[Chem. Pharm. Bull. 34(1) 401—405 (1986)]

# Saponins from Chinese Cucurbitaceous Plants: Solubilization of Saikosaponin-a with Hemslosides Ma2 and Ma3 and Structure of Hemsloside $H_1$ from *Hemsleya chinensis*

TOSHINOBU MORITA,[a] RUI-LIN NIE,[b] HIROKO FUJINO (née KIMATA),[a] KEIKO ITO,[a] NORIKO MATSUFUJI,[a] RYOJI KASAI,[a] JUN ZHOU,[b] CHENG-YIH WU,[b] NOBORU YATA[a] and OSAMU TANAKA*,[a]

*Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] Kasumi, Minami-ku, Hiroshima 734, Japan, Kunming Institute of Botany, Academia Sinica,[b] Kunming, Yunnan, China*

(Received June 13, 1985)

It was found that the water solubility of saikosaponin-a (**8**), a pharmacologically active saponin of Bupleuri radix, was significantly increased in the presence of hemslosides Ma2 (**2**) and Ma3 (**3**), bisdesmosides of oleanolic acid (**9**) isolated from a Chinese folk medicine, rhizomes of *Hemsleya macrosperma* (Cucurbitaceae). The structure of a new saponin named hemsloside $H_1$ (**7**), isolated from rhizomes of *H. chinensis* together with several known saponins, was elucidated to be β-gentiobiosyl oleanolate 3-*O*-[β-glucopyranosyl-(1→2)]-[α-arabinopyranosyl-(1→3)]-β-glucopyranouroside.

**Keywords**——*Hemsleya macrosperma*; *Hemsleya chinensis*; Cucurbitaceae; Chinese folk medicine; oleanolic acid-saponin; saikosaponin-a; solubilizing effect; bisdesmoside

The plants of the genus *Hemsleya* (Cucurbitaceae) which are abundant in Yunnan and Sichuan, China, are used as Chinese herbal medicines. Recently, isolation and structure elucidation of three new oleanolic acid-saponins (bisdesmosides), hemslosides Ma1(**1**), Ma2(**2**) and Ma3(**3**) from rhizomes of *H. macrosperma* C. Y. WU were reported.[1] The structures of these saponins are closely related to chikusetsusaponins-IV(**4**), -IVa(**5**) and -V(**6**, =ginsenoside-Ro from *Panax ginseng* C. A. MEYER[2]), saponins of rhizomes of *Panax japonicus* C. A. MEYER (Araliaceae),[3] and other Chinese[4] and Himalayan wild *Panax* spp.[5] Isolation of four saponins, **1**, **3**, **5** and a new saponin(**7**) from rhizomes of *H. chinensis* COGN. was also reported.[1] The present paper deals with the solubilization of saikosaponin-a(**8**), a pharmacologically active saponin of the root of *Bupleurum falcatum* L., by **2** and **3**. The structure determination of the new saponin(**7**), now named hemsloside $H_1$, is also described.

It has been found that the solubility of monodesmosides of pericarps of *Sapindus mukurossi* GAERTN. in water is remarkably increased by the bisdesmosides which coexist in this plant.[6] Like these monodesmosides, an active principle of Bupleuri radix, **8**(monodesmoside), is known to be sparingly soluble in water. Recently, it has been reported that the water solubility of **8** is significantly increased in the presence of **6**,[7] a bisdesmoside of *Panax ginseng* and *P. japonicus* which are sometimes used together with Bupleuri radix in the Kanpo decoction. As mentioned above, the bisdesmosides isolated from *Hemsleya* spp. have a close structural relationship to **6**; **3** is the 3′-*O*-α-arabinopyranosyl derivative of **6** and **2** possesses a β-xylopyranosyl moiety instead of the β-glucopyranosyl group in **6**. In view of the above relationship between structure and solubilizing effect, the increase of the solubility of **8** in the presence of **2** and **3** was investigated.

