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BIOSYNTHESIS OF RUBIJERVINE IN *VERATRUM GRANDIFLORUM*

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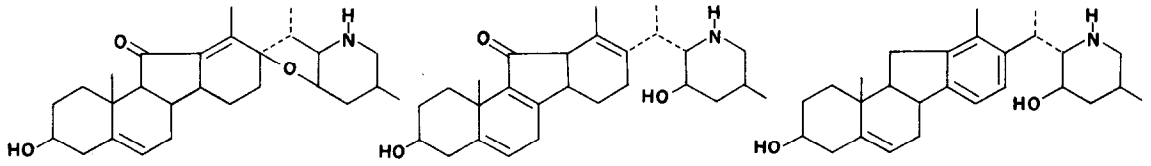
Key Word Index—*Veratrum grandiflorum*; Liliaceae; *Veratrum* alkaloid; biosynthesis; 22,26-epiminocholestane alkaloid; hakurirodine; rubijervine.

Abstract—The rhizome of dormant *Veratrum grandiflorum* accumulated hakurirodine, a new 22,26-epiminocholestane alkaloid. This alkaloid, derived from verazine but not from etioline, was found to be a key intermediate in the biosynthesis of rubijervine.

INTRODUCTION

Among the alkaloids identified specifically from the genera *Veratrum*, *Fritillaria* and *Zygadenus* of the Liliaceae are the jerveratrum and ceveratrum alkaloids which have an interesting C-nor-D-homo-steroid skeleton and a strong pharmacological effect on vertebrates. The biogenesis of steroidal alkaloids and sapogenins has been studied by Tschesche [1–3] and Heftmann [4–5], who have found that the C_{27} carbon skeleton of solanidine, tomatidine, diosgenin and related compounds is a glycoside and is converted by illumination to jervine (**2a**) and veratramine (**3**), two typical *Veratrum* alkaloids. On the other hand, Kaneko *et al.* identified several alkaloids and sterols; for example, etioline (**4**) [17, 28], verazine (**5**) [19, 28], teinemine (**6**) [20], dormantinone (**7**) [21], and dormantinol (**8**) [21], which accumulated in the budding veratrum plant as major constituents, and proposed the biogenetic pathway of solanidine (**1**) in the *Veratrum* plant, based on the presence of these alkaloids and sterols.



(2a) Jervine (2b) Isojervine (3) Veratramine

supplied from cholesterol. Identification of cholesterol in higher plants with the aid of GLC [6] has been reported recently. The formation of *Veratrum* alkaloids from the normal steroidal skeleton was proposed by Narayanan [7] and Leete [8], and their view was based on the results of chemical conversion of normal steroids to C-nor-D-homo-steroids [9–13].

Kaneko *et al.* [14] reported that solanidine (**1**) accumulates in etiolated *Veratrum grandiflorum* as

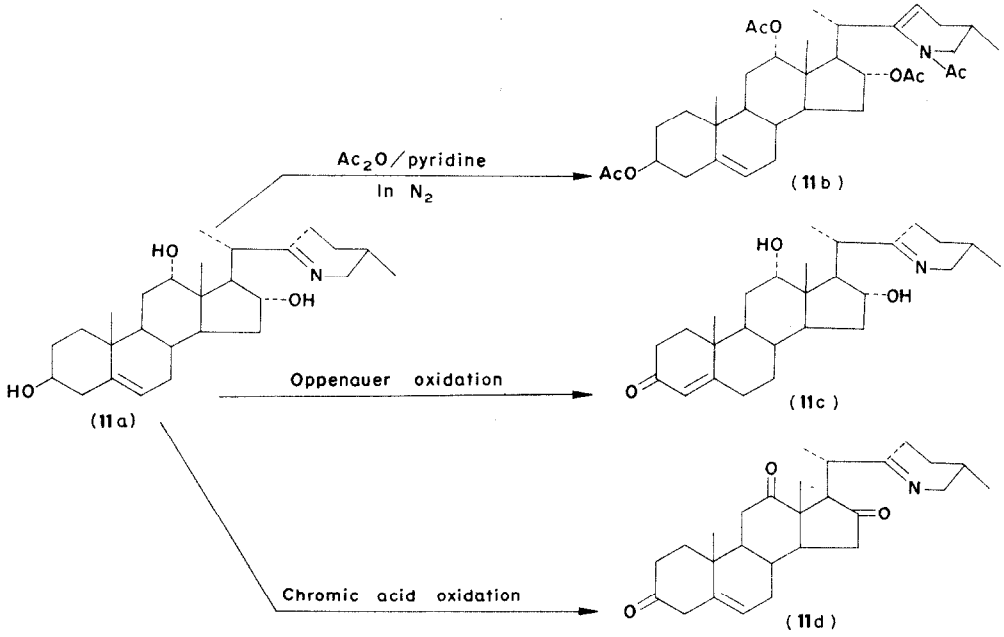
With respect to the biosynthesis of rubijervine, Kaneko at first assumed that the hydroxylation of solanidine (**1**) at C-12 to yield rubijervine (12α-OH) (**9a**) and epirubijervine (12β-OH) (**10**) is catalyzed by a nonstereo-specific oxidase or by two different enzymes under illuminated conditions, and the one epimer, epirubijervine (**10**), is converted into C-nor-D-homo-steroidal alkaloid and the other, rubijervine (**9a**), is accumulated in the rhizome. Recently, rubijervine (**9a**) was isolated from dor-

