# New Acylated Iridoid Glucosides from *Vitex altissima*†

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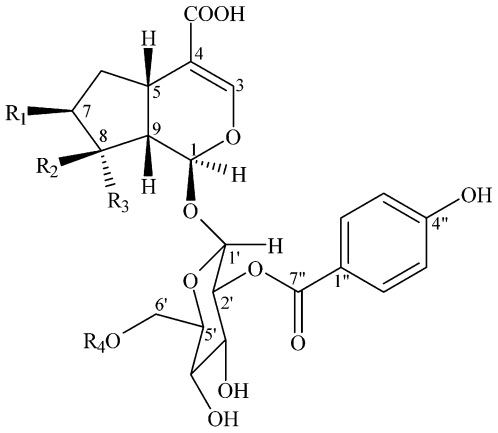
Six new iridoid glucosides, 6′-*O*-*trans*-feruloylnegundoside (**1**), 6′-*O*-*trans*-caffeoylnegundoside (**2**), 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (**3**), 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (**4**), 2′-*O*-*p*-hydroxybenzoyl gardoside (**5**), and 2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (**6**), along with two known iridoids, agnuside and negundoside, have been isolated from the ethyl acetate extractive of the leaves of *Vitex altissima*. The structures of these compounds were elucidated on the basis of spectral data interpretation. These isolates did not exhibit significant 5-lipoxygenase enzyme inhibitory activity, but compounds **2**−**4** showed potent antioxidant activity by both the superoxide (NBT riboflavin photoreduction) free-radical-scavenging and DPPH-radical-scavenging methods. Compounds **1**, **2**, and negundoside were evaluated in a rat paw edema assay.

The genus *Vitex* (Verbenaceae) consists of trees and shrubs, found in tropical and subtropical regions.[1] About 14 species are found in India.[2] *Vitex* species have been reported to exhibit a broad range of bioactivities, namely, antiviral,[3] anticancer,[4] antiinflammatory,[5] analgesic,[6] hepatoprotective,[7] antibacterial,[8] and potential effects on menopausal symptoms.[9] A large number of iridoids,[10−13] monoterpenes,[14] diterpenoids,[15,16] flavonoids,[17,18] and steroids[19] have been isolated from *Vitex* species.

*Vitex altissima* L. is a moderate- to large-sized tree found in the Eastern Ghats and Deccan Plateau in India.[20] The leaves are reported to be useful in the treatment of rheumatism.[21] No detailed phytochemical investigations have been reported on this species, previously. Our present study on the leaves of *V. altissima* has resulted in the isolation of several new mono- and diacylated iridoid glucosides. We report in this paper the isolation and structural elucidation of six new acylated iridoid glucosides (**1**−**6**) along with two known iridoids, agnuside and negundoside. The structures of the new compounds were established on the basis of the interpretation of NMR (COSY, HMQC, HMBC, and NOESY) and mass spectral data. The isolated compounds have been evaluated for their 5-lipoxygenase enzyme inhibitory and antioxidant activities.




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **1** | H | OH | $CH_3$ | *trans*-feruloyl |
| **2** | H | OH | $CH_3$ | *trans*-caffeoyl |
| **3** | OH | $=CH_2$ | | *trans*-caffeoyl |
| **4** | OH | H | $CH_3$ | *trans*-caffeoyl |
| **5** | OH | $=CH_2$ | | H |
| **6** | OH | H | $CH_3$ | H |

## Results and Discussion

Dried and powdered leaves of *V. altissima* were extracted with *n*-hexane, ethyl acetate, methanol, and 70% methanol, successively. The ethyl acetate extractives, which exhibited potent antiinflammatory activity, were purified by silica gel column chromatography followed by reversed-phase ($C_{18}$) preparative HPLC, to afford six new acylated iridoid glucosides (**1**−**6**) along with two known iridoids, agnuside and negundoside.