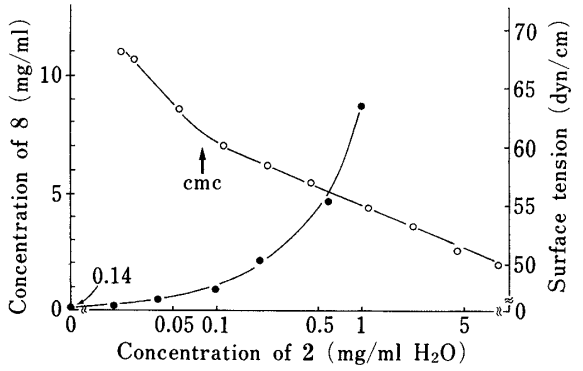



Fig. 1. Solubility Curve of Saikosaponin-a (**8**) in Hemsloside Ma2 (**2**) Solution and Surface Tension of Hemsloside Ma2 (**2**) Solution

—●—, solubility curve; —○—, surface tension at 15 °C (in $H_2O$).

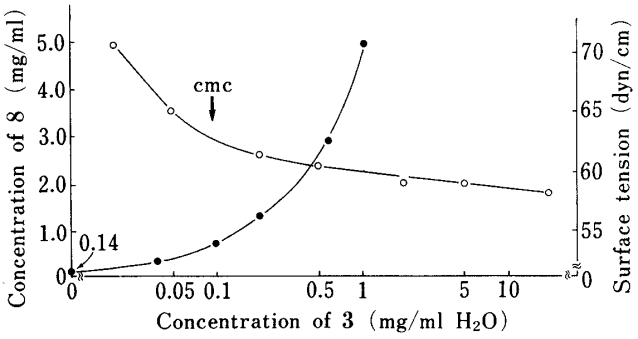



Fig. 2. Solubility Curve of Saikosaponin-a (**8**) in Hemsloside Ma3 (**3**) Solution and Surface Tension of Hemsloside Ma3 (**3**) Solution

—●—, solubility curve; —○—, surface tension at 22 °C (in $H_2O$).

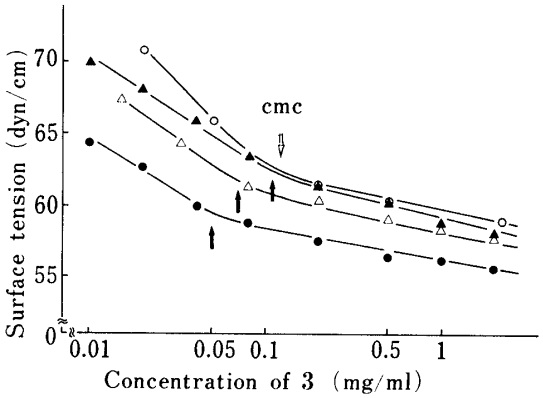
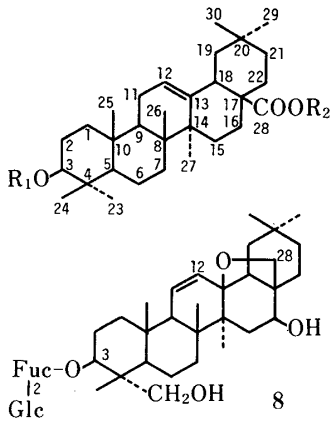



Fig. 3. Surface Tension of Hemsloside Ma3 (**3**) Solution at 25 °C

—○—, $H_2O$; —●—, pH 4.2; —△—, pH 5.1; —▲—, pH 5.5.
Ionic strength, 0.02. Buffer, $CH_3COOH$–$CH_3COOK$–KCl.

| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | –GlcUA$^3$—Ara(p) | –Glc |
| **2** | –GlcUA$^2$< Xyl, $^3$< Ara(p) | –Glc |
| **3** | –GlcUA$^2$< Glc, $^3$< Ara(p) | –Glc |
| **4** | –GlcUA$^4$—Ara(f) | –Glc |
| **5** | –GlcUA | –Glc |
| **6** | –GlcUA$^2$—Glc | –Glc |
| **7** | –GlcUA$^2$< Glc, $^3$< Ara(p) | –Glc$^6$—Glc |
| **9** | –H | –H |
| **10** | –GlcUA$^2$< Glc, $^3$< Ara(p) | –H |




GlcUA, β-D-glucuronic acid; Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl; Fuc, β-D-fucopyranosyl; Ara(p or f), α-L-arabino(pyranosyl or furanosyl)

Chart 1

The solubility curve of **8** in an aqueous solution of **2** or **3** and the surface tension of solutions of both bisdesmosides are shown in Figs. 1 and 2. The critical micelle concentration (cmc) of both bisdesmosides is similar to that of **6** and an increase of the solubility of **8** in the presence of both bisdesmosides became apparent near the cmc. However, the solubilizing effects of **2** and **3** were found to be significantly higher than that of **6**. The concentration of **8** in

TABLE I. $^{13}$C-NMR Chemical Shifts (in $C_5D_5N$)

| | Aglycone moieties | | | | Sugar moieties | | |
|---|---|---|---|---|---|---|---|
| | **3**[1] | **7** | **10** | | **3**[1] | **7** | **10** |
| C-1 | 38.8 | 38.7 | 38.6 | 3-GlcUA 1 | 105.2[a] | 105.4[a] | 105.4[a] |
| C-2 | 26.4 | 26.6 | 26.5 | 2 | 79.1 | 79.2 | 79.2 |
| C-3 | 89.6 | 89.6 | 89.6 | 3 | 86.1 | 86.1 | 86.0 |
| C-4 | 39.5 | 39.6 | 39.6 | 4 | 72.8 | 72.9 | 72.9 |
| C-5 | 55.7 | 55.8 | 55.7 | 5 | 77.2 | 77.3 | 77.2 |
| C-6 | 18.5 | 18.5 | 18.4 | 6 | 171.7 | 171.9 | 172.0 |
| C-7 | 33.2 | 33.1 | 33.3 | Glc 1 | 103.7[a] | 103.8[a] | 103.8[a] |
| C-8 | 39.9 | 39.9 | 39.7 | 2 | 76.3 | 76.5 | 76.4 |
| C-9 | 48.0 | 48.0 | 47.9 | 3 | 78.8[b] | 78.4[b] | 78.5[b] |
| C-10 | 36.9 | 36.9 | 36.9 | 4 | 72.5 | 72.5 | 72.4 |
| C-11 | 23.7 | 23.7 | 23.8 | 5 | 77.8[b] | 77.8[b] | 77.8[b] |
| C-12 | 122.5 | 122.8 | 122.5 | 6 | 63.3 | 63.3 | 63.3 |
| C-13 | 144.1 | 144.1 | 144.8 | Ara 1 | 105.2[a] | 105.2[a] | 105.2[a] |
| C-14 | 42.1 | 42.1 | 42.1 | 2 | 71.4 | 71.5 | 71.5 |
| C-15 | 28.0 | 27.9 | 27.9 | 3 | 74.6 | 74.7 | 74.7 |
| C-16 | 23.7 | 23.7 | 23.8 | 4 | 69.6 | 69.6 | 69.6 |
| C-17 | 47.0 | 47.0 | 46.6 | 5 | 67.8 | 67.8 | 67.7 |
| C-18 | 41.7 | 41.7 | 42.0 | 28-Glc 1 | 95.6 | 95.7 | — |
| C-19 | 46.4 | 46.3 | 46.6 | 2 | 74.1 | 75.2 | — |
| C-20 | 30.8 | 30.8 | 30.9 | 3 | 78.8[b] | 78.4[b] | — |
| C-21 | 34.1 | 34.0 | 34.1 | 4 | 71.1 | 71.5 | — |
| C-22 | 32.5 | 32.5 | 33.3 | 5 | 79.1[b] | 78.7[b] | — |
| C-23 | 28.0 | 28.3 | 28.3 | 6 | 62.2 | 69.4 | — |
| C-24 | 16.6 | 16.7 | 16.7 | Glc 1 | — | 105.3[a] | — |
| C-25 | 15.5 | 15.5 | 15.4 | 2 | — | 73.9 | — |
| C-26 | 17.4 | 17.5 | 17.3 | 3 | — | 78.0[b] | — |
| C-27 | 26.1 | 26.1 | 26.2 | 4 | — | 70.9 | — |
| C-28 | 176.3 | 176.5 | 180.1 | 5 | — | 78.6[b] | — |
| C-29 | 33.2 | 33.1 | 33.3 | 6 | — | 62.6 | — |
| C-30 | 23.7 | 23.4 | 23.8 | | | | |