mant veratrum plant, and its content exceeded the expectation based on the above hypothesis. Dormant *Veratrum* plant was kept in the dark and cold in order to accelerate budding, as described previously [15]. From this result, the biosynthesis of rubijervine seems to be carried out in the absence of light, which may be indispensable for C-nor-D-homo rearrangement. In this paper, we report the presence of a biosynthetic pathway to rubijervine (**9**a) which branches from that of solanidine (**1**), based on the isolation of a new key intermediate and from isotope studies.

RESULTS AND DISCUSSION

TLC analysis of the alkaloids in dormant, budding and etiolated veratrum plants showed that a new alkaloid-G increases in the rhizome during the cold treatment and decreases significantly after growing in the dark under natural conditions. The rhizome of budding *Veratrum* plant gave a glycoside mixture and crude alkaloids were obtained as described previously [16]. Tertiary alkaloids, obtained from the crude alkaloid mixture by Jacobs' method [24], were separated by alumina column chromatography. Elution of the column with ethyl acetate–benzene gave solanidine (**1**), verazine (**5**), etioline (**4**), rubijervine (**9**a), and *iso*jervine (**2**b) fractions. Subsequent elution with chloroform-methanol gave the alkaloid-G fraction; rechromatography gave pure alkaloid-G (**11**a), mp 194–197°, $[\alpha]_D^{19}$ −163°. This was found to be a steroidal alkaloid with the formula, $C_{27}H_{43}O_3N$, from elemental analysis and mass spectrum (M^+ 429). The IR spectrum of **11**a showed absorptions for hydroxyl groups at 3375 and 1060 cm^{-1}, and C=N groups at 1655 cm^{-1}, which was supported by UV absorption at 240 nm (ε, 196).

Compound **11**a was assumed to be 22,26-epiminocholestane derivative since in the mass spectrum, the base peak, *m/e* 125, is elucidated as the fragment ion of a McLafferty rearrangement [25], as occurs with etioline [17] (**4**) and verazine [18, 19] (**5**), and its *O,O,O,N*-tetraacetate (**11**b) displayed an enamide function with IR absorptions at 1665 and 1540 cm^{-1} (C=C–NAc), and UV maximum at 234 nm (ε, 9500). The NMR spectrum showed signals of two tertiary methyl groups at δ 0·61 and 0·96, two secondary methyl groups at 0·92 and 1·20 (*d*, *J* 6 Hz), three hydroxymethines at 3·16, 3·54, and 4·04, and one olefinic proton at 3·54. From the assignment of the hydroxymethine at δ 3·54 as 3α-H and the olefinic proton at δ 5·34 as C-6, and from the colour response to $SbCl_3$, compound **11**a was assumed to have a Δ^5-3β-hydroxy structure. This was confirmed by its conversion by



Scheme 1. Conversions of hakurirodine.

