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REVIEW

Chemical studies on monoterpenoid indole alkaloids from medicinal plant resources *Gelsemium* and *Ophiorrhiza*

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Abstract We have proved that the plant of origin of ''Yakatsu'', one of the ancient medicines stored in Shosoin Repository, is *Gelsemium elegans* (Loganiaceae). Exhaustive investigation of the alkaloids in this plant as well as its closely related plant, *G. sempervirens*, resulted in the isolation of more than 50 compounds, including new gelsedine-, sarpagine-, and yohimbane-type alkaloids. Pharmacological study of representative and new *Gelsemium* alkaloids revealed that the gelsedine-type alkaloids exhibit potent cytotoxic activity against the A431 human epidermoid carcinoma cell line. It was found that *Ophiorrhiza pumila* (Rubiaceae) produces a remarkable anti-tumor alkaloid, camptothecin, and its related alkaloids, including new compounds that might be the biogenetic precursors of camptothecin. Chemical investigation of callus cultures, regenerated plants, and hairy roots of *O. pumila* revealed that the regenerated plants and the hairy roots produce almost the same alkaloids, including camptothecin, as do the wild-type plants.

Keywords Indole alkaloid · *Gelsemium* · Gelsedine · Anti-tumor · Camptothecin · *Ophiorrhiza*

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Introduction

Numerous monoterpenoid indole alkaloids having rich structural diversity have been isolated from natural resources. Many of them possess remarkable bioactivities and have been utilized as key lead compounds in drug development.

For many years, we have been conducting the chemical investigation of biologically active alkaloids produced by botanical medicinal resources. Some of our recent studies on the monoterpenoid indole alkaloids from genera *Gelsemium* and *Ophiorrhiza* are described herein.

Studies on alkaloids in *Gelsemium* plants

The genus *Gelsemium*, which belongs to the Loganiaceae, comprises three species: *G. elegans* Benth., *G. sempervirens* Ait., and *G. rankinii* Small, from which more than 50 indole alkaloids have been isolated [1, 2]. *Gelsemium elegans*, which is distributed over Southeast Asia, is known as a toxic plant and has been used in traditional Chinese medicine as a remedy for certain kinds of skin ulcers. Recently, some pharmacological effects, including analgesic [3], anti-inflammatory [4], and anti-tumor [5] effects, of *G. elegans* alkaloids have been reported. *Gelsemium sempervirens* and *G. rankinii* grow in the southeastern part of the United States of America, the former having been used in the treatment of neuralgia, migraine and spasmodic disorders such as asthma and whooping cough. The *Gelsemium* alkaloids have markedly diverse and complex architectures and are classified into six types (Fig. 1), based on their chemical structures, i.e.,

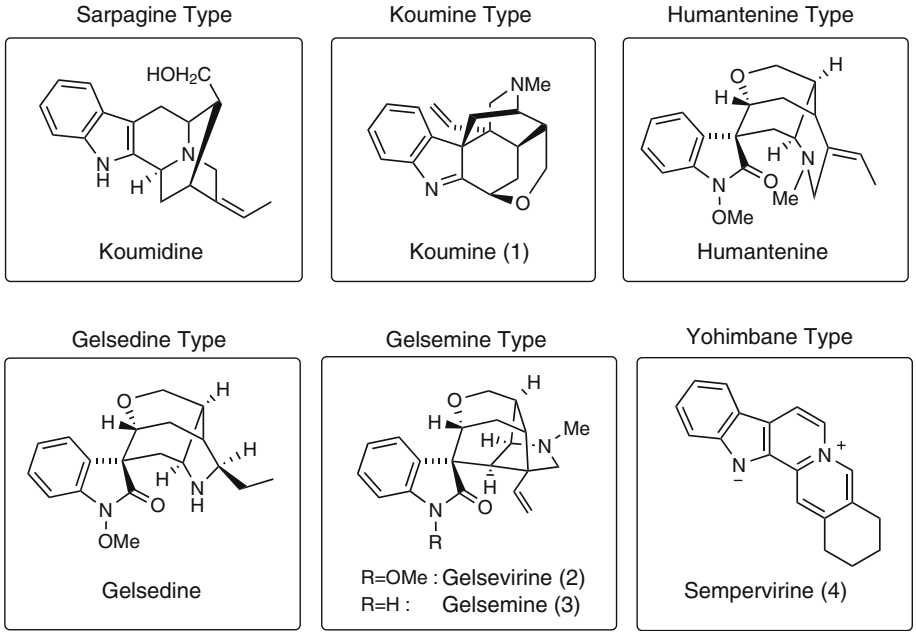


Fig. 1 Representative *Gelsemium* alkaloids and alkaloids **1**–**4** isolated from Yakatsu stored in Shosoin Repository

sarpagine, koumine, humantenine, gelsedine, gelsemine, and yohimbane.

Chemical study on ''Yakatsu'' stored in Shosoin Repository

''Yakatsu'' is one of the 60 kinds of medicines stored in Shosoin Repository, with accompanying dedicatory record, ''Shuju-yaku-cho'' (Memorandum of Medicines), in A.D. 756. Prior to the first scientific investigation in 1948, Yakatsu, listed last in the record, had been thought to have been scattered or lost. During the first scientific investigation, however, the possibility that a bundle of woody roots with the code name ''N-127, Uyaku-no-zoku'' might be Yakatsu was pointed out. On the occasion of the second scientific investigation, we isolated and identified the alkaloid constituents from this 1,250-year-old medicine. As a result, we succeeded in isolating four indole alkaloids, koumine (**1**), gelsevirine (**2**), gelsemine (**3**), and sempervirine (**4**), in their pure states from the crude base obtained from the MeOH extract of sample N-127, Uyaku-no-zoku (Fig. 1) [6]. The identified alkaloids were proof that the plant of origin of this medicine was a species of *Gelsemium*. The isolation of koumine (**1**), an alkaloid characteristic of the Asian species, *G. elegans*, indicated that the plant species was *G. elegans*. This result demonstrated that the medicine is, indeed, the Yakatsu listed in the dedicatory record and that the plant of origin was *G. elegans*, endemic to the southern part of China.

