Bioactive Norlignan Glucosides from *Curculigo capitulata*

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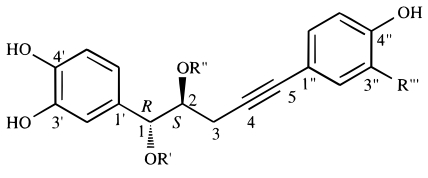
Chemical investigation of *Curculigo capitulata* yielded two new norlignans, (+)-(1*R*,2*S*)- and (−)-(1*S*,2*S*)-1-*O*-butylnyasicosides (**1**, **2**), and the known nyasicoside (**3**) from the BuOH-soluble fraction of the rhizomes. An alternative study devoid of *n*-BuOH yielded two additional novel norlignans, 3″-dehydroxynyasicoside (**4**) and 1-*O*-methylnyasicoside (**5**), in addition to **3**. The pharmacological studies indicated that **1** and **3** possessed potent activity against ouabain-induced arrhythmia in the heart preparations of guinea pig.

Curculigo capitulata (Lour.) O. Kuntze (Amaryllidaceae), alias *Curculigo recurvata*, is widely distributed in southern and southwestern China, Malaysia, India, Australia, and Taiwan. This folk medicine has been used as a tonic and in the treatment of dysmenorrhea and rheumatism.[1] Recent studies have revealed that this plant is rich in a novel acetylenic norlignan, nyasicoside (**3**).[2] This compound contains a (1*R*)-1-hydroxycatechol moiety and might possess biological activity related to (−)-epinephrine, which has a similar skeleton. To evaluate this proposal, we reinvestigated this plant and report here the outcome of this study.

Results and Discussion

The EtOH extract of the rhizome was partitioned into fractions soluble in *n*-hexane, $CHCl_3$, *n*-BuOH, and H_2O. From the *n*-BuOH-soluble fraction, three norlignans, **1**−**3**, were isolated by the combination of Amberlite XAD-2 and low-pressure RP-8 column chromatography. Among them, **3** was identified as nyasicoside by comparing its physical data with those previously reported.[2]



Compd	R′	R″	R‴
1	butyl	β-D-glc	OH
2 (1*S*)	butyl	β-D-glc	OH
3	H	β-D-glc	OH
4	H	β-D-glc	H
5	Me	β-D-glc	OH
6	butyl	H	OH
7 (1*S*)	butyl	H	OH
8	H	H	OH

Compounds **1** and **2** have the same molecular formula $C_{27}H_{34}O_{11}$ (negative FAB $[M - H]^-$ *m*/*z* 533), being 56 amu more than that of **3**. This additional molecular weight corresponds to a butyl group, evidenced in the ^{1}H-NMR spectra of the butoxyl signals at δ 0.88 (t, 3H, J = 7.3 Hz, H-4), 1.37 (m, 2H, H-3), 1.54 (m, 2H, H-2), and 3.35 (m, 2H, H-1), clarified by a COSY-45 spectrum. Except for this difference, both spectra are closely similar to that of **3** (Table 1). For instance, **1** displayed signals for two ABX systems belonging to the aromatic protons, protons of a β-glucosyl moiety ($\delta_{H\text{-}1}$ 4.57, d, J = 7.8 Hz; $\delta_{H\text{-}2}$ 3.30, $\delta_{H\text{-}3}$ 3.40, $\delta_{H\text{-}4}$ 3.35, $\delta_{H\text{-}5}$ 3.40, $\delta_{H\text{-}6}$ 3.64 and 3.82), and four aliphatic protons at δ 4.49 (d, H-1), 4.10 (m, H-2), 2.30 (dd, H-3), and 2.67 (dd, H-3). These assignments were made by analyzing the COSY-45 spectrum, incorporating HMQC data. The placement of 1-*O*-butyl and 2-*O*-β-Glc was made from the observation of the three-bond coupling of H-1 to C-1 of the butyl group, anomeric proton to C-2, and H-2 to the anomeric carbon in the HMBC spectrum (Figure 1). These data and the optical rotation, $[\alpha]^{23}_D$ +29.0° for **1** and −41.0° for **2**, suggested that **1** and **2** are diastereoisomers.

