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Equipe Chimie des Substances Naturelles, Institut de Chimie Moléculaire de Reims, UMR CNRS 7312, BP 1039, 51687 Reims Cedex 2, France.

Abstract. The reaction of catharanthine with sodium borohydride in methylene chloride was claimed to produce an isomer of the title compound. A spectroscopic re-examination of the product has revealed that it is catharanthine borane.

Key words: catharanthine, isocatharanthine, borane, ^{11}B NMR

Introduction

Catharanthine **1** is the second most abundant alkaloid from *Catharanthus roseus* and a building block in the synthesis of the important anti-cancer drugs such as vinblastine, vincristine and navelbine (Potier et al, 1975, Kutney et al, 1975, Mangeney et al, 1979, Sears and Boger, 2015). The role of catharanthine in the biosynthesis of these dimeric alkaloids was first suggested by Harley-Mason and Atta-ur-Rahman (Harley Mason and Atta-ur-Rahman, 1967, Atta-ur-Rahman, 1971). At that time, the synthetic strategy involved coupling vindoline with carbomethoxy-cleavamines **2** derived from catharanthine by reduction under acid conditions (Kutney et al, 1970). For yet unclear reasons, there were controversies about the various transformations of catharanthine and an article from the Karachi group added to the confusion (Atta-ur-Rahman et al, 1978). This article described the reaction of catharanthine with sodium borohydride in methylene chloride, which is a rather unusual solvent for these reductions. It probably was expected to produce the cleavamines but, under these conditions, full transformation into a faster moving spot was obtained and following spectroscopic analyses, this compound was declared to be an isomer of catharanthine and named isocatharanthine. It is difficult to imagine isomers that do not involve deep-seated rearrangements. Furthermore, the reaction was also said to be reversible and the new compound reverted to catharanthine on standing.

Results and discussion

This aroused our curiosity, and we ran the reaction under the same conditions. It did not go to completion but, besides the starting material catharanthine, a single other faster moving spot was observed. The new compound **3** was purified by flash chromatography and, as reported in the literature, its spectroscopic characteristics were similar to those of catharanthine, although the melting point was higher (190°, decomposition). The ^{1}H NMR spectrum showed minor but definitive differences from the spectrum of catharanthine. For example, the olefinic proton appeared at δ 6.00 instead of 5.97 ppm and H-21 at 4.45 *vs* 4.23 in the starting material. Examination of the COSY and HSQC experiments showed that the gross structure of catharanthine was conserved in the new compound and that no rearrangement had occurred. The ^{13}C NMR spectrum however, showed important differences, especially for the resonances of the atoms surrounding the basic nitrogen, which were strongly deshielded (+ 21 ppm for C-5, + 13.5 for C-3 and + 3.2 for C-21). Clearly something had happened around this nitrogen atom. Our attention was drawn to a broad hump, centred around 1.6 ppm, which deformed the baseline of the ^{1}H NMR spectrum and which raised the possibility of a borane derivative of catharanthine. A ^{11}B NMR spectrum of the molecule was recorded under ^{1}H saturation and showed a peak at – 8.8 ppm, corresponding to an amine borane signal. An ^{1}H-^{15}N HMBC experiment was run to confirm the location of the boron and it showed a ^{15}N resonance at – 351 ppm in line with the expectations for an amine nitrogen linked to boron (Marian

and Gastreich, 2001). Infrared spectroscopy showed the typical bands of BH bonds at 2350 cm^{-1}. MS did not show the expected molecular ion corresponding to $C_{21}H_{28}BN_2O_2$ but a [M-2] with the appropriate ratio of isotopes for the boron atom. This is not totally unexpected for amine boranes (Kulkarny and Ramanchandran, 2017) and this loss of dihydrogen has been rationalized (Abboud et al, 2012). Structure **3** is thus proposed for the supposed "isocatharanthine" (equation 1).