GlcUA, β-D-glucuronic acid; Glc, β-D-glucopyranosyl; Ara, α-L-arabinopyranosyl. *a, b*) Assignments in any column may be interchanged, though those given here are preferred.

a saturated aqueous solution at 37 °C is 0.14 mg/ml. It was found that 1 ml of 0.1 % aqueous solution of **6** could dissolve 3.4 mg of **8**,[7] while 0.1% solutions of **2** and **3** dissolved 8.7 and 5.0 mg of **8**, respectively.

Figure 3 shows the influence of pH on the surface tension of a solution of **3** (a kind of anionic surfactant) at the same ionic strength, indicating that the surface tension decreased with increase of the hydrogen ion concentration, as in the case of **6**.[7] The solubilizing effect of **1** could not be determined because of its low solubility in water.

A new saponin, **7** was isolated from rhizomes of *H. chinensis* in a yield of 0.41%. The carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum of **7** indicated that **7** is a bisdesmoside of the same sapogenin, oleanolic acid (**9**), as that of **1**—**6**. Glucose, arabinose and glucuronic acid were identified in the acid hydrolysate of **7**. On alkaline saponification, **7** yielded a monodesmoside (**10**) which was proved to be identical with the prosapogenin obtained from **3**.[1] Recently, the selective hydrolysis of ester-type glycosyl linkages with LiI and 2,6-lutidine in methanol was reported.[8] By means of this new procedure, an ester-type glycosyl linkage of acidic tri- and diterpenes can be selectively cleaved without decomposition of the reducing terminal of the resulting sugar moiety to give an anomeric mixture of methyl

glycosides along with an aglycone or a pro-aglycone in quantitative yield. Treatment of **7** with this reagent afforded a methyl glycoside which was identified as methyl gentiobioside ($\alpha$ and $\beta$ anomeric mixture). The $^{13}$C-NMR spectrum of the sugar moiety of **7** (anomeric carbon signal at $\delta$ 95.7) indicated the presence of a $\beta$-gentiobiosyl ester. It follows that **7** should be formulated as a $\beta$-gentiobiosyl ester of **10**.

Chemical studies on other Chinese cucurbitaceous plants are in progress.

## Experimental

**General Procedures**——Nuclear magnetic resonance (NMR) spectra were taken on JEOL JNM PFT-100 (proton nuclear magnetic resonance ($^1$H-NMR) at 100 MHz and $^{13}$C-NMR at 25.15 MHz), JEOL FX-100 ($^1$H-NMR at 99.55 MHz and $^{13}$C-NMR at 25.00 MHz) and JEOL GX-270 ($^1$H-NMR at 270 MHz and $^{13}$C-NMR at 67.80 MHz) spectrometers in $C_5D_5N$ with tetramethylsilane (TMS) as an internal standard.

Optical rotations were measured with a Union automatic digital polarimeter at 27 °C in MeOH.

