Pergamon 0031-9422(94)00853-1 Phytochemistry, Vol. 39, No. 1, pp. 111–114, 1995

0031-9422/95 $9.50 + 0.00

CAERULEOSIDES A AND B, BIS-IRIDOID GLUCOSIDES FROM *LONICERA CAERULEA**

KOICHI MACHIDA, JUNKO ASANO and MASAO KIKUCHI†

Tohoku College of Pharmacy, 4-4-1 Komatsushima, Aobaku, Sendai, Miyagi 981, Japan

(*Received in revised form* 14 *September* 1994)

Key Word Index—*Lonicera caerulea*; Caprifoliaceae; bis-iridoid glucosides; caeruleosides A, B; acetal linkage; HSQC-ROESY.

Abstract—Two new bis-iridoid glucosides, named caeruleosides A and B, were isolated from the leaves of *Lonicera caerulea*. Their structures have been determined by chemical methods and by 1D and 2D NMR analysis. The compounds consist of secologanin attached through acetal bonds to C-4′ and C-6′ of the sugar part of loganin and sweroside, respectively.

INTRODUCTION

In a previous paper [1], we reported on the isolation of protocatechuic acid, quercetin, quercetin-3-rhamnoside, quercetin-3-glucoside, eriodictyol-7-glucoside, methyl chlorogenate, 7-ketologanin, epivogeloside and (6*R*, 9*R*)-3-oxo-α-ionol β-D-glucoside from the leaves of *Lonicera caerulea* L. var *emphyllocalyx* Nakai [2–5], a plant which grows in Japan. In the course of further studies on the constituents of the above plant, two new bis-iridoid glucosides along with four known iridoid glucosides have been isolated. The new compounds, caeruleosides A (**5**) and B (**7**), consist of secologanin attached through acetal bonds to C-4′ and C-6′ of the sugar part of loganin and sweroside, respectively, and this structural feature has so far not been seen in bis-iridoids before.

RESULTS AND DISCUSSION

The isolation and purification of the compounds are described in detail in the Experimental. Compounds **1** (loganin) [6, 7], **2** (secologanin) [6], **3** (secologanin dimethyl acetal)‡ [7] and **4** (sweroside) [6] were identified by comparison of various diagnostic data with reported values and authentic samples.

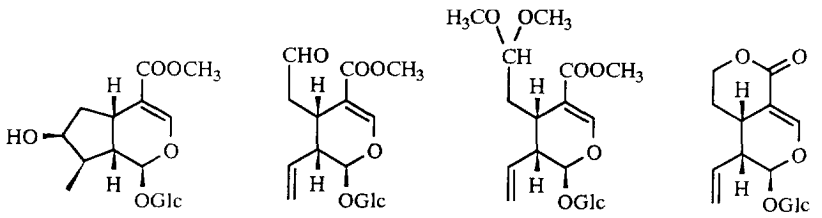
Caeruleoside A (**5**) was obtained as an amorphous powder, $[\alpha]_D$ − 144.4°. The FAB-mass spectrum exhibited ions at *m/z* 761 $[M + H]^+$ and 783 $[M + Na]^+$. The ^{1}H NMR spectrum of **5** showed two sets of signals, one set similar to **1** and another to **2**, indicating a dimeric structure. However, the ^{1}H NMR spectrum of **5** lacked the signal from the C-7 aldehyde proton of **2** and instead showed an acetal methine signal at δ4.70 from H_{SC}-7. Other chemical shifts, except for the signals owing to the glucosyl moieties, were coincident with those of **1** and **2**. The ^{13}C NMR spectrum of **5** contained a set of signals almost identical to those assigned to **3** except for the signals owing to C_{SC}-6 and 7. The other set of signals, corresponding to **1**, showed a larger deviation with respect to the absorptions assigned to the C_{LO}-3′, C_{LO}-4′, C_{LO}-5′ and C_{LO}-6′. A comparison of the ^{13}C NMR spectrum of the loganin moiety of **5** with that of **1** showed shifts of − 3.7, + 10.2, − 10.1 and + 6.5 ppm at C_{LO}-3′, C_{LO}-4′, C_{LO}-5′ and C_{LO}-6′, respectively, and the other signals were coincident. The results showed that **5** had a dimeric iridoid structure like that shown in the figure with acetal bonds linking the aldehyde group (C_{SC}-7) of the secologanin moiety to C-4′ and C-6′ of the sugar part of the loganin moiety. Further proof of the linkage was obtained from the HMBC spectrum as follows; H_{SC}-7 was correlated with C_{LO}-4′ and C_{LO}-6′, and H_{LO}-6′B was correlated with C_{SC}-7. In addition, the ^{13}C NMR chemical shifts of loganin moiety in **5** were in good agreement with those of 4′,6′-propylidene loganin (**6**) which we had prepared as a model compound (see Experimental). Thus, the planar structure of **5** was established.

