*Phytochemistry*, Vol. 20, No. 8, pp. 1997–2001, 1981.
Printed in Great Britain.

0031–9422/81/081997–05 $02.00/0


# TWO CAGE-TYPE LUPIN ALKALOIDS FROM *SOPHORA FRANCHETIANA**

SHIGERU OHMIYA†, KIMIO HIGASHIYAMA†, HIROTAKA OTOMASU†, JOJU HAGINIWA‡ and ISAMU MURAKOSHI‡

† Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo, 142, Japan; ‡ Faculty of Pharmaceutical Sciences, University of Chiba, Yayoi-cho 1-33, Chiba, 260, Japan

(*Revised received* 5 *January* 1981)

**Key Word Index**—*Sophora franchetiana*; Leguminosae; alkaloid; lupin alkaloid; (−)-tsukushinamine-A; (−)-tsukushinamine-B; tsukushinamine-C; (−)-anagyrine; (−)-baptifoline; (±)-ammodendrine; variations of alkaloid content.

**Abstract**—Two new cage-type lupin alkaloids, (−)-tsukushinamine-B and tsukushinamine-C, have been isolated from the fresh epigeal parts of *Sophora franchetiana*, along with (−)-cytisine, (−)-*N*-formylcytisine, (−)-rhombifoline, (−)-anagyrine, (−)-baptifoline and (±)-ammodendrine, as well as (−)-tsukushinamine-A. The structures of these novel tsukushinamine-type lupin alkaloids were determined by spectroscopic data and partly by a chemical reaction. Variations of the alkaloid contents in the seeds, seedlings and various parts of *S. franchetiana* were also examined.

## INTRODUCTION

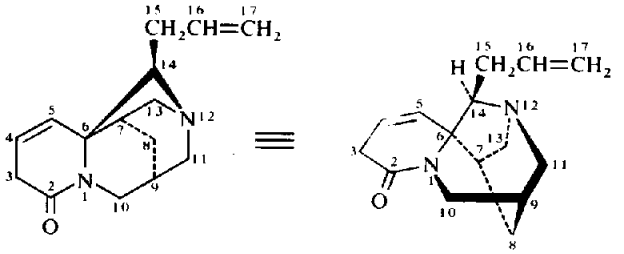
During the course of our studies on lupin alkaloids in leguminous plants [1–4], Murakoshi and Ohmiya *et al.* [5] have recently reported evidence for the presence of a novel cage-type lupin alkaloid, (−)-tsukushinamine-A (**1**), in the roots and the epigeal parts of *Sophora franchetiana*, which is locally native but a very rare shrub in Japan. Its proposed structure has also been confirmed by X-ray analysis as **1**, including the absolute configuration at C-6, C-7, C-9 and C-14 [6].

From the same sources, we have now isolated two new cage-type lupin alkaloids, (−)-tsukushinamine-B (**2**) and tsukushinamine-C (**3**), as the isomers of (−)-tsukushinamine-A (**1**). This paper describes the structural elucidation of these two lupin alkaloids and the relative variations of the alkaloid content in the seeds, seedlings and various parts of *S. franchetiana*.

## RESULTS AND DISCUSSION

From the 75% EtOH extract of the freshly harvested epigeal parts of *S. franchetiana*, two new cage-type lupin alkaloids, (−)-tsukushinamine-B (**2**) and tsukushinamine-C (**3**), were isolated in 0.0015 and 0.0005% yields of the fr. wt, respectively, along with (−)-cytisine (**4**), (−)-*N*-formylcytisine, (−)-rhombifoline (**7**), (−)-anagyrine (**5**), (−)-baptifoline (**6**) and (±)-ammodendrine, as well as (−)-tsukushinamine-A (**1**) [5,6].

(−)-Tsukushinamine-B (**2**) was obtained as an oily product, $[\alpha]_D^{25}$ −144.4° (EtOH), with a $M^+$ (92%) at *m/z* 244.158 for $C_{15}H_{20}N_2O$ (calc. 244.158) and prominent fragments at *m/z* (relative intensity) 229 (10), 203 (87), 160 (61), 146 (36), 122 (27), 98 (36), 97 (81), 96 (100) and 82 (67), indicative of a lupin alkaloid containing the same moiety as **1**, as described in a previous paper [5]. As expected, the chemical shifts of the $^{13}C$ NMR signals of **2** were in good agreement with those of **1**, as shown in Table 1.