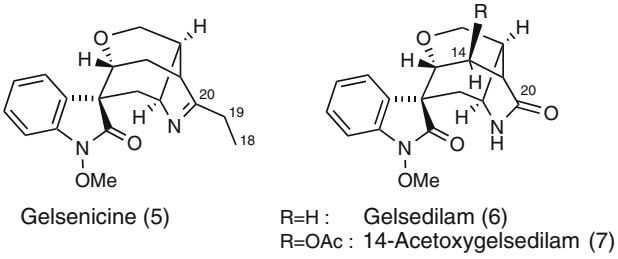


Fig. 2 Structures of gelsenicine (**5**) and new alkaloids **6** and **7** isolated from *Gelsemium elegans*

Alkaloids in *Gelsemium elegans*

We next carried out a chemical investigation of the alkaloid constituents of *G. elegans*, the plant of origin of Yakatsu, as well as its related plant, *G. sempervirens*, and isolated more than 50 compounds, including new gelsedine-, sarpagine-, and yohimbane-type alkaloids, as follows.

From the leaves of *G. elegans* were isolated new types of gelsenicine-related oxindole alkaloids (**6–9**). Gelsedilam (**6**) [7] possessed two carbon atoms fewer than those of common *Gelsemium* alkaloids (Fig. 2). Its ^{1}H and ^{13}C NMR data were very similar to those of the gelsedine-type alkaloid, gelsenicine (**5**), except for the lack of proton and carbon signals ascribable to the C18 and C19 positions. Furthermore, in addition to the carbonyl carbon due to the oxindole nucleus, an sp^2 quaternary carbon at δ 179.8 was observed in the ^{13}C NMR spectra. From the above data, compound **6** was

deduced to be an 18,19-nor-20-lactam compound biogenetically derived from gelsenicine (**5**). The structure of **6** with a lactam ring and its absolute configuration were established by the partial synthesis of **6** from gelsenicine (**5**) (Fig. 3). Compound **5** was treated with trichloroethoxycarbonyl (Troc) chloride to give enamine carbamate **10**. The double bond that migrated to the C19–C20 position was oxidatively cleaved in two steps [(a) OsO_4, (b) $Pb(OAc)_4$] to yield lactam **12**. It was found that treatment of **10** with *m*-CPBA also afforded lactam **12**. Finally, removal of the N_b-Troc group gave gelsedilam (**6**). The 14-acetoxy derivative of **6**, 14-acetoxygelsedilam (**7**), was also isolated (Fig. 2). The new alkaloids, gelsedilam (**6**) and 14-acetoxygelsedilam (**7**) are unprecedented 18,19-nor-type monoterpenoid indole alkaloids.

Gelsefuranidine (**8**) [7] is a derivative of 14-hydroxygelsenicine (**13**), having a 2-substituted furan residue and a propenyl group (Fig. 4). The structure, including the absolute configuration, was confirmed by condensation of 14-hydroxygelsenicine (**13**) and furfural under acidic conditions. Gelsefuranidine (**8**) is the first example of a monoterpenoid indole alkaloid having a furan residue on the side chain.

Gelseiridone (**9**) [7], whose structure was deduced by spectroscopic analysis, is a new type of alkaloid having a nitrogen–carbon linkage between a gelsenicine-type monoterpenoid indole alkaloid that possesses an α, β-unsaturated ketone residue as well as the N_b-C20 seco-form and a monoterpene unit having an iridoid skeleton (Fig. 4). From a biogenetic point of view, **9** would have originated from 14-hydroxygelsenicine (**13**) and 7-deoxygelsemide (**14**), a coexisting iridoid in this plant.

Gelsemoxonine was first isolated by Lin et al. from *G. elegans* in 1991 [8]. On the basis of spectroscopic analysis, it was proposed to be an unusual N_b-C20 seco-oxindole gelsedine-type alkaloid (**15**). However, during the reinvestigation of the chemical constituents of *G. elegans*, we found that gelsemoxonine (**16**) had an unusual azetidine unit (Fig. 5) [9]. Acetylation of gelsemoxonine under ordinary conditions gave an unexpected diacetylated derivative. This finding raised doubts regarding the reported structure **15** of gelsemoxonine. The HMBC spectrum was measured at –30°C in pyridine-d_5 by utilizing the phenomenon that the signal of N_b-H in **16** becomes sharp and well defined at low temperature. As a result, clear correlations

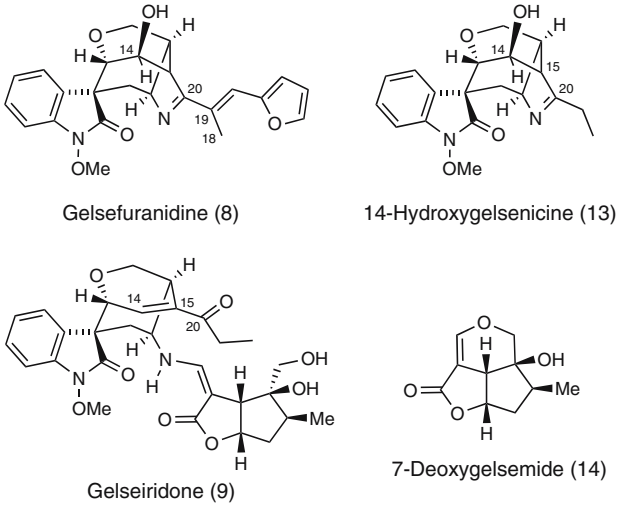


Fig. 4 Structures of new alkaloids **8** and **9** and known compounds **13** and **14** isolated from *Gelsemium elegans*

