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NEPETANUDOSIDES AND IRIDOID GLUCOSIDES HAVING NOVEL STEREOCHEMISTRY FROM *NEPETA NUDA* SSP. *ALBIFLORA*

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Key Word Index—*Nepeta nuda* ssp. *albiflora*; Labiatae; nepetanudosides B, C and D; iridoid glucoside.

Abstract—From the aerial parts of *Nepeta nuda* ssp. *albiflora*, three new iridoid glucosides, nepetanudosides B, C and D, were isolated together with the known compound, velpetin. The structures of the new compounds were elucidated by spectral and chemical analyses.

INTRODUCTION

The plants belonging to the genus *Nepeta* are known to contain iridoid glucosides such as (1*R*, 5*R*, 8*S*, 9*S*)-deoxyloganic acid [1–3] and velpetin (**8**) [4] with an unusual stereochemistry. Recently, we isolated nepetanudoside A (**9**) from *N. nuda* ssp. *albiflora* collected in Northern Anatolia and elucidated its structure [5]. In a continuation of our studies, we examined the glycosidic constituents of this plant collected in East Anatolia and isolated three new iridoid glucosides, nepetanudosides B (**1**), C (**4**) and D (**6**), together with the known compound, velpetin (**8**) [4]. This paper deals with the structure elucidation of the new compounds.

RESULTS AND DISCUSSION

Nepetanudoside B (**1**), $[\alpha]_D$ −28.8°, was isolated as an amorphous powder and the molecular formula was determined as $C_{16}H_{22}O_9$ based on its negative-ion high-resolution FAB mass spectrum. The ^1H and ^{13}C NMR (Table 1) spectra of **1** were very similar to those of 10-deoxygeniposidic acid (**10**) [6] except for the distinct differences in the resonances due to C-1 and C-1′; both carbon atoms resonated 4.3 and 4.4 ppm downfield compared with those reported for **10** [6]. A similar relationship was also observed between nepetanudoside A (**9**) and mussaenoside [5]. Thus, the structure was presumed to be **1** in which the aglucone portion is the enantiomer of 10-deoxygeniposidic acid (**10**). This was supported by the ^1H–^1H COSY spectrum and the NOE correlations (data not shown).

Nepetanudoside B (**1**) gave the tetraacetate methyl ester (**3**) by the usual acetylation method, followed by methylation with ethereal diazomethane. This compound was identical to the authentic sample [1], which was prepared by dehydration of nepetanudoside A tetraacetate (**11**). Thus, the structure of nepetanudoside B was unequivocally established as **1**.

Nepetanudoside C (**4**), $[\alpha]_D$ −15.6°, was obtained as

Table 1. ^{13}C NMR data (δ)* for nepetanudoside B (**1**), C (**4**) and D (**6**), and 10-deoxygeniposidic acid (**10**)

C	1	4	6	10†
1	101.9	102.4	101.9	97.6
3	153.4	164.8	165.3	151.3
4	113.1	125.6	123.3	113.0
5	35.7	32.7	33.4	35.9
6	39.6	38.4	30.1[c]	39.8
7	127.9	128.1	31.9[c]	127.9
8	140.2	139.2	150.1	140.3
9	50.5	50.5	46.2	49.8
10	16.2	15.6	110.2	16.3
11	171.3	193.5	193.2	171.0
1′	104.5	104.6	104.6	100.1
2′	75.2	75.2	75.2	74.9
3′	78.4[a]	78.4[b]	78.4[d]	78.1[e]
4′	71.4	71.2	71.2	71.7
5′	78.2[a]	78.1[b]	78.0[d]	78.4[e]
6′	62.5	62.5	62.5	62.9

*Measured in CD_3OD.
†Data taken from Inoue *et al.* [6].
[a–e]May be reversed.