**Determination of Solubilizing Effects**——A saturated aqueous solution of **8** was prepared by incubation of an excess of **8** in water at 37 °C for 24 h, followed by filtration through a 0.5 $\mu$m filter (Millipore Corporation). A saturated solution of **8** in an aqueous solution of bisdesmoside (**2** or **3**) was prepared as follows. A solution of an excess of **8** in MeOH containing the bisdesmoside was concentrated to complete dryness and the residue was incubated in water (5 ml) at 37 °C for 24 h. Each saturated solution was filtered as described above. The content of **8** in each saturated solution was determined by thin layer chromatography (TLC)-densitometry according to the methods of Kimata *et al.*[9]

**Measurement of Surface Tension**——Surface tension was determined with Wilhelmy type tensiometer (Shimadzu surface tensiometer, type ST-1).

**Properties of 7**——The separation of **7** was reported in our previous paper.[1]

**7** (yield: 0.41%): a white powder (reprecipitated from MeOH–EtOAc), $[\alpha]_D^{27}$ +2.9° ($c$ = 1.32, MeOH). *Anal.* Calcd for $C_{59}H_{94}O_{28} \cdot 5/2H_2O$: C, 54.66; H, 7.70. Found: C, 54.52; H, 7.59.

Mass spectra (MS) were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV. Trimethylsilylation for MS: A methyl ester (a few mg) of **7** prepared by treatment with $CH_2N_2$ was heated with *N*-trimethylsilylimidazole (5 drops) in a sealed micro-tube at 80 °C for 2 h. The reaction mixture was diluted with $H_2O$ and then extracted with *n*-$C_6H_{14}$. The $C_6H_{14}$ layer was washed with $H_2O$ and concentrated to dryness by blowing $N_2$ gas over it at room temperature. The residue was subjected to MS. The trimethylsilyl ether of **7** exhibited fragment ions at $m/z$ 349 [Ara(TMSi)$_3$], 259 (349–TMSiOH), 451[Glc(TMSi)$_4$], 361 (451 − TMSiOH), 829 [Glc(TMSi)$_3$-Glc(TMSi)$_4$] and 583 [Glc(TMSi)$_4$-O-$CH_2$-$\overset{+}{C}$H-OTMSi](characteristic fragment ion due to hexose$^{6}$-hexose).

**Methanolysis of 7 and Identification of the Resulting Monosaccharides**——A few mg of **7** was heated with 9.7% dry HCl–MeOH in a sealed micro-tube at 70 °C for 3 h. The reaction mixture was neutralized with $Ag_2CO_3$ and then filtered. The filtrate was concentrated to dryness by blowing $N_2$ gas over it at room temperature. For analysis by gas-liquid chromatography (GLC), the residue was trimethylsilylated by the same procedure as that used for MS (*vide supra*). GLC: on a Shimadzu GC-6A gas chromatograph; 2.6 mm × 2 m glass column of 2% SE-30 on Chromosorb W(AW-DMCS); detector, FID; injection temperature, 200 °C; column temperature, 160 °C; carrier gas, $N_2$ (40 ml/min). Methyl glucoside, methyl arabinoside and methyl glucuronide were identified by comparison of the retention times with those of authentic samples.

**Alkaline Hydrolysis of 7**——**7** (600 mg) was heated with 10% KOH in MeOH–$H_2O$ (1 : 1) (15 ml) at 80 °C for 3 h. The reaction mixture was diluted with $H_2O$ and acidified to pH 5 with aq. acetic acid, then extracted with 1-BuOH saturated with $H_2O$. The BuOH layer was washed with $H_2O$ and concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel (solvent, $CHCl_3$–MeOH–$H_2O$ (6 : 4 : 1, homogeneous)) to give **10** (200 mg), a white powder(reprecipitated from MeOH–EtOAc), $[\alpha]_D^{27}$ +28.8° ($c$ = 1.20, MeOH). The identification was confirmed by comparison of the thin layer chromatographic behavior [on Kieselgel 60 $F_{254}$ (Merck); solvent, $CHCl_3$–MeOH–$H_2O$ (6 : 4 : 1, homogeneous)], $^1$H-NMR and $^{13}$C-NMR spectra, optical rotation and MS (as the TMSi derivative of **10** after methylation with $CH_2N_2$) with those of an authentic sample.