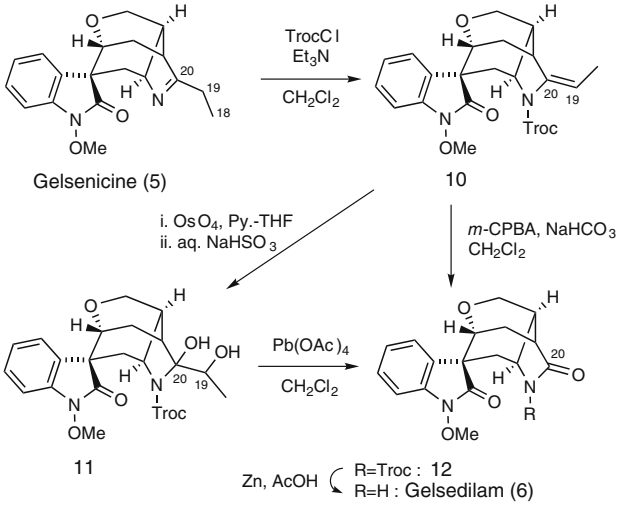


Fig. 3 Chemical transformation of gelsenicine (**5**) into gelsedilam (**6**)

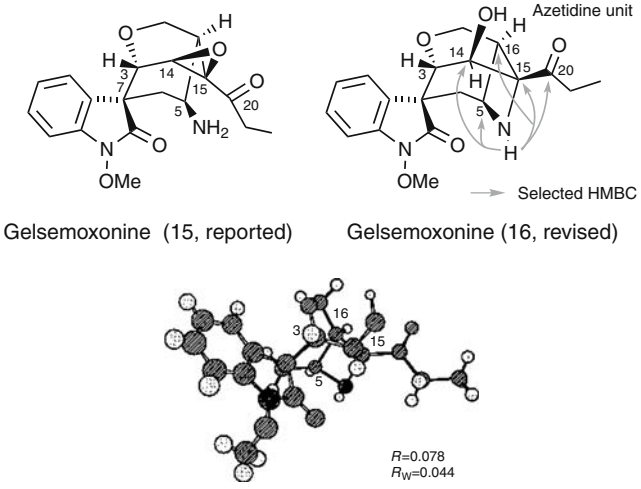
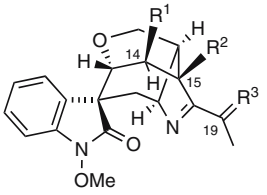
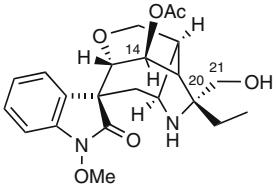


Fig. 5 Structures of gelsemoxonine and selected HMBC correlations and X-ray structure of gelsemoxonine



R^1=OAc, R^2=H, R^3=H_2 : 14-Acetoxygelsenicine (17)
R^1=OH, R^2=OH, R^3=H_2 : 14,15-Dihydroxygelsenicine (18)
R^1=OAc, R^2=OH, R^3=H_2 : 14-Acetoxy-15-hydroxygelsenicine (19)
R^1=OH, R^2=H, R^3=O : 14-Hydroxy-19-oxogelsenicine (20)



14-Acetoxygelselegine (21)

Fig. 6 Structures of new alkaloids **17**–**21** isolated from *Gelsemium elegans*

between N_b-H and the two carbon atoms, that is, the oxymethine carbon at C14 and the ketone carbon at C20, were observed. Formula **15** cannot explain these long-range couplings that enabled us to construct an azetidine ring consisting of N_b, C15, C16 and C5 positions. Finally, structure **16**, with an azetidine unit, was confirmed by X-ray crystallographic analysis. This is the first example of azetidine-containing monoterpenoid indole alkaloids.

Some gelsedine-type alkaloids (**17**–**21**), possessing an oxygen function at the C14 position or C14 and C15 positions, were isolated from the leaves of *G. elegans* (Fig. 6) [9, 10]. 14,15-Dihydroxygelsenicine (**18**) [9] was presumed to be the biosynthetic precursor of gelsemoxonine (**16**).

Alkaloids in *Gelsemium sempervirens*

Five new sarpagine-type alkaloids, gelsempervine-A (**22**), -B (**23**), -C (**24**), -D (**25**), and 19*Z*-16-*epi*-voacarpine (**26**), were isolated from the dried radix of *G. sempervirens* Ait. (Fig. 7) [11]. The structure of gelsempervine-A (**22**), with a 3-oxo, i.e., 2-acyl indole nucleus, was determined by spectroscopic analysis and chemical conversion from the known sarpagine-type alkaloid, 16-*epi*-voacarpine (**27**). However, perusal of the UV spectrum of **22** revealed typical absorptions of the indole nucleus in MeOH. The 2-acyl indole alkaloids are known to exhibit a characteristic absorption at around 310 nm. In order to investigate this unusual