Table 1. Variation in the alkaloids during the life cycle of *V. grandiflorum* (mg/100 g of dry plants)

Stage	Part	Solanidine	Verazine	Etioline	Rubijervine	Hakurirodine
Budding	Terrestrial part	150	90	70	8	—
	Rhizome	—	8	—	17	67
Etiolated (20 days)	Rhizome	—	—	—	13	28
1 Month after budding	Terrestrial part	41	—	—	10	—
	Root and rhizome	—	20	—	26	38
2 Months after budding	Terrestrial part	25	...	...	5	—
	Root and rhizome	—	70	—	10	13

Oppenauer oxidation to an α,β-unsaturated oxo-derivative (**11**c) having UV absorption at 240 nm (ε, 14000).

Chromic acid oxidation of **11**a gave a trione (**11**d) which showed IR absorption of a five-membered ring ketone at 1745 cm^{-1}, so that one of the remaining two hydroxyl groups is located on ring *D*. Compound **11**a was converted into solanidane derivative by the procedure of Schreiber *et al.* [26–28]. The product was identical with 5α-solanidane-3,12-dione (**12**), derived from rubijervine (**9**a) by catalytic hydrogenation followed by Kiliani's oxidation. Thus **11**a is 3,12,16-trihydroxylated and the configuration at C-25 is S.

The hydroxyl group at C-12 was assigned the α orientation from the NMR spectrum of **11**a which showed narrow triplet signal of 12β-H at δ 3·16 (*J* 2 Hz) coupled with C-11 methylene group. The hydroxyl group at C-16 was assigned an α orientation since the unresolved signal of a hydroxymethine at δ 4·04, enveloped by other signals, shifted to 4·84 as a clear triplet (*J* 6 Hz) by acetylation, indicating that it is coupled with 17α- and 15α-H with β orientation. The NMR spectrum of the tetra-acetate (**11**b) indicated a newly produced olefinic signal of enamide moiety at δ 5·12 as a broad singlet. From this evidence, **11**a is designated as (25S)-22,26-epiminocholesta-5,22(*N*)-diene-3β,12α,16α-triol and named hakurirodine [28] after the Japanese drug name of hakurirokon for *V. grandiflorum*.

Up to the present time, several 22,26-epiminocholestane derivatives have been reported e.g., etioline [17] (**4**) and verazine [19] (**5**) from *V. grandiflorum*, verazine [18] (**5**) from *V. album*, tomatillidine [25] from *Solanum tomatillo*, and solacongestidine [27], solafloridine [27], and 23-oxo [27] - and 24-oxo-solacongestidine [27] from *S. congestiflorum*. Although etioline (**4**), verazine (**5**), and hakurirodine (**11**a) are the main alkaloids in dormant and budding veratrum plants, the others are minor components. It is very interesting that the accumulation of (**4**), (**5**), and (**11**a) is related to the biosynthesis of solanidine (**1**) and rubijervine (**9**a). Variation in the content of rubijervine (**9**a) and hakurirodine (**11**a) during the life cycle of *V. grandiflorum* is summarized in Table 1.

Since rubijervine (**9**a) has been reported to be a minor component [21, 22, 30–34] it may be regarded as the alkaloid which is metabolized rapidly during the growth of the veratrum plant and does not accumulate significantly. Since it has

Table 2. Incorporation of acetate [1-^{14}C] into *Veratrum* alkaloids

	Terrestrial part		Rhizome and root	
	Weight (mg)	Radioactivity (dpm/mg)	Weight (mg)	Radioactivity (dpm/mg)
Dry wt	100×10^3		250×10^3	
Crude extract	15×10^3		40×10^3	
Crude alkaloid	$4{\cdot}5 \times 10^3$		10×10^3	
Solanidine	500	390·2	5	23·1
Verazine	90	783·0	40	64·0
Etioline	200	324·0	—	
Rubijervine	18	31·0	30	13·5
Hakurirodine	—		30	12·3

Table 3. Incorporation of labeled verazine into rubijervine and hakurirodine (total dpm)

Stage	Dormant				Budding	
Incubation period	6 hr		24 hr		24 hr	
	[^{14}C]-verazine	[^{14}C]-etioline	[^{14}C]-verazine	[^{14}C]-etioline	[^{14}C]-verazine	[^{14}C]-etioline
Fed precursor	15660 (100%)	6480 (100%)	15660 (100%)	6480 (100%)	15660 (100%)	6480 (100%)
Hakurirodine	72 (0·46%)	0 —	133 (0·85%)	3 —	0 —	0 —
Rubijervine	30 (0·19%)	0 —	67 (0·43%)	0 —	0 —	0 —
Veratramine	0 —	0 —	0 —	0 —	0 —	0 —

