Nepetaracemosides A and B, Iridoid Glucosides from *Nepeta racemosa*

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From the aerial parts of *Nepeta racemosa*, two new iridoid glucosides, nepetaracemosides A and B, which are related to nepetalactone and dehydronepetalactone, were isolated. The structures of the new compounds were elucidated by spectral and chemical analyses.

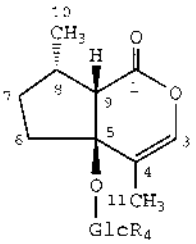
Key words *Nepeta racemosa*; Labiatae, nepetaracemoside A, nepetaracemoside B; iridoid glucoside

Several iridoid glucosides having novel stereochemistry[1—5] and monoterpene lactones, such as nepetalactone,[6—8] have been isolated from the plants of genus *Nepeta* (Labiatae). In continuation of studies on the constituents of plants used in Turkish traditional medicine and related practices, we examined the glycosidic constituents of *Nepeta racemosa*, collected in East Anatolia, Turkey and isolated two new glucosides, nepetaracemosides A (**1**) and B (**3**), which are related to nepetalactone and dehydronepetalactone. This paper deals with the isolation and structural elucidation of these new compounds.

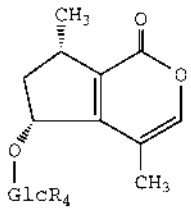
The methanolic extracts of the aerial parts of *N. racemosa* were fractionated as described in the Experimental section. From the *n*-BuOH soluble fraction, nepetaracemosides A (**1**) and B (**3**) were isolated as amorphous powders.

Nepetaracemoside A (**1**), $[\alpha]_D$ +27.8°, was formulated as $C_{16}H_{24}O_8$ based on its positive-ion high-resolution (HR) FAB-MS. The ^{1}H-NMR spectrum showed the presence of a secondary methyl group [δ 0.91 (3H, d, J=7.3 Hz)], a methyl group on a double bond [δ 1.67 (3H, d, J=1.5 Hz)] and a proton on a double bond [δ 6.66 (1H, d, J=1.5 Hz)] which were long range-coupled with the vinylic methyl group. Besides the signals due to the β-glucopyranosyl moiety, the ^{13}C-NMR spectrum showed signals due to two methyl groups, two methylene groups, two methine groups, an oxygenated quaternary carbon atom (δ 85.2), a lactonic carbon (δ 172.6) and a trisubstituted double bond (Table 1). The planar structure was deduced from analysis of the ^{1}H–^{1}H correlated spectroscopy (COSY) spectrum. Starting from δ 3.39 (1H, d, J=11.4 Hz, H-9), cross peaks were followed to δ 2.76 (H-8), 1.15 (H_1-7), and 2.24 (H_1-6), successively. The cross peaks between H-8 and a methyl signal at δ 0.91 (3H, d, J=7.3 Hz) and between H-3 (δ 6.66) and a vinylic methyl group (δ 1.67) were also observed. Thus, a planar structure **1** was deduced for nepetaracemoside A. The presumption was supported by the results of ^{1}H–^{13}C long range COSY spectrum (J=8 Hz), which are shown in Fig. 1. The relative stereochemistry was then examined by differential nuclear Overhauser enhancement (NOE) experiments for the tetraacetate (**2**) which was prepared by conventional acetylation with acetic anhydride and pyridine. On irradiation at δ 3.46 (H-9), differential NOEs for the signals at δ 2.78 (H-8) and 4.45 (H-1′) were observed. On the other hand, with respective irradiation at δ 2.78 and 4.45, differential NOEs were observed for the signal at δ 3.46. The results clearly demonstrate the cis relationships between H-8, H-9 and 5-*O*-β-glucopyranosyl group. The absolute stereochemistry was presumed to be as shown based on the comparison of the circular dichroism (CD) spectrum of **1** ($\Delta\varepsilon_{243}$ +1.48) with that of *cis*,*cis*-nepetalactone (**5**)[9] ($\Delta\varepsilon_{235}$ +2.05). Thus, the structure of nepetaracemoside A should be represented as **1**.