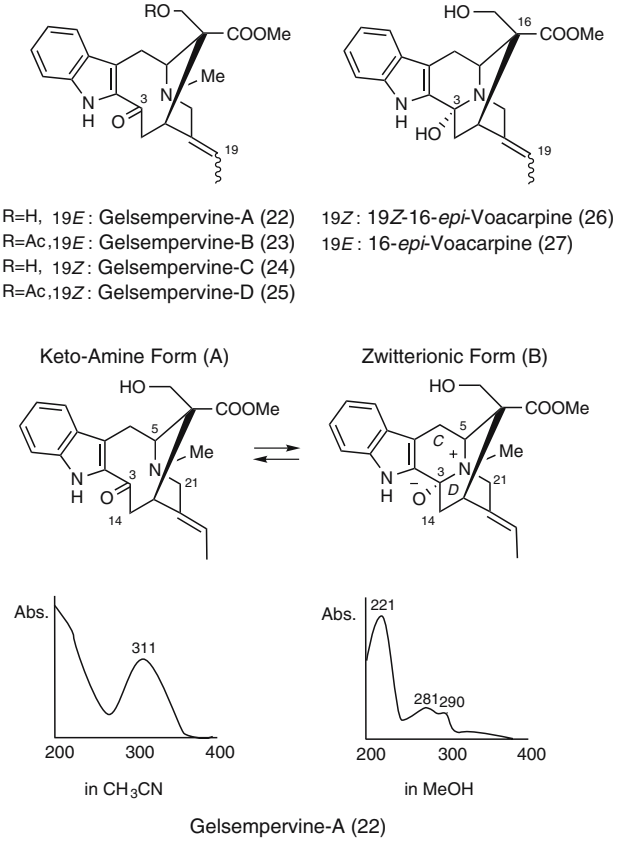


Fig. 7 Structures of new sarpagine-type alkaloids **22**–**26** isolated from *Gelsemium sempervirens* and 16-*epi*-voacarpine (**27**), and two structural forms and UV spectra of gelsempervine-A (**22**)

observation in the UV spectrum, we next measured UV as well as NMR spectra in protic or aprotic solvents. As a result, we found that **22** exhibited the typical absorption patterns of indole and 2-acyl indole alkaloids in MeOH and CH_3CN, respectively. Quite interestingly, the signals of the protons and carbons at C5 and C21 in CD_3OD were observed at low fields compared with those in CD_3CN. On the other hand, the signals of H_2-14 were observed at lower fields in CD_3CN than in CD_3OD. These spectroscopic data suggest that gelsempervine-A (**22**) existed exclusively as a *C/D* ring-opening structure with the keto-amine form (A), in such aprotic solvents as CD_3CN, and as a trans-annular structure with the zwitterionic form (B), in such protic solvents as CD_3OD. The above-described phenomena in the UV and NMR spectra of compound **22** were also observed in three new compounds, gelsempervine-B (**23**), -C (**24**), and -D (**25**). Therefore, it was found that 2-acyl sarpagine-type alkaloids possessing an N_b-methyl group take a keto-amine form or a trans-annular form in solution, depending on the solvent.

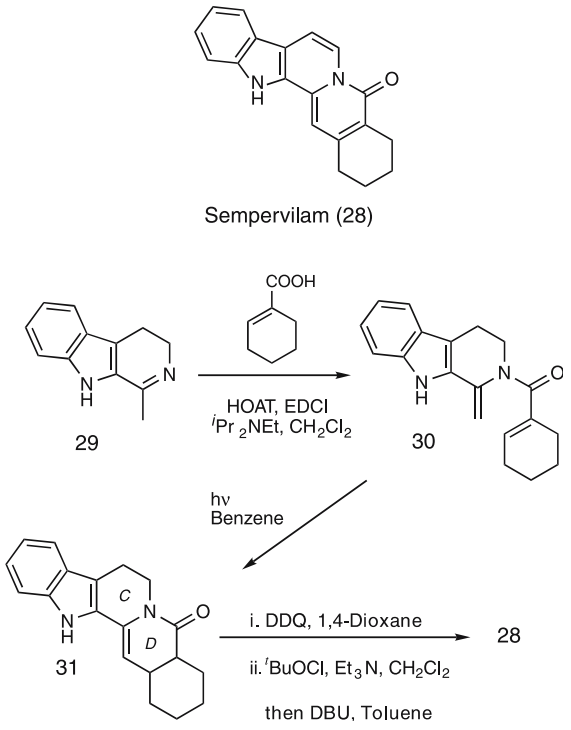


Fig. 8 Structure and synthesis of sempervilam (**28**) isolated from *Gelsemium sempervirens*

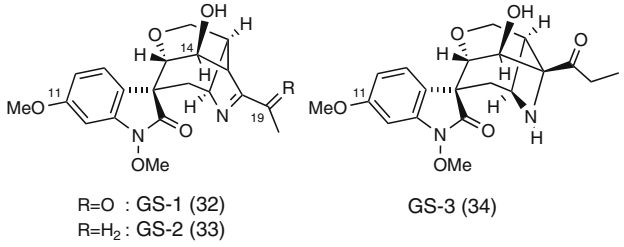


Fig. 9 Structures of new alkaloids **32**–**34** isolated from *Gelsemium sempervirens*

Sempervilam (**28**), a yohimbane-type alkaloid, was isolated from the dried radix of *G. sempervirens* (Fig. 8) [11]. The total synthesis of **28** was achieved using 3,4-dihydroharman (**29**). Compound **29** was condensed with cyclohexene-1-carboxylic acid using 1-hydroxy-7-azabenzotriazole (HOAT) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI) to give amide **30**. Photocyclization of **30** gave cycloadduct **31**. Oxidation of the *D*-ring with DDQ, and aromatization of the *C*-ring by treatment with ^{t}BuOCl and then with DBU afforded **28**.

Three gelsedine-type alkaloids, GS-1 (**32**), GS-2 (**33**), and GS-3 (**34**), were isolated from the dried stems of *G. sempervirens* (Fig. 9) [12]. GS-2 (**33**) was also isolated from the fresh leaves of this plant [12].

