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# Lonijaposides, novel cerebrosides from *Lonicera japonica*☆

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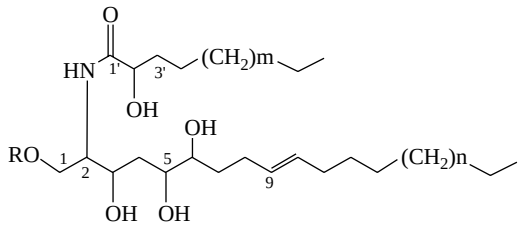
**Abstract**—Six novel cerebrosides, lonijaposides $A_1$–$A_4$, $B_1$ and $B_2$ (**1–6**) have been isolated from the flowers of *Lonicera japonica*. Their structures were established on the basis of 1D, 2D (DEPT, HMQC, HMBC and COSY) NMR, ESI-QTOF-MS/MS and chemical evidence.


## 1. Introduction

The flowers and buds of *Lonicera japonica* Thunb., are used in Chinese herbal medicine for latent-heat-clearing, antipyretic, detoxicant and anti-inflammatory ailments.[1] This plant is known for its properties as an anti-inflammatory agent in Korea since ancient times and is widely used for respiratory infections, diabetes mellitus and rheumatoid arthritis.[2] Iridoid glucosides,[3–5] flavonoids[6–8] and saponins[9,10] have previously been reported from the plant. Cerebrosides are a unique class of secondary metabolites, some of which are reported to have anti-tumour, anti-HIV-1, neuritogenic, hepatoprotective, immunosuppressive, immunostimulatory, anti-ulcerogenic, anti-fungal and antimicrobial activities.[11] It has been proved that the polarity of the cerebrosides is associated with the presence of extra hydroxyls in the sphingoid base and plays a key role in the neuritogenic activities.[12] Moreover, cerebrosides have been observed to be correlated to the tolerance of some plants to chilling stresses.[11] In continuation of our work for a search of novel molecules from the plant bioresource of the western Himalayas,[8,13–15] we now report the isolation and structural elucidation of six new cerebrosides, designated as lonijaposides $A_1$–$A_4$, $B_1$ and $B_2$, from the flowers of *L. japonica*. To the best of our knowledge this is the first report of novel cerebrosides from the genus *Lonicera* (Fig. 1).

## 2. Results and discussion

Column chromatography of methanol extract of fresh flowers over silica gel afforded a molecular species LJC-1 as amorphous white solid. The IR spectrum of LJC-1 showed absorption bands at 3350 and 3220, 1620 and 1540, and 1665 $cm^{-1}$ indicated the presence of hydroxyl, amide and olefinic functions, respectively. The $^1H$ NMR spectrum of LJC-1 (Table 1) showed the presence of two terminal methyls at $\delta$ 0.73 (6H, t, $J=7.2$ Hz), methylenes at $\delta$ 1.18 (br s), an amide proton signal at $\delta$ 8.46 (1H, d, $J=8.7$ Hz), signals of a *trans*-olefinic bond at $\delta$ 5.38 (1H, br dt, $J=16.5$, 6.5 Hz) and $\delta$ 5.40 (1H, br dt, $J=16.5$, 6.5 Hz) and six characteristic signals of geminal protons to hydroxyl groups were also observed at $\delta$ 4.50 (1H, m), 4.40 (1H, dd, $J=10.5$, 4.5 Hz), 4.33 (1H, dd, $J=10.5$, 4.7 Hz), 4.23 (1H, m), 4.10 (2H, m). Another signal at low field was observed at $\delta$ 5.01 (1H, m) for a methine proton vicinal to the nitrogen atom of the amide group. The data indicated a phytosphingolipid structure.[16,17] To further confirm, $^{13}C$ NMR spectra of LJC-1 (Table 1) showed one quaternary carbon at $\delta$ 176.5 (CONH), two olefinic methine carbons at $\delta$ 132.1 and 131.9 (C=C), five methines at $\delta$ 54.5 (CHNH), 78.1 (CHOH), 74.2 (CHOH), 74.1 (CHOH), 73.7 (CHOH) and one methylene at $\delta$ 63.2 ($CH_2OH$). The geometry (*E*) of the double bond in the unsaturated long chain base portion was determined on the basis of the $^{13}C$ NMR chemical shift




