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11-DEOXOJERVINE AS A PRECURSOR FOR JERVINE BIOSYNTHESIS IN *VERATRUM GRANDIFLORUM*

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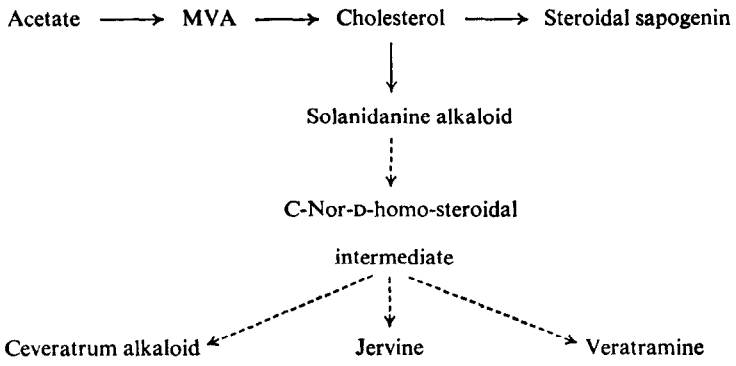
Abstract—Experimental data show that 11-deoxojervine-^{14}C (I) is converted into jervine (II) but not to veratramine (III) in the growing *Veratrum* plant. Moreover, non-radioactive 11-deoxojervine (I) inhibits the incorporation of acetate-1-^{14}C into jervine.

INTRODUCTION

THE BIOGENESIS of steroidal alkaloids and steroidal sapogenins is presently under investigation in several laboratories.[1-5] The biosynthetic pathway has now been elucidated as follows:



In our previous paper,[5] it was shown that acetate-1-^{14}C and cholesterol-^{14}C are incorporated into jervine (II) and veratramine (III), and that the addition of jervine (II) at the site of synthesis is very effective in stimulating veratramine (III) production.

We have now examined whether 11-deoxojervine (I) participates in the biosynthesis of jervine (II) and veratramine (III) in *Veratrum grandiflorum*. Indeed, it appears to be a precursor of II. Also it seems possible to convert 11-deoxojervine (I) into veratramine (III) by the breakdown of ether linkage and aromatization of the D-ring.

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[3] E. HEFTMANN, E. R. LIEBER and R. D. BENNETT, *Phytochem.* **6**, 225 (1967).
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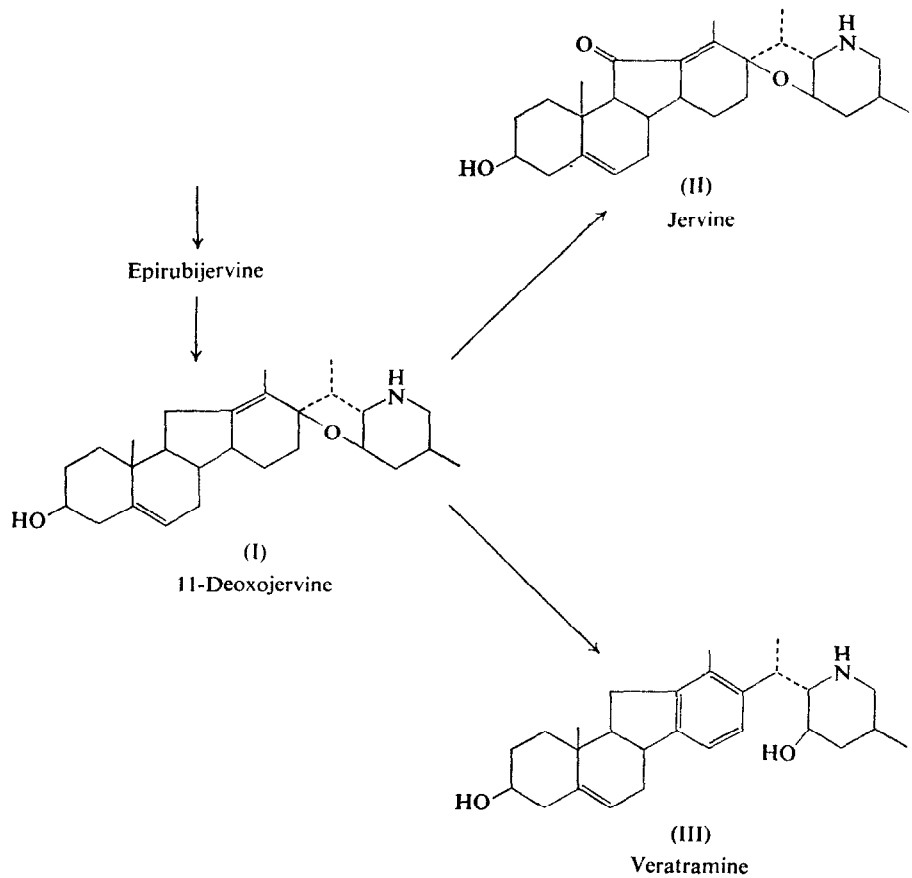