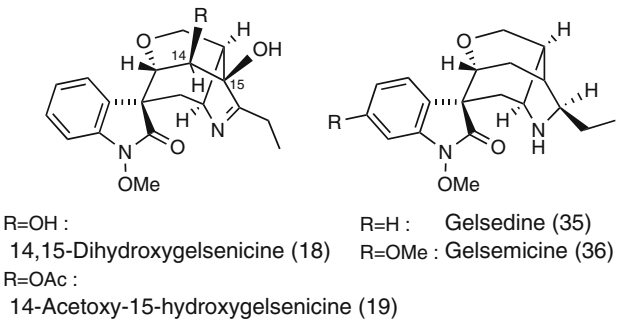


Fig. 10 Gelsedine-type alkaloids possessing cytotoxic activity against A431 human epidermoid carcinoma cells

Evaluation of cytotoxicity of *Gelsemium* alkaloids

The cytotoxic effects of 14 *Gelsemium* alkaloids [sarpagine type, 19*Z*-akuammidine; koumine type, koumine (**2**); humantenine type, humantenine, 11-methoxyhumantenine; gelsedine type, gelsenicine (**5**), 14-hydroxygelsenicine (**13**), 14-acetoxygelsenicine (**17**), 14,15-dihydroxygelsenicine (**18**), 14-acetoxy-15-hydroxygelsenicine (**19**), GS-2 (**33**), gelsedine (**35**), gelsemicine (**36**), gelsemoxonine (**16**); gelsemine type, gelsemine (**3**)] on some tumor cells were evaluated. Among them, the gelsedine-type alkaloids, 14,15-dihydroxygelsenicine (**18**), 14-acetoxy-15-hydroxygelsenicine (**19**), gelsedine (**35**), and gelsemicine (**36**) showed relatively strong cytotoxic effects on the A431 human epidermoid carcinoma cell line (Fig. 10) [10]. Their cytotoxicities were compared with that of a positive control, cisplatin (EC_{50}=3.5 μM), a drug used for the treatment of skin cancer. In particular, 14,15-dihydroxygelsenicine (**18**) showed high cytotoxicity (EC_{50}=250 nM). Further chemical and pharmacological studies on *Gelsemium* alkaloids are in progress in our laboratories.

Chemical studies on alkaloids in *Ophiorrhiza* plants distributed in Japan and on callus, regenerated plants and hairy roots of *Ophiorrhiza pumila*

Camptothecin (**37**) [13] is a well-known monoterpenoid indole alkaloid possessing remarkable anti-tumor activity and was isolated for the first time from *Camptotheca acuminata* (Nyssaceae) [14]. To date, several camptothecin-producing plants, including *Ophiorrhiza mungos* [15] and *O. filistipula* [16] (Rubiaceae), have been reported. Medicinal chemistry studies of camptothecin have been actively performed [17], and, at present, two semi-synthetic camptothecins, topotecan and irinotecan, are used clinically as anti-tumor agents.

Alkaloids of *Ophiorrhiza* plants distributed in Japan

In order to discover new camptothecin-related alkaloids, investigations of the constituents of *Ophiorrhiza* plants that are distributed in Japan, that is, *O. pumila* Champ., *O. liukiuensis* Hayata, and *O. japonica* Bl., were carried out. As a result, it was found that *O. pumila* produces camptothecin (**37**) and its related alkaloids (**38**–**43**) [18–21], whereas *O. japonica* produces *β*-carboline-type alkaloids (**45**–**52**) and no camptothecin-related alkaloids (Fig. 11) [22, 23]. Another *Ophiorrhiza* plant, *O. liukiuensis*, was found to produce both camptothecin-related alkaloids (**37**, **38**, **40**–**42**, **44**) and *β*-carboline-type alkaloids (**45**, **48**, **49**, **52**) [24].

From *O. pumila*, new alkaloids, pumiloside (**38**) [18], (3*S*)- and (3*R*)-deoxypumilosides (**39** and **40**) [18, 21] and the first natural glyco-camptothecin, chaboside (**43**) [19, 20], were isolated. Pumiloside (**38**) and deoxypumilosides (**39** and **40**) showed UV absorptions indicative of a quinolone or a quinoline chromophore, respectively. In their ^{1}H NMR spectra, a set of three protons on a vinyl group, an acetal proton, and one sugar unit containing an anomeric proton were observed, respectively, which are very similar to those of strictosamide (**41**). The above data indicated that pumiloside (**38**) and deoxypumilosides (**39** and **40**) are hybrid-type compounds possessing the partial structures of both camptothecin (**37**) and strictosamide (**41**), which is established as a biosynthetic intermediate of camptothecin (**37**). Therefore, they seemed to be plausible biogenetic precursors of camptothecin (**37**). To establish these structures, including the absolute configurations, we synthesized three new camptothecinoids (**38**–**40**) starting from tryptamine (**53**) and secologanin (**54**) (Fig. 12). Condensation of **53** and **54** via the Pictet–Spengler reaction under acidic conditions, followed by *D*-ring closure by treatment with alkali, gave strictosamide (**41**) having 3*S* and vincoside lactam (**55**) having 3*R* stereochemistry, respectively. Pumiloside tetra-acetate (**56**) was prepared from **41** by a three-step procedure that included acetylation of the hydroxyl groups in the glucose unit, oxidative bond cleavage at C2–C7, and ring closure between C2 and C6 to give a quinolone ring. Removal of the protecting