**1** (6*R*:7*R*:9*S*:14*R*)

* Part of this work was presented at the 22nd Symposium on the Chemistry of Natural Products of Japan, Fukuoka, 26 October 1979 (symposium papers, p. 525).

Table 1. $^{13}C$ NMR data for the tsukushinamine-type alkaloids **1** and **2** in $C_6D_6$*

| Carbon | 1 | 2 |
|---|---|---|
| 2 | 167.2 *s* | 165.8 *s* |
| 3 | 32.1 *t* | 32.1 *t* |
| 4 | 119.2 *d* | 119.5 *d* |
| 5 | 129.6 *d* | 127.2 *d* |
| 6 | 70.0 *s* | 71.9 *s* |
| 7 | 46.8 *d* | 47.7 *d* |
| 8 | 31.5 *t* | 31.3 *t* |
| 9 | 28.6 *d* | 28.9 *d* |
| 10 | 51.4 *t* | 48.6 *t* |
| 11 | 52.4 *t* | 61.0 *t* |
| 13 | 61.2 *t* | 56.7 *t* |
| 14 | 75.7 *d* | 74.5 *d* |
| 15 | 32.1 *t* | 35.5 *t* |
| 16 | 135.8 *d* | 137.6 *d* |
| 17 | 115.8 *t* | 115.4 *t* |

*TMS was used as the internal standard and chemical shifts were given in ppm ($\delta$) relative to the internal standard. $^{13}C$–$^1H$ correlations are based on selective $^1H$-decoupled $^{13}C$ NMR measurements.

Furthermore, the $^1H$ NMR spectrum of **2** ($C_6D_6$) exhibited signals at $\delta$ 2.82 (2 H, *br s*, 3-$H_2$), 3.17 (1 H, *d*, $J$ = 13.5 Hz, 10-$H_\beta$), 4.33 (1 H, *dd*, $J$ = 13.5 and 7.5 Hz, 10-$H_\alpha$), 4.9–5.1 (2 H, *m*, 17-$H_2$), 5.03 (1 H, *dt*, $J$ = 10.5 and 1 Hz, 5-H), 5.22 (1 H, *dt*, $J$ = 10.5 and 3 Hz, 4-H) and 5.92 (1 H, *m*, 16-H) which were characteristic of **1** [5, 6]. These spectroscopic patterns of **2** were very similar to those of **1**, suggesting that **2** also belongs to the cage-type lupin alkaloid of the tsukushinamine-A (**1**) type. This assumption was confirmed further by the other $^1H$ NMR signals for **2**: from the results of appropriate decoupling experiments and comparison with that of **1**, the multiplet centred at $\delta$ 1.96 (2 H) was assigned to the allylic methylene protons (15-$H_2$), the broad singlet at 1.69 (1 H) to the C-7 methine proton and the broad singlet at 1.28 (3 H) to the C-8 methylene and C-9 methine protons. The spectrum also revealed the double quartet at $\delta$ 3.07 (1 H, $J$ = 10, 5.5 and 2.5 Hz, 14-H). This signal collapsed to a doublet ($J$ = 2.5 Hz) on irradiation at $\delta$ 1.96 due to 15-$H_2$ but was not altered on each irradiation at the signals due to 7-H, 8-$H_2$ and 9-H. These spectroscopic data indicated that the allyl group in **2** was linked to C-14 as found in the case of **1**.

Therefore, (−)-tsukushinamine-B (**2**) was presumed to be a diastereomer of **1** differing in the configuration of the allyl group at C-14. In addition, the configuration of the allyl group of **2** was supported by the $^1H$ NMR analysis, which revealed two pair of geminal-coupled signals corresponding to either the methylene group at C-11 or C-13 from their chemical shifts and coupling characteristics: one resonating at $\delta$ 2.30 (1 H, *dd*, $J$ = 12 and 2.5 Hz, 13-$H_\alpha$) and 2.63 (1 H, *br d*, $J$ = 12 Hz, 13-$H_\beta$), the other at 2.43 (1 H, *br d*, $J$ = 13 Hz, 11-$H_\beta$) and 2.83 (1 H, *br dd*, $J$ = 13 and 4 Hz, 11-$H_\alpha$). The broad doublet at $\delta$ 2.63 of the former pair collapsed to a slightly broadened doublet ($J$ = 12 Hz) on irradiation at $\delta$ 1.69 due to 7-H, which caused no change of the later pair, and to a double doublet ($J$ = 12 and 4 Hz) on irradiation at $\delta$ 1.28 due to 9-H and 8-$H_2$, although in the decoupling experiments the counterpart, the signal at $\delta$ 2.30, did not show any change.

