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NOTES

SYNTHESIS OF [METHYL-^{2}H]-LABELLED AJMALICINE, YOHIMBINE, TABERSONINE AND CATHARANTHINE

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SUMMARY

Deuterium labelled analogues of ajmalicine, yohimbine, tabersonine and catharanthine were synthesized by base catalyzed transesterification of the methyl group in deuterated methanol.

Key words: Deuterium, transesterification, ajmalicine, yohimbine, tabersonine, catharanthine

INTRODUCTION

The Madagascan periwinkle *Catharanthus roseus* (L.) G. Don produces more than 70 different compounds and many of them are medicinally important (1). The production of Catharanhus alkaloids in plant cell cultures has been widely studied in order to find more economical methods to produce them for pharmaceutical purposes. In the phytochemical research alkaloid content of samples originated from plants and cultured plant cells of *C. roseus* has been analyzed using HPLC combined with UV (2), fluoresence (3) or electrochemical (4) detection. Because of the complex alkaloid content of the samples more selective thermospray HPLC mass spectrometric (MS) detection has also been applied for several of the compounds (5, 6). However, due to the lack of reliable internal standards, all the methods tend to be unreliable, when absolute alkaloid levels in series of biological samples of varying size and consistence should be determined. In order to overcome this in the MS method, we started studying possibilities to prepare ^{2}H-labelled analogues of the compounds.

Many of the *Catharanthus* alkaloids contain a methyl ester moiety (Figs. 1, 2), which may be changed by transesterification with alcohol, when strong mineral acid or base is used as catalyst (7, Fig. 2). We applied this principle in order to synthesize ^{2}H-labelled ajmalicine, yohimbine, tabersonine, catharanthine and serpentine.

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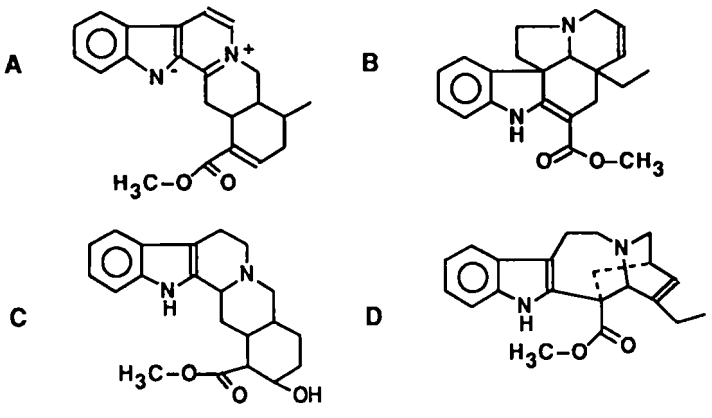

Figure 1. Chemical structures of serpentine (A), tabersonine (B), yohimbine (C) and catharanthine (D).

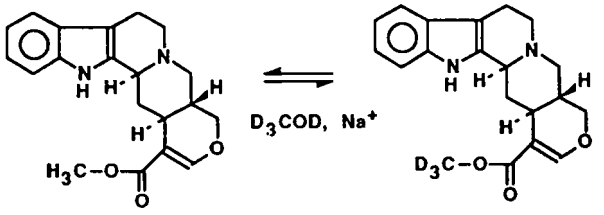

Figure 2. Transesterification of methyl group of ajmalicine in deuterated methanol.

EXPERIMENTAL

Catharanthine was purified from *C. roseus* leaves and identified as described earlier (6, 8). Tabersonine hydrocloride was generously provided by Prof. W. G. W. Kurz (Plant Biotechnology Institute, National Research Council, Saskatoon, Canada). Ajmalicine hydrocloride was purchased from Sigma (St Louis, MO) and yohimbine was a gift from Torkel Berglung (Tekniska Högskolan, Stockholm, Sweden). [^{2}H]-Methanol (min. 99 % D) was purchased from E. Merck (Darmstadt, FRG).

Transesterification of the alkaloids

Ajmalicine, yohimbine, tabersonine, or catharanthine (10, 2.3, 2.3, and 0.4 mg respectively) was dissolved in 1 to 2 ml of perdeuterated methanol containing 0.1 M NaOH. The solution was heated in a closed reaction tube at 70^{o}C with a Pierce Reacti-therm heating module (Rockford, IL).

The reactions were followed by determining the ratio of the original and transmethylated alkaloids in the medium. For this purpose 20 µl aliquots of the reaction solutions were neutralized with 0.1 M HCl and intensities of the respective M^+ ions were recorded as described below. When intensity of the original mass ion in each case was decreased below 2 % of that of the

trideuterated analogue, the solution was cooled to 20°C, the pH was adjusted to 8 with 0.1 M HCl, and methanol was evaporated in air stream. After this each product was washed once with 1.5 ml of water (adjusted to pH 8 with 0.1 M NaOH) and dried at 70°C.

Characterization of the products

The low resolution electron ionization mass spectra of the compounds were routinely recorded with a Jeol JMS D300 magnetic sector mass spectrometer. The electron beam energy applied for the determination of isotopic purity was 18 eV, ionization current was 300 μA and ion source temperature was 230°C. Samples were admitted using a solids insertion probe.