Compound **1** was obtained as a pale yellow amorphous powder. Its molecular formula, $C_{33}H_{36}O_{15}$, was deduced from microanalytical and LC-MS data [*m/z* 671 $(M - H)^-$]. The IR spectrum of **1** showed absorptions at 1633 (enol-ether) and 1698 $cm^{-1}$ (α,β-unsaturated ester). The $^1H$ and $^{13}C$ NMR spectral data of **1** showed signals characteristic of an iridoid.[22] The $^1H$ NMR spectrum showed the presence of a methyl group (3H, δ 1.23, s, $H_3$-10) attached to an oxygen-bearing carbon, a trisubstituted olefinic proton at δ 7.11 (1H, d, $J = 1.0$ Hz) assignable to H-3, and a doublet at δ 5.31 (1H, $J = 4.0$ Hz), characteristic of H-1 of an iridoid nucleus. These spectral data indicated the presence of a mussaenosidic acid skeleton[23] in **1**. The presence of a sugar moiety was revealed by the signals observed in the $^1H$ NMR (between 3.55 and 5.01 ppm) and $^{13}C$ NMR (between 97.9 and 64.2 ppm) spectra.[22] The β-configuration of the sugar unit is consistent with the coupling constant observed for the anomeric proton (H-1′, δ 5.01, d, $J = 8.0$ Hz). A set of signals [δ 7.85 (2H, d, $J = 8.5$ Hz) and 6.83 (2H, d, $J = 8.5$ Hz)], characteristic of a *p*-hydroxybenzoyl unit,[10] were also present. In addition, the $^1H$ NMR spectrum showed the presence of a *trans*-feruloyl moiety[24] constituted by a methoxyl group (δ 3.88, s, 3H), a 1,2,4-trisubstituted phenyl unit [δ 7.18, (1H, d, $J = 2.0$ Hz), 7.06 (1H, dd, $J = 8.5$, 2.0 Hz), and 6.78 (1H, d, $J = 8.5$ Hz)], and two *trans*-coupled

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**Table 1.** $^1$H NMR Data of Compounds **1**−**6** (in $CD_3OD$, 500 MHz)[a]

| position | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| aglycon | | | | | | |
| 1 | 5.31 d (4.0) | 5.31 d (4.0) | 5.24 d (4.5) | 5.29 d (4.0) | 5.35 d (3.5) | 5.40 br s |
| 3 | 7.11 d (1.0) | 7.11 d (0.5) | 7.09 br s | 7.09 br s | 7.03 br s | 7.03 br s |
| 4 | | | | | | |
| 5 | 2.93 m | 2.93 m | 2.95 m | 2.83 m | 2.97 br s | 2.86 br s |
| 6 | 2.12 m | 2.14 m | 1.94 m | 1.92 m | 2.03 m | 1.93 br s |
| | 1.33 m | 1.34 m | 1.83 m | 1.74 m | 1.85 m | 1.82 br s |
| 7 | 1.60 m (2H) | 1.63 m (2H) | 4.26 br s | 3.70 m | 4.30 br s | 3.72 br s |
| 8 | | | | 2.02 dd (14.0,7.0) | | 2.04 d (5.5) |
| 9 | 2.12 dd (9.0,4.0) | 2.14 dd (9.0,4.0) | 2.86 br s | 2.45 m | 2.84 br s | 2.48 br s |
| 10 | 1.23 s | 1.23 s | 5.25 br s<br>5.22 br s | 0.96 d (7.5) | 5.29 br s | 1.00 d (7.0) |
| glucose | | | | | | |
| 1′ | 5.01 d (8.0) | 5.01 d (8.0) | 4.99 d (8.0) | 4.99 d (8.0) | 4.97 d (8.5) | 4.95 d (8.0) |
| 2′ | 4.94 dd (9.0,8.0) | 4.95 dd (9.0,8.0) | 4.95 dd (9.0,8.0) | 4.94 dd (9.5,8.0) | 4.90 dd (9.5,8.0) | 4.88 dd (9.0,8.0) |
| 3′ | 3.73 dd (9.5,9.0) | 3.73 dd (9.5,9.0) | 3.71 dd (9.0,9.0) | 3.69 m | 3.71 m | 3.68 m |
| 4′ | 3.55 dd (9.5,9.0) | 3.54 dd (9.5,9.0) | 3.51 dd (9.5,9.0) | 3.52 dd (9.5,9.0) | 3.41 m | 3.39 m |
| 5′ | 3.69 m | 3.68 m | 3.67 m | 3.67 m | 3.69 m | 3.67 m |
| 6′ | 4.56 dd (12.0,2.0) | 4.55 dd (12.0,2.0) | 4.53 dd (11.5,2.0) | 4.54 dd (12.0,2.0) | 3.66 m | 3.65 m |
| | 4.41 dd (12.0,5.5) | 4.40 dd (12.5,6.0) | 4.42 dd (12.0,6.0) | 4.42 dd (12.0,6.0) | 3.93 d (12.0) | 3.93 d (12.0) |
| acyl moieties | | | | | | |
| 1″ | | | | | | |
| 2″ and 6″ | 7.85 d (8.5) | 7.85 d (8.5) | 7.85 d (8.5) | 7.84 d (8.5) | 7.84 br d (7.5) | 7.83 br d (7.5) |
| 3″ and 5″ | 6.83 d (8.5) | 6.80 d (8.5) | 6.80 d (8.5) | 6.81 d (8.5) | 6.80 br d (7.5) | 6.80 br d (7.5) |
| 4″ | | | | | | |
| 1‴ | | | | | | |
| 2‴ | 7.18 d (2.0) | 7.05 d (2.0) | 7.05 d (2.0) | 7.05 br s | | |
| 3‴ | | | | | | |
| 4‴ | | | | | | |
| 5‴ | 6.78 d (8.5) | 6.78 d (8.5) | 6.78 d (8.5) | 6.77 d (8.5) | | |
| 6‴ | 7.06 dd (8.5,2.0) | 6.95 dd (8.5,2.0) | 6.95 dd (8.5,2.0) | 6.95 dd (8.5,1.5) | | |
| 7‴ | 7.64 d (16.0) | 7.58 d (16.0) | 7.58 d (16.0) | 7.59 d (15.5) | | |
| 8‴ | 6.40 d (16.0) | 6.30 d (16.0) | 6.30 d (16.0) | 6.31 d (15.5) | | |
| $-OCH_3$ | 3.88 s | | | | | |