**1:** R = H, m = 9, n = 11
**2:** R = H, m = 9, n = 9
**3:** R = H, m = 10, n = 11

**Figure 1.** Structures of lonijaposide $A_1$ (**1**), $A_2$ (**2**) and $A_3$ (**3**).

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*Keywords*: *Lonicera japonica*; Cerebrosides; Lonijaposides $A_1$–$A_4$, $B_1$, $B_2$.
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**Table 1**. $^{1}H$ and $^{13}C$ NMR (300 and 75.6 MHz) of LJC-1, **1** and **4**

| | Compounds | | | | | |
|---|---|---|---|---|---|---|
| | LJC-1 | | **1** | | **4** | |
| No. | $\delta_C$ | $\delta_H$ m (*J* Hz) | $\delta_C$ | $\delta_H$ m (*J* Hz) | $\delta_C$ | $\delta_H$ m (*J* Hz) |
| 1 | 63.2 | 4.33dd (10.5, 4.7)<br>4.40dd (10.5, 4.5) | 63.2 | 4.33dd (10.5, 4.7)<br>4.41dd (10.5, 4.5) | 70.9 | 4.40dd (10.3, 3.5)<br>4.71dd (10.3, 6.1) |
| 2 | 54.2 | 5.01m | 54.2 | 5.01m | 54.9 | 5.00m |
| 3 | 78.1 | 4.23m | 78.2 | 4.23m | 78.0 | 4.21m |
| 4 | 35.3 | 2.30m | 35.4 | 2.30m | 35.4 | 2.30m |
| 5 | 74.1 | 4.10m | 74.1 | 4.10m | 74.3 | 4.00m |
| 6 | 74.2 | 4.10m | 74.2 | 4.10m | 74.3 | 4.10m |
| 7 | 33.3 | 2.05 | 33.4 | 2.05 | 33.3 | 2.01 |
| 8 | 34.5 | 1.88–2.05m | 34.4 | 1.88–2.05m | 34.7 | 1.89–2.03m |
| 9 | 131.9 | 5.38 br dt (16.5, 6.5) | 131.9 | 5.38 br dt (16.5, 6.5) | 131.9 | 5.33 br dt (16.5, 6.5) |
| 10 | 132.1 | 5.40 br dt (16.5, 6.5) | 132.1 | 5.40 br dt (16.5, 6.5) | 132.2 | 5.46 br dt (16.5, 6.5) |
| 11 | 34.2 | 1.88–2.05m | 34.4 | 1.88–2.05m | 34.7 | 1.89–2.03m |
| 12 | 33.3 | 2.05m | 33.4 | 2.05m | 33.3 | 2.05m |
| $(CH_2)_n$ | 30.7–31.5 | 1.18 br s | 30.7–31.5 | 1.18 br s | 30.6–31.2 | 1.23 br s |
| 24 | 30.8 | 1.18m | 30.8 | 1.18m | 30.6 | 1.23m |
| 25 | 24.1 | 1.18m | 24.0 | 1.18m | 24.1 | 1.23m |
| 26 | 15.5 | 0.73t (7.2) | 15.5 | 0.73t (7.2) | 15.5 | 0.67t (7.0) |
| NH | | 8.46d (8.7) | | 8.46d (8.7) | | 8.60d (8.7) |
| 1′ | 176.5 | | 176.4 | | 175.4 | |
| 2′ | 73.7 | 4.50m | 73.6 | 4.50m | 73.6 | 4.62m |
| 3′ | 36.9 | 1.88m | 36.8 | 1.88m | 37.0 | 1.90m |
| 4′ | 27.0 | 1.64m | 27.0 | 1.64m | 27.3 | 1.66m |
| $(CH_2)_m$ | 30.7–31.5 | 1.18 br s | 30.7–31.5 | 1.18 br s | 30.6–31.2 | 1.23 br s |
| 13′ | 30.8 | 1.18 | 30.8 | 1.18 | 30.6 | 1.23 |
| 14′ | 24.1 | 1.18m | 24.0 | 1.18m | 24.1 | 1.23m |
| 15′ | 15.5 | 0.73t (7.2) | 15.5 | 0.73t (7.2) | 15.5 | 0.67t (7.0) |
| 1′ | | | | | 106.0 | 4.97d (7.8) |
| 2′ | | | | | 75.9 | |
| 3′ | | | | | 78.8 | |
| 4′ | | | | | 72.0 | 3.67–4.50 |
| 5′ | | | | | 79.7 | |
| 6′ | | | | | 65.4 | |

value (34.2, 34.5) of the methylene carbon adjacent to the olefinic carbon, which must be observed at $\delta \approx 27$ in (*Z*) isomers and at $\delta \approx 32$ in (*E*) isomers. All these spectral data revealed that the compound LJC-1 possessed two aliphatic chains containing one double bond and five hydroxyl groups and suggesting it to be a phytosphingosine type sphingolipid.[16,17] The positive and negative charged HRESI-QTOF-MS of LJC-1 gave three protonated $[M+H]^+$ and three deprotonated $[M-H]^-$ molecular ion peaks at *m/z* 684.6121, 656.5798, 698.6278 and 682.5965, 654.5655, 696.6133, respectively. This led to the conclusion that LJC-1 is a mixture of three cerebrosides (**1**–**3**), which was further confirmed by methanolysis of LJC-1.

