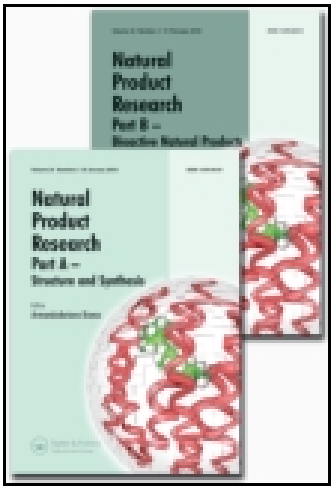

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Rattan L. Sharma [a] , Anil Prabhakar [b] , Kanaya L. Dhar [b] & Anand Sachar [a]

[a] Department of Chemistry , University of Jammu , Jammu-180004, India

[b] Indian Institute of Integrative Medicine (IIIM) , Jammu Tawi-180001, India

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A new iridoid glycoside from *Vitex negundo* Linn (Verbenacea)

Rattan L. Sharma[a]*, Anil Prabhakar[b], Kanaya L. Dhar[b] and Anand Sachar[a]

[a]*Department of Chemistry, University of Jammu, Jammu-180004, India;* [b]*Indian Institute of Integrative Medicine (IIIM), Jammu Tawi-180001, India*

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Five compounds have been isolated from the leaves of *Vitex negundo* Linn (Verbenacea) and their structures established by spectral analysis. Compound **1** has been identified and characterised as a new iridoid with a novel structure which has never before been reported in literature from *Vitex* or any other source. Its structure has been established as 1,4a,5,7a-tetrahydro-1-β-D-glucosyl-7-(3′,4′-dihydroxybenzoyloxymethyl)-5-ketocyclopenta[c]pyran-4-carboxylic acid. Other compounds, **2**, **3**, **4** and **5**, have been identified as luteolin-7-*O*-β-D glucoside, nishindaside, negundoside, and agnuside, respectively. Compound **2**, a flavone-*O*-glycoside, is being reported from this plant for the first time, and compounds **3**, **4** and **5** are structurally known iridoids.

Keywords: *Vitex negundo*; new iridoid glycoside

1. Introduction

The genus *Vitex* belongs to the family Verbenacea, which includes 80 genera and about 800 species. *Vitex* is a genus of trees and shrubs widely found in the tropic and warm region. *Vitex negundo* Linn is a shrub which is quite prolific in India, occurring up to an altitude of ~1500 m. The leaf extract of *V. negundo* has been reported to exhibit a wide range of pharmacological activities, including mosquito-repellant activity (D. Hebbalkar, G. Hebbalkar, Sharma, Joshi, & Bhat, 1992), hepato protective action (Avadhoot & Rana, 1991), anti-inflammatory activity (Chawala, Sharma, Handa, & Dhar, 1991), antigenotoxic effects (Balboa & Lim-Sylianco, 1993), antihistamine release properties (Rimando et al., 1987), rehumatoid arthritis action (Kishor & Banerjee, 1988), activity of cattrachea (Dayrit, Lapid, Cagampang, & Lagurin, 1987), central nervous system (CNS) depressant activity (Gupta, Mazumder, & Bhawal, 1999), and antifilarial activity (Bhargava, 1986) *in vitro*. From the leaves, twigs, seeds and roots of *V. negundo*, a large number of compounds with varied structural ranges have been reported, including gluconononitol (Ghosh & Krishna, 1936), camphene, α-pinene, citral and β-caryophyllene (Masilungan, 1955), casticin, orientin, isorientin, corymbosin and various new flavonoid glycosides (Achari, Chowdhury, Dutta, & Pakrashi, 1984; Banerji, Chadha, & Malshet 1969; Banerji, Das, Chakrabarty, & Jha, 1988; Dayrit et al., 1987; Ferdous et al., 1984; Hänsal, Leuckert, Rimpler, & Schaaf, 1965; Sirait, Rimpler, & Hänsal, 1962), a diterpenoid, three triterpenoids, various long-chained unsaturated fatty acids

*Corresponding author. Email: rlsharma_hod@rediffmail.com

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(Vishnol, Shoeb, Kapil, & Popli, 1983), five iridoid glycosides, aucubin, agnuside, nishindaside, negundoside and 6-p-hydroxybenzoyl mussaenosidic acid (Dayrit & Lagurin, 1994), and a lignan (Chawala, Sharma, Handa, & Dhar, 1992). Now, we report one new iridoid (**1**); a flavonoid glycoside, luteolin-7-*O*-β-D-glucoside (**2**); and three known iridoids: nishindaside (**3**), negundoside (**4**) and agnuside (**5**) from the methanolic extract of leaves of *V. negundo*. The methanolic extract has been found to exhibit very potent antifeedant, anti-inflammatory, analgaesic and anti-convulsant activities.