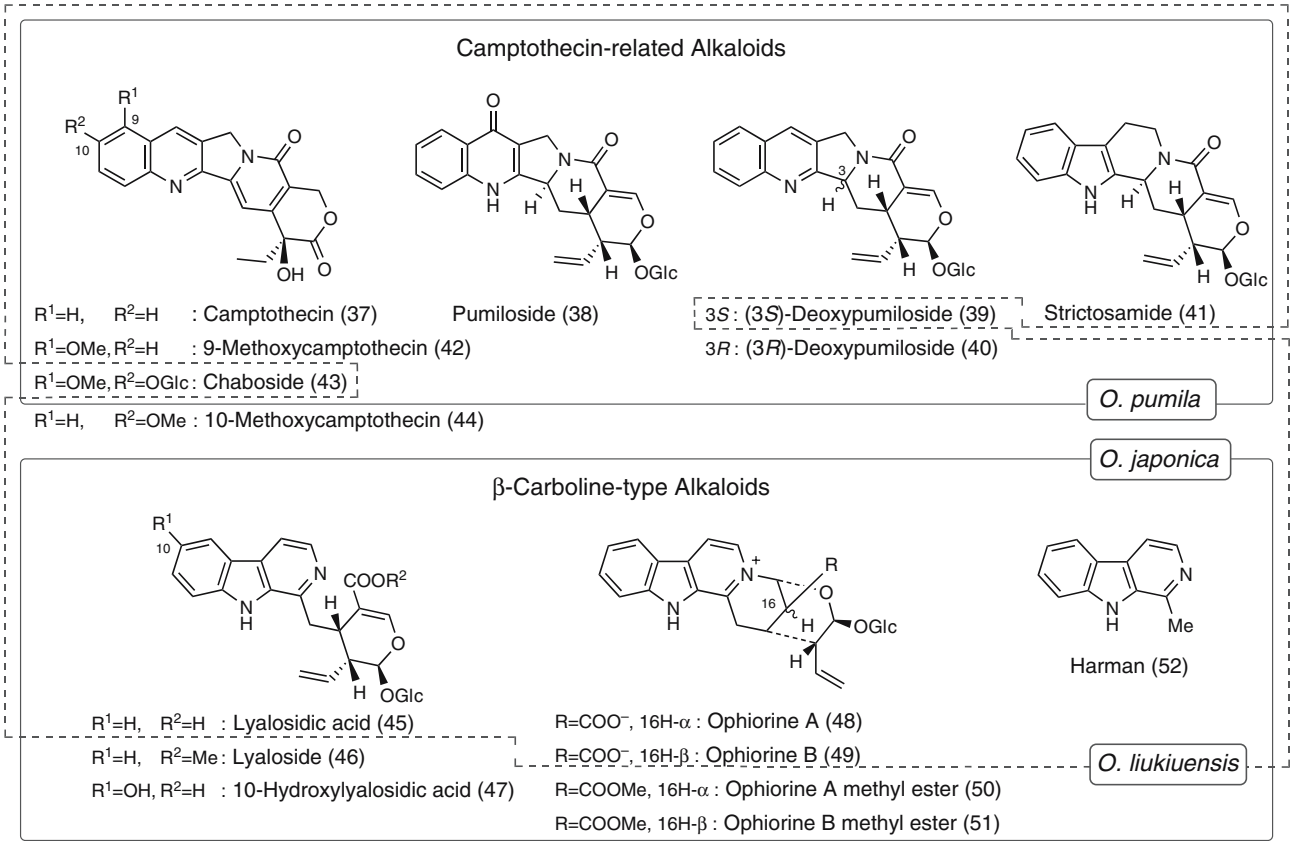


Fig. 11 Alkaloids isolated from *Ophiorrhiza* plants distributed in Japan

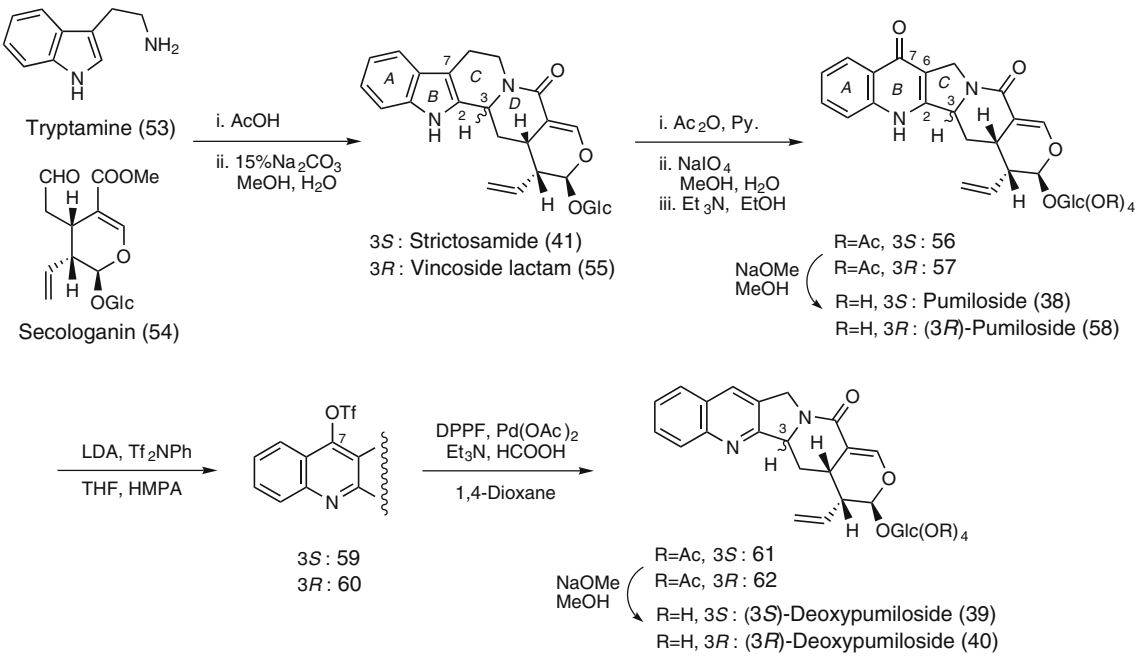


Fig. 12 Synthesis of pumiloside (**38**) and (3*S*)- and (3*R*)-deoxypumilosides (**39** and **40**)

group of **56** gave pumiloside (**38**) having 3*S* stereochemistry. Next, the oxygen function at the C7 position in **56** was removed in two steps as follows. Compound **56** was treated with lithium diisopropylamide (LDA) and then with *N*-phenyltrifluoromethanesulfonimide. The enol triflate **59** thus obtained was treated with $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)-ferrocene (DPPF), Et_3N and HCOOH in dioxane to afford deoxygenated compound **61**. Deacetylation of **61** gave (3*S*)-deoxypumiloside (**39**). The same procedure was applied to vincoside lactam (**55**) to give (3*R*)-deoxypumiloside (**40**). The spectroscopic data of synthetic pumiloside (**38**) and deoxypumilosides (**39** and **40**), including the CD spectra, were identical with those of the natural compounds, respectively, thereby establishing the structures of these new alkaloids.