[a] Chemical shifts ($\delta$) are in ppm, and coupling constants ($J$ in Hz) are given in parentheses.

olefinic protons located at $\delta$ 7.64 (1H, d, $J$ = 16.0 Hz) and 6.40 (1H, d, $J$ = 16.0 Hz).

The $^1$H NMR (Table 1) and $^{13}$C NMR (Table 2) data of **1** were found to be similar to those of negundoside,[10,11] except for the additional signals of a feruloyl moiety present in **1**. The attachment of the feruloyl moiety on C-6′ was determined on the basis of the observed downfield shift of C-6′ ($\delta_C$ 64.2) in **1** in comparison with negundoside ($\delta_C$ 62.7). The position of the linkage of the feruloyl moiety at C-6′ was confirmed by the analysis of the HMBC data. In the HMBC spectrum (Figure 1), the methylene (H-6′) protons [$\delta$ 4.56 (1H, dd, $J$ = 12.0, 2.0 Hz) and 4.41 (1H, dd, $J$ = 12.0, 5.5)] of the glucose showed a correlation with the ester carbonyl ($\delta$ 169.0, C-9‴) of the feruloyl moiety. On the basis of the above, the structure of this compound was deduced as 6′-*O*-*trans*-feruloylnegundoside (**1**). The stereochemistry of **1** was assumed to be the same as in negundoside, on the basis of the similar chemical shifts and coupling constants observed[10,11] and the same sign of optical rotation.

Compound **2** was isolated as a pale yellow amorphous powder. The molecular formula $C_{32}H_{34}O_{15}$ was deduced from microanalytical and LC-MS [*m/z* 657 (M − H)$^-$] data. The $^1$H NMR and $^{13}$C NMR spectral data (Tables 1 and 2) of **2** closely resembled those of **1** except for the signals due to the feruloyl moiety. The absence of a methoxyl signal in the $^1$H NMR spectrum of **2** and the difference of 14 mass units in comparison with **1** were consistent with the presence of a hydroxyl group in place of a methoxyl in **2**. Close scrutiny of the $^{13}$C NMR data of **2** showed the presence of a caffeoyl moiety[25] constituted by two olefinic carbon signals ($\delta$ 147.3 and 114.7) and signals of a 1,2,4-trisubstituted phenyl unit ($\delta$ 149.6, 146.8, 127.6, 123.1, 116.5, and 115.1). The linkage of the caffeoyl moiety with the glucose unit at C-6′ was confirmed by the observed downfield shift of C-6′ ($\delta_C$ 64.1) and the HMBC correlations between the ester carbonyl ($\delta$ 169.0, C-9‴) of the caffeoyl moiety and H-6′ [$\delta$ 4.55 (1H, dd, $J$ = 12.0, 2.0 Hz) and 4.40 (1H, dd, $J$ = 12.5, 6.0 Hz)]. On the basis of the above, the structure of this compound was assigned as 6′-*O*-*trans*-caffeoylnegundoside (**2**).