2. Experimental

2.1. *Plant material*

Vitex negundo leaves were collected from Trivandrum (Kerala State in India) in the month of August (1997) and authenticated by Dr B.K. Kapahi, Head of the Botany Division, Regional Research Laboratory (CSIR), Jammu.

2.2. *Isolation*

Air-dried leaves of *V. negundo* (2.25 kg) from Trivandrum were extracted thrice in a percolator at room temperature with 18.0 L of petroleum ether (60–80°C), and kept for over 24 h, affording a defatted mass which was dried under high vacuum and charged in a percolator for extraction with 11.25 L of methanol again at room temperature and kept for over 24 h to yield a dry brown solid (360 g). Si-gel CC of 50 g of this residue over 300 g of gel gave, on elution with (EtOAc–MeOH 49 : 1), 0.22 g of **5**, with (EtOAc–MeOH 19 : 1) 0.16 g of **3**, with (EtOAc–MeOH 9 : 1) 0.11 g of **4** and 0.465 g of **1** and with (EtOAc–MeOH 17 : 3) 0.065 g of **2**.

2.3. *Characterisation and spectral analysis of compounds 1–5*

Compound ***1***: White needles, m.p. 224°C, R_f 0.65 solvent system ($EtOAc : MeOH : H_2O :: 100 : 17 : 13$), $[\alpha]_D$ −24 (MeOH *ca.* 0.121), analysed for $C_{23}H_{24}O_{14}$; IR (KBr on a Shimadzu IR-435) bands: 1715, 1602 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$, TMS int. std.) δ 3.25 to 4.85 (10H, methylene and methine protons geminal to hydroxyl, C-1 methine proton and C-10 methylene protons of the iridoid nucleus, i.e. CH-1, CH-1′, CH-2′, CH-3′, CH-4′, CH-5′, CH_2-6′, CH_2-10), 6.34 (1H, s, H-2″), 6.45 (1H, s, H-7), 6.62 (1H, s, H-3), 6.86 (1H, d, H-5″), 7.37 (1H, d, H-6″); ^{13}C-NMR – see Table 1; MS: *m*/*z*, 563 ($M^+ + 39$) on a FAB system confirming the molecular formula with M^+ at *m*/*z* 524. The ^{1}H-NMR data for the heptaacetate of **1** is explained in the Results and discussion section.

Compound ***2****:* Colourless crystals, m.p. 253−254°C, R_f 0.63 solvent system ($CHCl_3 : MeOH : H_2O :: 65 : 35 : 13$), analysed for $C_{21}H_{20}O_{11}$; UV_{max} (MeOH) 254 and 348 nm, shifted to 274,389 and 393 nm after addition of $AlCl_3$ + HCl solution; IR (KBr on a Shimadzu IR-435) bands: 3435 (highly H-bonded hydroxyl bands spread out beneath CH-stretching), 1686 (C=O) and 1605 (Ar); ^{1}H-NMR (90 MHz DMSO-d_6, TMS internal standard) did not provide much information and hence the compound was subjected to acetylation. ^{1}H-NMR of the heptaacetate in $CDCl_3$ has been explained in the Results and discussion section.

Compound ***3****:* Amorphous powder, m.p. 179°C (with decomposition), R_f 0.48 ($CHCl_3$: MeOH : : 85 : 15), $[\alpha]_D$ −83° (MeOH ca. 0.975) analysed for $C_{23}H_{36}O_{12}$; IR (KBr on a Shimadzu 1R-435) bands: 1740, 1710 cm^{-1}; ^{1}H-NMR (90 MHz, CD_3OD, TMS int. std.): δ 3.41 (3H, s, OMe); 4.50 (1H, d, H-1′, 8 Hz), 4.56 (1H, d, 1-H, 8 Hz), 4.95 (1H, d, 3-H, 6 Hz), 5.76 (1H, s, 7-H) 6.85 (2H, dd, 3″-H and 5″-H, 8 Hz), 7.85 (2H, dd, 2″-H and 6″-H, 8 Hz); ^{13}C-NMR – see Table 2; MS: *m*/*z* 504 (M^+), 138, 120, 94, 64.

