Nepetacilicioside, a New Iridoid Glucoside from *Nepeta cilicia*

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Received October 30, 1995[⊗]

A new iridoid glucoside, nepetacilicioside (**1**), was isolated from the aerial parts of *Nepeta cilicia* together with the known compound velpetin. The structure of the new compound was elucidated on the basis of spectroscopic and chemical evidence.

Several iridoid glucosides having an unusual stereochemistry—such as (1*R*,5*R*,8*S*,9*S*)-deoxyloganic acid,[1] velpetin,[2] and nepetanudosides A−D[3,4]—have been isolated from plants belonging to the genus *Nepeta* (Labiatae). These iridoid glucosides are featured in the enantiomeric stereochemistry in the aglycon portion compared to that of the usual iridoid glycosides.[5] In continuation of phytochemical studies on the glycosidic constituents of the genus *Nepeta*, we investigated the constituents of *Nepeta cilicia* Boiss. ex Benth. and isolated a new iridoid glucoside, nepetacilicioside (**1**), together with the known compound velpetin.[2] This paper deals with the isolation and structure elucidation of the new compound.

Nepetacilicioside (**1**) was isolated as an amorphous powder from the MeOH extract of the aerial parts of *N. cilicia*. On the basis of its high-resolution negative ion FABMS, the molecular formula was determined as $C_{16}H_{22}O_9$. Spectral data showed the presence of an aldehyde group conjugated with a double bond [UV λ_{max} (ϵ) 250 (11 005); IR ν_{max} 1660 and 1627 cm^{-1}; ^{1}H NMR δ 9.23 (1H, s) (H-11) and 7.36 (1H, s) (H-3); ^{13}C NMR δ 193.74 (d), 165.00 (d) and 123.06 (s) (Table 1)], an acetalic group [δ_H 5.49 (1H, d, $J = 3.4$ Hz) (H-1); δ_C 101.72 (d)], a trisubstituted double bond [δ_H 5.50 (1H, br s) (H-7); δ_C 130.63 (d) and 144.00 (s)], a methyl group at a double bond [δ_H 1.87 (3H, d, $J = 1.0$ Hz) (H-10); δ_C 15.47 (q)], and a secondary hydroxyl group [δ_H 4.45 (1H, br s) (H-6); δ_C 80.56 (d)] in addition to the presence of a β-glucopyranosyl moiety. The ^{13}C-NMR spectrum further showed the presence of two methine groups (Table 1). Thus, nepetacilicioside was presumed to be an iridoid glucoside having an aldehyde group at C-4. The overall structure **1** was elucidated for nepetacilicioside by analysis of the ^{1}H−^{1}H COSY spectrum. By following the cross peaks from H-1 to δ 3.31 (H-9), 2.98 (H-5), H-6, H-7, and H-10, the connectivities C-1 → C-9 → C-5 → C-6 → C-7 → C-8 → C-10 were established. The connectivity of C-11 → C-4 → C-3 was also observed in the spectrum by the allylic coupling between H-11 and H-3. The relative stereochemistry was then elucidated by the NOE correlations (Figure 1) detected by differential NOE experiments. The absolute stereochemistry was inferred as shown by the similar CD spectrum [$\Delta\epsilon_{251}$ −10.7] with that [$\Delta\epsilon_{252}$ −14.1] of nepetanudoside C.[4] Acetylation of nepetacilicioside (**1**) gave the pentaacetate **2**, $C_{26}H_{32}O_{14}$. The acetate was hydrogenated over Pd-C to give compound **3**, which was identical with the hydrogenation product of nepetanudoside C tetraacetate (**4**),[4] having a known absolute stereochemistry. Thus, the structure of nepetacilicioside, including absolute stereochemistry, was unequivocally established as **1**.

Table 1. ^{13}C-NMR Data[a] for Nepetacilicioside (**1**)

carbon no.		carbon no.	
1	101.72	1′	104.59
3	165.00	2′	75.07
4	123.06	3′	78.42[b]
5	41.92	4′	71.12
6	80.56	5′	77.83[b]
7	130.63	6′	62.62
8	144.00		
9	49.78		
10	15.47		
11	193.74		

[a] Measured in CD_3OD at 100 MHz. [b] Values may be interchanged.