The configurations of H_{LO}-1, H_{LO}-5, H_{LO}-7, H_{LO}-8, H_{LO}-9 and H_{SC}-1, H_{SC}-5, H_{SC}-9 were confirmed by the NOESY spectrum. The only remaining problem was to settle the stereochemistry of the acetal methine proton (C_{SC}-7). The NOEs (measured at 70° in D_2O) observed between H_{SC}-7 and H_{LO}-4′ and H_{LO}-6′A suggested that H_{SC}-7 was β with respect to the six-membered acetal ring, with the six-membered acetal ring in the chair form. That is, these results proved that H_{SC}-7 and each of H_{LO}-4′ and

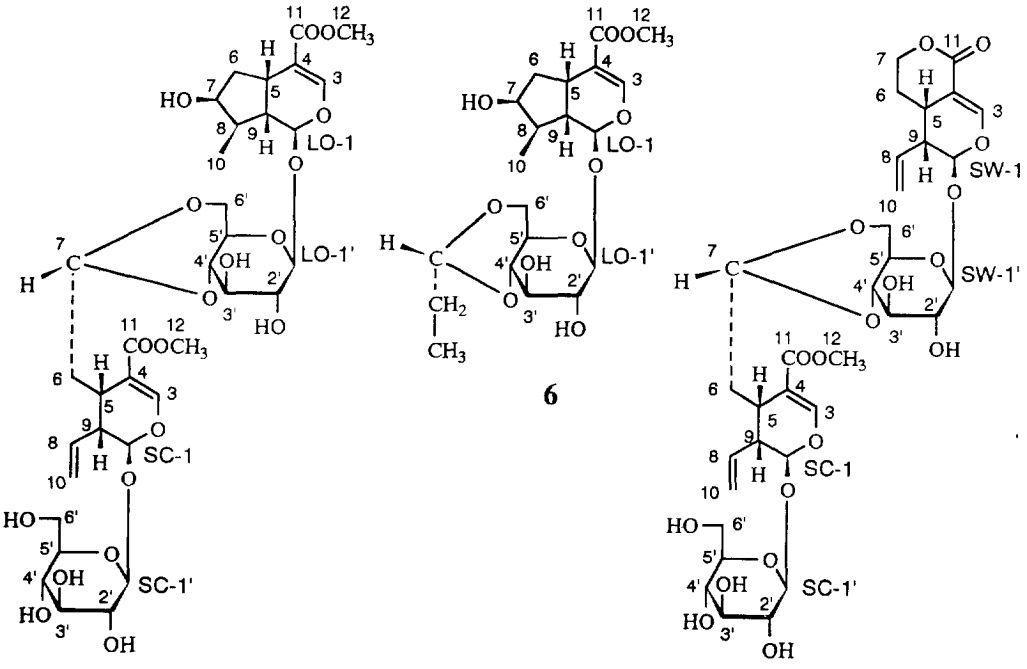
*Part 3 in the series, 'Analysis of the Components of *Lonicera* Species'. For part 2 see ref. [1].

†Author to whom correspondence should be addressed.

‡Compound **3** may be an artifact formed from **2** during the extraction and isolation processes.



1 2 3 4



5 6 7

H_{LO}-6'A are in 1,3-diaxial arrangements. Further evidence were obtained by the HSQC-ROESY cross peaks between H_{SC}-7 and C_{LO}-4' and C_{LO}-6'A, between H_{LO}-4, and C_{SC}-7, and between H_{LO}-6'A and C_{SC}-7 (Fig. 1). The structure of caeruleoside A was, therefore, established as **5**.

Caeruleoside B(**7**) was obtained as an amorphous powder, $[\alpha]_D$ − 101.4°. The ^1H and ^{13}C NMR spectra of **7** lacked the signals owing to the loganin moiety of **5** and instead showed the signals owing to the sweroside moiety. Furthermore, the ^1H and ^{13}C NMR chemical shifts in the sugar carbon region of the sweroside moiety of **7** were in good agreement with those of the loganin moiety of **5**. These facts suggested that the structure of **7** was an acetalic dimer of sweroside and secologanin and, as in **5**, the secologanin moiety was linked to C_{SW}-4' and C_{SW}-6' of the sweroside moiety via acetal oxygens at C_{CS}-7. On the basis of the above data, the structure of caeruleoside B was established as **7**. The ^{13}C NMR assignments of **1** and **3**–**7** are listed in Table 1.