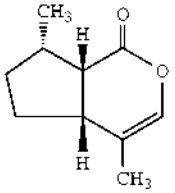
Nepetaracemoside B (**3**) was obtained as an amorphous powder, $[\alpha]_D$ −43.6°. Its molecular formula was determined to be $C_{16}H_{22}O_8$ based on its negative-ion HR-FAB-MS and was two mass units less than that of nepetaracemoside A (**1**). The ^{1}H-NMR spectrum showed the presence of a secondary methyl group [δ 1.29 (3H, d, J=6.8 Hz)], a methyl group on a double bond [δ 2.12 (3H, br s)] and an olefinic proton [δ 7.38 (1H, br s)]. The ^{13}C-NMR spectrum (Table 1) showed, in addition to the signals due to a β-glucopyranosyl moiety, the presence of two methyl groups, a methylene group, two



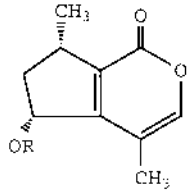
1 R=H
2 R=COMe



3 R=H
4 R=COMe



5



6 R=H
7 R=*p*-Bromobenzoyl

Glc : β-D-glucopyranosyl

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Table 1. ^{13}C-NMR Data[a] for Nepetaracemosides A (**1**) and B (**3**)

Carbon	**1**	**3**
1	172.6	163.9
3	141.5	148.9
4	113.5	158.0
5	85.2	116.9
6	37.9	84.9
7	31.6	41.5
8	38.5	37.9
9	55.2	133.1
10	17.9	20.4
11	11.4	12.8
1′	99.9	106.0
2′	74.7	75.3
3′	78.1	78.0
4′	70.8	71.6
5′	77.9	78.2
6′	61.9	62.8

a) Measured at 100 MHz for CD_3OD solution.

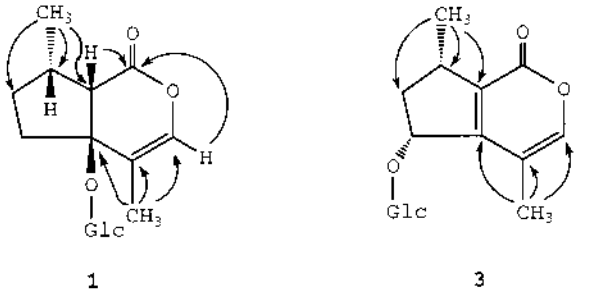

Fig. 1. The Selected ^{1}H–^{13}C long-Range Correlations (J=8 Hz) of Nepetaracemosides A **1** and B **3**

methine groups, one of which bears an oxygen atom, trisubstituted and tetrasubstituted double bonds and a lactonic carbon atom (δ 163.9). These data, coupled with the UV absorption maximum at 299 nm ($\log\varepsilon$ 3.75),[8] suggested that the structure of nepetaracemoside B (**3**) is a dehydro-nepetalactone type carbon skeleton to which a secondary β-glucopyranosyloxy group was introduced. The planar structure was elucidated by interpretation of the ^{1}H–^{1}H COSY spectrum. Starting from the signal at δ 5.05 (H-6), cross peaks were followed to δ 2.65 (H_1-7), 1.95 (H_1-7), 3.02 (H-8) and 1.29 (H_3-10), successively. Cross peaks between H-3 (δ 7.38) and a vinylic methyl group (δ 2.12) were also observed. Thus, the glucopyranosyloxy group should be located at C-6, therefore the planar structure **3** was deduced for nepetaracemoside B. Additionally, results of the ^{1}H–^{13}C long-range COSY spectrum (J=8 Hz), which are summarized in Fig. 1, support this discussion. The relative stereochemistry was determined as shown in formula **3** based on the results of differential NOE experiments for the tetraacetate (**4**). On irradiation at δ 3.08 (H-8), an NOE was observed for the signal at δ 2.57 (H-7β), and an NOE for H-6 (δ 4.94) was observed on irradiation at δ 2.57. On the other hand, with respective irradiation at δ 1.34 (H_3-10) and 4.94, NOEs for the signals at δ 1.90 (H-7α) and 4.75 (H-1′) were observed, respectively. The results clearly show the *cis*-relationships between H-8, H-7β and H-6. Enzymatic hydrolysis of **3** with β-glucosidase gave the aglucone (**6**). Compound **6** was then converted to its *p*-bromobenzoate (**7**) which showed exciton coupling ($\Delta\varepsilon_{245}$ −6.6)[10] in the CD spectrum, suggesting that the absolute stereochemistry at C-6 is an *R*-configuration. Thus, the structure of nepetaracemoside B was elucidated as **3**.

