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NEPETANUDOSIDE, AN IRIDOID GLUCOSIDE WITH AN UNUSUAL STEREOSTRUCTURE FROM *NEPETA NUDA* SSP. *ALBIFLORA*

YOSHIO TAKEDA,* YUKA MORIMOTO, TAKASHI MATSUMOTO,

Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770, Japan

GISHO HONDA, MAMORU TABATA, TETSURO FUJITA,

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

HIDEAKI OTSUKA,

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan

EKREM SEZIK, and ERDEM YESILADA

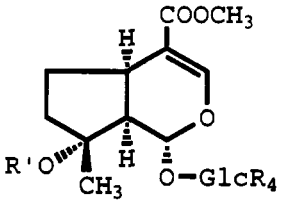
Faculty of Pharmacy, Gazi University, Ankara 06330, Turkey

ABSTRACT.—A new iridoid glucoside with an unusual stereochemistry, named nepetanudoside, was isolated from the aerial parts of *Nepeta nuda* ssp. *albiflora*. The structure was elucidated on the basis of spectroscopic and chemical evidence.

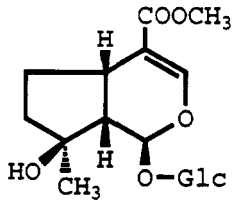
Nepetalactone and its stereoisomers have been isolated from plants belonging to the genus *Nepeta* (Labiatae) and are known to induce peculiar physiological activity in felines (1). To the best of our knowledge, no reports have appeared on the chemical constituents of *Nepeta nuda* L. ssp. *albiflora* (Boiss.) Gams. We now report the isolation and structural elucidation of a new iridoid glucoside, nepetanudoside [**1**], from the aerial parts of this plant collected in northern Turkey.

RESULTS AND DISCUSSION

Nepetanudoside [**1**], $[\alpha]^{28.5}D$ +83.7° (c=0.37, H_2O) was isolated as a crystalline precipitate, mp 248–250° (dec) from a MeOH extract of the title plant, according to procedures described in the Experimental section. The molecular formula was determined as $C_{17}H_{26}O_{10}$ on the basis of negative-ion hrfabms. It showed an absorption maximum at 239 nm (ε 8525) in the uv spectrum and bands at 3400 (br) (hydroxyl), 1690 (ester), and 1630 (double bond) cm^{-1} in the ir spectrum. The ^{13}C-nmr spectrum showed data very similar to those reported for mussaenoside [**4**] (2–4), except for notable (4 ppm) differences in the shifts for C-1 and C-1′. Acetylation of **1** with a mixture of Ac_2O and pyridine gave the pentaacetate **2**, $C_{27}H_{36}O_{15}$ and the tetraacetate **3**, $C_{25}H_{34}O_{14}$. In the ^1H-^1H-COSY spectrum of **3** the connectivities for the protons on C-1→C-9→C-5→C-6→C-7 were demonstrated by following the cross-peaks from δ 5.14 (1H, d, J=3.9 Hz, H-1) to δ 2.25 (1H, dd, J=9.8 and 3.9 Hz, H-9), δ 3.15 (1H, m, H-5), δ 2.32 (1H, m,