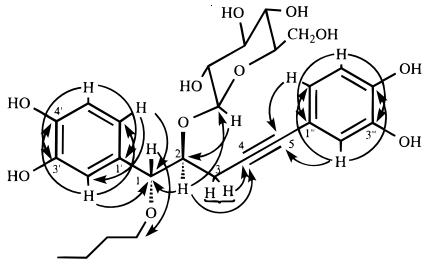


Figure 1. Major HMBC correlations of **1**.

The stereochemistry for **1** and **2** was elucidated by examination of their CD spectra. It has been established that the CD spectrum of (1*R*,2*S*)-nyasicoside (**3**) displays two positive Cotton effects near 250 nm and 280 nm.[3] The CD curve of **1** being almost superimposable to **3** suggested **1** to be (1*R*,2*S*)-1-*O*-butylnyasicoside. Nevertheless, the CD curve of **2** showing two negative Cotten effects might indicate (1*S*,2*S*)-stereochemistry. Enzymatic hydrolysis of **1**−**3** with β-glucosidase in acetate buffer solution (pH = 5.5)[2] gave the corresponding aglycons **6**−**8**, respectively. The superimposable CD curves and similar ^{1}H-NMR data between **6** and **8** (Table 1) supported **6** to be (1*R*,2*S*)-1-*O*-butylnyasicol and confirmed **1** to be 1-*O*-butylnyasicoside. Because the optical rotation ($[\alpha]^{25}_D$ +3.8°, **6**; +35.0°, **7**) and ^{1}H-NMR data of **6** and **7** are different, both compounds are diastereoisomers. However, the CD curve of **7** being a mirror image of **6** would indicate that the chromophore substitution at C-1 chirality plays the dominant role in determining the Cotton effect in the CD spectrum like that observed in flavanones and 3-hydroxyflavanones.[4]

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Table 1. ^{1}H-NMR Data for Compounds **1**−**5** (δ/ppm, J in Hz) in CD_3OD

proton	1	2	3	4	5
1	4.49 d (5.6)	4.49 d (7.3)	4.65 d (7.3)	4.65 d (7.3)	4.35 (d, 6.3)
2	4.10 m[a]	4.05 m	4.01 m	4.01 m	4.10 m
3	2.30 dd (6.1, 17.0)	2.54 dd (4.9, 17.1)	2.30 dd (5.8, 17.2)	2.32 dd (4.5, 17.3)	2.27 dd (5.6, 17.2)
	2.67 dd (5.1, 17.0)	2.74 dd (6.9, 17.1)	2.57 dd (4.5, 17.2)	2.58 dd (5.5, 17.3)	2.66 dd (4.9, 17.2)
2′	6.86 d (1.6)	6.87 d (1.2)	6.90 d (1.7)	6.90 d (1.6)	6.85 d (1.8)
5′	6.74 d (8.0)	6.73 d (8.0)	6.73 d (8.0)	6.74 d (8.1)	6.76 d (8.0)
6′	6.71 dd (1.6, 8.0)	6.72 dd (1.2, 8.0)	6.74 dd (1.7, 8.0)	6.77 dd (1.6, 8.1)	6.75 dd (1.8, 8.0)
2″	6.83 d (1.9)	6.81 d (1.8)	6.81 d (1.9)	7.22 d (8.6)	6.82 d (1.9)
3″				6.69 d (8.6)	
5″	6.67 d (8.2)	6.65 d (8.2)	6.65 d (8.2)	6.69 d (8.6)	6.66 d (8.1)
6″	6.76 dd (8.2, 1.9)	6.75 dd (8.2, 1.9)	6.77 dd (1.9, 8.2)	7.22 d (8.6)	6.72 dd (8.1, 1.9)
1-*O*-butyl					
1	3.35 m	3.35 m			
2	1.54 m	1.50 m			
3	1.37 m	1.37 m			
4	0.88 t (7.3)	0.85 t (7.2)			
1-OMe					3.24 s
2-*O*-Glc					
1	4.57 d (7.7)	4.53 d (7.8)	4.61 d (7.6)	4.61 d (7.6)	4.59 d (7.6)
2	3.30 m	3.30 m	3.30 m	3.30 m	3.30 m
3	3.40 m	3.40 m	3.40 m	3.40 m	3.40 m
4	3.35 m	3.35 m	3.35 m	3.35 m	3.35 m
5	3.40 m	3.40 m	3.40 m	3.40 m	3.40 m
6	3.64 dd (5.4, 11.8)	3.60 dd (5.3, 11.8)	3.68 dd (5.1, 11.8)	3.69 dd (5.0, 11.8)	3.65 dd (5.4, 12.0)
	3.82 dd (2.0, 11.8)	3.79 dd (1.8, 11.8)	3.86 dd (2.0, 11.8)	3.87 dd (1.4, 11.8)	3.86 dd (2.2, 12.0)

[a] Data with multiplicity "m" were overlapped or poorly resolved signals whose chemical shifts were assigned from COSY-45 or HMQC spectra.