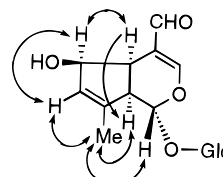

Figure 1. NOE correlation for nepetacilicioside (**1**) detected by differential NOE experiments.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: ^{1}H- and ^{13}C-NMR spectra, JEOL JNM EX-400 FT-NMR spectrometer (400 and 100 MHz, respectively), with TMS as an internal standard; specific rotation, JASCO DIP-360 digital polarimeter; HRFABMS; JEOL JMS SX-102 mass spectrometer with PEG-400 as a matrix; UV

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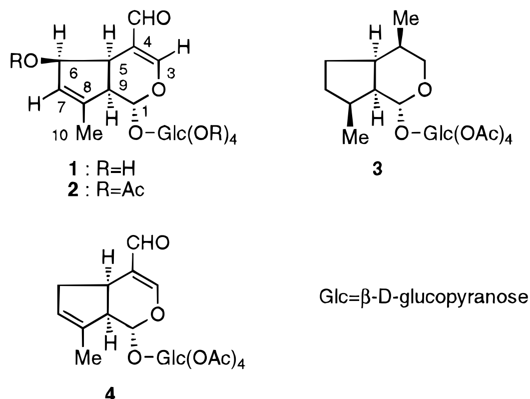
⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0163-3864(96)00334-5 CCC: $12.00 © 1996 American Chemical Society and American Society of Pharmacognosy



spectra, JASCO V-520 UV/vis spectrophotometer; IR, Shimadzu IR-400 spectrophotometer or Perkin-Elmer 1720 infrared FT spectrophotometer; and CD spectra, JASCO DP-720 spectrophotometer. The following experimental conditions were used for chromatography: column chromatography, Si gel 60 (230−400 mesh, Merck); TLC and preparative TLC, precoated Si gel plate 60 F_{254} (0.25 and 0.5 mm in thickness, respectively); and HPLC, column Cosmosil 10 C_{18} (20 mm i.d. × 250 mm), detection 230 nm, solvent MeOH-H_2O 3:7, 6 mL·min^{-1}.

Plant Material. Plant material was collected in the suburbs of Kahrman Maras, Turkey, on May 31, 1990, and identified as *N. cilicia* Boiss. ex Benth. by the authors (G.H. and E.S.). Voucher specimens (90 C 028) are deposited in the Herbaria of the Faculty of Pharmaceutical Sciences, Kyoto University, and at the Faculty of Pharmacy, Gazi University.

Isolation. The dried aerial parts (255 g) of *N. cilicia* were extracted (2×) with MeOH (3 L) at room temperature for 3 weeks. The combined MeOH extract was concentrated *in vacuo*. The residue was dissolved in 90% MeOH (400 mL), and the solution was washed with *n*-hexane (400 mL × 3). The 90% MeOH layer was concentrated *in vacuo*, the resultant residue suspended in H_2O (300 mL), and the suspension extracted with EtOAc (300 mL × 3). The EtOAc extract was dried and evaporated *in vacuo* to give a residue (2.64 g). The aqueous layer was extracted with *n*-BuOH (300 mL × 3). The *n*-BuOH extract was evaporated *in vacuo* to give a residue (1.33 g) that was chromatographed on Si gel (100 g). $CHCl_3$ (400 mL), $CHCl_3$−MeOH (19:1, 300 mL), $CHCl_3$−MeOH (9:1, 300 mL), $CHCl_3$−MeOH (17:3, 300 mL), and $CHCl_3$−MeOH (4:1, 500 mL) were passed successively through the column. After 1 L of eluate was collected, 10-mL fractions were collected. Fractions 20−28 gave a residue (76.4 mg) on evaporation, which was purified by HPLC to give nepetacilicioside (**1**) (27.0 mg) as an amorphous powder. Fractions 32−41 gave a residue (91.6 mg) that was purified by HPLC to give velpetin (21.9 mg). Velpetin was identified by comparison of the spectral data with those reported.[2]

Nepetacilicoside (1): $[\alpha]^{25.5}_{D}$ + 72.4° (*c* 0.98, MeOH); UV (MeOH) λ_{max} (ϵ) 250 (11 005): IR (KBr) ν_{max} 3350, 1660, and 1627 cm^{-1}; ^1H NMR (CD_3OD) δ 1.87 (3H, d, J = 1.0 Hz, Me-10), 2.98 (1H, dd, J = 7.3, 2.0 Hz, H-5), 3.23 (1H, dd, J = 7.8, 7.8 Hz, H-2′), ca 3.31 (H-9), 3.67 (1H, dd, J = 11.7, 3.4 Hz, H-6′), 3.85 (1H, br d, J = 11.7 Hz, H-6′), 4.45 (1H, br s, H-6), 4.61 (1H, d, J = 7.8 Hz, H-1′), 5.49 (1H, d, J = 3.4 Hz, H-1), 5.50 (1H, br s, H-7), 7.36 (1H, s, H-3) and 9.23 (1H, s, H-11); ^{13}C NMR: see Table 1; CD $\Delta\epsilon_{251}$ −10.7 (MeOH, 3.58 × 10^{-5} M); negative ion HRFABMS m/z 357.1192 [M − H]$^-$, $C_{16}H_{21}O_9$ requires 357.1185.