Compound **3** was isolated as a pale yellow amorphous powder. The molecular formula, $C_{32}H_{32}O_{15}$, was deduced from microanalytical and LC-MS [*m/z* 655 (M − H)$^-$] data. The spectral data of **3** were very similar to those of **2** except for the signals due to the cyclopentane ring of the iridoid nucleus. The $^1$H NMR spectrum of **3** showed the presence of exocyclic methylene protons ($\delta$ 5.25, brs, 1H and 5.22, brs, 1H, H-10) and a signal ($\delta$ 4.26, brs, 1H, H-7) attributable to an allylic hydroxymethine proton. These findings were supported further by the $^{13}$C NMR signals at $\delta$ 73.8 (C-7), 152.7 (C-8), and 112.7 (C-10). In the HMBC spectrum (Figure 2), the olefinic protons ($\delta$ 5.25, brs, 1H and 5.22, brs, 1H, H-10), showed correlations with a hydroxymethine carbon ($\delta$ 73.8, C-7). The absence of a tertiary methyl signal (3H, $\delta$ 1.20, s, $H_3$-10) present in **1** and **2** and the presence of an $\Delta^{8(10)}$ exocyclic methylene group and a 7-hydroxy group indicated the presence of a gardoside nucleus[26,27] in **3**. Thus, the structure of this compound was assigned as 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (**3**).

Compound **4** was isolated as a pale yellow amorphous powder. Its molecular formula, $C_{32}H_{34}O_{15}$, was deduced

**Table 2.** $^{13}C$ NMR Data of Compounds **1**−**6** (in $CD_3OD$, 75 MHz)

| position | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| aglycon | | | | | | |
| 1 | 95.4 | 95.3 | 96.8 | 96.1 | 96.5 | 96.1 |
| 3 | 150.6 | 151.3 | 151.5 | 151.0 | *a* | *a* |
| 4 | 113.6 | 113.6 | 113.0 | 113.8 | *a* | *a* |
| 5 | 31.8 | 31.8 | 31.7 | 31.6 | 30.7 | 31.3 |
| 6 | 30.3 | 30.3 | 40.4 | 40.8 | 40.5 | 40.9 |
| 7 | 40.7 | 40.8 | 73.8 | 79.3 | 73.9 | 79.4 |
| 8 | 80.2 | 80.2 | 152.7 | 44.7 | 153.2 | 44.8 |
| 9 | 52.2 | 52.2 | 44.8 | 42.7 | 45.2 | 42.8 |
| 10 | 24.6 | 24.5 | 112.7 | 14.3 | 112.5 | 14.4 |
| 11 | 170.0 | 170.1 | *a* | *a* | *a* | *a* |
| glucose | | | | | | |
| 1′ | 97.9 | 97.9 | 98.4 | 97.9 | 98.3 | 98.0 |
| 2′ | 74.8 | 74.8 | 74.9 | 74.9 | 75.1 | 75.0 |
| 3′ | 75.8 | 75.9 | 75.8 | 75.8 | 76.3 | 76.3 |
| 4′ | 71.7 | 71.7 | 71.8 | 71.9 | 71.8 | 71.8 |
| 5′ | 75.8 | 75.9 | 75.9 | 76.0 | 78.5 | 78.5 |
| 6′ | 64.2 | 64.1 | 64.3 | 64.2 | 62.7 | 62.8 |
| acyl moieties | | | | | | |
| 1″ | 122.2 | 122.1 | 122.2 | 122.2 | 122.3 | 122.3 |
| 2″ and 6″ | 132.9 | 132.9 | 132.9 | 132.9 | 132.9 | 132.9 |
| 3″ and 5″ | 116.1 | 116.1 | 116.2 | 116.2 | 116.2 | 116.2 |
| 4″ | 163.3 | 163.4 | 163.4 | 163.3 | 163.4 | 163.3 |
| 7″ | 167.3 | 167.3 | 167.4 | 167.4 | 167.5 | 167.4 |
| 1‴ | 127.6 | 127.6 | 127.6 | 127.7 | | |
| 2‴ | 111.7 | 115.1 | 115.2 | 115.2 | | |
| 3‴ | 151.3 | 146.8 | 146.8 | 146.8 | | |
| 4‴ | 149.3 | 149.6 | 149.7 | 149.6 | | |
| 5‴ | 116.5 | 116.5 | 116.5 | 116.6 | | |
| 6‴ | 124.2 | 123.1 | 123.1 | 123.1 | | |
| 7‴ | 147.2 | 147.3 | 147.3 | 147.3 | | |
| 8‴ | 115.2 | 114.7 | 114.8 | 114.8 | | |
| 9‴ | 169.0 | 169.0 | 169.0 | 169.0 | | |
| $-OCH_3$ | 56.4 | | | | | |

[a] Signals not observed.