Equation 1 near here

The formation of amine boranes of complex alkaloids is not unprecedented and the first example was condylocarpine borane, the structure of which, originally thought to be a methyl ammonium salt, was solved by X-ray crystallography (Wang and Paul, 1977). Later, during the course of a large-scale preparation of the Vinca alkaloid dimers, Szantai and his colleagues prepared the amine borane of anhydrovinblastine and they found that the adduct was much more stable than the base, which is not a general feature of these derivatives (Szantay et al, 1991). This stability is highly dependent on the nature of the amine as noticed by Kuehne (Kuehne and Zebovitz, 1987) and by us during attempts to prepare vincadifformine borane. Advantage was cleverly taken of this reaction to determine a configuration in a complex alkaloid mersiphylline A (Low *et al*, 2019). As far as preparation is concerned, we found that the metathesis conditions developed by Kulkarny and Ramanchandran, 2017 ($NaBH_4$, $NaHCO_3$, H_2O, THF) were far more convenient than the original approach. Exchange with the BH_3-THF complex was infructuous.

Owing to its reactivity and its susceptibility to relatively easy retro Diels Alder reactions, catharanthine may rearrange into a wealth of monoterpene indole alkaloids (Scott and Wei, 1974, Kan-Fan et al, 1974). The reaction suggested by Atta-ur-Rahman in 1978 did not yield the expected rearrangement products but simply catharanthine borane. This new compound and catharanthine were assayed for antimicrobial activities using a standard *in vitro* microbiological assay. Neither compound possesses significant antimicrobial activity (up to 200 µg/mL) against the Gram-negative bacteria *Escherichia coli* or *Pseudomonas aeruginosa* as well as Gram-positive *Staphylococcus aureus* except for catharanthine borane presenting a MIC of 200 µg/mL against *S. aureus*.

Experimental part

General

The optical rotation was determined in MeOH with an Anton Paar MCP 5100 polarimeter at 25°C. Fourier transform-infrared (FT-IR) measurements was carried out using a Perkin Elmer Spectrum Two spectrometer. HR-ESI-MS experiments were performed using a SYNAPT G2 si, Waters instrument (Manchester, UK). NMR spectra were acquired in $CDCl_3$ on a Bruker AVIII500 instrument (^1H at 500 MHz, ^{11}B at 160 MHz, ^{13}C at 125 MHz and ^{15}N at 50.6 MHz). Standard pulse sequences and parameters were used to obtain 1D ^1H and ^{13}C spectra, and 2D COSY, HSQC and HMBC spectra.

Synthesis of catharanthine-borane

Catharanthine sulfate (300 mg, 0.70 mmole) was suspended in 5 mL CH_2Cl_2. Na_2CO_3 (1 g, 9.4 mmoles) and 2 mL H_2O were added and the suspension was stirred for 2 hours at room temperature. The organic layer was separated from the solids and 5 mL THF and 5 drops of water were added followed by 500 mg of $NaBH_4$ and 500 mg $NaHCO_3$ in small portions. The reaction mixture was stirred vigorously for 24 hours and the organic layer was separated, filtered and evaporated to give 280 mg of a white foam, which showed two spots on TLC (eluent was $CHCl_3$/MeOH: 98/2). The mixture was separated on 30 g of Kieselgel eluted by CH_2Cl_2. Catharanthine borane **2** was obtained as a white foam: 110 mg (45%). White solid; M.P. > 190° (dec.); $[\alpha]_D$ +56 (c 0.73, MeOH); IR (film) ν max 3368, 2958, 2350, 1729, 1460, 1434, 1170, 1091 cm^{-1} ; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm):