From the above $^{13}C$ and $^1H$ NMR spectroscopic data and from the results of examination of the molecular model, the signal at $\delta$ 2.63 was assigned to 13-$H_\beta$, which had a dihedral angle of *ca* 30° with 7-H and was in the coplanar '*W*' configuration with one of the C-8 methylene protons, and hence the signal at $\delta$ 2.30 to 13-$H_\alpha$ bearing the dihedral angle of *ca* 90° with 7-H. Irradiation at $\delta$ 3.07 due to 14-H collapsed the double doublet at 2.30 due to 13-$H_\alpha$ to a doublet ($J$ = 12 Hz) and vice versa, suggesting that the bond of 14-H–C-14–N-12–C-13–13-$H_\alpha$ had a coplanar '*W*' configuration. This stereochemistry was in agreement with the above configurational assignment for **2** as shown in the partial stereostructure (**2a**).

From the above results, it can therefore be concluded that the structure of (−)-tsukushinamine-B is represented as **2**.

Tsukushinamine-C (**3**) was obtained as an oily product and as a very minor component. Its mass spectrum also was very similar to those of (−)-tsukushinamine-A (**1**) and (−)-tsukushinamine-B (**2**), suggesting that **3** also belongs to the tsukushinamine-type alkaloids **1** and **2**. The $^1H$ NMR spectrum of **3** ($CDCl_3$) revealed signals at $\delta$ 3.39 (1 H, *br d*, $J$ = 13 Hz, 10-$H_\beta$) and 4.18 (1 H, *dd*, $J$ = 13 and 6.5 Hz, 10-$H_\alpha$) due to the C-10 methylene protons, characteristic of the tsukushinamine skeleton, and at 5.87 (1 H, *m*, 16-H), 5.08 (1 H, fine split *d*, $J$ = 10 Hz, 17-H) and 5.11 (1 H, fine split *d*, $J$ = 16 Hz, 17-H') for the vinyl protons of the allylic side chain, which were in accord with those observed in the cases of **1** and **2**.

**3** showed a UV absorption at 261.0 nm (EtOH). The $^1H$ NMR spectrum of **3** ($CDCl_3$) revealed signals for the cyclic double bond at 5.97 (1 H, *dt*, $J$ = 10 and 2 Hz, 3-H) and 6.46 (1 H, *dt*, $J$ = 10 and 4 Hz, 4-H) which both were shifted to a lower field than those of **1** and **2**. The spectrum also showed signals due to the C-5 methylene protons at $\delta$ 2.62 (2 H, *dd*, $J$ = 4 and 2 Hz, 5-$H_2$) which was coupled only with the cyclic olefine protons, indicating that the C-5 methylene carbon was contiguous to the quarternary carbon at the C-6 position. In addition, the $^1H$ NMR spectrum showed the signal due to 14-H at $\delta$ 2.88 (1 H, *t*, $J$ = 5.5 Hz) which was similar to that of **1**.

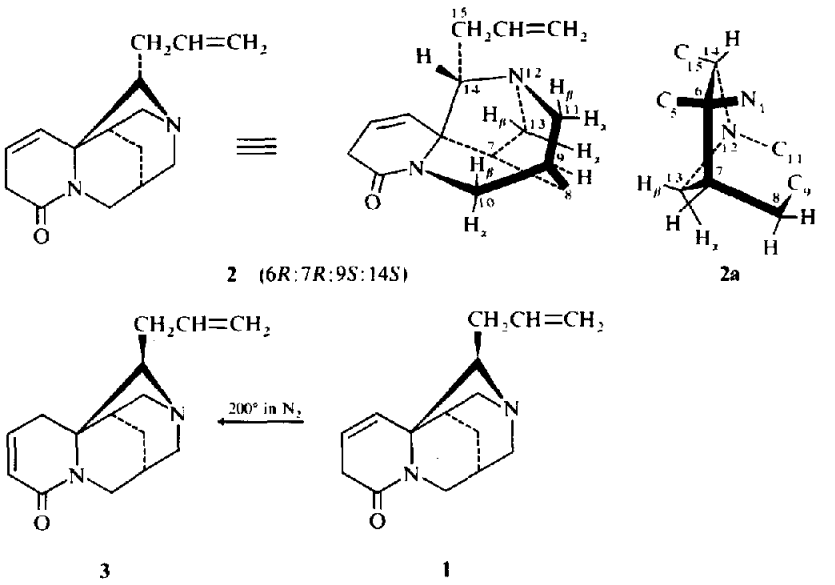
Consequently, tsukushinamine-C must have the structure shown as **3**, except that the $[\alpha]_D$ has not been obtained due to the shortage of material.