Experimental

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. IR spectra were measured on a Shimadzu IR-400 spectrophotometer or Perkin-Elmer 1720 IR FT spectrometer and UV spectra on a JASCO V-530SR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM EX-400 spectrometer at 400 and 100 MHz with tetramethylsilane as an internal standard. HR-FAB-MS was performed on a JEOL JMS SX-102 spectrometer with polyethyleneglycol-400 as a matrix. Column chromatography was performed on Silica gel 60 (230—400 mesh, Merck), and TLC and preparative TLC were performed on precoated silica gel plates 60 F_{254} (0.25 and 0.5 mm in thickness). HPLC was performed on octadecyl silica gel (Cosmoil 10C_{18}, Nakalai Tesque, Kyoto, ϕ=20 mm, L=250 mm) with a mixture of H_2O and MeOH at the flow rate of 6 ml min^{-1}, and the eluate was monitored by UV.

Plant Material Plant material was collected in Sarican village, East Anatolia, Turkey on 20th June, 1990, and identified as *Nepeta racemosa* Lam. by the authors (G. H. and E. S.). Voucher specimens (90E082) are deposited in the herbaria of the Graduate School of Pharmaceutical Sciences, Kyoto University and the Faculty of Pharmacy, Gazi University.

Isolation The dried aerial parts (900 g) of *N. racemosa* were extracted with MeOH (8 l) at room temperature for 2 weeks. The methanolic extract was concentrated *in vacuo*. The residue was dissolved in 90% MeOH (1.1 l) and the solution was washed with *n*-hexane (1 l×3). The 90% MeOH layer was concentrated *in vacuo*. The resultant residue was suspended in H_2O (1 l) and the suspension was extracted with EtOAc (1 l×3). The aqueous layer was extracted with *n*-BuOH (1 l×3). The *n*-BuOH extract was evaporated *in vacuo* to give a residue (14.2 g).

An aliquot (13.2 g) of the residue was chromatographed over silica gel (385 g). Then, $CHCl_3$ (1.25 l), $CHCl_3$–MeOH (97 : 3, 2.0 l; 19 : 1, 2.0 l; 93 : 7, 2.0 l; 9 : 1, 2.0 l; 22 : 3, 2.0 l; 17 : 3, 2.0 l; 4 : 1, 2.0 l; 3 : 1, 2.0 l; 7 : 3, 2.0 l) and MeOH (2.0 l) were passed successively through the column and 100 ml fractions were collected. Fractions 61—69 gave a residue (1.11 g) which was subjected to HPLC (MeOH–H_2O, 2 : 3) to give nepetaracemoside A (**1**) (539 mg) as an amorphous powder. Fractions 70—72 gave a residue (514 mg) which was subjected to HPLC (MeOH–H_2O 3 : 7) to give nepetaracemoside B (**3**) (67.0 mg). Fractions 73—76 gave a residue (568 mg) which was subjected to HPLC (MeOH–H_2O 3 : 7 and then 1 : 3) to give another aliquot of nepetaracemoside B (**3**) (151 mg).

Nepetaracemoside A (**1**): $[\alpha]_D^{23}$ +27.8° (c=0.81, MeOH). IR ν max (dry film) cm^{-1}: 3392, 1739, 1673, 1110, 1077. ^{1}H-NMR (CD_3OD) δ: 0.91 (3H, d, J=7.3 Hz, H_3-10), 1.15 (1H, m, H_1-7), 1.67 (3H, d, J=1.5 Hz, H_3-11), 1.99 (2H, m, H_1-6, H_1-7), 2.24 (1H, m, H_1-6), 2.76 (1H, m, H-8), 3.39 (1H, d, J=11.4 Hz, H-9), 3.65 (1H, dd, J=12.1, 3.7 Hz, H_1-6′), 3.77 (1H, dd, J=12.1, 2.6 Hz, H_1-6′), 4.19 (1H, d, J=7.7 Hz, H-1′), 6.66 (1H, q, J=1.5 Hz, H-3). ^{13}C-NMR: see Table 1. CD: $\Delta\varepsilon_{243}$ +1.48 (MeOH, 4.70×10^{-5} M). Positive-ion HR-FAB-MS *m/z*: 345.1559 $[M+H]^+$ (Calcd for $C_{16}H_{25}O_8$: 345.1549).