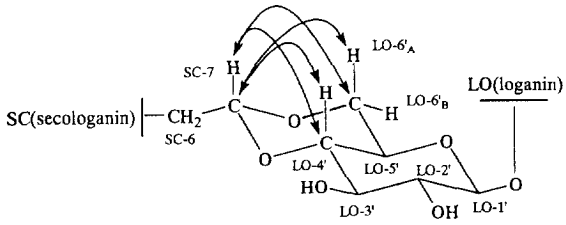


Fig. 1. The main HSQC–ROESY correlations of **5**.

Loganin and secologanin were each dissolved in methanol, methanol–water and methanol–water containing a small amount of acetic acid, and left at room temp. for 10 days. Caeruleoside A(**5**) could not be detected in any of the solutions by HPLC analysis and this established that caeruleoside A is not an artifact of the extraction and isolation procedures.

Table 1. ^{13}C NMR spectral data of **1**, **3**–**7** (in CD_3OD, at 67.8 MHz)

C	1	3	5	6	C	4	7
LO-1	97.7		98.2	98.2	SW-1	97.9	98.2
3	152.2		152.1	152.1	3	153.9	153.7
4	114.1		114.1	114.1	4	106.0	106.1
5	32.2		32.3	32.3	5	28.4	28.5
6	42.2		42.9	42.8	6	25.9	26.4
7	75.1		75.0	75.0	7	69.7	69.8
8	42.7		42.4	42.4	8	133.3	133.3
9	46.5		46.5	46.5	9	43.8	44.0
10	13.5		13.5	13.5	10	120.8	121.0
11	169.6		169.5[a]	169.5	11	168.5	168.3
12	51.7		51.7[b]	51.7			
LO-1′	100.1		100.9	100.9	SW-1′	99.7	100.4
2′	74.8		75.5	75.5	2′	74.7	75.4
3′	78.4		74.7	74.7	3′	78.4	74.6
4′	71.6		81.8	81.7	4′	71.5	81.8
5′	78.0		67.9	68.0	5′	77.9	68.0
6′	62.8		69.3	69.2	6′	62.7	69.2
SC-1		97.7	97.7		SC-1		97.7
3		153.1	153.7		3		153.9
4		111.5	111.5		4		111.5
5		29.3	29.6		5		29.6
6		33.1	35.2		6		35.2
7		104.2	102.7		7		102.7
8		135.6	135.8		8		135.8
9		45.1	45.4		9		45.4
10		119.8	119.8		10		119.8
11		170.0	169.3[a]		11		169.3
12		51.8	51.8[b]		12		51.8
SC-1′		99.9	100.0		SC-1′		100.0
2′		74.6	74.7		2′		74.7
3′		78.0	78.0		3′		78.0
4′		71.7	71.7		4′		71.7
5′		78.5	78.5		5′		78.5
6′		62.9	62.9		6′		62.9

Assignments were confirmed by DEPT, ^{1}H–^{1}H and ^{13}C–^{1}H COSY, and HMBC methods.

[a, b]Signals may be interchanged.

Caeruleoside A (**5**) and B (**7**) are the first examples of bis-iridoids which consist of two iridoid moieties joined through an acetal linkage.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 270 and 67.8 MHz, respectively, CD_3OD, TMS as int. standard. FAB-MS: Jeol JMS-DX 303 mass spectrometer; CC: Kieselgel 60 (Merck; 230–400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemical); Prep. HPLC: Tosoh HPLC system using a Cosmosil 5C_{18}-AR column (nacalai tesque, 10 mm i.d. × 25 cm) with UV detector.

Plant material. The leaves of *Lonicera caerulea* L. var *emphyllocalyx* Nakai were collected and identified by one of the authors (M. Kikuchi) from Bibai city, Hokkaido, Japan, June 1992. A voucher specimen (No. 5) is deposited in the laboratory of M. Kikuchi.