Since (−)-tsukushinamine-A (**1**) is transformed into tsukushinamine-C (**3**) by heating at 200° in a sealed tube under $N_2$, **3** is considered to be an artefact arising from **1** during the extraction and purification of the alkaloids.

The only cage-type lupin alkaloid possessing a tsukushinamine skeleton previously reported is (−)-tsukushinamine-A (**1**) from *S. franchetiana* [5, 6]. Therefore, **2** and **3** are the second and third examples of this type in nature.

Variations in alkaloid content in the various parts of *S. franchetiana* and at various stages of the seedling's growth are shown in Fig. 1.

The absolute configuration of (−)-tsukushinamine-type alkaloid(s) at the C-7 and C-9 positions are all the same as those of (−)-cytisine (**4**, 7*R*:9*S*), (−)-anagyrine (**5**, 7*R*:9*R*), (−)-baptifoline (**6**, 7*R*:9*R*) and (−)-rhombifoline (**7**, 7*R*:9*S*) [7–9], present in the same plant



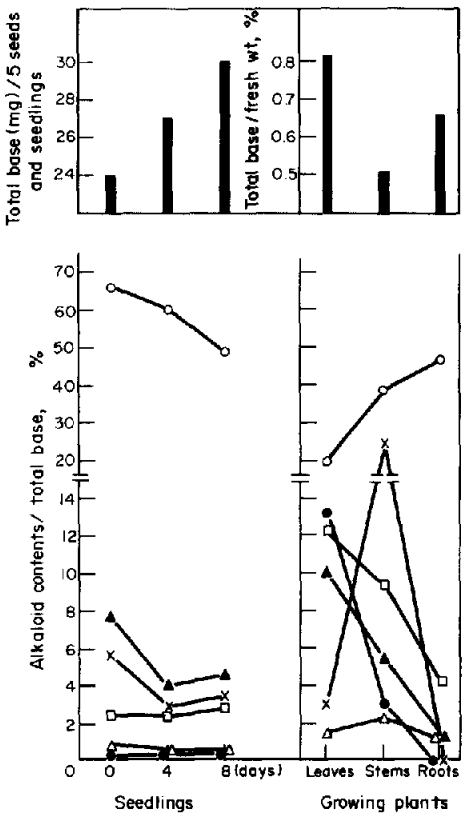




Fig. 1. Variations in alkaloid content in the seed, various stages of seedling growth and various parts of *Sophora franchetiana*. Alkaloid content was quantitatively estimated by HPLC as described in the Experimental. The seedlings were grown at 28° in the dark, and the plant was collected in May. ○—○, (−)-cytisine (**4**); ●—●, (−)-rhombifoline (**7**); △—△, (−)-*N*-formylcytisine; ×—×, (−)-anagyrine (**5**); □—□, (−)-baptifoline (**6**); ▲—▲, (−)-tsukushinamine-A (**1**).