The high resolution mass spectra were recorded using a VG 70-250SE magnetic sector mass spectrometer. The resolution of the instrument was adjusted to 10 000. The electron energy was 20 eV, ionization current 500 μA and the ion source temperature was 200°C. Samples were introduced to the mass spectrometer in a glass sample holder with a direct insertion probe. The probe temperature was raised from 30°C to 400°C in 5 minutes. Accurate mass measurements of the molecular ions were carried out automatically with the data system. Perfluorokerosene was used as the reference compound.

Chemical purity of the compounds was examined with thermospray HPLC-MS using a system developed for analysis of corresponding alkaloids in plant cell culture samples (6). 10 μg of each compound was chromatographed and the existense of ions between m/z 150 and m/z 500 in the eluent was screened to detect possible byproducts.

Suitability of the deuterated compounds for use as internal standards in mass spectrometric analyses was tested by injecting 50 ng of each to the HPLC/MS system. Single ion monitoring of ions at m/z 337, 340, 353, 355, 356 and 358 (protonated molecular ions of the original alkaloids and trideuterated analogues) was used to confirm the absence of overlapping ions. None were observed, and each of the labelled alkaloids eluted a few seconds faster than the respective originals.

[Methyl-^{2}H]-labelled ajmalicine. The yield was 3 mg (33 %). Low resolution MS: m/z 355 (M^+) (100 %), 184 (19), 156 (15). Accurate mass (M^+) 355.1983 (required for $C_{21}H_{21}{}^2H_3O_3N_2$ (M^+) 355.1975). The thermospray mass spectrum of deuterated ajmalicine showed MH^+ at m/z 356 as the base peak and no prominent fragment ions; retention time (Rt) 8:50. The chromatogram indicated formation of one major byproduct eluting 2 minutes earlier than ajmalicine. Its intensity of total ion current between m/z 150 and m/z 500 was 20 % of that of deuterated ajmalicine, and the mass spectrum indicated that the signal was produced primarily by ions at m/z 392 (presumably MH^+).

[Methyl-^{2}H]-labelled yohimbine. The yield was 1.6 mg (80 %). The low resolution MS of the synthesized compound show the molecular ion at m/z 357 as the base peak and no other abundant (over 3 %) ions. Accurate mass (M^+) 357. 2137, (required for $C_{21}H_{23}{}^2H_3O_3N_2$ 357.2132). Only the expected MH^+ ion at m/z 358 was observed by HPLC/MS (Rt 5:40).

[Methyl-^{2}H]-labelled tabersonine. The yield was 1.7 mg (81 %). Low resolution MS: m/z 339 (M^+) (90%), 232 (17), 135 (100), 122 (15), 121 (12), 107 (20). Accurate mass (M^+) 339.2021, required for $C_{21}\ H_{21}\ ^2H_3\ O_2\ N_2$ (M^+) 339.2026. Only the expected MH^+ ion at m/z 340 was observed by HPLC/MS (Rt 16:30).

[Methyl-^{2}H]-labelled catharanthine. The product did not crystallize and the deuterated catharanthine was stored in 50 % ethanol at -20 ^oC. Low resolution MS: m/z 339 (M^+) (75 %), 232 (17), 135 (100), 122 (18), 121 (18), 107 (24). Accurate mass 339.2021 (required for $C_{21}\ H_{21}\ ^2H_3\ O_2\ N_2$ 339.2026). Only the expected MH^+ ion at m/z 340 was observed by HPLC/MS (Rt 12:00).

[Methyl-^{2}H]-labelled serpentine. The samples taken for following the completion of transmethylation indicated major interference of other reactions. When the process was stopped after 1 h and the precipitated material was analyzed by low resolution MS, most abundant ions were seen at m/z 363, 364, 365, 366 and 367, and an other group of 5 ions was between m/z 349 and 353. The former group indicates significant addition of methyl to the original serpentine with varying degree of hydrogen exchange during the process. The latter ion group may in part represent transmethylated serpentine, but they may as well be fragment ions of the methyl adduct.

RESULTS AND DISCUSSION

Transmethylation (Fig. 2) of four of the target alkaloids could be succesfully performed under similar reaction conditions, but the times required for sufficient label transfer varied. Over 99 % of yohimbine and 98 % of ajmalicine was labelled in 30 min, while tabersonine required 4 h and catharanthine 20 h for > 99 % transmethylation. Although somewhat different amounts of the alkaloids were included in the reaction for practical reasons, the varying reactivity obviously relies upon differences in the structures of the compounds.

An important observation was that at least catharanthine could be kept in hot alkaline methanol for 20 h without any major degradation. This suggests that the method may be applied to a wider range of indolic alkaloids than was done now. On the other hand, the behaviour of serpentine shows that if the alkaloids contain functional groups reactive under the transmethylation conditions, the formation of unwanted side products may invalidate the approach. Serpentine is a quaternary amine (Fig. 1), which acts as a strong base in the alkaline methanol.This and position of the charged nitrogen in a heterocyclic system with delocalized π-bonds render various types of methyl additions / substitutions possible under the present conditions, but further study of these was beyond the scope of this work.

The transesterification with deuterated methanol is a convenient, easy, rapid and low cost method to prepare stable isotope labelled analogues of these alkaloids. The ^{2}H-labelled alkaloids have proved very useful in practice, since the standard deviations between samples were markedly reduced, when they were used as internal standards in HPLC-MS analysis of plant tissue samples during an ongoing work.

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