Table 1. ^{13}C NMR (DMSO-d_6) of compound **1** and its acetate (**1**).

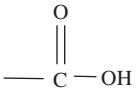
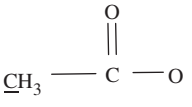
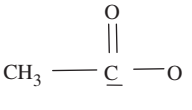
Carbon atom no.	Compound **1**	Acetate of **1**
	Chemical shift	Chemical shift
1	94.01	91.90
3	157.16	157.59
4	114.10	112.11
5	104.19	107.03
6	182.71	176.31
7	109.47	109.45
8	144.72	143.12
9	Embedded in DMSO-d_6	30.08
10		21.75
11	164.63	168.32
1′	98.25	95.82
2′	79.65	76.88
3′	82.16	74.81
4′	73.81	72.75
5′	73.84	70.02
6′	71.62	68.78
1″	119.92	129.72
2″	122.12	123.22
3″	146.52	153.81
4″	156.54	157.59
5″	116.95	122.04
6″	122.26	124.83
	164.10	168.10
		21.61, 21.71, 29.01
		168.01, 169.12, 169.22, 178.11

Table 2. ^{13}C NMR (CD_3OD) data of nishindaside (**3**).

Carbon atom no.	Chemical shift	Carbon atom no.	Chemical shift
1	98.5	1′	100.0
3	100.0	2′	74.9
4	30.1	3′	77.8
5	44.5	4′	71.2
6	80.6	5′	77.9
7	131.3	6′	62.5
8	149.3	1″	121.7
9	142.3	2″, 6″	132.6
10	63.0	3″, 5″	116.1
		4″	163.3
		OCH_3	56.1
		CO	167.6

Table 3. ^{13}C NMR (CD_3OD) data of negundoside (**4**).

Carbon atom no.	Chemical shift	Carbon atom no.	Chemical shift
1	94.8	1′	97.5
3	151.0	2′	75.5
4	113.3	3′	78.0
5	30.7	4′	71.6
6	30.0	5′	78.1
7	41.1	6′	62.5
8	79.5	1″	122.0
9	52.1	2″, 6″	132.6
10	24.2	3″, 5″	116.0
11	169.7	4″	163.3
		– COOH	167.0

*Compound **4**:* Colourless crystals, m.p. 162–164°C, R_f 0.32 ($CHCl_3$: MeOH : : 85 : 15), $^{25}[\alpha]_D$ −130 (MeOH, *ca.* 0.120), analysed for $C_{23}H_{28}O_{11;}$ IR (KBr on a Shimadzu 1R-435) bands: 3400, 1710, 1690, 1645, 1600, 1599, 1455 cm^{-1}; ^{13}C-NMR – see Table 3; MS: *m*/*z* 496 (M^+).

*Compound **5**:* Colourless crystals, m.p. 147–151°C, R_f 0.68 ($CHCl_3$: MeOH : : 85 : 15), analysed for $C_{22}H_{26}O_{11}$; 1R (KBr on a Shimadzu 1R-435) bands: 3400, 1707, 1645, 1590, 1455 cm^{-1}; ^{13}C-NMR – see Table 4; MS: *m*/*z* 466.44 (M^+).

2.3.1. *Acid hydrolysis of **1***

Acid hydrolysis was carried out with 45 mg of **1** with 7% H_2SO_4 in MeOH (30 mL) by refluxing for 12 h. A total of 20 mL of methanol was removed from the reaction mixture by distillation, 20 mL of H_2O added, the reaction mixture extracted with 75 mL of $CHCl_3$, dried on anhydrous Na_2SO_4 and the chloroform distilled off to yield the aglycon.

Table 4. ^{13}C NMR (CD_3OD) data of agnuside (**5**).