7.73 (br s, NH), 7.47 (1H, br d, J =6.7 Hz, H-9), 7.23 (1H, br d, J =6.7 Hz, H-12), 7.16 (1H, dt, J = 1.2, 7.6 Hz, H-11), 7.11 (1H, dt, J = 1.2, 6.7 Hz, H-10), 6.00 (1H, dq, J = 6.6, 1.5 Hz, H-15), 4.45 (1H, br s, H-21), 4.11 (1H, m, H-5), 3.63 (3H, s, OMe), 3.40 (1H, dd, J = 10.3, 17 Hz, H-6), 3.12 (2H, m, H-5, H-3), 2.95 (3H, m, H-15, H-6, H-3), 2.85 (1H, br t, J = 5 Hz, H-14), 2.60 (1H, ddq, J = 2, 18, 7.5 Hz, H-19), 2.14 (1H, ddq, J = 2, 18, 7.5 Hz, H-19), 1.74 (1H, d, J = 13 Hz, H-15), 1.6 (3H, hump, BH_3), 1.06 (3H, t, J = 7.5 Hz, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 172.5 (C=O), 147.8 (C-20), 135.2 (C-13), 134.5 (C-2), 127.3 (C-8), 124.9 (C-15), 122.4 (C-11), 120 (C-10), 118.2 (C-9), 114.5 (C-7), 110.8 (C-12), 71.6 (C-5), 66.4 (C-3), 64.7 (C-21), 52.8 (OMe), 52.4 (C-16), 31.5 (C-17), 30.5 (C-14), 27.7 (C-19), 20.3 (C-6), 10.1 (C-18); ^{11}B NMR ($CDCl_3$, 160 MHz, δ, ppm) – 8.8 (br s, $W_{1/2}$ = 270 Hz); ^{15}N NMR ($CDCl_3$, 50.6 MHz, δ, ppm) – 351; (+)-HRESIMS m/z 348.2123 (calcd for $C_{21}H_{26}{}^{10}BN_2O_2$: 348.2124).

Determination of minimal inhibitory concentrations

The antimicrobial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines M7-A2, using broth serial dilution in sterile 96-well microplates. The bacteria strains were grown on trypticase soy agar (Becton Dickinson) at 37°C for 24 h (*E. coli* ATCC28922, *P. aeruginosa* PAO1, *S. aureus* ATCCC25923) in MH2 broth. Inocula were prepared in MH2 by adjusting the turbidity at 623 nm to obtain 5 10^5 CFU/mL in each well. Compounds were solubilized in DMSO at a concentration of 10 mg/mL and 8 µL were transferred to each microplate well (in all cases concentrations of the desired molecules in DMSO do not exceed 2% of the total proportion), to obtain a two-fold serial dilution in 100 µL of broth and 100 µL of inocula were added to each well. Several wells were reserved for positive controls, inoculum viability and solvent effect. After 24 or 48 h incubation, growth was assayed by absorbance measurement at 623 nm with an IEMS Labsystem automatic plate reader. The MIC for each agent obtained from duplicate observations was the lowest concentration of compound allowing no measurable bacterial growth. The results are the mean of three independent measurements.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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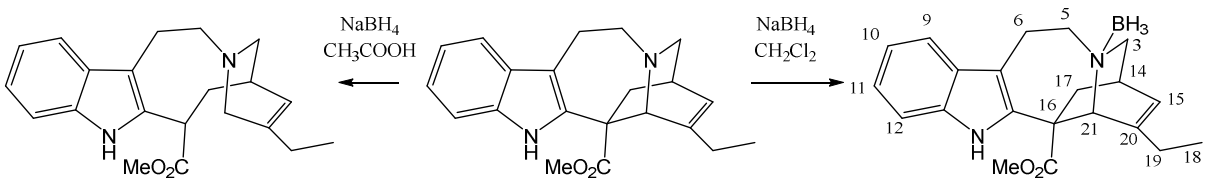
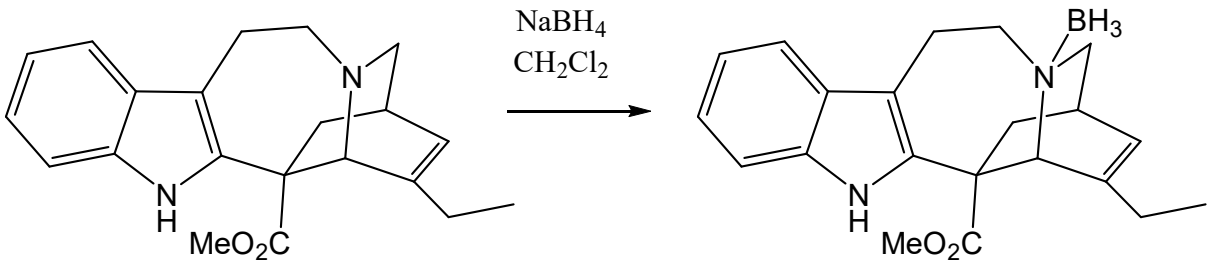


2 **1** **3**

Figure 1 : transformations of catharanthine **1**.



« Isocatharanthine » = Catharanthine-borane