Methanolysis of LJC-1 yielded a mixture of two fatty acid methyl esters (FAM) and two long chain bases (LCB). The fatty acid methyl esters (FAM) were identified by GC–MS as methyl 2-hydroxypentadecanoate and methyl 2-hydroxyhexadecanoate. The positive and negative mode HRESI-QTOF-MS of mixture of long chain bases gave sodiated $[M+Na]^+$ and deprotonated $[M-H]^-$ molecular ion peaks at *m/z* 466.3870 (calcd 466.3872), 438.3556 (calcd 438.3559) and 442.3889 (calcd 442.3897), 414.3579 (calcd 414.3584) corresponding to the molecular formulae $C_{26}H_{53}NO_4$ and $C_{24}H_{49}NO_4$, respectively. The above data suggested that LJC-1 comprised of three cerebroside (**1**–**3**) and among the three, two cerebrosides (**1** and **2**) had identical fatty acids with long chain bases of varying chain lengths ($C_{26}$ and $C_{24}$, respectively). The third cerebroside (**3**) contained long chain base identical to that of **1** but with a different fatty acid (2-hydroxy hexadecanoic acid). LJC-1 showed a single spot on normal phase TLC, but different retention times in reversed phase HPLC, thus revealing difference in their carbon chains. HPLC of LJC-1 showed the presence of **1** as a major constituent whereas other constituents (**2** and **3**) were in lesser amounts. Therefore, compound **1** was separated by reversed phase prep-HPLC for detailed analysis.

$^{1}H$ and $^{13}C$ NMR spectra of **1** (Table 1) were identical to those of LJC-1. The molecular formula of **1** was established as $C_{41}H_{81}NO_6$ by positively and negatively charged HRESI-QTOF-MS, which gave protonated $[M+H]^+$ and deprotonated $[M-H]^-$ molecular ion peaks at *m/z* 684.6121 (calcd 684.6142) and 682.5965 (calcd 682.5986).

Methanolysis of **1** yielded methyl 2-hydroxypentadecanoate identified by GC–MS. The existence of the 2-hydroxylpentadecanoyl moiety was also confirmed by the presence of EI-MS at *m/z* 286 $[M]^+$ and 256 $[M-15]^+$ as well as by the release of characteristic fragment ions at *m/z* 227 $[M-59]^+$ and 90 $[CH_2OHCOOCH_3]^+$. Therefore, the long chain base (LCB) was characterized as $C_{26}$-phytosphingosine having four hydroxyls, one double bond and an amino group.

The positively charged ESI-QTOF-MS/MS of **1** showed fragment ions at *m/z* 428 $[M-CH_3(CH_2)_{12}CHOHCONH]^+$,

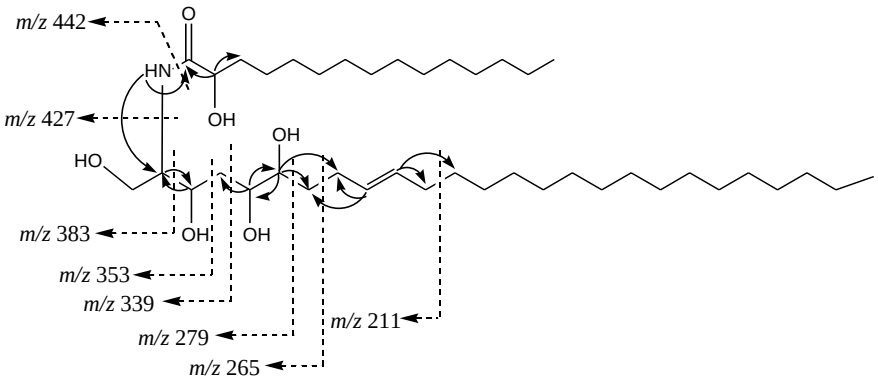
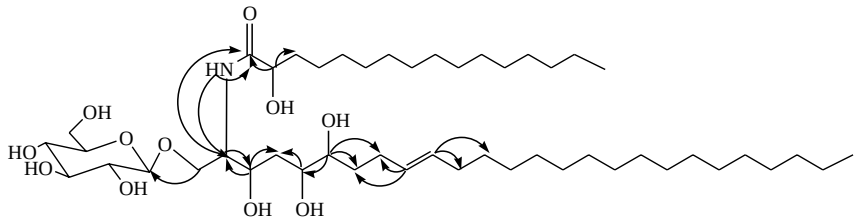