Nepetaracemoside B (**3**): $[\alpha]_D^{23}$ −43.6° (c=0.59, MeOH). UV λ_{max} (MeOH) nm (log ε): 299 (3.75). IR ν_{max} (film) cm^{-1}: 3386, 1703, 1638, 1075. ^{1}H-NMR (CD_3OD) δ: 1.29 (3H, d, J=6.8 Hz, H_3-10), 1.95 (1H, ddd, J=14.4, 3.6, 3.6 Hz, Hα-7), 2.12 (3H, br s, H_3-11), 2.65 (1H, ddd, J=14.4, 7.8, 7.8 Hz, Hβ-7), 3.02 (1H, m, H-8), 3.70 (1H, dd, J=11.7, 4.4 Hz, H_1-6′), 3.88 (1H, br d, J=11.7 Hz, H_1-6′), 4.49 (1H, d, J=7.8 Hz, H-1′), 5.05 (1H, dd, J=7.8, 3.6 Hz, H-6), 7.38 (1H, br s, H-3). ^{13}C-NMR: see Table 1. CD: $\Delta\varepsilon_{209}$ −12.9 (MeOH, 3.42×10^{-5} M). Negative-ion HR-FAB-MS *m/z*: 341.1205 $[M-H]^-$ (Calcd for $C_{16}H_{21}O_8$: 341.1236).

Nepetaracemoside A Tetraacetate (2) Nepetaracemoside A (**1**) (20.4 mg) was acetylated with a mixture of Ac_2O and pyridine (each 0.2 ml) overnight. After the addition of excess MeOH, the solvent was removed *in vacuo*. The residue was purified by silica gel (5 g) chromatography ($CHCl_3$) to give the tetraacetate (**2**) (29.1 mg) as an amorphous powder. IR ν_{max} ($CHCl_3$) cm^{-1}: 1730, 1705, 1660, 1210. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, d, J=7.8 Hz, H_3-10), 1.15 (1H, m, H_1-7), 1.59 (3H, d, J=1.5 Hz, H_3-11), 1.99 (6H, s, 2×OAc), 2.02, 2.10 (each 3H, s, 2×OAc), 2.78 (1H, m, H-8), 3.46 (1H, d, J=11.0 Hz, H-9), 3.59 (1H, ddd, J=9.5, 3.7, 2.2 Hz, H-5′), 4.03 (1H, dd, J=12.5, 2.2 Hz, H_1-6′), 4.29 (1H, dd, J=12.5, 3.7 Hz, H_1-6′), 4.45 (1H, d, J=8.1 Hz, H-1′), 4.99 (1H, dd, J=8.1, 9.5 Hz, H-2′), 5.05 (1H, dd, J=9.5, 9.5 Hz, H-4′), 5.16 (1H, dd, J=9.5, 9.5 Hz, H-3′), 6.57 (1H, br d, J=1.5 Hz).

Negative-ion HR-FAB-MS *m/z*: 511.1806 $[M-H]^-$ (Calcd for $C_{24}H_{31}O_{12}$: 511.1816).