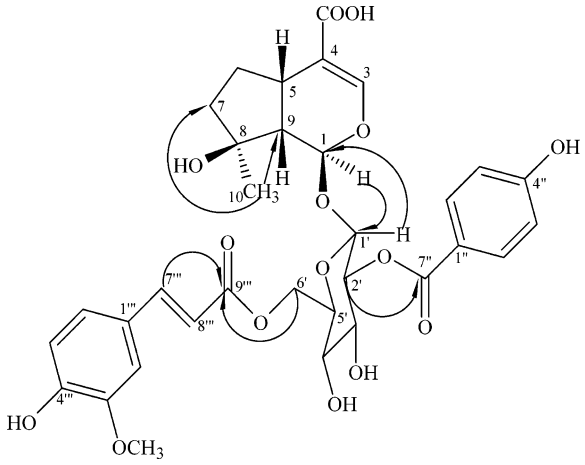

**Figure 1.** Selected HMBC connectivities for **1**.

from microanalytical and LC-MS [*m/z* 657 $(M - H)^-$] data. The spectral data (Tables 1 and 2) of **4** were very similar to those of **2** except for the signals due to the cyclopentane ring of the iridoid nucleus. The $^1H$ NMR spectrum showed the presence of a secondary methyl signal at $\delta$ 0.96 (3H, d, $J$ = 7.5 Hz, $H_3$-10), a hydroxymethine proton (1H, $\delta$ 3.70 m, H-7), and a methine proton signal at $\delta$ 2.02 (1H, dd, $J$ = 14.0, 7.0 Hz, H-8). The $^{13}C$ NMR data (Table 2) supported the presence of a secondary methyl ($\delta$ 14.3, C-10) at C-8 ($\delta$ 44.7) and a hydroxymethine carbon ($\delta$ 79.3, C-7). In the HMBC spectrum (Figure 3), the methyl group ($\delta$ 0.96, d, $J$ = 7.5 Hz, $H_3$-10) showed a correlation with a hydroxymethine carbon ($\delta$ 79.3, C-7). The above data supported the presence of an 8-epiloganic acid nucleus[28,29] in **4**. The

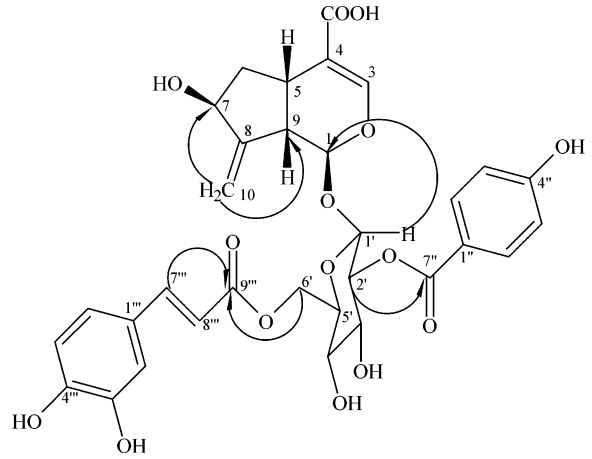

**Figure 2.** Selected HMBC connectivities for **3**.

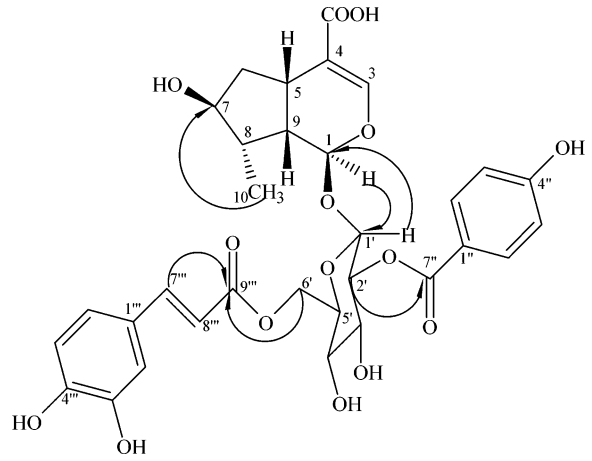

**Figure 3.** Selected HMBC connectivities for **4**.

chemical shifts of the glucose and the aromatic moieties of **4** and **2** were nearly identical. Thus, the structure of the compound was derived as 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (**4**).