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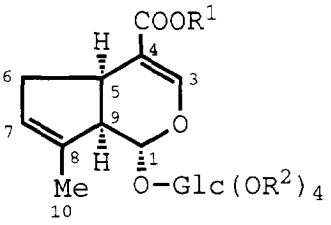
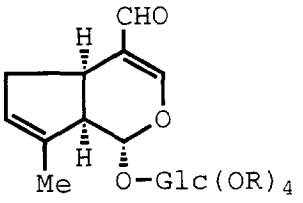
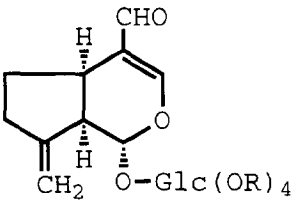


(1) $R^1=R^2=H$
(2) $R^1=H$; $R^2=Ac$
(3) $R^1=Me$; $R^2=Ac$



(4) R=H
(5) R=Ac



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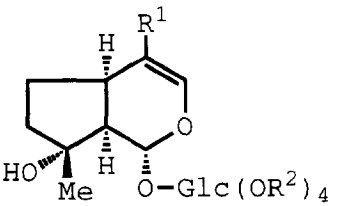
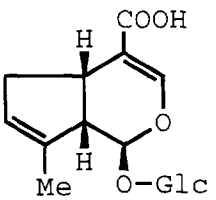
(7) R=Ac



(8) $R^1=CHO$; $R^2=H$
(9) $R^1=COOMe$; $R^2=H$
(11) $R^1=COOMe$; $R^2=Ac$



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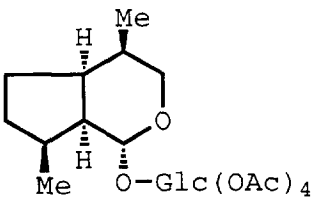

(12)

Glc=β-D-Glucopyranose

an amorphous powder and the molecular formula was determined as $C_{16}H_{22}O_8$, i.e., one oxygen atom less than that of nepetanudoside B (**1**). Acetylation of **4** with a mixture of acetic anhydride and pyridine gave the tetraacetate **5**. The NMR spectra suggested that the structure differs from **1** only by the presence of an aldehyde group at C-4. In addition, the absorption maximum in the UV spectrum showed a bathochromic shift to 251 nm, which is characteristic for the iridoid glucosides having an aldehyde group at C-4. Thus, the structure of nepetanudoside C was suggested to be a C-4 aldehyde congener of nepetanudoside B (**1**). The absolute stereochemistry was proved to be the same as that of **1** by comparison of the CD spectra. Thus, the structure of nepetanudoside C was elucidated as **4**.

Nepetanudoside D (**6**), $[\alpha]_D$ +22.6°, was also obtained as an amorphous powder and the molecular formula was determined to be the same as that of nepetanudoside C (**4**). The ^1H and ^{13}C NMR spectra suggested that nepetanudoside D was an isomer of **4** with an exocyclic 8, 10-double bond. Catalytic hydrogenation of the tetraacetate (**7**) over Pd-C gave **12**, which was identical to the hydrogenation product of **5**. Thus, the structure of nepetanudoside D was elucidated as **6**.

EXPERIMENTAL

General. NMR: ^1H (200 or 400 MHz) and ^{13}C (50 or 100 MHz), TMS as int. standard; FAB-MS: matrix, PEG-400; CC: silica gel 60 (230–400 mesh, Merck); TLC and prep. TLC: precoated silica gel plates 60 F_{254} (0.25 and 0.5 mm); HPLC: column, M & S pack C-18 B, detection 230 nm, solvent: MeOH–H_2O, (6.5 ml min^{-1}).

Plant material. Plant material was collected in East Anatolia in June, 1990 and identified as *Nepeta nuda* L. ssp. *albiflora* (BOISS.) GAMS. by the authors (G. H. and E. S.). Voucher specimens (90 E 028) are deposited in the Herbaria of the Faculty of Pharmaceutical Sciences,

Kyoto University and the Faculty of Pharmacy, Gazi University.

Isolation. The dried aerial parts (795 g) of *N. nuda* ssp. *albiflora* were extracted (×2) with MeOH (9 l) at room temp. for 2 weeks. The combined methanolic extract was concd *in vacuo*. The residue was dissolved in 90% MeOH (440 ml) and the soln was washed with *n*-hexane (400 ml × 3). The 90% MeOH layer was concd *in vacuo*, the resultant residue suspended in H_2O (400 ml) and the suspension extracted with EtOAc (400 ml × 3). The aq. layer was partitioned with *n*-BuOH (400 ml × 3). The *n*-BuOH layer was evapd *in vacuo* to give a residue (11.7 g). Analiquot (10.0 g) of the *n*-BuOH extracts was chromatographed over silica gel (370 g). Two litres each of $CHCl_3$, $CHCl_3$–MeOH (97:3), $CHCl_3$–MeOH (19:1), $CHCl_3$–MeOH (93:7), $CHCl_3$–MeOH (9:1), $CHCl_3$–MeOH (22:3), $CHCl_3$–MeOH (17:3), $CHCl_3$–MeOH (4:1) and $CHCl_3$–MeOH (7:3) were passed successively through the column and 100 ml frs were collected. Frs 72–78 gave a residue (333 mg) on evapn which was sepd by HPLC (MeOH–H_2O, 2:3) to give nepetanudosides C (**4**) (81.3 mg) and D (**6**) (20.4 mg). Frs 79–104 gave a residue (1.32 g) on evapn which was sepd by HPLC (MeOH–H_2O, 3:7) to give nepetanudoside B (**1**) (171 mg) and velpetin (**8**) (45.4 mg). Velpetin (**8**) was identified by comparison of its spectral data with those reported [4].