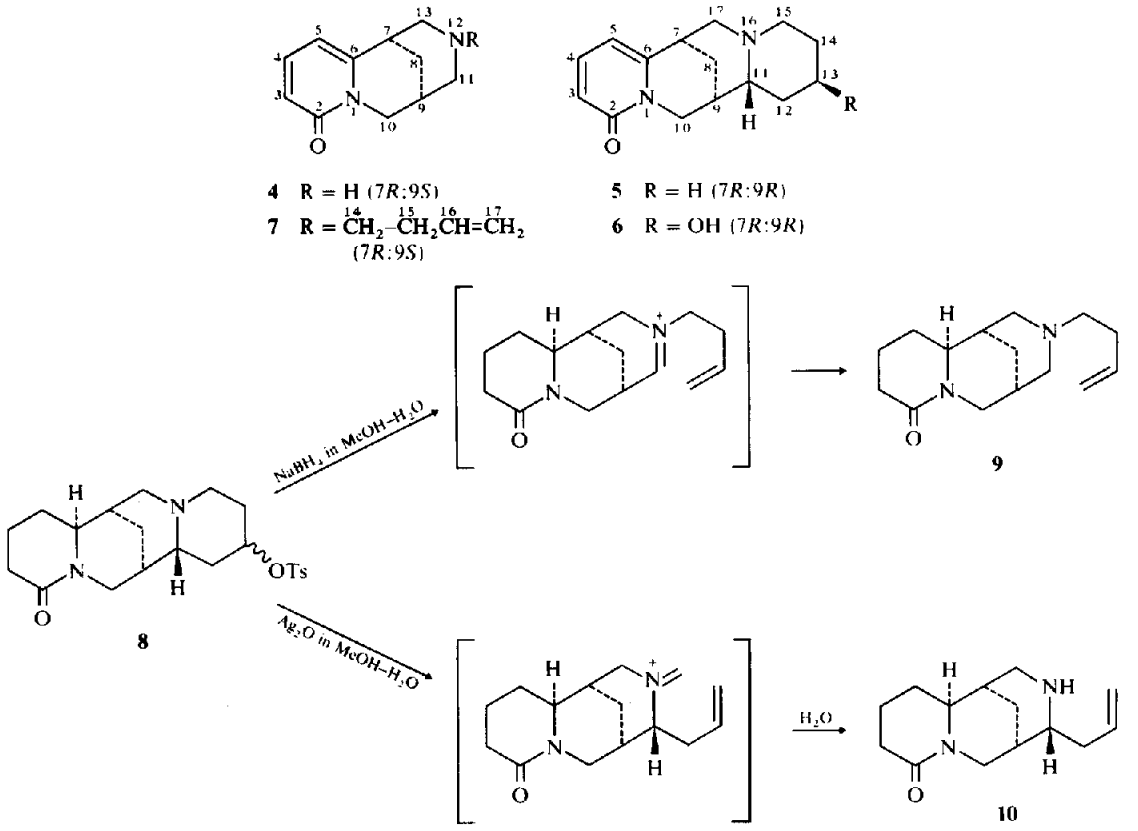
[5,6]. Additionally, the chemical conversion of the epimeric tosylates of 13-hydroxylupanine (**8**) into tetrahydrorhombifoline (**9**) and angustifoline (**10**) under mild conditions has been shown by Bohlmann and Schumann [10]. Reaction of this type conceivably might be implicated in the biosynthesis of certain tricyclic lupin alkaloids and tsukushinamine-type alkaloids from the tetracyclic lupin alkaloids. From this evidence, it might therefore be presumed that the tsukushinamine-type alkaloids **1**–**3** and (−)-rhombifoline (**7**) are derived from the anagyrine-type alkaloids, such as (−)-baptifoline (**6**) co-existing in the same plant.

Studies on the biosynthetic pathway leading to the formation of the tsukushinamines **1**–**3** and on a preliminary screening of the distribution of tsukushinamine-type lupin alkaloids in other plants are being undertaken in our laboratories.

## EXPERIMENTAL

*General procedures.* Mps were uncorr. The high and low resolution MS were measured at 70 eV. $^1$H NMR spectra were recorded at 100 MHz, $^{13}$C NMR spectra at 25 MHz. TMS was used in $C_6D_6$ or $CDCl_3$ as the int. standard. Chemical shifts are given in ppm ($\delta$) relative to the int. standard. Si gel G was employed for analytical TLC in the following solvent systems: 1, $CH_2Cl_2$–MeOH–28% $NH_4OH$ (90:9:1); 2, $CH_2Cl_2$-MeOH (4:1); and alkaloid spots were visualized by exposure to $I_2$ vapour or spraying with Dragendorff's reagent. Analytical HPLC was carried out with solvents 3, 15% MeOH · $Et_2O$–2.5% $NH_4OH$ (50:1) and 4, 15% MeOH · $Et_2O$–$H_2O$–25% $NH_4OH$ (500:10:3), using a LiChrosorb SI 100 (Merck, 10 $\mu$m, 0.3 × 50 cm) column employing a monitoring flow system (220 and 310 nm) coupled to the recorder at a flow rate of 1 ml/min. Prep. HPLC was performed on LiChrosorb SI 100 (10 $\mu$m, 0.5 × 50 cm or 1.0 × 50 cm) column monitoring with an UV detector. The chromatographic behaviour of the alkaloids is summarized in Table 2.

*Plant material. S. franchetiana* Dunn which was growing in Kumamoto and Miyazaki prefectures (southern island of Japan) was collected in May. Plant material was identified by Prof. J. Haginiwa, Faculty of Pharmaceutical Sciences, Chiba University




4 R = H (7*R*:9*S*)
7 R = $\overset{14}{CH_2}$–$\overset{15}{CH_2}\overset{16}{CH}$=$\overset{17}{CH_2}$ (7*R*:9*S*)

5 R = H (7*R*:9*R*)
6 R = OH (7*R*:9*R*)

8 9 10

and by Mr. M. Otomasu, Tsukigi Middle School, Kumamoto. Voucher specimens of *S. franchetiana* have been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan.