Compound **5** was obtained as a colorless amorphous powder. Its molecular formula, $C_{23}H_{26}O_{12}$, was deduced from microanalytical and LC-MS [*m/z* 493 $(M - H)^-$] data. The IR spectrum exhibited bands at 3368 (hydroxyl), 1703 (ester), 1636 (C=C), and 1603 and 1512 $cm^{-1}$ (aromatic). The NMR spectral data of **5** (Tables 1 and 2) were very similar to those of **3** except for the absence of signals due to a caffeoyl moiety. Thus, the structure of this compound was deduced as 2′-*O*-*p*-hydroxybenzoylgardoside (**5**).

Compound **6** was isolated as a colorless amorphous powder. The molecular formula of **6** was determined as $C_{23}H_{28}O_{12}$ on the basis of microanalytical and LC-MS [*m/z* 495 $(M - H)^-$] data. The spectral data (Tables 1 and 2) of **6** were identical with those of **4** except for the absence of signals of a caffeoyl moiety. Thus, the structure of this compound was assigned as 2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (**6**). The known compounds were identified as agnuside[11] and negundoside[10,11] by comparison of spectral data with literature data.

Significant 5-lipoxygenase enzyme inhibitory activity was not observed for the eight compounds isolated from *V. altissima*, even at a dose of 1000 $\mu$M. However, compounds **2**−**4** exhibited potent antioxidant activity, both in a superoxide free-radical scavenging test (NBT method) ($IC_{50}$ 24.3, 32.0, and 31.9 $\mu$M, respectively) and in a DPPH-radical scavenging test ($IC_{50}$ 15.2, 10.9, and 11.4 $\mu$M, respectively), in comparison to the known antioxidants,

butylated hydroxytoluene (BHT) (NBT, $IC_{50}$ 381 $\mu$M and DPPH, $IC_{50}$ 19 $\mu$M) and butylated hydroxyanisole (BHA) (NBT, $IC_{50}$ 966 $\mu$M and DPPH, $IC_{50}$ 18 $\mu$M). Moreover, compounds **1**, **2**, and negundoside were evaluated in a rat paw edema assay, at 200 mg/kg, with compound **1** being the most active (20% inhibition).

## Experimental Section

**General Experimental Procedures.** Melting points were recorded on a Mel-Temp apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Varian (Cary-50) and Perkin-Elmer BX FT-IR spectrophotometers, respectively. The $^1$H NMR and 2D spectra ($^1$H−$^1$H-COSY, $^1$H−$^1$H-NOESY) were recorded on a Varian Unity INOVA 500 MHz spectrometer with standard pulse sequences, and $^{13}$C NMR spectra were recorded on an Avance Bruker 300 MHz instrument. Mass spectra were recorded on an Agilent 1100 series LC/MSD, and elemental analysis was carried out on a Vario El Elementar instrument. Preparative HPLC was carried out on a Shimadzu HPLC system (LC-8A Pump, SPD-10A UV−visible detector) using a Luna $C_{18}$ (10 $\mu$m, 21.2 × 250 mm, Phenomenex) column. Silica gel (100−200 mesh, ACME) was used for open column chromatography.

**Plant Material.** The leaves of *Vitex altissima* L. were collected from the Seshachelam Hill Range (Tirumala Forest) of the Eastern Ghats (situated between east longitudes 79°24′29″ and north latitudes 13°36′54″ and 13°39′12″) in Andhra Pradesh, India, in January 2001. The plant was authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. Voucher specimens (VA-010222) are on deposit at the Herbarium of Sri Venkateswara University, Tirupati, India.