Nepetanudoside B (**1**). Amorphous powder, $[\alpha]_D^{25}$ −28.8° (MeOH, *c* 0.75); UV λ_{max} (MeOH): 236 (log ε 3.99); IR ν_{max} (KBr): 3327, 1684, 1634 cm^{-1}; ^1H NMR (CD_3OD): δ 1.85 (3H, *br s*, H_3-10), 2.10 (1H, *m*, H_1-6), 2.72 (2H, *m*, H_1-6 and H-9), 3.13 (1H, *m*, H-5), 3.69 (1H, *dd*, *J* = 11.9 and 4.7 Hz, H_1-6′), 3.84 (1H, *dd*, *J* = 11.9 and 1.8 Hz, H_1-6), 4.58 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.13 (1H, *d*, *J* = 5.6 Hz, H-1), 5.48 (1H, *m*, H-7), 7.43 (1H, *s*, H-3); ^{13}C NMR (CD_3OD): Table 1; CD $\Delta\varepsilon_{232}$ −11.4 (MeOH, 6.05×10^{-5} M); negative ion HRFAB-MS *m/z*: 357.1179 $[M-H]^-$ ($C_{16}H_{21}O_9$ requires 357.1185).

Nepetanudoside C (**4**). Amorphous powder, $[\alpha]_D^{25}$ −15.6° (MeOH, *c* 1.31); UV λ_{max} (MeOH): 251 nm (log ε 4.01); IR ν_{max} (KBr): 3387, 1686, 1626 cm^{-1}; ^1H NMR (CD_3OD): δ 1.83 (3H, *br s*, H_3-10), 2.16 (1H, *m*, H_1-6), 2.72 (1H, *m*, H_1-6), 2.89 (1H, *m*, H-9), 3.15 (1H, *m*, H-5), 3.68 (1H, *dd*, *J* = 12.0 and 3.8 Hz, H_1-6′), 3.85 (1H, *br d*, *J* = 12.0, H_1-6′), 4.61 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.40 (1H, *d*, *J* = 4.6 Hz, H-1), 5.45 (1H, *m*, H-7), 7.38 (1H, *s*, H-3), 9.19 (1H, *s*, H-11); ^{13}C NMR (CD_3OD): Table 1; CD $\Delta\varepsilon_{252}$ −14.1 (MeOH, 4.21×10^{-5} M): negative-ion HRFAB-MS *m/z*: 341.1270 $[M-H]^-$ ($C_{16}H_{21}O_8$ requires 341.1236).

Nepetanudoside D (**6**). Amorphous powder, $[\alpha]_D^{25}$ +22.6° (MeOH, *c* 0.83); UV λ_{max} (MeOH): 248 nm (log ε 4.01); IR λ_{max} (KBr): 3355, 1703, 1626 cm^{-1}; ^1H NMR (CD_3OD): δ 1.92 (2H, *m*, H_2-6), 2.31 (2H, *m*, H_2-7), 2.99 (1H, *m*, H-9), 3.24 (1H, *m*, H-5), 3.70 (1H, *dd*, *J* = 12.0 and 4.0 Hz, H_1-6′), 3.86 (1H, *br d*, *J* = 12.0, H_1-6′), 4.61 (1H, *d*, *J* = 8.0 Hz, H-1′), 5.11 (1H, *d*, *J* = 2.0 Hz, H_1-10), 5.81 (1H, *d*, *J* = 2.0 Hz, H_1-10), 5.48 (1H, *d*, *J* = 5.0 Hz, H-1), 7.38 (1H, *s*, H-3), 9.19 (1H, *s*, H-11); ^{13}C NMR (CD_3OD): Table 1; CD $\Delta\varepsilon_{253}$ −3.20 (MeOH, 4.80×10^{-5} M), negative-ion HRFAB-MS *m/z*: 341.1250 $[M-H]^-$ ($C_{16}H_{21}O_8$ requires 341.1236).