**Figure 2.** EI mass fragmentation pattern and important HMBC correlations of compound **1**.

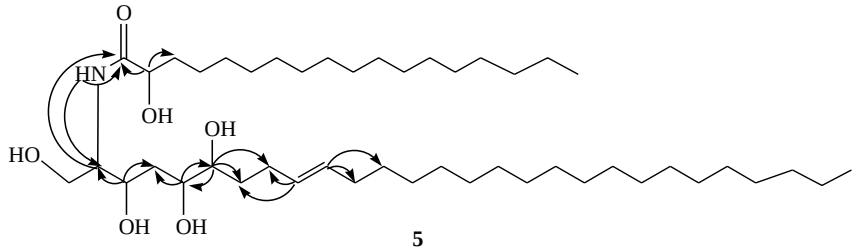
257 $[M-LCB]^+$, 383 $[428-CH_2CH_2OH]^+$, 300 $[257+CH_2CH_2OH]^+$, 282 $[300-H_2O]^+$ and 264 $[300-2\times H_2O]^+$. This further confirmed for 2-hydroxypentadecanoyl and $C_{26}$-long chain base in **1**. The fragmentation pattern of compound **1** in EI mode was consistent with the pattern obtained in ESI mode (Fig. 2). The presence of 2-amino and 1, 2′, 3, 5, 6 pentahydroxyl groups as well as 9, 10 double bond in the main chain was established by the analysis of $^1H$ and $^{13}C$ NMR spectroscopic data of **1**, which was unambiguously assigned by extensive 2D NMR ($^1H$–$^1H$ COSY, HMQC and HMBC) techniques. The $^1H$–$^1H$ COSY spectrum of **1** showed a pair of double doublets of oxygenated methylenes at $\delta$ 4.41 (1H, dd, $J=10.5$, 4.5 Hz) and 4.33 (1H, dd, $J=10.5$, 4.7 Hz) coupled to the nitrogen bearing methine signal at $\delta$ 5.01 (1H, m), which coupled further to the signal at $\delta$ 4.23 (1H, m). Olefinic protons at $\delta$ 5.38 (1H, br dt, $J=16.5$, 6.5 Hz) and $\delta$ 5.40 (1H, br dt, $J=16.5$, 6.5 Hz) showed coupling with methylene protons resonated at $\delta$ 1.88 (1H, m) and 2.05 (1H, m). In the HMBC spectrum of **1**, the signal at $\delta$ 4.50 correlated to the quaternary carbon and methylene carbon resonated at $\delta$ 176.5 and 36.9, respectively. The proton at $\delta$ 5.01 showed correlation with oxygenated methylene ($\delta$ 63.2) and oxygenated methines ($\delta$ 78.1). Thus, on the basis of the above evidence, compounds **1**, **2** and **3** were assigned structures as 1,3,5,6-tetrahydroxy-2-(2′-hydroxypentadecanoyl amino)-9-(*E*)-hexacosene named as lonijaposide $A_1$, 1,3,5,6-tetrahydroxy-2-(2′-hydroxypentadecanoyl amino)-9-(*E*)-tetracosene named as lonijaposide $A_2$ and 1,3,5,6-tetrahydroxy-2-(2′-hydroxyhexadecanoyl amino)-9-(*E*)-hexacosene named as lonijaposide $A_3$, respectively. The configuration at the chiral centers could not be established due to paucity of the compounds.

Compound **4** was obtained as a white solid showing $[M+Na]^+$ and $[M-H]^-$ peaks in positive and negative mode HRESI-QTOF-MS at *m/z* 882.6621 (calcd 882.6646) and 858.6668 (calcd 858.6671), respectively, corresponding to the molecular formula $C_{48}H_{93}NO_{11}$. The IR spectrum was similar to LJC-1 but in the $^1H$ NMR spectrum (Table 1) additional peaks due to the glucose moiety were observed. The anomeric proton showed a signal at $\delta$ 4.97 (d, $J=$ 7.8 Hz) and $J$ value suggested a β-configuration of the glucose unit. Other protons of glucose, geminal to hydroxyl groups resonated at $\delta$ 3.67–4.65. The $^{13}C$ NMR spectrum (Table 1) also revealed the presence of the sugar moiety, which showed an anomeric carbon at $\delta$ 106.0 and hydroxyl containing methine carbons at $\delta$ 75.9, 78.8, 72.0 and 79.7 and a signal at $\delta$ 65.4 ($CH_2OH$). The above observations suggested that **4** is a glycoside of **3**. It was also confirmed by ESI-QTOF-MS/MS of *m/z* 882, which showed prominent peak at *m/z* 720 due to the elimination of glucosyl moiety. The further fragmentation pattern observed was similar to compound **3**. The position of the glucose moiety at C-1 was evident by the downfield chemical shift of hydroxymethylene carbon at $\delta$ 70.9 in $^{13}C$ NMR spectrum by 7.8 ppm and further confirmed by HMBC spectrum in which the correlation was observed between the anomeric proton ($\delta$ 4.97) with the hydroxymethylene carbon ($\delta$ 70.9). In conclusion, glycoside **4** was assigned as 1-*O*-β-D-glucopyranosyl-3,5,6-trihydroxy-2-(2′-hydroxyhexadecanoyl amino)-9-(*E*)-hexacosene designated as lonijaposide $B_1$ (Fig. 3).