**Cleavage of Ester Glycoside Linkage of 7**——A mixture of **7** (150 mg) and anhydrous LiI (150 mg) in 2,6-lutidine (4 ml) and anhydrous MeOH (2 ml) was refluxed in an oil bath at 130 °C for 63 h. The reaction mixture was diluted with 50% MeOH (10 ml) and deionized with ion exchange resin (Amberlite MB-3), then concentrated to dryness. A suspension of the residue in $H_2O$ was subjected to column chromatography on reverse-phase highly porous polymer, DIAION HP-20 (Mitsubishi Chemical Ind., Ltd.) (solvent, $H_2O$ and then MeOH) to provide the $H_2O$ eluate (35 mg) (the methyl oligosaccharide = methyl gentiobioside, *vide infra*) and MeOH eluate. The MeOH eluate was purified by column chromatography on silica gel ( solvent, $CHCl_3$–MeOH–$H_2O$ (6 : 4 : 1, homogeneous)), followed by deionization with ion exchange resin (Amberlite MB-3), to afford **10** (27 mg). **10** was identified in the same way as described in the section on alkaline hydrolysis of **7** (*vide supra*). The methyl oligosaccharide thus obtained (a few mg)

was heated with 10% HCl in $H_2O$–dioxane (1 : 1) in a sealed micro-tube at 80 °C for 3 h. The reaction mixture was concentrated to dryness by blowing $N_2$ gas over it at room temperature. For GLC analysis, the residue was trimethylsilylated by the same procedure as used for MS (*vide supra*). GLC: On a Shimadzu GC-6A gas chromatograph; 2.6 mm × 1.5 m glass column of 5% SE-52 on Chromosorb W(AW-DMCS); detector, FID; injection temperature, 200 °C; column temperature, 180 °C; carrier gas, $N_2$ (40 ml/min). Glucose was identified by comparison with an authentic sample.

The trimethylsilyl ether of the methyl oligosaccharide exhibited fragment ions at *m/z* 451 [Glc(TMSi)$_4$], 361 (451 − TMSiOH), 829[Glc(TMSi)$_3$-Glc(TMSi)$_4$], and 583[Glc(TMSi)$_4$-O-$CH_2$-$\overset{+}{C}$H-OTMSi] (*vide supra*).

Sequence analysis by GC-MS:[10] A solution of the methyl oligosaccharide in dimethyl sulfoxide (DMSO) (0.5 ml) was treated with a saturated solution of *tert*-BuOK in DMSO (0.5 ml) and the mixture was sonicated at room temperature for 1 h. Then $CH_3I$(0.3 ml) was added with cooling, and the whole was further sonicated at room temperature for 1 h. The reaction mixture was diluted with $H_2O$ (3 ml) and extracted with $CHCl_3$ (2 ml × 3). The $CHCl_3$ layer was washed with $H_2O$ (3 ml × 3), dried and concentrated to dryness.

The resulting permethylether was treated with HCOOH (3 ml) at 100 °C for 1 h. The reaction mixture was evaporated to remove HCOOH, and the residue was treated with 3% HCl in $H_2O$ at 70 °C for 12 h. The reaction mixture was neutralized to pH 7 with Amberlite MB-3, then $NaBH_4$ (25 mg) in $H_2O$ (2 ml) was added. After standing at room temperature for 2 h, the mixture was acidified with ion exchange resin (DOWEX 50W-X2, $H^+$ form) and concentrated to dryness. Boric acid in the residue was removed by repeated (three times) co-distillation with MeOH. The resulting methylated alditol mixture was acetylated with $Ac_2O$–$C_5H_5N$ (1 : 1) (2 ml) at room temperature overnight, then toluene was added and the whole was subjected to azeotropic distillation. The residue was extracted with *n*-$C_6H_{14}$ and methylated alditol acetates in the *n*-$C_6H_{14}$ layer were analyzed by GC-MS.