Nepetanudoside B tetraacetate (**2**). Nepetanudoside B (**1**) (15.0 mg) was dissolved in a mixture of Ac_2O (0.15 ml) and pyridine (0.15 ml) and the soln was left at 4° for 18 hr. After addition of excess MeOH, the solvent was removed *in vacuo*. The residue was purified by prep. TLC [solvent: $CHCl_3$–MeOH (19:1)] to give the tetraacetate **2** (18.1 mg) which was crystallized on addition of EtOH. Needles, mp 165–166°, IR ν_{max} ($CHCl_3$) 2900, 1740, 1670, 1620, 1210, 1060 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.76 (3H, *br s*, H_3-10), 2.01, 2.02, 2.04, 2.09 (each 3H, *s*, 4 × OAc), 2.54 (1H, *br t*, *J* = 7.8 Hz, H-9), 2.80 (1H, *br dd*, *J* = 16.1 and 8.3 Hz; H_1-6), 3.16 (1H, *q*, *J* = 7.8 Hz, H-5), 3.79 (1H, *m*, H-5′), 4.15 (1H, *dd*, *J* = 12.5 and 2.2 Hz, H_1-6′), 4.29 (1H, *dd*, *J* = 12.5 and 5.1 Hz, H_1-6′), 4.85 (1H, *d*, *J* = 7.8 Hz, H-1), 4.89 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.08 (1H, *dd*, *J* = 7.8 and 9.3 Hz, H-3′), 5.54 (1H, *br s*, H-7), 7.55 (1H, *s*, H-3); negative-ion HRFAB-MS *m/z*: 525.1615 $[M-H]^-$ ($C_{24}H_{29}O_{13}$ requires 525.1608).

Nepetanudoside B tetraacetate methyl ester (**3**). Nepetanudoside B tetraacetate (**2**) (15.0 mg) was dissolved in Et_2O (3 ml) and an ethereal soln of CH_2N_2 was added until the yellow colour persisted. A few drops of HOAc were added to the soln and the solvent was evapd *in vacuo*. The residue was purified by prep. TLC (solvent: Et_2O) to give the methyl ester **3** (14.4 mg) as a syrup. $[\alpha]_D^{24}$ −21.4° (MeOH, *c* 0.72); negative-ion HRFAB-MS *m/z* 539.1746 $[M-H]^-$ ($C_{21}H_{31}O_{13}$ requires 539.1764). This compound was identified with an authentic sample of 1-*O*-β-D-tetra-acetylglucosyl-*ent*-10-deoxygenipin (**3**), $[\alpha]_D^{24}$ −21.5° (MeOH, *c* 1.11) by comparison of the IR, ^1H and ^{13}C NMR spectra.

Nepetanudoside C tetraacetate (**5**). Nepetanudoside C (**4**) (13.3 mg) was acetylated as above. The product was purified by prep. TLC (solvent: Et_2O) to give the tetraacetate **5** (15.3 mg) which was crystallized by addition of EtOH. Needles, mp 124–125°. IR ν_{max} ($CHCl_3$): 1760, 1680, 1635, 1380, 1250, 1070 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.76 (3H, *br s*, H_3-10), 2.02, 2.03, 2.04, 2.10 (each 3H, *s*, 4 × OAc), 2.62 (1H, *br t*, *J* = 6.4 Hz, H-9), 2.82 (1H, *br dd*, *J* = 16.1 and 7.8 Hz, H_1-6), 3.19 (1H, *q*, *J* = 7.8 Hz, H-5), 3.78 (1H, *m*, H-5′), 4.19 (*m*, *dd*, *J* = 12.1 and 2.4 Hz, H_1-6′), 4.28 (1H, *dd*, *J* = 12.1 and 4.6 Hz, H_1-6′), 4.90 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.02 (1H, *d*, *J* = 6.4 Hz, H-1), 5.07 (1H, *dd*, *J* = 9.3 and 6.4 Hz, H-2′), 5.14 (1H, *dd*, *J* = 9.8 and 9.3 Hz, H-4′), 5.21 (1H, *dd*, *J* = 9.3 and 9.3 Hz, H-3′), 5.52 (1H, *br s*, H-7), 7.17 (1H, *s*, H-3), 9.31 (1H, *s*, H-11); negative-ion HRFAB-MS *m/z*: 509.1626 $[M-H]^-$ ($C_{24}H_{29}O_{12}$ requires 509.1659).