Secondary metabolites in callus cultures, regenerated plants and hairy roots of *Ophiorrhiza pumila*

To establish an efficient method for the production of camptothecin (**37**), as well as finding novel secondary metabolites and a clue to clarifying the camptothecin biosynthetic pathway, we started related biotechnology research of *O. pumila* that was found to produce camptothecin-related alkaloids exclusively. As a result, we succeeded in obtaining callus cultures [25] from the leaves and shoots, regenerated plants [26, 27], and hairy roots [28] of *O. pumila*. From the investigation of their constituents, it was found that well-growing callus cultures produced anthraquinones that could not be detected in the wild-type plants, and the regenerated plants and the hairy roots produced almost the same types of alkaloids as did the wild-type plants. From the regenerated plants that were obtained from the callus cultures originally derived from the leaf segment, camptothecin (**37**) and a new alkaloid, 9-*β*-D-glucosyloxycamptothecin (**63**), together with five camptothecin-related alkaloids (**38**, **40**–**43**), were isolated. The structure, including the absolute configuration, of the new glucosyloxycamptothecin (**63**) was determined by chiral total synthesis (Fig. 13) [27]. The Friedländer condensation of the *A*-ring moiety **65** and *CDE*-ring counterpart **67** was adopted for the construction of the entire molecule. First, 6-glucosyloxy-2-aminobenzaldehyde (**65**), corresponding to the *A*-ring part of **63**, was prepared from known phenol **64** in two steps. The *CDE*-ring moiety **67** was prepared from known (*S*)-**66**. The condensation of **65** and **67** in AcOH-MeOH under reflux gave camptothecin skeleton **68**. Deacetylation of **68** afforded 9-*β*-D-glucosyloxycamptothecin (**63**).

Hairy roots of *O. pumila* were obtained from the regenerated plant stems by the direct inoculation of *Agrobacterium rhizogenes* (pRi15834, pGSGluc1) [28]. From the hairy roots cultivated on solid medium or in liquid medium, camptothecin (**37**) and its related alkaloids (**38**–**41**) were isolated, together with two new

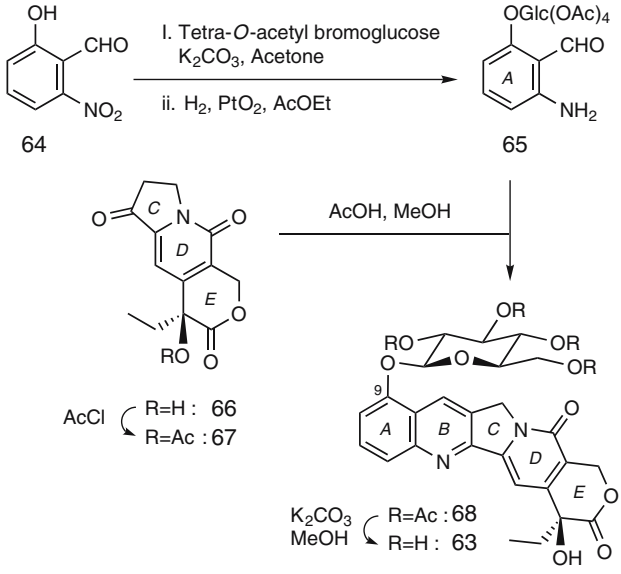


Fig. 13 Structure and synthesis of 9-β-D-glucosyloxycamptothecin (**63**)

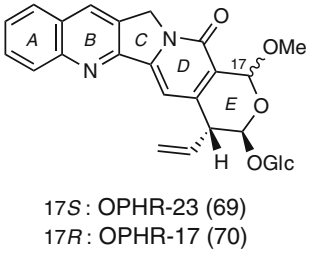
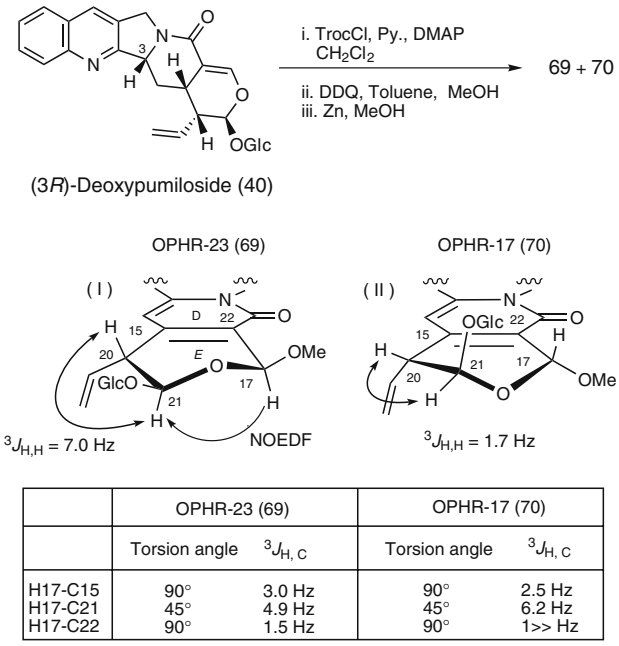


Fig. 14 Structure of OPHR-23 (**69**) and OPHR-17 (**70**)

alkaloids, OPHR-23 (**69**) and OPHR-17 (**70**) (Fig. 14) [29]. This result revealed that the hairy roots of *O. pumila* retained their alkaloid-producing ability. HPLC analysis of the liquid medium indicated that camptothecin (**37**), pumiloside (**38**) and (3*R*)-deoxypumiloside (**40**) were excreted into the culture medium [30, 31]. From spectroscopic analysis, new alkaloids (**69** and **70**) were determined to be a pair of diastereomers at the C17 position, having characteristic structures due to the *ABC*-ring of camptothecin (**37**) and the *CD*-ring of strictosamide (**41**), pumiloside (**38**), and deoxypumilosides (**39** and **40**), together with acetal and methoxy groups. To determine their structures, we carried out the chemical conversion from (3*R*)-deoxypumiloside (**40**) into the new alkaloids (Fig. 15). After protection of the hydroxyl groups in the glucose unit with Troc group, **40** was treated with DDQ in toluene–MeOH, followed by deprotection to give new alkaloids **69** and **70**. The configuration at the C17 position in