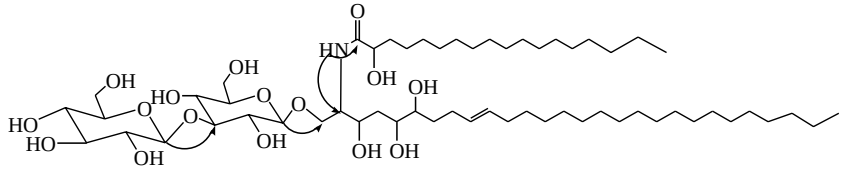
Compound **5** was also obtained as a white powder. The $^1H$ and $^{13}C$ NMR of **5** (Table 2) was found to be quite similar to LJC-1. However, its positive and negative HRESI-QTOF-MS gave sodiated $[M+Na]^+$ and deprotonated $[M-H]^-$ molecular ion peaks at *m/z* 776.6721 (calcd 776.6744) and 752.6759 (calcd 752.6768) corresponding to the molecular formula $C_{46}H_{91}NO_6$. The $^1H$ NMR spectrum of **5** showed the presence of two terminal methyls at $\delta$ 0.85 (6H, t, $J=$ 7.1 Hz), methylenes at $\delta$ 1.23 (56H, br s), an amide proton signal at $\delta$ 8.60 (1H, d, $J=8.7$ Hz), signals of *trans*-olefinic bond at $\delta$ 5.33 (1H, br dt, $J=16.5$, 6.5 Hz) and $\delta$ 5.46 (1H, br dt, $J=16.5$, 6.5 Hz) and six characteristic signals of geminal protons to hydroxyl groups were also observed at $\delta$ 4.62 (1H, m), 4.50 (1H, dd, $J=10.5$, 4.7 Hz), 4.35 (1H, dd, $J=10.5$, 4.5 Hz), 4.21 (1H, m), 4.10 (2H, m). A seventh signal at low field was observed at $\delta$ 5.03 (1H, m) identified for a methine proton vicinal to the nitrogen atom of the amide groups. The $^{13}C$ NMR spectra of **5** showed one quaternary carbon at $\delta$ 176.9 (CONH), two olefinic methine carbons at $\delta$ 132.1 and 132.3 (C=C), five methines at $\delta$ 54.9 (CHNH), $\delta$ 78.2 (CHOH), $\delta$ 74.1 (CHOH), $\delta$ 74.2 (CHOH), $\delta$ 73.6 (CHOH) and one methylene at $\delta$ 63.8 ($CH_2OH$). Methanolysis of **5** yielded methyl 2-hydroxyoctadecanoate and one long chain base. The positive HRESI-MS of LCB gave a sodiated $[M+Na]^+$ molecular ion peak at *m/z* 494.4166 (calcd 494.4185) corresponding to the molecular formula $C_{28}H_{57}NO_4Na$. Thus, the molecular mass of LCB together with $^1H$ and $^{13}C$ NMR spectroscopic data suggested $C_{28}$-phytosphingosine-type long chain base containing four hydroxyls, one double bond and an amino group. Therefore, the structure of **5** was assigned to be