CHART I. PROBABLE BIOSYNTHESIS OF JERVERATRUM ALKALOID.

RESULTS

According to the method described in our previous paper,[5] the radioactive jervine (II) was prepared from acetate-1-^{14}C, and then reduced by the Wolff–Kishner method. The radioactive 11-deoxojervine (I) was purified by TLC, and its specific activity was $1{\cdot}07 \times 10^{-7}$ dpm per mmole.

TABLE 1. INCORPORATION OF 11-DEOXOJERVINE-^{14}C INTO JERVINE IN *Veratrum grandiflorum*

	Jervine		Veratramine	
	dis/min/mmole $\times 10^{-6}$	Ratio of molar dilution (%)	dis/min/mmole $\times 10^{-6}$	Ratio of molar dilution (%)
11-Deoxojervine-^{14}C, 10 mg, $2{\cdot}7 \times 10^{7}$ dis/min	2·7	26·1	1·3	12·1

The ratio of molar dilution is expressed as $\frac{\text{dis/min/mmole of the product}}{\text{dis/min/mmole of the additional precursor}} \times 100$.

The incorporation of 11-deoxojervine-^{14}C into jervine (II) and veratramine (III) in *Veratrum* is shown in Table 1. In this table, the ratio of the conversion of 11-deoxojervine (I) to jervine (II) and veratramine (III) is represented as the ratio of molar dilution; this assumes that 1 mole of 11-deoxojervine (I) is converted into 1 mole of jervine (II) or veratramine (III), without breakdown of the steroidal skeleton. The conversion of 11-deoxojervine (I) to jervine (II) and veratramine (III) is 26·1% and 12·1%, respectively, so that I is an effective intermediate.

However, 11-deoxojervine-^{14}C may contaminate the radioactivity of jervine (II) and veratramine (III), since it cannot be removed completely during isolation and purification from both jervine (II) and veratramine (III). Therefore, after purification, the radioactive jervine (II) and veratramine (III) were diluted with 10 vol. of non-radioactive jervine (II) and

TABLE 2. DILUTION EFFECT ON THE RADIOACTIVITY OF JERVINE AND VERATRAMINE

	Radioactivity (dis/min/mg of alkaoid)	
	Jervine	Veratramine
Original alkaloid	1050	315
10-Fold dilution with non-radioactive jervine and veratramine and recrystallization	995	0
2-Fold dilution with 11-deoxojervine, separation by TLC and recrystallization	1370	—

The radioactivity of diluted jervine was expressed in terms of original jervine.

TABLE 3

Expt. No.	Composition of feeding solution	Radioactivity of jervine	
		dis/min/mmole × 10^{-5}	Ratio
1	Acetate-1-^{14}C (50 μC)	6·2	100
2	Acetate-1-^{14}C (50 μC) plus 10 mg 11-deoxojervine	1·5	24·2

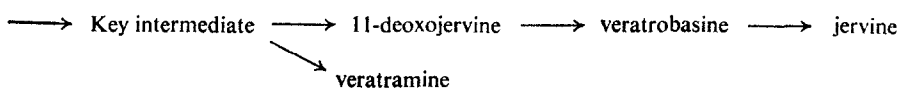
veratramine (III), and recrystallized. At this step, the radioactivity of veratramine (III) was completely lost, while the radioactivity of jervine (II) remained almost unchanged. Moreover, when jervine (II) was diluted with 2 vol. non-radioactive 11-deoxojervine (I), separated by a preparative TLC, and crystallized from methanol, its radioactivity increased (Table 2). Thus 11-deoxojervine (I) apparently inhibits the incorporation of acetate-1-^{14}C into jervine (II).