Carbon atom no.	Chemical shift	Carbon atom no.	Chemical shift	Carbon atom no.	Chemical shift
1	97.8	1′	100.1	1″	121.8
3	141.5	2′	74.5	2″, 6″	132.6
4	105.5	3′	78.0	3″, 5″	116.2
5	46.0	4′	71.2	4″	163.2
6	82.5	5′	77.8	CO	167.8
7	132.2	6′	62.5		
8	142.6				
9	48.5				
10	63.5				

The major moiety was identified as glucose on paper chromatogram by comparison with standard sugars in solvent system ($BuOH : AcOH : H_2O :: 4 : 1 : 5$) and sprayed with ninhydrin after drying.

2.3.2. *Alkaline hydrolysis of **1***

A total of 50 mg of **1** was refluxed with 5% alcoholic KOH (10 mL) for 3 h, half of the alcohol distilled off, 10 mL of H_2O added, the remaining quantity of alcohol again distilled off, the aqueous solution acidified to pH2 and extracted with the benzene. After removal of the solvent, the residue passed through silica gel and was eluted with ethyl acetate and alcohol (1 : 1) to yield brownish powder, m.p. 199–200°C.

2.3.3. *Acetylation of **1***

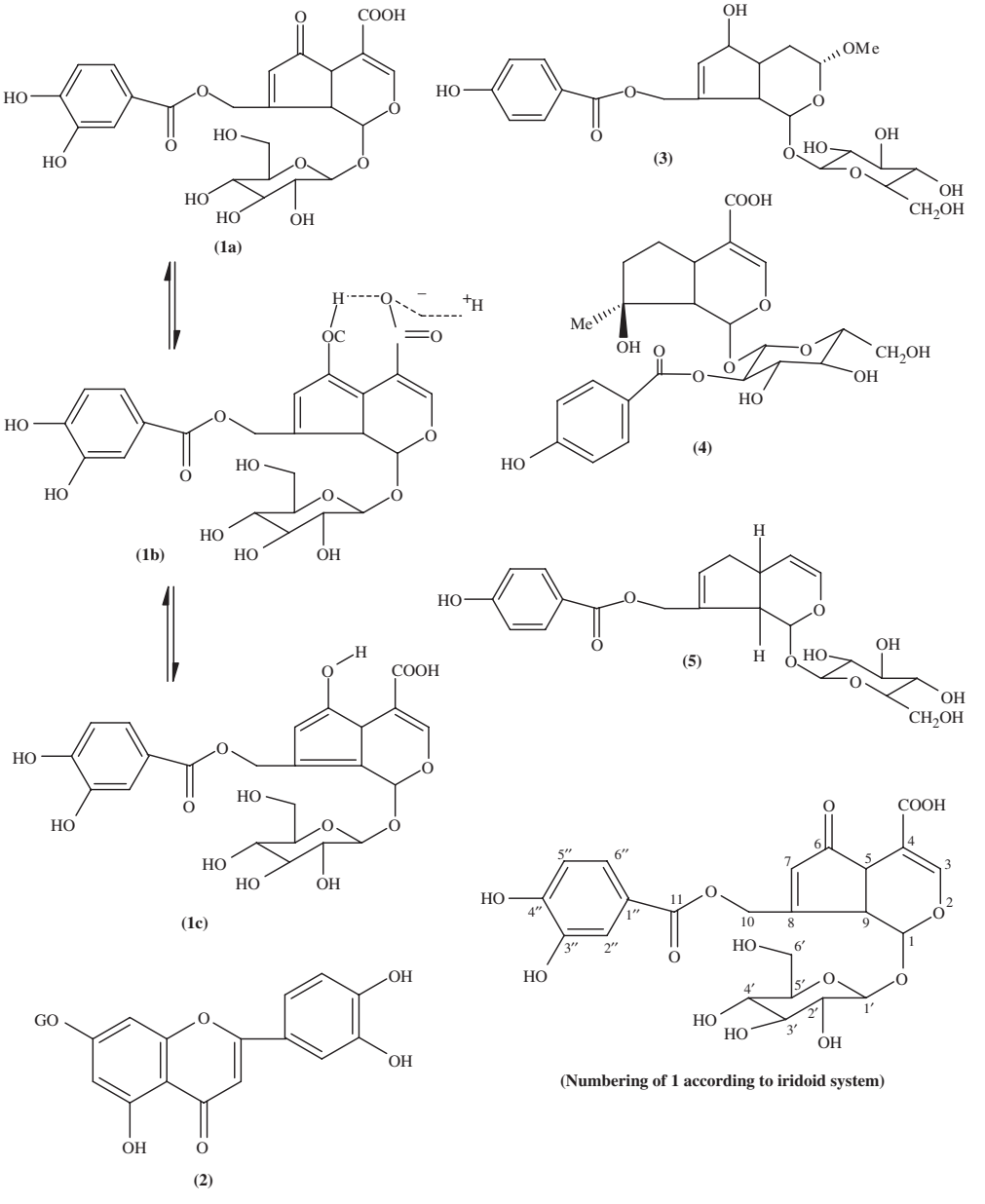
A total of 20 mg of **1** was dissolved in dry pyridine (2 mL); acetic anhydride (5 mL) was added and the reaction mixture kept under anhydrous conditions at room temperature. Progress of the reaction was monitored on TLC and further processed in the usual manner. The picture became clear when **1** formed a heptaacetate, when all the sugar protons were clearly visible at the appropriate position alongwith $OCOCH_3$ protons at δ.182 ($1 \times CH_3$), 2.01 ($3 \times CH_3$), 2.32 ($2 \times CH_3$) and 2.42 ($1 \times CH_3$).