**Extraction and Isolation.** The shade-dried and milled leaves of *V. altissima* (2.8 kg) were extracted successively with *n*-hexane (5 × 5 L), ethyl acetate (5 × 5 L), methanol (5 × 5 L), and 70% methanol (5 × 5 L) in a reflux apparatus. The combined extracts of each solvent were concentrated in vacuo, to give dark gummy residues of *n*-hexane (60 g), ethyl acetate (150 g), methanol (400 g), and 70% methanol (275 g). The ethyl acetate extractives, which showed potent antiinflammatory activity (39% inhibition against carrageenan-induced paw edema in rats, $p < 0.001$), were subjected to column chromatography over silica gel with different solvents of increasing polarity (*n*-hexane−EtOAc; $CHCl_3$−MeOH). The selected fractions were combined into 18 subfractions (1−18) based on TLC, using mixtures of $CHCl_3$−MeOH (9.6:0.4), $CHCl_3$−acetone (8:2), and EtOAc−MeOH (9:1) as solvent systems. Fraction 14 was rechromatographed over a silica gel column with increasing polarities of $CHCl_3$−MeOH to give compound **1** (500 mg). Fraction 15 was rechromatographed over a silica gel column using ethyl acetate as an eluent to yield compound **1** (300 mg) and compound **2** (800 mg). Fraction 16 was subjected to column chromatography over silica gel with increasing polarities of $CHCl_3$−MeOH mixtures as an eluent to give agnuside (118 mg) and compound **2** (1200 mg). Fraction 17 was rechromatographed over a silica gel column with increasing polarities of EtOAc−MeOH mixtures as eluent to give a mixture of **3** and **4** (935 mg) and negundoside (1700 mg). The mixture of **3** and **4** (200 mg) was subjected to reversed-phase preparative HPLC (Luna $C_{18}$, 10 $\mu$m, 250 × 21.2 mm, 20 mL/min) using $CH_3CN$−$H_2O$ (25:75) as solvent system to give compound **3** (45 mg, $t_R$ 11.2 min) and compound **4** (95 mg, $t_R$ 14.6 min). Fraction 18 was rechromatographed over a silica gel column and eluted with ethyl acetate to give a mixture of **5** and **6** (70 mg), which was subjected to reversed-phase preparative HPLC (Luna $C_{18}$, 10 $\mu$m, 250 × 21.2 mm, 20 mL/min), using $CH_3CN$−$H_2O$ (15:85) as solvent system, to give compound **5** (13 mg, $t_R$ 8.8 min) and compound **6** (20 mg, $t_R$ 14.0 min).

**6′-*O*-*trans*-Feruloylnegundoside (1):** pale yellow amorphous powder (MeOH); mp 155−156 °C; $[\alpha]_D^{25}$ −74.6° (*c* 0.25, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 247 (4.27), 327 (4.07) nm; IR (KBr) $\nu_{max}$ 3415, 1698, 1633, 1601, 1515, 1452, 1271, 1168, 1123, 980, 850 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; LC-MS (ESI, negative scan) *m/z* 671 (M − H)$^-$; *anal.* C 58.90%, H 5.26%, calcd for $C_{33}H_{36}O_{15}$, C 58.92%, H 5.35%.

**6′-*O*-*trans*-Caffeoylnegundoside (2):** pale yellow amorphous powder (MeOH); mp 168−169 °C; $[\alpha]_D^{25}$ −109.3° (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 249 (4.29), 331 (4.07) nm; IR (KBr) $\nu_{max}$ 3398, 1695, 1633, 1604, 1517, 1448, 1272, 1168, 1117, 980, 852 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; LC-MS (ESI, negative scan) *m/z* 657 (M − H)$^-$; *anal.* C 58.31%, H 5.11%, calcd for $C_{32}H_{34}O_{15}$, C 58.35%, H 5.16%.

**2′-*O*-*p*-Hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (3):** pale yellow amorphous powder (MeOH); mp 191−192 °C; $[\alpha]_D^{25}$ −32.6° (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 250 (4.33), 330 (4.17) nm; IR (KBr) $\nu_{max}$ 3406, 1698, 1633, 1604, 1516, 1449, 1272, 1168, 1113, 985, 852 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; LC-MS (ESI, negative scan) *m/z* 655 (M − H)$^-$; *anal.* C 58.50%, H 4.85%, calcd for $C_{32}H_{32}O_{15}$, C 58.53%, H 4.87%.

**2′-*O*-*p*-Hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (4):** pale yellow amorphous powder (MeOH); mp 158−160 °C; $[\alpha]_D^{25}$ −88.6° (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 249 (4.34), 330 (4.16) nm; IR (KBr) $\nu_{max}$ 3400, 1698, 1604, 1517, 1449, 1272, 1168, 1116, 977, 852 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; LC-MS (ESI, negative scan) *m/z* 657 (M − H)$^-$; *anal.* C 58.30%, H 5.13%, calcd for $C_{32}H_{34}O_{15}$, C 58.35%, H 5.16%.