Extraction and isolation. Fresh leaves of *L. caerulea* (0.4 kg) were extracted with MeOH at room temp. The MeOH extract was concd and the resultant aq. suspension was extracted with $CHCl_3$, Et_2O, EtOAc and *n*-BuOH, successively. The EtOAc-soluble fraction was concd under red. pres. to produce a residue (8.0 g). This residue was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30:10:1) and the eluate was separated into 10 fractions (frs 1–10). Fr. 3 was re-chromatographed on a Sephadex LH-20 column using MeOH–H_2O (1:1) and the eluate was separated into 5 fractions (frs 3-1–3-5). Fr. 3–1 was subjected to prep. HPLC (MeOH–H_2O, 1:1) to give **1** (200 mg), **2** (40 mg), **3** (230 mg), **4** (10 mg), **5** (7 mg) and **7** (4 mg).

Compounds **1**–**4** were identified by comparison of various data with reported values and authentic samples.

Caeruleoside A (**5**). Amorphous powder, $[\alpha]_D$ − 144.4° (MeOH; *c* 0.3). FAB-MS *m/z*: 761 $[M + H]^+$, 783 $[M + Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 234 (4.26); IR ν_{max}^{KBr}

cm^{-1}: 3387, 2929, 1698, 1635, 1441, 1387, 1293, 1078; ^1H NMR (270 MHz, CD_3OD): δ1.07 (3H, *d*, *J* = 6.6 Hz, H_{LO}-10), 1.57 (1H, *m*, H_{LO}-6A), 1.78 (1H, *m*, H_{SC}-6A), 1.80 (1H, *m*, H_{LO}-8), 1.98 (1H, *m*, H_{LO}-9), 2.01 (1H, *m*, H_{SC}-6B), 2.23 (1H, *m*, H_{LO}-6B), 2.68 (1H, *m*, H_{SC}-9), 3.02 (1H, *m*, H_{SC}-5), 3.13 (1H, *m*, H_{LO}-5), 3.18–3.36 (7H, *m*, H_{LO}-2′, H_{LO}-4′, H_{LO}-5′, H_{SC}-2′, H_{SC}-3′, H_{SC}-4′ and H_{SC}-5′), 3.50 (1H, *dd*, *J* = 10.1, 9.4 Hz, H_{LO}-6′A), 3.54 (1H, *t*, *J* = 9.3 Hz, H_{LO}-3′), 3.62 (1H, *dd*, *J* = 11.9, 6.3 Hz, H_{SC}-6′A), 3.68, 3.69 (each 3H, *s*, H_{LO}-12 and H_{SC}-12), 3.89 (1H, *dd*, *J* = 11.9, 1.7 Hz, H_{SC}-6′B), 4.03 (1H, *m*, H_{LO}-7), 4.13 (1H, *dd*, *J* = 10.1, 4.8 Hz, H_{LO}-6′B), 4.67 (1H, *d*, *J* = 7.6 Hz, H_{SC}-1′), 4.70 (1H, *m*, H_{SC}-7), 4.72 (1H, *d*, *J* = 7.9 Hz, H_{LO}-1′), 5.10 (1H, *d*, *J* = 4.6 Hz, H_{LO}-1), 5.24 (1H, *br d*, *J* = 9.7 Hz, H_{SC}-10A), 5.29 (1H, *br d*, *J* = 17.3 Hz, H_{SC}-10B), 5.54 (1H, *d*, *J* = 5.6 Hz, H_{SC}-1), 5.73 (1H, *ddd*, *J* = 17.3, 9.7, 8.6 Hz, H_{SC}-8), 7.37 (1H, *d*, *J* = 1.3 Hz, H_{LO}-3), 7.45 (1H, *d*, *J* = 1.0 Hz, H_{SC}-3); ^{13}C NMR: Table 1.

