, C_{15}–H), 5.36 (s, 1H, C_{17}–H), 5.27 (d, 1H, C_{15}–H), 4.91 and 4.68 (2d, $J_{6'-6'b}$ = 13, 2H, C_6–H_2), 3.85, 3.78 and 3.71 (3s, 9H, OCH_3, C_{16}–CO_2CH_3 and C_{16}–CO_2CH_3), 2.73 (s, 3H, N_a–CH_3), 2.1 (s, 3H, $OCOCH_3$), 1.08 and 0.70 (t, 3H, $J_{18,19}$ = 7, C_{19}–H_3 and C_{18}–H_3).

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