**2′-*O*-*p*-Hydroxybenzoylgardoside (5):** colorless amorphous powder (MeOH); mp 215−216 °C; $[\alpha]_D^{25}$ −41.3° (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 255 (3.88) nm; IR (KBr) $\nu_{max}$ 3368, 1703, 1636, 1603, 1512, 1271, 1170, 1079, 902, 852 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; LC-MS (ESI, negative scan) *m/z* 493 (M − H)$^-$; *anal.* C 55.82%, H 5.22%, calcd for $C_{23}H_{26}O_{12}$, C 55.87%, H 5.26%.

**2′-*O*-*p*-Hydroxybenzoyl-8-epiloganic acid (6):** colorless amorphous powder (MeOH); mp 219−220 °C; $[\alpha]_D^{25}$ −119.9° (*c* 0.75, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 255 (4.42) nm; IR (KBr) $\nu_{max}$ 3405, 1703, 1648, 1609, 1516,1275, 1171, 1074, 904, 856 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; LC-MS (ESI, negative scan) *m/z* 495 (M − H)$^-$; *anal.* C 55.61%, H 5.62%, calcd for $C_{23}H_{28}O_{12}$, C 55.64%, H 5.64%.

**Antiinflammatory Activity.** The *n*-hexane, ethyl acetate, methanol, and 70% methanol extracts of *V. altissima* were screened for their antiinflammatory activity using the carrageenan-induced rat paw edema model of Winter et al.[30] Wistar rats of either sex weighing between 180 and 220 g were divided into six groups, with each group consisting of six animals. One group served as negative control (received 1% Tween-80, 10 mL/kg), and a second group served as positive control (received 25 mg/kg, diclofenac sodium suspended in 1% Tween-80). The third, fourth, fifth, and sixth groups received 250 mg/kg of the hexane, ethyl acetate, methanol, and 70% methanol extracts suspended in 1% Tween-80, respectively, by the oral route. Edema was produced by injecting 0.1 mL (1% w/v in saline) of carrageenan solution into the subplantar region of the left hind paw of rats of all groups. Drug treatment was given 1 h prior to carrageenan injection. The paw volume was measured by a plethysmometer at zero and 3 h after carrageenan injection. The difference between the initial and the final paw volume gave the edema volume. The percent inhibition of paw edema was calculated by comparing the mean edema volume of treated group and control group (diclofenac sodium 62%; hexane extractives 15%; ethyl acetate extractives 39%; methanol extractives 13%, and 70% methanol extractives 20%). Compounds **1**, **2**, and negundoside (200 mg/kg) were screened for antiinflammatory activity in this model.

**5-Lipoxygenase Enzyme Inhibitory Activity.** Test compounds were screened for 5-lipoxygenase enzyme inhibitory activity by the modified ferric-xylenol orange peroxide assay of Gay et al.[31] The assay mixture contained 50 mM phosphate buffer (pH 6.3), 5-lipoxygenase, various concentrations of test substances, and linoleic acid (80 mM), in a total volume of 0.5 mL. After 5 min of incubation, to the above reaction mixture was added 0.5 mL of ferric-xylenol orange reagent (in perchloric acid) and absorbance was measured after 2 min at 585 nm on a spectrophotometer. Nordihydroguaiaretic acid (NDGA)

(100 $\mu$M) was used as a positive control (70% inhibition). Percent inhibition was calculated by comparing absorbance of test substances with that of the control. All the tests were run in triplicate and averaged.

**Superoxide Free-Radical-Scavenging Activity.** Superoxide radical-scavenging activity of the test compounds isolated from *V. altissima* was detemined by the method of McCord and Fridovich.[32] The assay mixture contained EDTA (6.0 $\mu$M), NaCN (3 $\mu$g), riboflavin (2 $\mu$M), NBT (50 $\mu$M), and various concentrations of the test substances in methanol and phosphate buffer (58 mM, pH 7.8), in a final volume of 3 mL. The tubes were shaken well, and the absorbance was measured before and after illumination at 560 nm. The percent inhibition of superoxide radical generation was measured by comparing the mean absorbance values of the control and those of the test substances. $IC_{50}$ values were obtained from the plot drawn of concentration in $\mu$M versus percentage inhibition.

**DPPH Radical-Scavenging Activity.** DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured by the method of Lamaison et al.[33] The reaction mixture contained $1.0 \times 10^{-4}$ mM methanolic solution of DPPH and various concentrations of the test substances and kept in a dark area for 50 min. The absorbance of the samples was measured on a spectrophotometer at 517 nm against a blank. All tests were run in triplicate and averaged.

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