**4**




**5**




**6**

**Figure 3.** Structures and selected HMBC (H→C) of lonijaposide $B_1$ (**4**), $A_4$ (**5**) and $B_2$ (**6**).

**Table 2**. $^1H$ and $^{13}C$ NMR (300 and 75.6 MHz) of **5** and **6**

| | Compounds | | | |
|---|---|---|---|---|
| | **5** | | **6** | |
| Position | $\delta_C$ | $\delta_H$ m (*J* Hz) | $\delta_C$ | $\delta_H$ m (*J* Hz) |
| 1 | 63.8 | 4.35dd (10.5, 4.5), 4.50dd (10.5, 4.7) | 70.5 | 4.30dd (10.5, 4.7), 4.52dd (10.5, 4.5) |
| 2 | 54.9 | 5.03m | 54.3 | 5.01m |
| 3 | 78.2 | 4.21m | 78.2 | 4.23m |
| 4 | 35.4 | 2.30m | 35.4 | 2.28m |
| 5 | 74.1 | 4.00m | 74.1 | 4.11m |
| 6 | 74.2 | 4.10m | 74.2 | 4.10m |
| 7 | 33.3 | 2.01 | 33.4 | 2.05 |
| 8 | 34.7 | 1.89–2.03m | 34.4 | 1.90–2.05m |
| 9 | 132.1 | 5.33 br dt (16.5, 6.5) | 131.9 | 5.37 br dt (16.5, 6.5) |
| 10 | 132.3 | 5.46 br dt (16.5, 6.5) | 132.1 | 5.40 br dt (16.5, 6.5) |
| 11 | 34.7 | 1.89–2.03m | 34.4 | 1.90–2.05m |
| 12 | 33.3 | 2.01m | 33.4 | 2.05m |
| 13–23 | 30.5–31.2 | 1.23 br s | 30.7–31.5 | 1.21 br s |
| 24 | 30.6 | 1.23m | 30.8 | 1.21m |
| 25 | 24.2 | 1.23m | 24.0 | 1.21m |
| 26 | 15.5 | 0.85t (7.1) | 15.5 | 0.75t (7.2) |
| NH | | 8.60d (8.7) | | 8.46d (8.7) |
| 1′ | 176.3 | | 176.4 | |
| 2′ | 73.6 | 4.62m | 73.6 | 4.50m |
| 3′ | 36.5 | 1.90m | 36.8 | 1.88m |
| 4′ | 27.3 | 1.66m | 27.0 | 1.64m |
| 5′–13′ | 30.6–31.2 | 1.23 br s | 30.7–31.5 | 1.18 br s |
| 14′ | 30.6 | 1.23 | 30.8 | 1.21 |
| 15′ | 24.1 | 1.23m | 24.0 | 1.21m |
| 16′ | 15.5 | 0.85t (7.1) | 15.5 | 0.75t (7.2) |
| 1″ | | | 105.9 | 4.97d (7.9) |
| 2″ | | | 75.2 | |
| 3″ | | | 76.4 | |
| 4″ | | | 79.8 | 3.90–4.52 |
| 5″ | | | 78.4 | |
| 6″ | | | 63.8 | |
| 1‴ | | | 105.0 | 4.68d (7.6) |
| 2‴ | | | 75.2 | |
| 3‴ | | | 78.0 | |
| 4‴ | | | 72.0 | 3.90–4.52 |
| 5‴ | | | 79.3 | |
| 6‴ | | | 65.4 | |

1,3,5,6-tetrahydroxy-2-(2′-hydroxyoctadecanoyl amino)-9-(*E*)-octacosene named as lonijaposide $A_4$ (Fig. 3).

Compound **6** was also obtained as a white solid. The HRESI-QTOF-MS in positive and negative mode gave sodiated $[M+Na]^+$ and deprotonated $[M-H]^-$ molecular ion peak at *m/z* 1100.7798 (calcd 1100.7801) and 1076.7810 (calcd 1076.7825), respectively. On the basis of molecular mass and $^1H$ and $^{13}C$ NMR spectral data (Table 2) the molecular formula of **6** was established as $C_{58}H_{111}NO_{16}$. The $^1H$ and $^{13}C$ NMR spectra were found to be identical with compound **5** except the presence of additional peaks of two sugar moieties. In $^1H$ NMR spectrum anomeric peaks of two glucose units were observed at $\delta$ 4.97 (1H, d, 7.9) and $\delta$ 4.68 (1H, d, 7.6) together with overlapping peaks of other carbons at $\delta$ 3.90–4.52. $^{13}C$ NMR spectrum also revealed the presence of two glucose moieties linked together in C1‴–C4″ pattern. C1‴–C4″ linkage in sugars was evidenced by downfield shift of C4″ carbons by 8 ppm and upfield shift of C3″ and C5″ carbons by 1–1.5 ppm. This linkage was further confirmed by HMBC correlation, which showed the correlation between anomeric proton ($\delta$ 4.68) of second glucose with C4″ carbon of first glucose. Thus, the above observations suggested that compound **6** was a diglycoside of **5**. The presence of two glucose units was further confirmed by ESI-QTOF-MS/MS of *m/z* 1100, which showed prominent peaks at *m/z* 938 and 776 due to the sequential elimination of two glucosyl moieties. This is the first report of presence of any diglycoside cerebroside in nature.[11] In conclusion, diglycoside **6** was assigned the structure as 1-*O*-β-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl-3,5,6-trihydroxy-2-(2′-hydroxyoctadecanoyl amino)-9-(*E*)-octacosene designated as lonijaposide $B_2$ (Fig. 3).

## 3. Experimental

### 3.1. General experimental procedure

Melting points were recoded on Barnstead Electrothermal melting point apparatus and are uncorrected. Optical rotations were determined on Horiba SEPA-300 polarimeter. IR spectra were recorded on a Perkin-Elmer 1760 FT-IR spectrometer with KBr disc. NMR spectra were recorded on a Bruker Avance-300 spectrometer. ESI-QTOF-MS was performed on QTOF-Micro, Waters Micromass and GC–MS was done on Shimadzu QP-2010 operating on EI mode at 70 eV with an ion source temperature of 200 °C; capillary column, BP20 (30 m×0.25 mm, 0.25 μm film thickness); carrier gas, He. HPLC was carried out on Waters prep LC 4000 system. Silica gel (60–120 mesh, Merck) was used for column chromatography. Pre-coated silica gel 60 $F_{254}$ (Merck) plates were used for TLC. All other chemicals used were produced by Merck India Ltd.