Nepetacilicioside Pentaacetate (2). Nepetacilicioside (**1**) (12.2 mg) was dissolved in a mixture of Ac_2O (0.1 mL) and pyridine (0.1 mL), and the solution was left at 4 °C for 18 h. After addition of excess MeOH, the solvent was removed *in vacuo*, and the residue was purified by preparative TLC (solvent: Et_2O) to give the pentaacetate (**2**) (18.7 mg), which was crystallized on addition of EtOH: mp 120−121 °C; IR ($CHCl_3$) ν_{max} 1750, 1680, 1640, 1230, 1070, 1040, and 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.83 (3H, br s, Me-10), 2.02 (3H), 2.05 (6H), 2.08 (3H), 2.10 (3H) (each s, 5 × OAc), 3.00 (1H, brt, J = 7.3 Hz, H-9), 3.23 (1H, dd, J = 7.3, 4.3 Hz, H-5), 3.77 (1H, m, H-5′), 4.22 (2H, m, H-6′), 4.87 (1H, d, J = 8.3 Hz, H-1′), 5.05 (1H, dd, J = 9.6, 8.3 Hz, H-2′), 5.12 (1H, dd, J = 9.6, 9.6 Hz, H-3′), 5.16 (1H, d, J = 7.3 Hz, H-1), 5.21 (1H, dd, J = 9.6, 9.6 Hz, H-4′), 5.45 (1H, m, H-7), 5.65 (1H, m, H-6), 7.13 (1H, s, H-3), and 9.30 (1H, s, H-11); negative ion HRFABMS m/z 567.1723 [M − H]$^-$, $C_{26}H_{31}O_{14}$ requires 567.1714.

Catalytic Hydrogenation of Nepetacilicioside Pentaacetate (2). Nepetacilicioside pentaacetate (**2**) (18.5 mg) was dissolved in MeOH (3 mL), and 5% Pd−C (18.5 mg) was added. The mixture was hydrogenated for 1 h at room temperature. After the catalyst was removed, the filtrate was concentrated *in vacuo* to give a residue that was purified by Si gel (7 g) chromatography with Et_2O as eluent, collecting 4 mL fractions. Fractions 6−8 gave compound **3** (4.7 mg) as colorless needles: mp 123−125 °C; IR ($CHCl_3$) δ_{max} 1750 and 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (3H, d, J = 6.8 Hz, Me-11), 0.96 (3H, d, J = 6.4 Hz, Me-10), 1.17 (1H, m, H-7), 1.36 (1H, dd, J = 10.5, 6.4 Hz, H-9), 1.43 (1H, m, H-4), 1.51 (1H, m, H-6), 1.68 (1H, m, H-6), 1.81 (1H, m, H-5) 1.90 (1H, m, H-7), 1.94 (1H, m, H-8), 2.01 (3H), 2.03 (6H), 2.08 (3H) (each s, 4 × OAc), 3.34 (1H, dd, J = 11.7, 4.6 Hz, H-3), 3.55 (1H, dd, J = 11.7, 11.7 Hz, H-3), 3.77 (1H, m, H-5′), 4.12 (1H, dd, J = 12.2, 2.2 Hz, H-6′), 4.28 (1H, dd, J = 12.2, 4.8 Hz, H-6′), 4.71 (1H, d, J = 7.8 Hz, H-1′), 4.86 (1H, br s, H-1), 5.03 (1H, dd, J = 9.8, 7.8 Hz, H-2′), 5.09 (1H, dd, J = 9.8, 9.8 Hz, H-4′), and 5.22 (1H, dd, J = 9.8, 9.8 Hz, H-3′); negative ion HRFABMS m/z 499.2180 [M − H]$^-$, $C_{24}H_{35}O_{11}$ requires 499.2179. This compound was identified with the sample derived from nepetanudoside C tetraacetate (**4**)[4] by mixed melting point determination and comparisons of IR and ^1H NMR spectra.

Acknowledgment. The authors thank the Cooperative Center of the University of Tokushima for NMR spectra. This work was supported in part by a grant (No. 02041048) from the Ministry of Education, Science, Sports and Culture, Japan.

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NP960334V