Nepetaracemoside B Tetraacetate (4) Nepetaracemoside B (**3**) (18.6 mg) was acetylated as above and the product was purified by preparative TLC [$CHCl_3$–MeOH (49 : 1)] to give the tetraacetate (**4**) (24.1 mg) as an amorphous powder. IR ν_{max} ($CHCl_3$) cm^{-1}: 1760, 1720, 1650, 1240—1210, 1040. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, d, J=6.8 Hz, H_3-10), 1.90 (1H, ddd, J=14.4, 4.0, 4.0 Hz, H_α-7), 2.57 (1H, ddd, J=14.4, 7.6, 7.6 Hz, H_β-7), 3.08 (1H, m, H-8), 3.74 (1H, m, H-5′), 4.21 (2H, m, H_2-6′), 4.75 (1H, d, J=8.0 Hz, H-1′), 4.94 (1H, dd, J=7.6, 4.0 Hz, H-6), 5.02 (1H, dd, J=8.0, 9.2 Hz, H-2′), 5.09 (1H, dd, J=9.2, 9.2 Hz, H-4′), 5.22 (1H, dd, J=9.2, 9.2 Hz, H-3′), 7.20 (1H, br s, H-3). Negative-ion HR-FAB-MS *m/z*: 509.1686 $[M-H]^-$ (Calcd for $C_{24}H_{29}O_{12}$: 509.1659).

Enzymatic Hydrolysis of Nepetaracemoside B (3) Nepetaracemoside B (**3**) (25.0 mg) was dissolved in H_2O (3 ml), and β-glucosidase (from Almond, Toyobo, Japan) (10.4 mg) was added to the solution. The mixture was incubated at 37 °C for 24 h. After dilution with H_2O (10 ml), the solution was extracted with Et_2O (10 ml×3). The Et_2O extract was washed with saturated aq.NaCl solution, dried and evaporated *in vacuo* to give the aglucone (**6**) (9.7 mg) as a colorless oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 3375, 1690, 1630. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, d, J=6.8 Hz, H_3-10), 1.59 (1H, dd, J=14.0, 4.4 Hz, H_a-7), 2.08 (3H, d, J=1.5 Hz, H_3-11), 2.71 (1H, dt, J=14.0, 7.8 Hz, H_b-7), 3.11 (1H, m, H-8), 5.11 (1H, dd, J=7.8, 4.4 Hz, H-6), 7.23 (1H, s, H-3); ^{13}C-NMR ($CDCl_3$) δ: 12.2 (C-11), 20.4 (C-10), 36.4 (C-8), 42.1 (C-7), 75.2 (C-6), 114.0 (C-5), 131.4 (C-9), 147.6 (C-3), 156.9 (C-4), 161.6 (C-1). Negative ion HR-FAB-MS *m/z*: 179.0682 $[M-H]^-$ (Calcd for $C_{10}H_{11}O_3$: 179.0708).

***p*-Bromobenzoate (7) of Nepetaracemoside B Aglucone (6)** Nepetaracemoside B aglucone (**6**) (4.0 mg) was dissolved in pyridine (0.5 ml) and *p*-bromobenzoyl chloride (15.5 mg) was added to the solution. After stirring overnight at room temperature, the reaction was quenched with H_2O (20 ml). The reaction mixture was extracted with $CHCl_3$ (15 ml×2). The $CHCl_3$ extract was washed with 1 N HCl aq. solution, 1 N NaOH aq. solution and H_2O, successively, dried and evaporated *in vacuo*. The residue was purified by silica gel (3 g) column chromatography with $CHCl_3$ as an eluent to give the *p*-bromobenzoate (**7**) (1.1 mg) as an oil. UV λ_{max} (MeOH) nm (log ε): 243.5 (4.41). ^{1}H-NMR ($CDCl_3$): δ 1.15 (1H, dd, J=10.3, 6.4 Hz, H_1-7), 1.40 (3H, d, J=6.8 Hz, H_3-10), 1.92 (3H, br s, H_3-11), 2.86 (1H, m, H_1-7), 3,24 (1H, m, H-8), 6.27 (1H, dd, J=8.1, 3.7 Hz, H-6), 7.61, 7.90 (each 2H, d, J=8.5 Hz, *p*-substituted benzene ring). CD: $\Delta\varepsilon_{245}$ −6.6 and $\Delta\varepsilon_{221}$ −0.75 (6.05×10^{-5} M, MeOH). HR-EI-MS *m/z*: 362.0153, 364.0140 $[M]^+$ (Calcd for $C_{17}H_{15}O_4$ Br: 362.0154, 364.0133).

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