### 3.2. Plant material

The fresh flowers of *L. japonica* were collected from IHBT, Palampur, India during May 2004. A voucher specimen (No. 5909) has been deposited in the Herbarium of IHBT, Palampur, India.

### 3.3. Extraction and isolation

The fresh flowers (400 g) of *L. japonica* were extracted successively, with *n*-hexane (3×1500 mL) and EtOAc (3×1000 mL) and remaining material was air dried and powdered. The powdered flower material was extracted at room temperature with MeOH (3×800 mL). Evaporation of each solvent in vacuo, yielded *n*-hexane extract (1.36 g), EtOAc (5.00 g) and MeOH extract (33.00 g). The MeOH extract (33.00 g) was subjected to column chromatography over silica gel (60–120 mesh) and eluted with $CHCl_3$, $CHCl_3$–MeOH (95/5), (90/10), (85/15), (80/20), (70/30), (50/50), (30/70), (10/90) and MeOH to give a total of 150 fractions (100 mL each). Fractions 42–45, eluted with $CHCl_3$–MeOH (85/15) were evaporated and the resulting residue (95 mg) was crystallized in MeOH to give white solid LJC-1 (55 mg), which showed a single spot on TLC ($CHCl_3$/MeOH, 90:10). HPLC of LJC-1 (solvent MeOH/$H_2O$; 40:60; flow rate 7.0 mL/min; column: LichroCART 250×10 mm, Lichrosphere$^{100}$ RP18, particle size 10 μm) showed 3 peaks. Using these conditions, 40 mg of LJC-1 was separated by HPLC to give compound **1** (19.3 mg). Fractions 50–53, eluted with $CHCl_3$–MeOH (85/15) were evaporated and the white residue on crystallization in MeOH yielded compound **5** (22.4 mg). Fractions 56–68 eluted with $CHCl_3$–MeOH (80/20 and 70/30), were mixed and evaporated, and the resultant white residue (92 mg) was rechromatographed over silica gel (60–120 mesh), which on elution with $CHCl_3$–MeOH (85/15), (80/20) and (70/30) provided compound **4** (20 mg) in $CHCl_3$–MeOH (80/20) and compound **6** (23 mg) in $CHCl_3$–MeOH (70/30).

**3.3.1. LJC-1.** Amorphous powder; IR (KBr) $\nu_{max}$ 3350, 3220, 1665, 1620, 1540 $cm^{-1}$; $^1H$ and $^{13}C$ NMR see Table 1; positive-ion HRESI-QTOF-MS *m/z*: 684.6121 $[M+H]^+$, 656.5798 $[M+H]^+$, 698.6278 $[M+H]^+$ and negative-ion HRESI-QTOF-MS *m/z*: 682.5965 $[M-H]^-$, 654.5655 $[M-H]^-$, 696.6133 $[M-H]^-$.

**3.3.2. Methanolysis of LJC-1.** LJC-1 (10 mg) was refluxed with 1.5 mL of 1 M HCl in 82% aqueous MeOH for 12 h. The reaction mixture was cooled and extracted with *n*-hexane. Hexane layer was concentrated to give a mixture of two fatty acid methyl esters, which could be identified as methyl 2-hydroxypentadecanoate and methyl 2-hydroxyhexadecanoate by GC–MS showing molecular ion peaks at *m/z* 272 and 286, respectively, and characteristic fragments at *m/z* 213 $[M-59]^+$, 90 $[CH_2OHCOOCH_3]^+$ and 227 $[M-59]^+$, 90 $[CH_2OHCOOCH_3]^+$, respectively. The MeOH–$H_2O$ phase was neutralized with $Ag_2CO_3$, filtrated, and the filtrate was concentrated in vacuo to give a mixture of two long chain bases, which on ESI-QTOF-MS generated molecular ion peaks at *m/z* 466.3870 $[M+Na]^+$, 438.3556 $[M+Na]^+$ and 442.3889 $[M-H]^-$, 414.3579 $[M-H]^-$, respectively.