Preparation of 4′,6′-propylidene loganin (**6**). Loganin (57 mg) was suspended in CH_3CH_2CHO (0.05 ml), dry $ZnCl_2$ (23 mg) added and the mixture stirred at room temp. for 3 hr. The mixture was extracted with EtOAc. The EtOAc extract was chromatographed on a silica gel column (hexane–Me_2CO, 3:2) to give amorphous **6** (10 mg). $[\alpha]_D$ − 87.0° (MeOH; *c* 0.3). FAB-MS *m/z*: 431 $[M + H]^+$, 453 $[M + Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 234 (3.96); ^1H NMR (270 MHz, CD_3OD): δ0.94 (3H, *t*, *J* = 7.6 Hz, H-3‴), 1.07 (3H, *d*, *J* = 6.9 Hz, H-10), 1.60 (3H, *m*, H-6A and H-2‴), 1.81 (1H, *ddd*, *J* = 9.2, 6.9, 4.6 Hz, H-8), 2.00 (1H, *ddd*, *J* = 9.3, 9.1, 4.6 Hz, H-9), 2.23 (1H, *ddd*, *J* = 14.2, 7.9, 1.6 Hz, H-6B). 3.10 (1H, *m*, H-5), 3.19–3.34 (3H, *m*, H-2′, H-4′ and H-5′), 3.52 (1H, *dd*, *J* = 10.1, 9.6 Hz, H-6′A), 3.55 (1H, *t*, *J* = 9.0 Hz, H-3′), 3.68 (3H, *s*, H-12), 4.02 (1H, *m*, H-7), 4.14 (1H, *dd*, *J* = 10.1, 4.4 Hz, H-6′B), 4.52 (1H, *m*, H-1‴), 4.72 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.11 (1H, *d*, *J* = 4.6 Hz, H-1), 7.38 (1H, *d*, *J* = 1.3 Hz, H-3); ^{13}C NMR: Table 1.

Caeruleoside B (**7**). Amorphous powder, $[\alpha]_D$ − 101.4° (MeOH; *c* 0.1). FAB-MS *m/z*: 729 $[M + H]^+$, 751 $[M + Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 234 (4.12): ^1H NMR (270 MHz, CD_3OD): δ1.78 (2H, *m*, H_{SW}-6), 1.80 (1H, *m*, H_{SC}-6A), 2.02 (1H, *m* H_{SC}-6B), 2.68 (2H, *m*, H_{SW}-9 and H_{SC}-9), 3.03 (1H, *m*, H_{SC}-5), 3.20 (1H, *m*, H_{SW}-5), 3.10–3.40 (7H, *m*, H_{SW}-2′, H_{SW}-4′, H_{SW}-5′, H_{SC}-2′, H_{SC}-3′, H_{SC}-4′ and H_{SC}-5′), 3.53 (2H, *m*, H_{SW}-3′ and H_{SW}-6′A), 3.64 (1H, *dd*, *J* = 11.9, 5.9 Hz, H_{SC}-6′A), 3.70 (3H, *s*, H_{SC}-12), 3.89 (1H, *m*, H_{SC}-6′B), 4.14 (1H, *dd*, *J* = 10.2, 4.6 Hz, H_{SW}-6′B), 4.39 (2H, *m*, H_{SW}-7), 4.67 (1H, *d*, *J* = 7.9 Hz, H_{SC}-1′), 4.70 (1H, *m*, H_{SC}-7), 4.76 (1H, *d*, *J* = 7.9 Hz, H_{SW}-1′), 5.28 (4H, *m*, H_{SW}-10 and H_{SC}-10), 5.39 (1H, *d*, *J* = 1.7 Hz, H_{SW}-1), 5.53 (1H, *m*, H_{SW}-8), 5.54 (1H, *d*, *J* = 5.9 Hz, H_{SC}-1), 5.73 (1H, *m*, H_{SC}-8), 7.45 (1H, *s*, H_{SC}-3), 7.58 (1H, *d*, *J* = 2.3 Hz, H_{SW}-3); ^{13}C NMR: Table 1.

Acknowledgements—We are grateful to Mr Y. Kumaki, Jeol Ltd, for measurement of the HSQC-ROESY spectra; our thanks are also due to Dr S. Suzuki, Dr K. Hisamichi and Mr S. Sato of this college for measurements of the mass and NMR spectra.

REFERENCES

1. Machida, K., Asano, J., Matsuda, N. and Kikuchi, M. (1993) *Tohoku Yokka Daigaku Kenkyu Nempo* **40**, 105.
2. Hayashi, Y., Furusato, K. and Nakanura, T. (1985) *Illustrated Tree in Colour*, p. 691. Hokuryukan, Tokyo.
3. Makino, T. (1979) *Makino's New Illustrated Flora of Japan*, p. 597. Hokuryukan, Tokyo.
4. Ohwi, J. and Kitagawa, M. (1983) *New Flora of Japan*, p. 1411. Shibundo, Tokyo.
5. Okuyama, S. (ed.) (1979) *Terasaki's Illustrated Flora of Japan*, p. 750, Heibonsha, Tokyo.
6. Kikuchi, M., Matsuda, N., Takahashi, Y. and Machida, K. (1991) *Tohoku Yakka Daigaku Kenkyu Nempo* **38**, 53.
7. Kawai, H., Kuroyanagi, M. and Ueno, A. (1988) *Chem. Pharm. Bull.* **36**, 3664.