*Extraction and isolation of alkaloids.* From the 75% EtOH extract of the freshly harvested epigeal parts (323 g) of *S. franchetiana*, the crude alkaloid (1.8 g) was obtained in a yield of 0.56% of the fr. wt as a pale yellow oil. The alkaloid fraction (1.8 g) was chromatographed on a Si gel (Merck, type 60, 230–400 mesh, 50 g) column with $CH_2Cl_2$–MeOH–28% $NH_4OH$ as eluant with increasing MeOH and 28% $NH_4OH$ contents, 100 ml fractions being collected: 1, 1.5% MeOH · $CH_2Cl_2$–28% $NH_4OH$ (500:0.4) (fractions 1–15); 2, 2.3% MeOH · $CH_2Cl_2$–28% $NH_4OH$ (500:0.7) (fractions 16–30); 3, 3% MeOH · $CH_2Cl_2$–28% $NH_4OH$ (500:1) (fractions 31–35); 4, 4% MeOH · $CH_2Cl_2$–28% $NH_4OH$ (500:1.5) (fractions 36–40); 5, 8% MeOH · $CH_2Cl_2$–28% $NH_4OH$ (500:3) (fractions 41–50). (−)-Rhombifoline (7, 128 mg) was obtained from fractions 6–10 as an oily product, which was almost pure. (−)-Anagyrine (5, 74 mg) and (−)-tsukushinamine-B (2, 5 mg)

Table 2. Physical constants and chromatographic behaviours of the lupin alkaloids isolated from *Sophora franchetiana**

| Alkaloids | mp | $[\alpha]_D$† | $R_f$ Si gel TLC‡ | | $R_t$ (min) HPLC‡ | |
|---|---|---|---|---|---|---|
| | | | Solvent 1 | Solvent 2 | Solvent 3 | Solvent 4 |
| (−)-Anagyrine (5) | oil | −165.3° | 0.65 | 0.57 | 8.4 | — |
| (−)-Baptifoline (6) | 210° | −137.2° | 0.30 | 0.27 | 24.3 | 16.0 |
| (−)-Cytisine (4) | 155° | −116.7° | 0.35 | 0.16 | 47.5 | 29.3 |
| (−)-*N*-Formylcytisine | 170–172° | −232.6° | 0.42 | 0.52 | 35.8 | 30.5 |
| (−)-Rhombifoline (7) | oil | −232.4° | 0.67 | 0.76 | 4.5 | — |
| (±)-Ammodendrine | oil | 0 | 0.38 | 0.17 | 51.7 | 22.5 |
| (−)-Tsukushinamine-A (1) | oil | −72.3° | 0.55 | 0.35 | 24.2 | 12.5 |
| (−)-Tsukushinamine-*B* (2) | oil | −144.4° | 0.66 | 0.57 | 11.5 | — |
| Tsukushinamine-C (3) | — | —§ | 0.57 | 0.35 | 26.0 | — |

* Plant was collected in May.
† EtOH solution.
‡ Solvents 1–4 for TLC and HPLC are described in Experimental.
§ Optical rotation has not been obtained due to the shortage of material.

were isolated successively from fractions 11–15 by prep. HPLC using 10% MeOH · $Et_2O$–0.42% $NH_4OH$ (500:6) as eluant. Fractions 16–20 were subjected to prep. HPLC with solvent 3 as eluant to yield (−)-*N*-formylcytisine (36 mg) along with (−)-anagyrine (210 mg). (−)-Tsukushinamine-A (**1**, 110 mg), tsukushinamine-C (**3**, 1.5 mg) and (−)-cytisine (**4**, 153 mg) in the residue of fractions 21–35 also were separated by prep. HPLC with solvent 3. (−)-Cytisine (569 mg) was present mainly in fractions 36–38. (±)-Ammodendrine (21 mg), the main alkaloid of fractions 39 and 40, was purified by prep. HPLC with solvent 4. From the residue of fractions 41–50, (−)-baptifoline (**6**, 207 mg) was obtained as colourless needles after recrystallization from $C_6H_6$.