Nepetanudoside D tetraacetate (**7**). Nepetanudoside D (**6**) (8.0 mg) was acetylated as above and the product was purified by prep. TLC (solvent: Et_2O) to give the

tetraacetate **7** as an amorphous powder. IR ν_{max} ($CHCl_3$): 1755, 1670, 1640, 1230 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.015, 2.023, 2.04, 2.10 (each 3H, *s*, 4 × OAc), 2.23 (1H, *m*), 2.36 (2H, *m*), 2.70 (1H, *br t*, J = 6.8 Hz, H-9), 2.99 (1H, *q*, J = 7.6 Hz, H-5), 3.78 (1H, *m*, H-5′), 4.18 (1H, *dd*, J = 12.5 and 2.4 Hz, H_1-6′), 4.30 (1H, *dd*, J = 12.5 and 4.9 Hz, H_1-6′), 4.86 (1H, *d*, J = 7.8 Hz, H-1′), 5.02 (1H, *d*, J = 6.8 Hz, H-1), 5.05–5.17 (4H, *m*, H-2′, H-4′ and H_2-10), 5.21 (1H, *dd*, J = 9.3 and 9.3 Hz, H-3′), 7.18 (1H, *s*, H-3), 9.31 (1H, *s*, H-11); negative-ion HRFAB-MS *m/z*: 509.1641 $[M-H]^-$ ($C_{24}H_{29}O_{12}$ requires 509.1659).

Catalytic hydrogenation of nepetanudoside D tetraacetate (**7**). Nepetanudoside D tetraacetate (**7**) (13.0 mg) was dissolved in MeOH (3 ml) and 5% Pd-C (9.5 mg) was added. The mixt. was hydrogenated for 1.5 hr at room temp. After removing the catalyst, the filtrate was concd *in vacuo* to give a residue which was purified by silica gel (3 g) CC with Et_2O as eluent to give the hydrogenation product **12** (3.5 mg) as needles, mp 122–123°. IR ν_{max} ($CHCl_3$) 1750 and 1230 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.76 (3H, *d*, J = 6.8 Hz, H_3-11), 0.96 (3H, *d*, J = 6.4 Hz, H_3-10), 1.17 (1H, *m*, H_1-7), 1.36 (1H, *dd*, J = 10.5 and 6.4 Hz, H-9), 1.43 (1H, *m*, H-4), 1.51 (1H, *m*, H_1-6), 1.68 (1H, *m*, H_1-6), 1.81 (1H, *m*, H-5), 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 and 4.6 Hz, H_1-3), 3.55 (1H, *dd*, J = 11.7 and 11.7 Hz, H_1-3), 3.77 (1H, *m*, H-5′), 4.12 (1H, *dd*, J = 12.2 and 2.2 Hz, H_1-6′), 4.28 (1H, *dd*, J = 12.2 and 4.8 Hz, H_1-6′), 4.71 (1H, *d*, J = 7.8 Hz, H-1′), 4.86 (1H, *br s*, H-1), 5.03 (1H, *dd*, J = 9.8 and 7.8 Hz, H-2′), 5.09 (1H, *dd*, J = 9.8 and 9.8 Hz, H-4′) and 5.22 (1H, *dd*, J = 9.8 and 9.8 Hz, H-3′); negative ion HRFAB-MS *m/z*: 499.2167 $[M-H]^-$ $C_{24}H_{35}O_{11}$ requires 499.2179).

Catalytic hydrogenation of nepetanudoside C tetraacetate (**5**). Nepetanudoside C tetraacetate (**5**) (15.0 mg) was dissolved in MeOH (3 ml) and 5% Pd-C (16.0 mg) was added. The mixt. was hydrogenated for 2 hr at room temp. The reaction mixt. was treated as before to give the hydrogenation product **12** (7.3 mg) as needles, mp 120–121°, negative-ion HRFAB-MS *m/z* 499.2178 $[M-H]^-$ ($C_{24}H_{35}O_{11}$ requires 499.2179). This compound was identified with the sample derived from nepetanudoside D tetraacetate (**7**) by mmp determination and comparisons of the IR and ^1H NMR spectra.

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