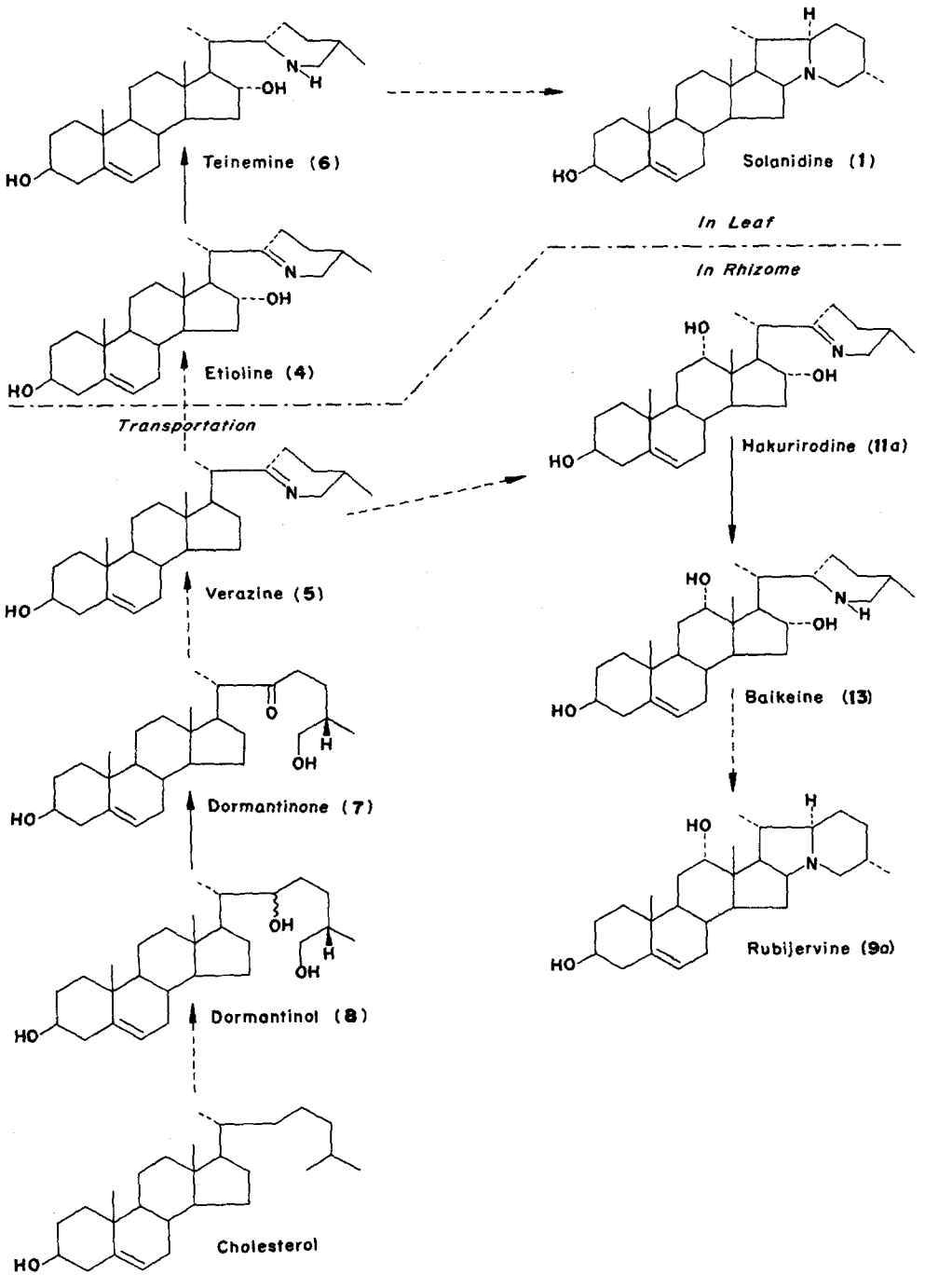
a related chemical structure, hakurirodine (**11**a) could be the precursor of rubijervine (**9**a). Isotope studies were planned to clarify this. In spite of the fact that (**11**a) accumulates in the dormant rhizome, it is impossible to feed a labeled compound in dormant *Veratrum* plant by the cotton-wick method [14] because of the slow uptake. Therefore, acetate [1-^{14}C] was fed to the budding plant by the cotton-wick method in a cold room. The content and specific activities of isolated alkaloids are summarized in Table 2. From this experiment, it was confirmed that the site of steroidal alkaloid synthesis moves from the rhizome to the aerial part after budding. There was no incorporation of acetate [1-^{14}C] into rubijervine (**9**a) and hakurirodine (**11**a), indicating that branching to the biosynthetic pathway of rubijervine (**9**a) from that of solanidine (**1**) occurs only during dormancy and the synthetic ability is lost during budding. Although there was no labeling of (**11**a), etioline (**4**) and verazine (**5**) were labeled sufficiently for a refeeding experiment. This also showed that the dormant plant is suitable for biosynthetic experiments of rubijervine (**9**a) and, therefore it was used for the following experiment.