GC-MS was taken on a Shimadzu GCMS-7000S; 2.6 mm × 1.5 m glass column of 5% ECNSS-M on Chromosorb W; injection temperature, 200 °C; column temperature, 190 °C; carrier gas, He (40 ml/min); ionization voltage, 70 eV. The permethyl ether of the methyl oligosaccharide(=methyl gentiobioside) yielded 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol ($t_R$ 2.67 min) and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol ($t_R$ 6.07 min), corresponding to the terminal glucoside and 6-linked glucoside, respectively.

**Acknowledgement** We are grateful to Wakunaga Pharmaceutical Co. Ltd., Hiroshima, for use of the tensiometer and for financial support for one of the authors (R.-L. Nie's study in Hiroshima University). This study was supported by a Grant-in-Aid for Scientific Research (To O. Tanaka No. 58430024, 1983—1984) from the Ministry of Education, Science and Culture of Japan.

## References

1) R.-L. Nie, T. Morita, R. Kasai, J. Zhou, C.-Y. Wu and O. Tanaka, *Planta Medica*, **50**, 322 (1984).
2) S. Sanada, N. Kondo, J. Shoji, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.*, **22**, 421 (1974).
3) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968); N. Kondo, J. Shoji, N. Nagumo and N. Komatsu, *ibid.*, **89**, 846 (1969); N. Kondo, Y. Marumoto and J. Shoji, *Chem. Pharm. Bull.*, **19**, 1103 (1971); T.-D. Lin, N. Kondo and J. Shoji, *ibid.*, **24**, 253 (1976); T. Morita, O. Tanaka, H. Kohda, *ibid.*, **33**, 3852 (1985).
4) T. Morita, R. Kasai, O. Tanaka, J. Zhou, T.-R. Yang and J. Shoji, *Chem. Pharm. Bull.*, **30**, 4341 (1982); T. Morita, R. Kasai, H. Kohda, O. Tanaka, J. Zhou and T.-R. Yang, *ibid.*, **31**, 3205 (1983); T.-R. Yang, Z. D. Jiang, M. Z. Wu, J. Zhou and O. Tanaka, *Acta Pharm. Sinica*, **19**, 232 (1984).
5) N. Kondo, J. Shoji and O. Tanaka, *Chem. Pharm. Bull.*, **21**, 2702 (1973); N. Kondo and J. Shoji, *ibid.*, **23**, 3282 (1975); O. Tanaka, T. Morita, R. Kasai, J. Kinouchi, S. Sanada, Y. Ida and J. Shoji, *ibid.*, **33**, 2323 (1985).
6) H. Kimata, T. Nakashima, S. Kokubun, K. Nakayama, Y. Mitoma, T. Kitahara, N. Yata and O. Tanaka, *Chem. Pharm. Bull.*, **31**, 1998 (1983).
7) H. Kimata, N. Sumida, N. Matsufuji, T. Morita, K. Ito, N. Yata and O. Tanaka, *Chem. Pharm. Bull.*, **33**, 2849 (1985).
8) K. Ohtani, K. Mizutani, R. Kasai and O. Tanaka, *Tetrahedron Lett.*, **25**, 4537 (1984).
9) H. Kimata, N. Fujioka, O. Tanaka and Y. Miyazaki, *Shoyakugaku Zasshi*, **34**, 311 (1980).
10) H. Bjorndal, C. G. Hellerqvist, B. Lindberg and S. Svensson, *Angew. Chem. Int. Ed. Engl.*, **9**, 610 (1970); H. Bjorndal, B. Lindberg, A. Pilotti and S. Svensson, *Carbohydr. Res.*, **15**, 339 (1970); P. E. Jansson, L. Kenne, H. Liedgren, B. Lindberg and J. Lonngren, *Chem. Comm. Univ. Stockholm*, **8**, 21 (1976).