Hence, **7** is (1*S*,2*S*)-1-*O*-butylnyasicol, and **2** is (1*S*,2*S*)-1-*O*-butylnyasicoside.

Another extraction and separation procedure avoiding *n*-BuOH was undertaken. The EtOH extract was divided into fractions soluble in $CHCl_3$ and H_2O. Separation of H_2O-soluble fraction via similar methods as indicated above yielded **3** as the major component together with two novel norlignans **4** and **5**. Compounds **1** and **2** were not isolated, nor detected, by RP-8 TLC. Thus, these two compounds may have been artifacts produced by isolation utilizing BuOH, although the exposure of **3** to BuOH at pH 3.5 for 3 days at 50 °C and for one month at 25 °C failed to produce either **1** or **2**.

Compound **4** showed $[M - H]^-$ at m/z 461.1410 (calcd 461.1448) in the negative HRFABMS for a molecular formula $C_{23}H_{26}O_{10}$, being 16 amu fewer than that of **3**. Its ^{1}H-NMR spectrum showed close resemblance to **3** in the aliphatic region but shows one AA′XX′ (δ 6.69 and 7.22, J_{AX} = 8.6 Hz) and one ABX systems in the aromatic region instead of two ABX systems as in **3** (Table 1). These two spectral data indicated **4** to be an analogue of **3** lacking a hydroxy substitution at either C-3′ or C-3″. Further comparison of their NMR data (Tables 1 and 2.) suggested the absence of 3″-OH in **4**. This suggestion and the similarity of its CD curve to **3** established **4** to be 3″-dehydroxynyasicoside.

Compound **5** showed $[M - H]^-$ at m/z 491.1538 (calcd 491.1553) in the negative HRFABMS, suggesting a molecular formula $C_{24}H_{28}O_{11}$, being 14 amu more than that of **3**. Its ^{1}H-NMR spectrum showed close resemblance to **3** except for an additional MeO singlet at δ 3.24 (Table 1), which reflected in the ^{13}C-NMR a signal at δ 57.2 (q) (Table 2). These data and a similar CD curve to that of **3** established **5** to be 1-*O*-methyl nyasicoside.

The ^{1}H-NMR data (Table 1) and ^{13}C-NMR data (Table 2) of compounds **1**−**5** were assigned by using **1**−**3** as model compounds whose assignments were made by analysis of 2D NMR spectra including COSY, HMQC, and HMBC (Figure 1). Among them, C-1′ and C-1″ of **3** were assigned unambiguously at δ 133.3 and 116.05 from their respective coupling to H-5′ (δ 6.73, d) and H-5″ (δ 6.65, d), revising the previous reported data.[2]