2.3.4. *Acid hydrolysis of **2***

A total of 30 mg of **2** was refluxed with 7% H_2SO_4 in MeOH (25 mL) for 14 h and worked out, as in the case of the hydrolysis of **1**. The aglycon obtained has m.p. ~300°C (dec) and was analysed as $C_{15}H_{10}O_6$; MS: m/z 286 (M^+). It was identified as luteolin, m.p. 328–330°C (dec). The sugar was identified as glucose by comparison with the standard sugar.

2.3.5. *Acetylation of **2***

A total of 30 mg of **2** was dissolved in 3 mL of dry pyridine; 5 mL of Ac_2O was added and refluxed for 1 h. After completion of the reaction, the product was worked up as usual to give heptaacetate, m.p. 224–226°C, and analysed for $C_{35}H_{34}O_{18}$.



3. Results and discussion

Compound 1: $C_{23}H_{24}O_{14}$, responds positively to Wieffering field test, giving blue colouration, which indicates it as an iridoid. On acid hydrolysis, it gave a sugar and an aglycon. The sugar moiety was identified as glucose on paper chromatogram by comparison with standard sugars. Alcoholic KOH hydrolysis and repeated chromatography of the acidified product yielded an acid which was identified as

protocatechuic acid, m.p. 200°C. ^{1}H-NMR of the acid showed two doublets (H-5) at δ 6.88 and (H-6) at 7.37 and a singlet (H-2) at 6.40. ^{1}H-NMR of **1** showed glucose methylene and methine protons geminal to different hydroxyl groups between δ 3.25 and 4.85. The singlets at δ 6.45 and 6.62 could be assigned to C-7 and C-3 olefinic protons of the iridoid nucleus. Two doublets, one for each proton at δ 6.86 and 7.37 were assigned to 5″ and 6″ protons of the aromatic nucleus alongwith one proton singlet at δ 6.34 due to 2″ proton. The picture became clear when compound **1** formed a heptaacetate where all the sugar protons were clearly visible at the appropriate positions, along with $OCOCH_3$ protons at δ 1.82 ($1 \times CH_3$), 2.01 ($3 \times CH_3$), 2.32 ($2 \times CH_3$) and 2.42 ($1 \times CH_3$). Two downfield signals of $COCH_3$ at δ 2.32 and 2.42 are due to two phenolic acetates and one due to an enolic acetate of C-6 position of the iridoid nucleus, respectively. The unusual upfield acetate proton at δ 1.82 indicates the close proximity of shielding the aromatic ring as benzoate at C-10 methylene. The two doublets due to 5″ and 6″ protons moved downfield to δ 7.41 and 7.73, as expected, and singlets due to C-7, C-3 and C-2″ protons appeared at δ 6.61, 7.37 and 7.69, respectively. The methylene and methine protons attached to oxygen function appeared between δ 3.6 and 5.75 as expected. ^{13}C NMR showed a signal at δ 182.7, which was assigned to C-6 carbon atom. This position is unique in having a partial allylic carbonyl character and partially enolic =C(OH) – carbon function. The structure with more enolic character due to strong hydrogen bonding with –COOH is borne by the fact that C-9 has moved upfield in acetate to δ 30.08 and C-10 metheylene carbon to 21.7 due to the allylic position. The carboxylic and ester carbonyl appeared as expected at δ 164.1 and 164.6 in compound **1**. Keeping in view the above data and discussions, structures 1_a, 1_b and 1_c can be assigned to compound **1**. Since the molecules have a partial keto carbonyl (182.7), the structure 1_c is ruled out because its keto forms cannot show the presence of one olefinic proton at δ 6.45. Structure 1_b is tautomeric to structure 1_a, and both appear to correctly represent the structure of compound **1**. This structure is being represented for the first time from a natural source and is new to the literature.