Since the *Veratrum* plant originally contains a small amount of 11-deoxojervine (I), as will be described later, the radioactivity in Expt. 1 (Table 3) seems to be lowered to a certain degree by the presence of the material. Although the content of jervine (II) in each plant depends on the weight of its rhizome, the mean value of the incorporation of acetate-1-^{14}C into jervine (II) calculated for five plants, is $6{\cdot}17 \pm 0{\cdot}87$ dis/min/mmole × 10^{-5}. The data in Table 3 are therefore significant and show that 11-deoxojervine (I) inhibits the incorporation of acetate-1-^{14}C into jervine (II); this is in good agreement with the result of incorporation of 11-deoxojervine-^{14}C (I) into jervine (II) (see Tables 1 and 2).

11-Deoxojervine (I) was isolated from *Veratrum grandiflorum* by Masamune.[6] Jervine (II) isolated by the method of Jacobs[7] was submitted to TLC on Silica Gel HF_{254}, with hexane–$HNEt_2$–EtOH (8:1:1). The spot of jervine (II) quenched u.v. fluorescence, whereas a second spot was observed by spraying with H_2O and, after drying, it gave an olive-green colour with $SbCl_3$. This second spot was identical in R_f and colour with synthetic 11-deoxojervine (I) obtained by the reduction of jervine (II). Preparative TLC of 3·3 g of crude jervine (II) from *Veratrum* and extraction of the band corresponding to 11-deoxojervine (I) with CH_2Cl_2–MeOH–NH_4OH (10:10:0·1) gave a fraction which crystallized from acetone to m.p. 236–237°; yield, 32 mg. I.r., mass and NMR spectra of this compound were identical with those of the reduced product of jervine (II). (Found: C, 78·60; H, 9·92; N, 3·32. Calc. for $C_{27}H_{41}O_2N$:C, 78·78; H, 10·04; N, 3·40%.) The mixed m.p. with 11-deoxojervine (I), obtained by the reducting of jervine (II), did not show any depression.

DISCUSSION

Our new results indicate that the product after C-nor-D-homo rearrangement of epirubijervine can be converted into jervine (II) and veratramine (III), and the key intermediate may be converted into 11-deoxojervine (I) and then to jervine (II). Moreover this system is inhibited by the externally added jervine (II), as described previously.[5] The key intermediate has not yet been isolated and new techniques which include the leaf-slice method[8,9] or cell-free enzyme system in *Veratrum* plant may be necessary for accomplishing this.



EXPERIMENTAL

Preparation of Radioactive Jervine

Twenty *Veratrum* plants were cultivated fed with acetate-1-^{14}C, and jervine was isolated as described previously.[5] From this compound, 280 mg of radioactive jervine was prepared.

Wolff–Kishner Reduction of Jervine

The Wolff–Kishner reduction of jervine[6] was carried out as follows: a mixture of 1·3 ml of freshly distilled diethylene glycol and 0·4 g of Na metal was refluxed in a 50-ml of two-necked flask at 170° under anhydrous condition. To the cooled mixture, 2·5 ml of anhydrous hydrazine[10] was added, the mixture was refluxed at 170–180° for a few min, 200 mg radioactive jervine was added to the cooled solution, and refluxed at 170–180° for several hr. An aliquot of this mixture was taken at 30-min intervals and checked by TLC. After 1·5 hr, jervine disappeared completely. The reflux temperature was gradually raised to 200°, the excess of hydrazine was distilled off, and the refluxing was continued for another 1 hr. The cooled mixture was added with 12 ml of H_2O, then extracted with CH_2Cl_2 (3 × 100 ml). The CH_2Cl_2 extract was evaporated and the residue was crystallized from acetone. The residue of the crystallization from acetone was purified by preparative TLC on Silica Gel HF_{254} with hexane–EtOH–$HNEt_2$ (8:1:1). The band of 11-deoxojervine was extracted with CH_2Cl_2–MeOH–NH_4OH (10:10:0·1). The two types of crystals were combined and recrystallized from acetone and 60 mg of radioactive 11-deoxojervine was obtained (yield 30%), m.p. 233–236° (reported m.p. 236–238°). Yield 40–65%.

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The cultivation of *Veratrum* plant, administration of the radioactive and non-radioactive compounds, and isolation of jerveratrum alkaloids followed the method described previously.[5] 10 mg of synthesized 11-deoxojervine-^{14}C was dissolved in 5·0 ml of citric acid buffer, pH 4·5, and was fed to plant, as described previously for non-radioactive jervine.[5] The radioactivities of all the samples were determined by liquid scintillation counter, as described previously.[5]