Table 2. ^{13}C-NMR Assignments for Compounds **1**−**5** (δ/ppm)[a] in CD_3OD

carbon	1	2	3	4	5
1	83.9 d	83.9 d	76.4 d	76.5 d	85.9 d
2	80.0 d	81.4 d	82.5 d	82.6 d	79.7 d
3	22.5 t	21.8 t	22.7 t	22.8 t	22.4 t
4	84.8 s	85.2 s	84.4 s	84.9 s	84.5 s
5	83.6 s	83.4 s	83.7 s	83.5 s	83.8 s
1′	131.4 s	131.6 s	133.3 s	133.3 s	130.6 s
2′	116.2 d	116.1 d	115.6 d	116.1 d	116.06 d
3′	146.0 s	145.9 s	146.1 s	146.1 s	146.0 s
4′	146.2 s	146.0 s	146.2 s	146.2 s	146.4 s
5′	115.9 d	115.8 d	116.08 d	115.6 d	116.04 d
6′	120.7 d	120.7 d	120.1 d	120.1 d	120.9 d
1″	116.1 s	116.2 s	116.05 s	115.8 s	116.1 s
2″	119.5 d	119.5 d	119.5 d	134.0 d	119.5 d
3″	146.1 s	146.0 s	145.9 s	116.2 d	146.3 s
4″	146.9 s	146.9 s	146.8 s	158.4 s	146.9 s
5″	116.2 d	116.2 d	116.2 d	116.2 d	116.2 d
6″	124.9 d	124.9 d	124.9 d	134.0 d	124.9 d
1-*O*-butyl					
1	70.2 t	70.1 t			
2	32.9 t	33.0 t			
3	20.4 t	20.5 t			
4	14.2 q	14.3 q			
1-OMe					57.2 q
2-*O*-Glc					
1	103.2 d	103.8 d	103.2 d	103.2 d	102.8 d
2	74.9 d	74.9 d	74.9 d	74.9 d	74.9 d
3	77.7 d	77.8 d	77.7 d	77.8 d	77.7 d
4	71.6 d	71.6 d	71.4 d	71.4 d	71.6 d
5	78.1 d	78.9 d	78.0 d	78.1 d	78.1 d
6	62.8 t	62.8 t	62.5 t	62.5 t	62.7 t

[a] Multiplicities were obtained from DEPT experiments.

Pharmacological study of **1**−**5** on rat cardiac tissues indicated that they had no apparent effect on both contractile tension and heart rate at the dose range of 0.3 to 30 μM (Table 3). Nevertheless, further study indicated these compounds were effective to a certain extent against the arrhythmic effect induced by ouabain

Table 3. Effects of Compounds **1**−**5** on Contractile Tension and Heart Rate of Rat Cardiac Tissues[a]

assay item	concn (μM)	compound 1	2	3	4	5
heart rate (RA)	0.3	100	100	100	100	
	1	100	99.0 ± 1.0[b]	98.8 ± 1.3[b]	97.5 ± 2.5	100
	3	99.0 ± 1.0[b]	98.4 ± 1.0[c]	97.4 ± 2.7	97.5 ± 2.5	103.0 ± 1.5
	10	99.8 ± 1.4	98.4 ± 1.0[c]	98.5 ± 3.0	98.5 ± 3.0	103.0 ± 1.5
	30	97.6 ± 3.3	97.4 ± 1.1[d]	98.5 ± 3.0	98.5 ± 3.0	103.0 ± 1.5
tension (RA)	0.3	94.0 ± 4.2	95.5 ± 2.7[c]	96.8 ± 2.0[c]		
	1	91.0 ± 3.2[d]	94.3 ± 6.6	94.5 ± 4.0[b]	97.5 ± 2.5	99.0 ± 3.8
	3	89.6 ± 3.2[d]	91.6 ± 5.9[b]	92.0 ± 2.8[d]	98.3 ± 1.8	99.0 ± 3.8
	10	86.2 ± 3.2[d]	87.8 ± 7.5[c]	83.3 ± 8.6[c]	94.5 ± 3.4	100.3 ± 4.2
	30	83.6 ± 3.4[d]	86.4 ± 8.4[c]	78.0 ± 9.0[d]	94.8 ± 5.3	101.7 ± 4.9
tension (LA)	0.3	98.8 ± 1.3	98.8 ± 2.8	108.3 ± 8.3		
	1	96.0 ± 2.4[c]	95.3 ± 6.8	105.8 ± 5.9	106.8 ± 6.8	103.0 ± 1.7
	3	91.2 ± 4.0[d]	101.0 ± 10.6	104.5 ± 8.7	111.5 ± 11.5	101.3 ± 4.7
	10	89.4 ± 6.4[c]	105.0 ± 13.8	100.3 ± 10.2	119.3 ± 19.3	102.3 ± 14.8
	30	92.5 ± 7.5	103.8 ± 13.1	98.0 ± 14.3	116.3 ± 22.9	89.7 ± 30.8
tension (RV)	0.3	99.0 ± 1.0	102.0 ± 1.2[c]	92.3 ± 4.8[c]		
	1	95.8 ± 3.1[b]	100.0 ± 2.9	84.8 ± 6.7[d]	98.4 ± 1.0	106.7 ± 4.1
	3	96.8 ± 3.1	100.4 ± 3.3	83.6 ± 8.0[d]	99.2 ± 2.7	106.3 ± 9.0
	10	95.4 ± 6.4	96.8 ± 3.7	78.2 ± 8.8[d]	95.2 ± 3.4	104.3 ± 9.8
	30	88.2 ± 8.5[b]	92.2 ± 4.5[c]	74.6 ± 9.4[d]	94.2 ± 6.0	99.3 ± 4.8