*Identification of alkaloids.* Some physical data and chromatographic behaviour of the alkaloids isolated from *S. franchetiana* are listed in Table 2. The known alkaloids, (−)-anagyrine (**5**), (−)-cytisine (**4**), (−)-*N*-formylcytisine, and (−)-baptifoline (**6**), were identified by mp, colour reaction, TLC, HPLC, and by comparison of the IR, MS, and NMR with those of authentic samples, as described in our previous papers [1–4]. (±)-Ammodendrine showed physical and spectral data identical to the lit. [11]. (−)-Rhombifoline (**7**), $[\alpha]_D^{26}$ −232.4° (EtOH, $c = 0.74$), was characterized by the following spectroscopic data: MS (probe) *m/z* (rel. int.): 244 $[M]^+$ (4), 203 $[M - CH_2CH{=}CH_2]^+$ (100), 160 (16), 146 (10), 98 (10), 58 (74). $^1H$ NMR ($C_6D_6$): $\delta$ 1.08 (2 H, *m*, 8-$H_2$), 1.5–2.1 (7 H, *m*), 2.19 (1 H, *br s*, 7-H), 2.38 (1 H, *br d*, $J = 9.5$ Hz, 11- or 13-$H_{eq}$), 2.48 (1 H, *br d*, $J = 9.5$ Hz, 11- or 13-$H_{eq}$), 3.69 (1 H, *dd*, $J = 15.5$ and 7.5 Hz, 10-$H_\alpha$), 4.05 (1 H, *d*, $J = 15.5$ Hz, 10-$H_\beta$), 4.82 (1 H, fine split *d*, $J = 17$ Hz, 17-H), 4.87 (1 H, fine split *d*, $J = 9.5$ Hz, 17-H'), 5.39 (1 H, *dd*, $J = 6.5$ and 1.5 Hz, 5-H), 5.48 (1 H, *m*, 16-H), 6.53 (1 H, *dd*, $J = 9.5$ and 1.5 Hz, 3-H), 6.81 (1 H, *dd*, $J = 9.5$ and 6.5 Hz, 4-H).

(−)-*Tsukushinamine-B* (**2**). Colourless oil, $[\alpha]_D^{25}$ −144.4° (EtOH, $c = 0.98$). MS (probe) *m/z* (rel. int.): 244.158 ($[M]^+$, calc. for $C_{15}H_{20}N_2O$ 244.158) (92), 229 (10), 203 (87), 160 (61), 146 (36), 122 (27), 98 (36), 97 (81), 96 (100), 82 (67). $^1H$ NMR ($C_6D_6$): $\delta$ 1.28 (3 H, *br s*, 9-H and 8-$H_2$), 1.69 (1 H, *br s*, 7-H), 1.96 (2 H, *m*, 15-$H_2$), 2.30 (1 H, *dd*, $J = 12$ and 2.5 Hz, 13-$H_\alpha$), 2.43 (1 H, *br d*, $J = 13$ Hz, 11-$H_\beta$), 2.63 (1 H, *br d*, $J = 12$ Hz, 13-$H_\beta$), 2.83 (1 H, *br dd*, $J = 13$ and 4 Hz, 11-$H_\alpha$), 2.82 (2 H, *br s*, 3-$H_2$), 3.07 (1 H, *dq*, $J = 10$, 5.5 *and* 2.5 Hz, 14-H), 3.17 (1 H, *d*, $J = 13.5$ Hz, 10-$H_\beta$), 4.33 (1 H, *dd*, $J = 13.5$ and 7.5 Hz, 10-$H_\alpha$), 4.9–5.1 (2 H, *m*, 17-$H_2$), 5.03 (1 H, *dt*, $J = 10.5$ and 1 Hz, 5-H), 5.22 (1 H, *dt*, $J = 10.5$ and 3 Hz, 4-H), 5.92 (1 H, *m*, 16-H). The $^{13}C$ NMR data are shown in Table 1.