Compound 2: $C_{21}H_{20}O_{11}$ m.p. 253–254°C responded to $FeCl_3$ and Shinoda test for flavanoids. On acid hydrolysis it gave an aglycon analysed for $C_{15}H_{10}O_6$, m.p. 279–278°C and glucose. Compound **2** showed UV_{max} (CH_3OH) at 348 and 254 and shifted to 274, 389 and 393 nm after the addition of $AlCl_3 + HCl$ solution, while sodium acetate solution addition showed no significant shift, indicating that the 7-oxygen function is either absent or bound with the carbon function, while 5-OH is present with a strong hydrogen bond with C=O function. ^{1}H-NMR of **2** in DMSO-d_6 did not provide much information, so the compound was subjected to acetylation. The ^{1}H-NMR of the heptaacetate in $CDCl_3$ showed four singlets (one for each proton) at δ 7.37 (slightly meta coupled), 6.65, 6.59 (meta coupled) and 6.46 (meta coupled) due to C-2′, C-3, C-6 and C-8 protons, respectively. Sugar protons, as usual, centred at δ 3.94, 4.23, 5.18 and 5.32. Acetate protons (each 3H) were centred at δ 2.43, 2.35 and 2.33 due to three phenolic $OCOCH_3$ protons and δ 2.07 due to four $OCOCH_3$ of the tetracetate sugar moiety. Anomeric protons of a sugar moiety at δ 5.31 in ^{1}H-NMR and δ 98.2 in ^{13}C NMR showed the glycosidic linkage to be β. The assignments are in agreement with luteolin-7-*O*-glucoside, m.p. 254°C. This is the first report of this compound from this plant.

Compound 3: $C_{23}H_{36}O_{12}$, m.p. 179°C gave a positive Wieffering field test, indicating it to be an iridoid. The absence of characteristic bands at 1645 and 1650 cm^{-1} ruled out the possibility of the O–C (3)=C (4) moiety which is usually present in the iridoid. In ^{1}H-NMR, a singlet at δ 3.41 integrating for three protons was assigned to a methoxy group

attached to the iridoid nucleus. Two doublets at δ 4.95 ($J = 6$ Hz) and 4.56 ($J = 8$ Hz) integrating for one proton each were assigned to acetate protons. The chemical shift and coupling constant of a doublet at δ 4.50 indicated it to be a β-glycoside. A broad singlet at δ 5.76 integrating for one proton indicated the presence of a trisubstituted double bond in the cyclopentane ring. Two symmetrical double doublets at δ 6.85 and 7.85 ($J = 8$ Hz) integrating for two protons each were assigned to aromatic protons. This indicated the presence of a monohydroxy benzoyl group in the molecule. In ^{13}C NMR spectrum, a total of 21 signals were recorded in proton and noise decoupled mode. In single frequency off resonance decoupled spectrum, one of the signals appeared as one quartet, 3 signals as triplets, 13 as doublets and 4 as singlets. The IR, ^{1}H-NMR, ^{13}C NMR and mass spectral data were observed to be in total conformity with nishindaside. So, compound **3** was assigned the structure of nishindaside.

Compounds **4** and **5** were also found to be iridoids and their spectral data were in conformity with negundoside and agnuside, respectively. Hence compound **4** was assigned the structure of negundoside and **5** of agnuside.

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