* Contractions of left atrial (LA) and right ventricular (RV) strips were elicited by electrical stimulations driven at 2 Hz. Spontaneous contraction of right atria (RA) and heart rate were measured. Mean values (% of control: heart rate 242 ± 7 beats/min; twitch tension RA 121 ± 10 mg, LA 140 ± 1 mg, RV 225 ± 17 mg) were obtained from four to five experiments, [b]$p < 0.05$, [c]$p < 0.01$, [d]$p < 0.001$, compared with the respective control by Student's *t*-test.

Table 4. Effects of Compounds **1**−**5** Against Ouabain-Induced Arrhythmia in Guinea Pig's Heart Preparations[a]

cardiac tissues	concn. (μM)	compound 1	2	3	4	5
RA	1	±			−	+
	3	±		±	−	+
	10		−	±	−	
	30		+		−	
LA	1	±	±	−	±	±
	3	++	+	++	±	−
	10					
	30					
RV	1	−	−	−	−	−
	3	±	−	−	−	±
	10		−	±	−	
	30				−	

[a] Contractions of left atrial (LA) and right ventricular (RV) strips were elicited by electrical stimulations dirven at 2 Hz. Spontaneous contraction of right atria (RA) and heart rate were measured. Mean values of control are as follows: heart rate 186 ± 6 beats/ min; twitch tension RA 336 ± 42 mg, LA 381 ± 48 mg, and RV 317 ± 49 mg. "−" denotes ineffective in reversion of cardiac arrhythmia. "±" and "+" denote partially effective in some or most of the preparations ($n \geq 3$), respectively. "++" denotes that the reversion of cardiac arrhythmia to normal rhythm can sustain for more than 10 min.

(0.6 μM) in guinea pig's heart preparations (Table 4). Of these, compounds **1** and **3** are most potent. In left atria, these two compounds can normalize completely the ouabain-induced arrhythmia at dose of 3 μM. Quinidine, the commonly used antiarrhythmic drug, exhibits similar activity at dose of 30−60 μM.[5] The mechanism of the antiarrhythmic effect of these compounds remains to be clarified.

Experimental Section

General Experimental Procedures. The physical data of the isolated compounds were obtained from the following instruments: Perkin-Elmer 1760-X IR-FT spectrometer; Hitachi 150-20 UV; JASCO J-710 spectropolarimeter; JEOL JMX-HX110 mass spectrometer (FAB); Bruker AMX-400 spectrometer using solvent peak (MeOH-d_4) as reference standard, 2D NMR spectra were recorded by using Bruker's standard pulse program in the HMQC and HMBC experiments, $\Delta = 1$ s and $J = 140$ Hz and 8 Hz, respectively, the correlation maps consisted of 512 × 1K data points per spectrum, each composed of 16 to 64 transients.

Plant Material. The rhizomes of *C. capitulata* (Lour.) O. Kuntze were collected in November 1993, in the suburban mountain of Taipei, Taiwan. A specimen was authenticated by Prof. C.-F. Hsien, Department of Botany, National Taiwan University. A voucher herbarium specimen is deposited at the School of Pharmacy, National Taiwan University.

Extraction and Isolation. The dried powder of the rhizome (5.10 kg) was extracted with 95% EtOH (7 L × 5). Concentration of the EtOH extract gave a residue (678 g) that was soluble in aqueous 80% MeOH solution (770 mL), and then partitioned with *n*-hexane (1 L × 3) to give the *n*-hexane-soluble fraction (33 g). The aqueous 80% MeOH layer was evaporated to remove residual MeOH, and then distilled H_2O (300 mL) was added. This aqueous solution was partitioned with $CHCl_3$ (1 L × 3) and *n*-BuOH (800 mL × 5) to get a $CHCl_3$-soluble (13 g) and an *n*-BuOH-soluble fraction (620 g). Part of the *n*-BuOH-soluble fraction (10.39 g) was passed through an Amberlite XAD-2 column (200 g) eluted in order with 30%, 50% MeOH in H_2O, and MeOH to give a 30% MeOH fraction (2.72 g), a 50% MeOH fraction (1.96 g), and a MeOH fraction (0.81 g). The MeOH fraction was then separated by repeated lobar RP-8 column (E. Merck, B type) with MeOH−H_2O (56:44) and MeOH−H_2O (54:46) as eluent to afford **1** (28 mg) and **2** (20 mg). Similarly, the 30% MeOH fraction (2.10 g) yielded **3** (280 mg) eluted with MeOH−H_2O (3:7).