	OPHR-23 (69)		OPHR-17 (70)	
	Torsion angle	$^3J_{H,C}$	Torsion angle	$^3J_{H,C}$
H17-C15	90°	3.0 Hz	90°	2.5 Hz
H17-C21	45°	4.9 Hz	45°	6.2 Hz
H17-C22	90°	1.5 Hz	90°	1>> Hz

Fig. 15 Chemical transformation of (3*R*)-deoxypumiloside (**40**) into OPHR-23 (**69**) and OPHR-17 (**70**) and stereochemical analysis at C-17 in **69** and **70**

OPHR-23 (**69**) and OPHR-17 (**70**), a pair of diastereomers at C17, was determined to be *S* and *R*, respectively, by NMR techniques, including differential NOE experiments and PFG J-HMBC 2D spectroscopy.

Camptothecin (**37**), which has a quinoline nucleus, is biogenetically derived from strictosamide (**41**), as was proven by Hutchinson et al. [32]. However, the biosynthetic pathway from strictosamide (**41**) to **37**, which comprised (i) transformation of an indole 6-5-6 ring system of the *ABC*-ring into a quinoline 6-6-5 ring system, (ii) oxidation of the *D*-ring to a pyridone, and (iii) removal of a glucose unit and structural conversions in the *E*-ring, is not yet clear. The existence of pumiloside (**38**) and (3*S*)-deoxypumiloside (**39**) suggests the possibility that the conversion of the *ABC*-ring of strictosamide (**41**) would be the next step after the formation of strictosamide (**41**), followed by *D*-ring oxidation and transformation of the *E*-ring (Fig. 16).

The biosynthetic pathway of camptothecin (**37**) from [1-^{13}C]glucose was investigated by in vivo tracer experiments using the hairy roots of *O. pumila* [33]. From the hairy roots that were grown in the presence of [1-^{13}C]glucose, ^{13}C-enriched camptothecin was isolated, and the ^{13}C incorporated positions were determined by ^{13}C NMR measurements. The ^{13}C labeling pattern of camptothecin (**37**) isolated from the hairy roots clearly showed that the secologanin moiety was

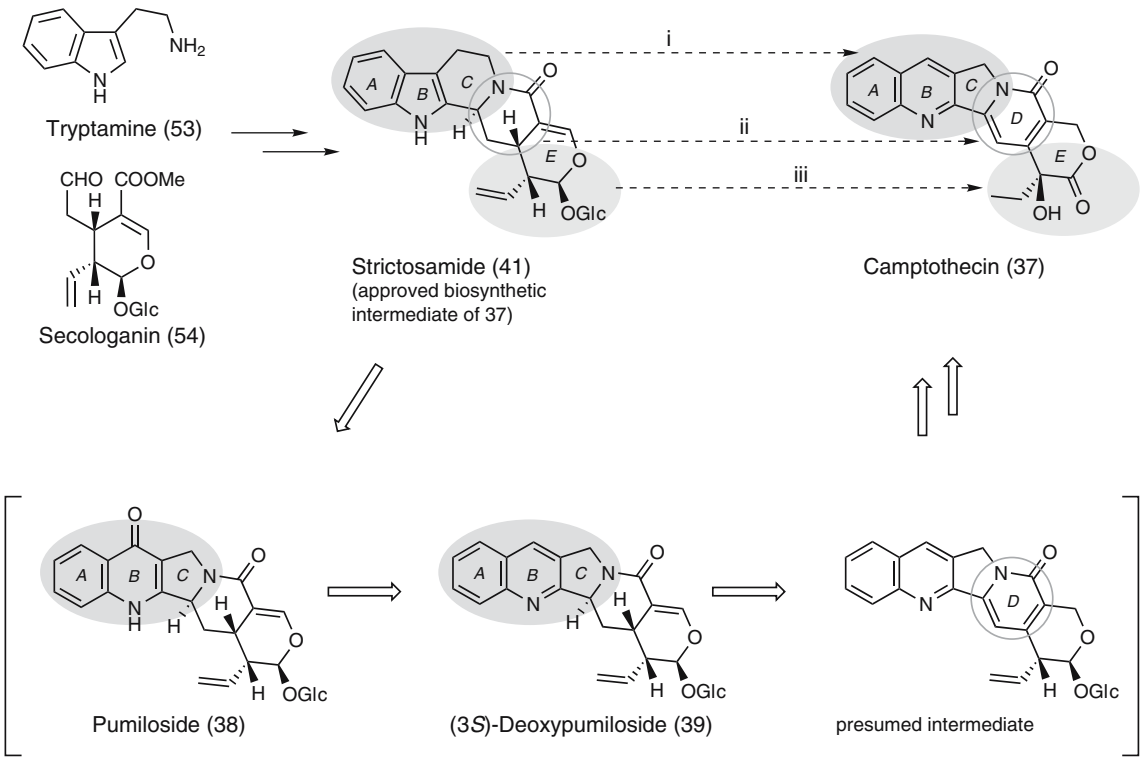


Fig. 16 Speculated biogenetic pathway of camptothecin (**37**) from strictosamide (**41**)

synthesized via the 2*C*-methyl-D-erythritol 4-phosphate (MEP) pathway, and not via the mevalonate pathway. Further studies are needed to clarify the complete features of camptothecin biosynthesis.

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