The slice method [35, 36] developed by Goodwin and Barton, was used with labeled etioline (**4**) and verazine (**5**) to determine the branching point from the biosynthetic pathway of solanidine (**1**). After incubation of the dormant rhizome slice with labeled compound, hakurirodine (**11**a) and rubijervine (**9**a) were isolated by the usual procedure and their activities were determined (Table 3).

It was found that only verazine, and not etioline, was incorporated into hakurirodine (**11**a) and rubijervine (**9**a). This result indicates the presence of two biosynthetic pathways in the dormant plant for solanidine (**1**) and rubijervine (**9**a), branching from verazine (**5**). On the other hand, in the budding rhizome slice, neither labeled verazine (**5**) nor etioline (**4**) was incorporated into (**11**a) and (**9**a). This result agrees with the failure of incorporation of acetate [1-^{14}C] into these alkaloids after budding, and with the accumulation of verazine (**5**) in the rhizome and etioline (**4**) in the aerial part during dormancy.

Table 4. Tertiary alkaloids in the rhizome of budding *V. grandiflorum*

Fract. no.	Solvent system	Volume (l)	Constituents	Weight (mg)
1	C_6H_6–EtOAc (1:1)	10	Solanidine	Trace
2	C_6H_6–EtOAc (1:1)		Verazine	130
3	C_6H_6–EtOAc (1:1)		Etioline	Trace
4	C_6H_6–EtOAc (1:1)		Rubijervine	300
5	C_6H_6–EtOAc (1:1)		*Iso*jervine	
6	$CHCl_3$	2	*Iso*jervine	7000
7	$CHCl_3$–MeOH (99:1)	4	Alkaloid-G	4000
8	$CHCl_3$–MeOH (9:1)	2	Residue	



Scheme 2. Biogenetic pathway of solanidanine alkaloids in dormant *Veratrum grandiflorum*.

From these data, a biogenetic pathway of solanidanine alkaloids in the dormant veratrum plant can be suggested (Scheme 2). The presence of baikeine (**13**), isolated from *V. grandiflorum* by Itoh [37], supports our scheme.

EXPERIMENTAL

M.ps are uncorr. Optical rotations were measured in $CHCl_3$ solution; NMR spectra were determined, in $CDCl_3$ with TMS as an internal standard; IR spectra in Nujol mull and UV spectra in EtOH. Radioactivity was determined on an Aloka LSC-501 liquid scintillation counter in toluene scintillator. TLC were performed on Si gel HF_{254} (Merck, Type 60), alumina (Merck, neutral 2-3) was used for column chromatography.

Isolation of crude alkaloid. The rhizome of budding *V. grandiflorum*, collected in early April 1972 at Teine, Hokkaido, Japan, was used. The dried rhizome (1·8 kg) was extracted with ammoniacal MeOH–$CHCl_3$ (1:1) to yield crude glycoside (274 g), and 76 g of crude alkaloid was obtained from this after HCl hydrolysis, as described previously [16]. Secondary alkaloids (10 g) (mainly veratramine) were obtained by Jacobs' method [24]. The tertiary alkaloids (43 g) were chromatographed with 30 vol of alumina, and the column was eluted successively with EtOAc–C_6H_6 (1:1), $CHCl_3$, $CHCl_3$–MeOH (99:1), and $CHCl_3$–MeOH (9:1) (Table 4).