The dried powder of the rhizome (1.02 kg) of the second crop of plant materials, collected in the same place in January 1995, was percolated with 95% EtOH

(7 L × 5). The EtOH extract (110 g) was partitioned between $CHCl_3$ (1 L × 3) and H_2O (1 L) to give a $CHCl_3$-soluble fraction (8 g). The aqueous layer after removal of residual $CHCl_3$ via condensation was passed through an Amberlite XAD-2 column (800 g, × 5) to get a 30% MeOH fraction (35 g), a 50% MeOH fraction (3.6 g), and a MeOH fraction (0.98 g). Further separation by a lobar RP-8 column (B type) eluted with MeOH−H_2O (3:7) yielded **3** (2.04 g, 1.41% w/w), **4** (111 mg), and **5** (47 mg) from a 4.96-g portion of the 30% MeOH fraction.

(+)-1-*O*-Butylnyasicoside (1): amorphous powder; $[\alpha]^{23}_D$ +19.0° (*c*, 1.0, MeOH); IR (KBr) ν max: 3400, 2960, 2940, 2870, 1600, 1520, 1440, 1360, 1280, 1080, 870, 815, 780 cm^{-1}; UV (MeOH) λ max (log ϵ) 257 (4.39), 288 (4.07) nm; CD (MeOH) $[\theta]_{308}$ 0°, $[\theta]_{289}$ +8380°, $[\theta]_{270}$ +6130°, $[\theta]_{249}$ +20 340°, $[\theta]_{233}$ +3820°, $[\theta]_{211}$ +53 120°; ^{1}H-NMR and ^{13}C-NMR spectral data, see Table 1 and Table 2; FABMS (neg) *m/z* $[M - H + TG]^-$ 641 (50), $[M - H]^-$ 533 (100), 459 (13), 409 (19), 279 (56).

(−)-1-*O*-Butylnyasicoside (2): amorphous powder; $[\alpha]^{23}_D$ −41.0° (*c* 1.0, MeOH); IR (KBr) ν max 3400, 2960, 2940, 2870, 1600, 1520, 1445, 1360, 1285, 1070, 870, 820, 780 cm^{-1}; UV (MeOH) λ max (log ϵ) 257 (4.20), 285 (3.98) nm; CD (MeOH) $[\theta]_{310}$ −400°, $[\theta]_{287}$ −5390°, $[\theta]_{270}$ −4250°, $[\theta]_{258}$ −10 720°, $[\theta]_{250}$ −12 480°, $[\theta]_{233}$ −3980°; ^{1}H-NMR and ^{13}C-NMR spectral data, see Table 1 and Table 2; FABMS (neg) *m/z* $[M - H + TG]^-$ 641 (34), $[M - H]^-$ 533 (79), 389 (100), 279 (50).

Nyasicoside (3): CD (MeOH); $[\theta]_{337}$ 0°, $[\theta]_{327}$ +210°, $[\theta]_{304}$ +2020°, $[\theta]_{300}$ +2180°, $[\theta]_{285}$ +5100°, $[\theta]_{270}$ +4040°, $[\theta]_{252}$ +11 420°, $[\theta]_{231}$ +1960°, $[\theta]_{210}$ + 31 570°; ^{1}H-NMR and ^{13}C-NMR spectral data, see Table 1 and Table 2; HMBC data, H-2 to C-1, C-4, and Glc C-1, H-3 to C-4, H-2′ to C-1, C-4′, C-6′, H-5′ to C-1′, C-3′, H-6′ to C-1 and C-2′, H-2″ to C-5, C-4″, and C-6″, H-5″ to C-1″ and C-3″, H-6″ to C-5, Glc H-1 to C-2, Glc H-3 to Glc C-2 and Glc C-4, Glc H-5 to Glc C-1 and Glc C-3.