**3.3.3. Lonijaposide $A_1$ (1).** Amorphous powder; mp 136–138 °C; $[\alpha]_D^{24}$ +13.3 (*c* 0.0011, pyridine); $^1H$ and $^{13}C$ NMR see Table 1; positive and negative-ion HRESI-QTOF-MS *m/z*: 684.6121 $[M+H]^+$(calcd 684.6142) and 682.5965 $[M-H]^-$ (calcd 682.5986), respectively; positive-ion ESI-MS/MS *m/z* (%): 666 $[M-H_2O]^+$(100), 648

$[M-2\times H_2O]^+$(32), 630 $[M-3\times H_2O]^+$(10), 428 $[M-CH_3\ (CH_2)_{12}CHOHCONH]^+$(10), 383 $[428-CH_2CH_2OH]^+$(17), 301 $[256+CH_2CH_2OH]^+$(28), 283 $[301-H_2O]^+$(57), 265 $[301-2\times H_2O]^+$(48). Lonijaposide $A_1$ (**1**) was methanolyzed as per the method described for LJC-1 to yield methyl 2-hydroxypentadecanoate as fatty acid methyl ester.

**3.3.4. Lonijaposide $A_4$ (5).** Amorphous powder; 128–130 °C; [α] +14.5 (*c* 0.0011, pyridine); $^1H$ and $^{13}C$ NMR see Table 1; positive and negative-ion HRESI-QTOF-MS *m/z*: 776.6721 $[M+Na]^+$ (calcd 776.6744) and 752.6759 $[M-H]^-$ (calcd 752.6768), respectively; positive-ion ESI-MS/MS *m/z* (%): 758 $[M+Na-H_2O]^+$(100), 740 $[M+Na-2\times H_2O]^+$(40), 722 $[M+Na-3\times H_2O]^+$(15), 478 $[M+Na-CH_3(CH_2)_{15}CHOHCONH]^+$(12), 433 $[478-CH_2CH_2OH]^+$(15), 321 $[CH_3(CH_2)_{15}CHOHCONH+Na]^+$(6), 366 $[321+CH_2CH_2OH]^+$(9), 348 $[366-H_2O]^+$(25). Lonijaposide $A_4$ (**5**) was methanolyzed as per the method described for LJC-1 to yield methyl 2-hydroxy-octadecanoate as fatty acid methyl ester.

**3.3.5. Lonijaposide $B_1$ (4).** Amorphous powder; mp 120–121 °C; [α] +8.9 (*c* 0.0011, pyridine) $^1H$ and $^{13}C$ NMR see Table 1; positive and negative-ion HRESI-QTOF-MS *m/z*: 882.6621 $[M+Na]^+$ (calcd 882.6646) and 858.6668 $[M-H]^-$ (calcd 858.6671), respectively; positive-ion ESI-MS/MS *m/z* (%): 720 $[M+Na\text{-}glu]^+$(20); 702 $[M+Na\text{-}glu-H_2O]^+$(18), 684 $[M+Na\text{-}glu-2\times H_2O]^+$(32), 666 $[M+Na\text{-}glu-3\times H_2O]^+$(10), 450 $[M+Na\text{-}glu-CH_3(CH_2)_{13}CHOHCONH]^+$(10), 405 $[450-CH_2CH_2OH]^+$(17), 315 $[CH_3(CH_2)_{13}CHOHCONHCH_2CH_2OH]^+$(28), 297 $[315-H_2O]^+$(57), 279 $[315-2\times H_2O]^+$(48). Lonijaposide $B_1$ (**4**) was methanolyzed as per the method described for LJC-1 to yield methyl 2-hydroxyhexadecanoate as fatty acid methyl ester.

**3.3.6. Lonijaposide $B_2$ (6).** Amorphous powder; mp 114–115 °C; $[\alpha]_D^{24}$ +9.3 (*c* 0.0011, pyridine) $^1H$ and $^{13}C$ NMR see Table 1; positive and negative-ion HRESI-QTOF-MS *m/z*: 1100.7798 $[M+Na]^+$ (calcd 1100.7801) and 1076.7810 $[M-H]^-$ (calcd 1076.7825), respectively; positive-ion ESI-MS/MS *m/z* (%): 938 $[M+Na\text{-}glu]^+$(9), 776 $[M+Na\text{-}2\times glu]^+$(20), 758 $[M+Na\text{-}2\times glu-H_2O]^+$(6), 722 $[M+Na\text{-}2\times glu-3\times H_2O]^+$(4), 478 $[M+Na\text{-}2\times glu-CH_3(CH_2)_{15}CHOHCONH]^+$(10), 306 $[CH_3(CH_2)_{15}CHOHCO+Na]^+$(9), 433 $[478-CH_2CH_2OH]^+$(17), 366 $[CH_3(CH_2)_{15}CHOHCONHCH_2CH_2OH+Na]^+$(28), 348 $[366-H_2O]^+$(27), 330 $[366-2\times H_2O]^+$(48), 347 $[2\times glu+Na]$ (100). Lonijaposide $B_2$ (**6**) was methanolyzed as per the method described for LJC-1 to yield methyl 2-hydroxyoctadecanoate as fatty acid methyl ester.

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