Hakurirodine (**11a**). Rechromatography of G-fraction and recrystallization several times from acetone gave needles (1·2 g), mp 194–197°, $[\alpha]_D^{19}$ −163° (*c*, 0·71 in $CHCl_3$). MS *m/e* 429 (M^+), 411 ($M^+ - H_2O$), 396 (M^+-H_2O-Me), 378 (M^+-$2H_2O$-Me), 164, 163, 126, 125 (base peak), 98. IR ν_{max}^{Nujol} cm^{-1}: 3375 (OH), 1655 (C=N), 1060 (C–O). UV λ_{max}^{EtOH} 240 nm (ε, 196), end absorption. NMR (δ): 0·61 (3H, *s*, 18-Me), 0·92 (3H, *d*, *J* 7 Hz, sec-Me), 0·96 (3H, *s*, 19-Me), 1·20 (3H, *d*, *J* 7 Hz, sec-Me), 2·92 (1H, *q*, *J* 11, 18 Hz, 26α-H), 3·16 (1H, *t*, *J* 2 Hz, 12β-H), 3·54 (1H, *m*, 3α-H), 3·64 (1H, *d*, *J* 18 Hz, 26β-H), 4·04 (1H, *m*, 16β-H), 5·34 (1H, *d*, *J* 3·5 Hz, C_6-olefinic proton). *Anal.* Calcd. for $C_{27}H_{43}O_3N$: C, 75·48; H, 10·09; N, 3·26. Found: C, 75,25; H, 10·02; N, 3·25. Hakurirodine acetate crystallized from MeOH as needles (**11b**), mp 137–138°. MS: *m/e* 597 (M^+), 582 (M^+-Me) 554 (M^+-MeCO), 523 (M^+-Me-$MeCO_2$), 522 (M^+-Me-$MeCO_2H$), 511 (M^+-2 × CH_3CO), 479 (M^+-2 × CH_3CO_2), 477 (M^+-2 × $MeCO_2H$), 453 (M^+-Me-3 × MeCO), 435 (M^+-4 × MeCO), 420 (M^+-Me-4 × CH_3CO) 204, 166, 165 (base peak), 150. IR ν_{max}^{Nujol} cm^{-1}: 1720 (OAc), 1665 and 1640 (C=C–NAc), 1260 and 1020 (C–O–C). UV λ_{max}^{EtOH}: 234 nm (ε, 9500). NMR (δ): 0·75 (3H, *s*, 18-Me), 0·90 (3H, *d*, *J* 7 Hz, sec-Me), 0·98 (3H, *s*, 19-Me), 1·16 (3H, *d*, *J* 7 Hz, sec-Me), 2·01 (6H, *s*, 2 × OAc), 2·09 (3H, *s*, OAc), 2·15 (3H, *s*, NAc), 3·60 (2H, *m*, 26-H_2), 4·60 (1H, *m*, 3α-H), 4·84 (1H, *t*, *J* 6 Hz, 16β-H), 5·12 (2H, *br. s*, 12β-H and C_{23}-olefinic proton), 5·40 (1H, *d*, *J* 3·5 Hz, C_6-olefinic proton). *Anal.* Calcd for $C_{35}H_{51}O_7N$: C, 70·32; H, 8·60; N, 2·34. Found: C, 70·08; H, 8·47; N, 2·42.

Oppenauer oxidation of hakurirodine (**11a**). This was carried out using the standard procedure. The product (**11c**) was obtained as needles, mp 235–238°. Mass spectrum: *m/e* 427 (M^+), 409 (M^+-H_2O), 394 (M^+-H_2O-Me), 376 (M^+-$2H_2O$-Me), 286, 162, 125 (base peak), 98. IR ν_{max}^{Nujol} cm^{-1}: 3375 (OH), 1675 and 1620 (C=C–C=O), 1655 (C=N), 1060 (C–O). UV λ_{max}^{EtOH}: 242 nm (ε, 14000). NMR (δ): 0·56 (3H, *s*, 18-Me), 0·86 (3H, *d*, *J* 7 Hz, sec-Me), 1·05 (3H, *s*, 19-Me), 1·16 (3H, *d*, *J* 7 Hz, sec-Me), 2·90 (1H, *q*, *J* 11, 18 Hz, 26α-H), 3·16 (1H, *br. s*, 12β-H), 3·62 (1H, *d*, *J* 18 Hz, 26β-H), 4·04 (1H, *m*, 16β-H), 5·69 (1H, *s*, C_4-olefinic proton). *Anal.* Calcd. for $C_{27}H_{41}O_3N$: C, 75·83; H, 9·66; N, 3·28. Found: C, 75·68; H, 9·45; N, 3·12.