3″-Dehydroxynyasicoside (4): amorphous powder; $[\alpha]^{23}_D$ −2.0° (*c* 1.0, MeOH); UV (MeOH) λ max (log ϵ) 255 (4.18), 282 (3.80) nm; $[\alpha]^{23}_D$ −2.0° (*c* 1.0, MeOH); IR (KBr) ν max 3400 (br m, OH), 2950, 1605, 1515, 1450, 1360, 1290, 1085, 1035, 840, 820 cm^{-1}; CD (MeOH) $[\theta]_{340}$ −200°, $[\theta]_{322}$ +150°, $[\theta]_{311}$ −310°, $[\theta]_{302}$ +230°, $[\theta]_{300}$ +220°, $[\theta]_{283}$ +3970°, $[\theta]_{268}$ +2540°, $[\theta]_{243}$ +10 430°, $[\theta]_{226}$ −850°; $[\theta]_{215}$ +3220°, $[\theta]_{207}$ +15 280°; ^{1}H-NMR and ^{13}C-NMR spectral data, see Table 1 and Table 2; FABMS (neg) *m/z* $[M - H]^-$ 461 (24), 443 (8), 371 (21), 331 (13), 297 (17), 281 (42), 263 (45), 249 (16), 205 (17), 183 (100), 181 (18), 150 (16), 137 (21).

1-*O*-Methylnyasicoside (5): amorphous powder; $[\alpha]^{23}_D$ +22.0° (*c* 1.0, MeOH); IR (KBr) ν max 3400 (br m, OH), 2940, 1603, 1520, 1445, 1360, 1285, 1070, 820, 780 cm^{-1}; UV (MeOH) λ max (log ϵ) 257 (4.25), 286 (3.92) nm; CD (MeOH) $[\theta]_{329}$ 0°, $[\theta]_{322}$ +250°, $[\theta]_{314}$ 0°, $[\theta]_{301}$ +2110°, $[\theta]_{285}$ +5300°, $[\theta]_{272}$ +4030, $[\theta]_{251}$ +12 030°, $[\theta]_{231}$ +2550°, $[\theta]_{210}$ +32 510°; ^{1}H-NMR and ^{13}C-NMR spectra data, see Table 1 and Table 2; FABMS (neg) *m/z* $[M - H]^-$ 491 (100), 470 (15), 459 (25), 390 (22), 327 (48), 297 (21), 279 (79), 255 (24), 243 (22).

Hydrolysis of Norlignans. (+)-1-*O*-Butylnyasicol (6). β-Glucosidase (15 mg) was added to the solution (7.5 mL) of **1** (40 mg) in acetate buffer (pH 5.5).[2] The solution was maintained at 37 °C for 4 days and extracted with EtOAc (40 mL × 3). The combined organic layer was dried over Na_2SO_4 and evaporated, and the residue was separated by low pressure column (RP-8) [MeOH−H_2O (30:70)] to give amorphous **6** (2 mg): $[\alpha]^{25}_D$ +3.8° (*c* 0.2, MeOH); UV (MeOH) λ max (log ϵ) 257 (4.17), 286 (3.92) nm; CD (MeOH) $[\theta]_{328}$ 0°, $[\theta]_{319}$ +318°, $[\theta]_{312}$ 0°, $[\theta]_{302}$ +2310°, $[\theta]_{284}$ +6160°, $[\theta]_{271}$ +4750°, $[\theta]_{250}$ +15 360°, $[\theta]_{231}$ +3650°, $[\theta]_{211}$ +37 280°; ^{1}H-NMR (MeOH-d_4) δ 6.80 (2H, d, J = 1.9 Hz, H-2′ and 2″), 6.74 (1H, d, J = 8.1 Hz, H-5′), 6.74 (1H, dd, J = 1.9, 8.1 Hz, H-6″), 6.69 (1H, dd, J = 1.9, 8.1 Hz, H-6′), 6.66 (1H, d, J = 8.1 Hz, H-5″), 4.20 (1H, d, J = 6.5 Hz, H-1), 3.75 (1H, m, H-2), 3.35 (2H, m, H-1 of *O*-Bu), 2.50 (1H, dd, J = 5.1, 16.8 Hz, H-3), 2.24 (1H, dd, J = 5.9, 16.8 Hz, H-3), 1.55 (2H, m, H-2 of *O*-Bu), 1.37 (2H, m, H-3 of *O*-Bu), 0.87 (3H, t, J = 7.4 Hz, H-4 of *O*-Bu); FABMS (neg) *m/z* $[M - H]^-$ 371.