(−)-*Tsukushinamine-A* (**1**). Colourless oil, $[\alpha]_D^{25}$ −72.3° (EtOH, $c = 0.56$). MS (probe) *m/z* (rel. int.): 244.155 ($[M]^+$, calc. for $C_{15}H_{20}N_2O$ 244.158) (100), 229 (8), 203 (73), 160 (40), 146 (25), 98 (31), 97 (73), 96 (79), 82 (61). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1640 (lactam C=O). $^1H$ NMR ($C_6D_6$): $\delta$ 1.22 (2 H, *br s*, 8-$H_2$), 1.28 (1 H, *br s*, 9-H), 1.80 (1 H, *br s*, 7-H), 2.14 (2 H, *m*, 15-$H_2$), 2.44 (2 H, *br s*, 11-$H_2$), 2.62 (2 H, *br s*, 13-$H_2$), 2.64 (1 H, *t*, $J = 7.5$ Hz, 14-H), 2.82 (2 H, *br s*, 3-$H_2$), 3.24 (1 H, *d*, $J = 13.5$ Hz, 10-$H_\beta$), 4.0 (1 H, *dd*, $J = 13.5$ and 6 Hz, 10-$H_\alpha$), 4.90 (1 H, fine split *d*, $J = 10$ Hz, 17-H), 4.92 (1 H, *dt*, $J = 10$ and 1.5 Hz, 5-H), 5.05 (1 H, fine split *d*, $J = 17$ Hz, 17-H'), 5.16 (1 H, *dt*, $J = 10$ and 3 Hz, 4-H), 5.78 (1 H, *m*, 16-H). The $^{13}C$ NMR data and other spectroscopic data are shown in Table 1 and in a previous paper in detail [5].

*Tsukushinamine-C* (**3**). The $[\alpha]_D$ of **3** has not been measured yet because of the limited amount of material. MS (probe) *m/z* (rel. int.): 244.158 ($[M]^+$, calc. for $C_{15}H_{20}N_2O$ 244.158) (18), 229 (3), 203 (100), 160 (28), 146 (17), 122 (11), 98 (17), 97 (22), 96 (43), 82 (26). UV $\lambda_{max}^{EtOH}$ nm: 261. $^1H$ NMR ($CDCl_3$): $\delta$ 1.72 (2 H, *m*, 8-$H_2$), 2.07 (1 H, *m*, 9-H), 2.40 (3 H, *m*, 7-H and 15-$H_2$), 2.62 (2 H, *dd*, $J = 4$ and 2 Hz, 5-$H_2$), 2.88 (1 H, *t*, $J = 5.5$ Hz, 14-H), 2.9–3.2 (4 H, *m*, 11- and 13-$H_2$), 3.39 (1 H, *br d*, $J = 13$ Hz, 10-$H_\beta$), 4.18 (1 H, *dd*, $J = 13.5$ and 6.5 Hz, 10-$H_\alpha$), 5.08 (1 H, fine split *d*, $J = 10$ Hz, 17-H), 5.11 (1 H, fine split *d*, $J = 16$ Hz, 17-H'), 5.87 (1 H, *m*, 16-H), 5.97 (1 H, *dt*, $J = 10$ and 2 Hz, 3-H), 6.46 (1 H, *dt*, $J = 10$ and 4 Hz, 4-H).

*Isomerization of* (−)-*tsukushinamine-A* (**1**) *into tsukushinamine-C* (**3**). **1** was converted into **3** on heating at 200° for 30 min in a sealed tube under $N_2$: the reaction product showed the presence of two compounds on HPLC with solvent 3 in the ratio of *ca* 5:1, which were found to be **1** and **3**, respectively, identical in all measurable respects as described previously.

*Acknowledgements*—We are very grateful to Prof. S. Okuda, Institute of Applied Microbiology, University of Tokyo, and to Prof. J. Bordner, Department of Chemistry, North Carolina State University at Raleigh, for their interests and encouragement during this work. We also are deeply indebted to Mr. M. Otomasu, Tsukigi Middle School, Kumamoto, for cordially providing the plant materials.

## REFERENCES

1. Ohmiya, S., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1979) *Phytochemistry* **18**, 649.
2. Murakoshi, I., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1979) *Chem. Pharm. Bull.* **27**, 144.
3. Murakoshi, I., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1979) *Phytochemistry* **18**, 699.
4. Ohmiya, S., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1980) *Chem. Pharm. Bull.* **28**, 546.
5. Ohmiya, S., Higashiyama, K., Otomasu, H., Murakoshi, I. and Haginiwa, J. (1979) *Chem. Pharm. Bull.* **27**, 1055.
6. Bordner, J., Ohmiya, S., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1980) *Chem. Pharm. Bull.* **28**, 1965.
7. Okuda, S., Tsuda, K. and Kataoka, H. (1961) *Chem. Ind.* 1115.
8. Okuda, S., Tsuda, K. and Kataoka, H. (1961) *Chem. Ind.* 1751.
9. Okuda, S., Tsuda, K. and Kataoka, H. (1965) *Chem. Pharm. Bull.* **13**, 491.
10. Bohlmann, F. and Schumann, D. (1965) *Chem. Ber.* **98**, 3133.
11. Fitch, W. L., Dolinger, P. M. and Djerassi, C. (1974) *J. Org. Chem.* **39**, 2974.