Chromic acid oxidation of hakurirodine (**11a**). To a soln of 36 mg of (**11a**) in 4 ml of Me_2CO, 0·9 ml of Kiliani's reagent was added dropwise at 0°. After the reaction mixture was allowed to stand for 50 min at 0°, 10 ml of EtOH was added to destroy the excess oxidant. The reaction mixture was evaporated to dryness *in vacuo* at room temp. and dissolved in a small amount of H_2O. The aq. soln was made alkaline with NaOH and extracted with $CHCl_3$. From the $CHCl_3$ phase, 5 mg of yellow powder product (**11d**) was obtained. IR ν_{max}^{Nujol} cm^{-1}: 1745 (five-membered ring ketone), 1715 (six-membered ring ketone), 1705 (six-membered ring ketone).

Conversion of hakurirodine (**11a**) *into* 5α-*solanidane*-3,12-*dione* (**12**). (a) *Hydrogenation of hakurirodine* (**11a**). A soln of 200 mg (**11a**) in 8 ml AcOH was hydrogenated over 250 mg of PtO_2 at atm. press. at 20°. It consumed 2 mol equiv. of H_2 (28 ml) during 3 hr. After the reaction mixture was diluted with H_2O with cooling, the catalyst was removed. The product was purified by preparative TLC ($CHCl_3$–EtOAc–MeOH, 3:3:2) to yield 195 mg of (**11e**), which was separated into the acetone-soluble fraction (R_f 0·3, $CHCl_3$–MeOH=5:1) (A) and the Me_2CO-insoluble fraction (R_f 0·2) (B). Recrystallization of the Me_2CO-insoluble fraction from MeOH–acetone gave needles, m.p. 233–235°. Mass spectrum: *m/e* 433 (M^+), 432 (M^+-1), 431 (M^+-2), 415 (M^+-H_2O), 413 (431-H_2O), 398 (431-H_2O-Me), 396 (431-H_2O-OH), 390, 286, 252, 224, 216, 150, 125, 99, 98 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3450 (NH), 3230 (OH), 1625 (NH), 1035 (C–O). NMR (in CD_3OD) (δ): 0·71 (3H, *s*, 18-Me), 0·90 (3H, *s*, 19-Me), 1·08 (6H, *d*, *J* 7 Hz, 2 × sec-Me), 3·50 (1H, *m*, 3α-H), 3·70 (1H, *br. s*, 12β-H), 4·20 (1H, *m*, 16β-H), no olefinic proton. The mass and IR spectrum of (A) + (B) was completely superimposable on those of (A) and, moreover, (A) was contained significantly in fraction (B), so that the next reaction was carried with this mixture. They may be (22*S*)- and (22*R*)- tetrahydrohakurirodine.

(b) *Chromic acid oxidation of tetrahydrohakurirodine* (**11e**). To a soln of 190 mg of (**11e**) in 20 ml of acetone, 2 ml of Kiliani's reagent was added at 0° and worked up in the usual way to produce 170 mg of an oil. It was purified by preparative TLC ($CHCl_3$:EtOAc:MeOH, 4:4:1) and 100 mg of an oil (**11f**) was isolated.