(−)-1-*O*-Butylnyasicol (7). Using the same conditions as in the preparation of **6**, **7** (1 mg) was obtained from hydrolysis of **2** (38 mg). The physical data of **7** are as follows: $[\alpha]^{25}_D$ +35.0° (*c* 0.1, MeOH); UV (MeOH) λ max (log ϵ) 257.0 (4.16), 284.6 (3.92) nm; CD (MeOH) $[\theta]_{329}$ 0°, $[\theta]_{323}$ +310°, $[\theta]_{313}$ 0°, $[\theta]_{288}$ −3850°, $[\theta]_{270}$ 0°, $[\theta]_{249}$ −9340°, $[\theta]_{232}$ −780°; ^{1}H-NMR (MeOH-d_4) δ 6.82 (1H, d, J = 1.9 Hz, H-2′), 6.81 (1H, d, J = 1.9 Hz, H-2″), 6.745 (1H, dd, J = 1.9, 8.1 Hz, H-6″), 6.74 (1H, d, J = 8.1 Hz, H-5′), 6.68 (1H, dd, J = 1.9, 8.1 Hz, H-6′), 6.65 (1H, d, J = 8.1 Hz, H-5″), 4.12 (1H, d, J = 6.3 Hz, H-1), 3.80 (1H, m, H-2), 3.35 (2H, m, H-1 of *O*-Bu), 2.60 (2H, d, J = 6.0 Hz, H-3), 1.52 (2H, m, H-2 of *O*-Bu), 1.38 (2H, m, H-3 of *O*-Bu), 0.88 (3H, t, J = 7.4 Hz, H-4 of *O*-Bu); FABMS (neg) *m/z* $[M - H]^-$ 371.

Nyasicol (8). Using the same conditions as in the preparation of **6**, **8** (9 mg) was obtained from hydrolysis of **3** (40 mg). The physical data of **8** are as follows: $[\alpha]^{25}_D$ +36.0° (*c* 1.0, MeOH); UV (MeOH) λ max (log ϵ) 257.8 (4.33), 283.8 (4.16) nm; CD (MeOH) $[\theta]_{320}$ +270°, $[\theta]_{313}$ 0°, $[\theta]_{302}$ +2190°, $[\theta]_{284}$ +6100°, $[\theta]_{270}$ +4860°, $[\theta]_{253}$ +14 510°, $[\theta]_{233}$ +3160°, $[\theta]_{211}$ +36 820°; ^{1}H-NMR (MeOH-d_4) δ 2.26 (1H, dd, J = 6.3, 17.0 Hz, H-3), 2.48 (1H, dd, J = 4.9, 17.0 Hz, H-3), 3.75 (1H, dt, J = 5.1, 6.6 Hz, H-2), 4.52 (1H, d, J = 6.6 Hz, H-1), 6.65 (1H, d, J = 8.1 Hz, H-5″), 6.75 (1H, dd, J = 1.9, 8.1 Hz, H-6″ or 6′), 6.80 (1H, d, J = 1.9 Hz, H-2″), 6.74 (1H, dd, J = 1.9, 8.1 Hz, H-6′ or 6″), 6.72 (1H, d, J = 8.1 Hz, H-5′), 6.86 (1H, d, J = 1.9 Hz, H-2′); FABMS (neg) *m/z* $[M - H]^-$ 315.

Assay on Contractions and Spontaneously Beating Heart Rate of Rat Cardiac Tissues. Right atrial, left atrial, and right ventricular strips (4 × 6 mm) were dissected from the hearts of male WKY rats (weighing 250−300 g) and placed in an organ bath containing 10 mL of Tyrode solution gassed with 95% O_2 and 5% CO_2. Contractions of electrically driven left atrial and right ventricular strips and heart rate in spontaneously beating right atria were measured by the method described previously.[6]

Evaulation of Antiarrhythmic Activity on Guinea Pig Heart. Cardiac arrhythmia of electrically driven left atrial or right ventricular strips of guinea pigs were induced by ouabain (0.6 μM). The antiarrhythmic activity of compounds **1**−**5** was tested after arrhythmia was induced.[7]

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