(c) *Hydrogenation of the oxidation product of tetrahydrohakurirodine* (**11f**). Hydrogenation of 100 mg of (**11f**) over 100 mg 10% Pd–C in 10 ml EtOAc–MeOH (10:1) under atm. press. at 20° resulted in consumption of 1 mol equiv. of H_2 (10 ml) in 2 hr. The product was purified by preparative TLC (C_6H_6–EtOAc 1:1 and 9:1) to yield 10 mg. Recrystallization from acetone gave needles (**12**), m.p. 228–231°, $[\alpha]_D^{19}$ 110° (*c*, 0·16, in $CHCl_3$). MS: *m/e* 411 (M^+), 410 (M^+-1), 396 (M^+-Me), 383, 368, 220, 204, 191, 178, 162, 151, 150 (base peak), 136, 98. IR ν_{max}^{Nujol} cm^{-1}: 1720 (C=O), 1700 (C=O). NMR (δ): 0·84 (3H, *d*, *J* 7 Hz, sec-Me), 1·04 (3H, *d*, *J* 7 Hz, sec-Me), 1·08 (3H, *s*, 18-Me), 1·12 (3H, *s*, 19-Me).

Conversion of rubijervine (**9a**) *into* 5α-*solanidane*-3, 12-*dione* (**12**). (a) *Hydrogenation of rubijervine* (**9a**). Hydrogenation of 100 mg of (**9a**) over 90 mg of PtO_2 in 6 ml of AcOH under atmospheric pressure at 20° and consumption of 1 mol equiv. of H_2 (11 ml) in 2 hr. Crystallization of the product from Me_2CO gave 100 mg plates (**9b**), m.p. 217–219°. MS: *m/e* 415 (M^+), 414 (M^+-1), 400 (M^+-Me), 396 (414-H_2O), 220, 178, 162, 151, 150 (base peak), 136, 98. IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 1040 (C–O). NMR (δ): 0·90 (3H, *s*, 18-Me), 0·92 (3H, *d*, *J* 7 Hz, sec-Me), 0·95 (3H, *s*, 19-Me), 0·98 (3H, *d*, *J* 7 Hz, sec-Me), 3·60 (1H, *m*, 3α-H), 3·72 (1H, *br. s*, 12β-H), no olefinic proton.

(b) *Chromic acid oxidation of dihydrorubijervine* (**9b**). A mixture of 90 mg of (**9b**), 20 ml of acetone and 1 ml of Kiliani's reagent was allowed to stand for 30 min at room temp and produced 40 mg of an amorphous substance. Recrystallization from Me_2CO gave plates (**12**), m.p. 230–232°, $[\alpha]_D^{19}$ 104° (*c*, 0·25 in $CHCl_3$). MS: *m/e* 411 (M^+) 410 (M^+-1), 396 (M^+-Me), 383, 368, 220, 204, 191, 178, 162, 151, 150 (base peak), 136 98. IR ν_{max}^{Nujol} cm^{-1}: 1720 (C=O), 1700 (C=O). NMR (δ): 0·76 (3H, *d*, *J* 7 Hz, sec-Me), 0·96 (3H, *d*, *J* 7 Hz, sec-Me), 1·00 (3H, *s*, 18-Me), 1·06 (3H, *s*, 19-Me).

Administration of acetate [1-^{14}C] *by the cotton-wick method.* Fifteen veratrum plants fed with 1 mCi of acetate [1-^{14}C] were cultivated in a cold dark room for 3 weeks and radioactive alkaloids were isolated and purified as described previously.

Administration of labeled verazine and etioline by the slice method. A soln of 20 mg of verazine (**5**) or etioline (**4**) in 2·0 ml of 1% H_3PO_4 was adjusted to pH 5·5–6 by the dropwise addition of 0·1 M Na_2HPO_4. When some turbidity appeared, the soln was clarified with a few drops of 10% Tween 80 soln. and final vol was made to about 15 ml. During this process, alkaloids remained unchanged. Dormant rhizome slices (3 × 3 × 0·5 mm) (40 g), prepared in a cold room, were added to this soln and incubated in flasks at 25° for 6 hr and 24 hr. The incubation was terminated by the addition of 20 ml of hot EtOH followed by boiling for several minutes. The content of the flasks was extracted with ammoniacal MeOH to yield about 0·5 g of crude alkaloids by the usual method. Hakurirodine (**11a**) and rubijervine (**9a**) were isolated and purified by column chromatography over 50 vol. of alumina and preparative TLC (hexane–Et_2NH–EtOH=9 : 0·75 : 0·75) followed by addition of 10 mg of respective carriers. Incubation of budding rhizome slices with radioactive compounds and isolation of alkaloids were made by the same procedure as above.

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