1 R=R′=H
2 R=R′=$COCH_3$
3 R=$COCH_3$, R′=H



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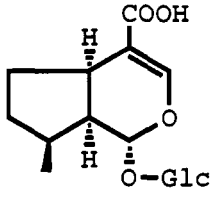
H_b-6), δ 1.43 (1H, m, H_a-6), and δ 1.71 (2H, m, H_2-7), successively. The results clearly showed that in the aglucone portion of the molecule, nepetanudoside [**1**] has the same planar structure as that of mussaenoside [**4**]. The relative stereochemistry was examined by nOe difference experiments. The results, summarized in Figure 1, demonstrate that the relative stereochemistry in the aglucone portion of nepetanudoside [**1**] is the same as that of mussaenoside [**4**]. Comparing the specific rotation of **1** to those of (*1R,5R,8S,9S*)-deoxyloganic acid [**5**] (5) and velpetin [**6**] (6), the absolute stereochemistry of nepetanudoside [**1**] in the aglucone portion was presumed to be the enantiomer of mussaenoside [**4**]. This presumption was confirmed by the following chemical evidence. Dehydration of the tetraacetate [**3**] by a mixture of phosphoryl chloride and pyridine gave the dehydrated product **7** [δ 1.75 (3H, d, J=1.5 Hz, H_3-10) and 5.53 (1H, br s)], which was deacetylated with a mixture of aqueous NaOH solution and MeOH followed by hydrolysis with β-glucosidase to give *ent*-10-deoxygenipin [**9**]. Acetylation with a mixture of Ac_2O and pyridine gave the monoacetate [**10**], $[\alpha]^{23}D$ −55.7° (c=1.99, $CHCl_3$), the spectral data of which were identical with those of 10-deoxygenipin acetate [**11**], $[\alpha]^{23}D$ +56.0° (c=1.12, $CHCl_3$), except for the specific rotation. The latter was prepared from 10-deoxygeniposide [**12**] (7) with known absolute stereochemistry via 10-deoxygenipin [**13**] (8). Thus, the structure of nepetanudoside was elucidated as **1**.

Iridoid glucosides which have an enantiomeric aglucone relative to the usual



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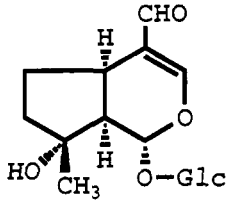

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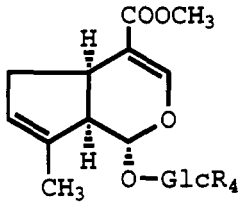
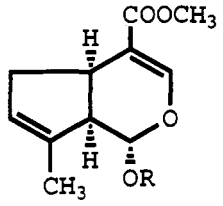
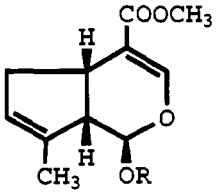


7 R=$COCH_3$
8 R=H

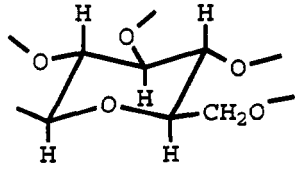


9 R=H
10 R=$COCH_3$



11 R=$COCH_3$
12 R=Glc
13 R=H

Glc=



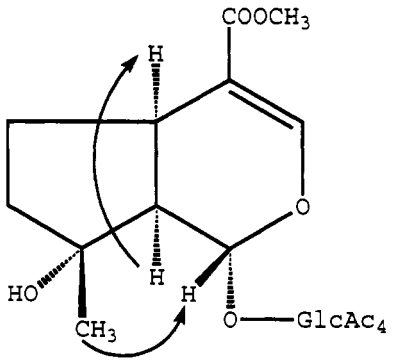


FIGURE 1. The results of nOe difference experiments for nepetanudoside tetraacetate [**3**].

situation (9) have been isolated only from plants belonging to the genus *Nepeta* (Labiatae) (5,6), and nepetanudoside [**1**] is the third example in this series.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Ir spectra were recorded on a Shimadzu IR-400 spectrometer or a Hitachi 260-10 spectrometer. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) nmr spectra were taken with a JEOL JNM EX-400 spectrometer using TMS as an internal standard and chemical shifts are given in δ (ppm) values. Ms were determined with a JEOL JMS SX-102 spectrometer. Uv spectra were recorded with a Hitachi 323 spectrometer. Optical rotations were taken with a Jasco DIP 360 digital polarimeter. Kieselgel 60 (230–400 mesh, Merck) was used for cc and precoated Si gel 60 F_{254} plates (0.25 mm) were used for tlc.

PLANT MATERIAL.—Plant material was collected in Destek, Turkey, on 2 July, 1991, and identified as *Nepeta nuda* ssp. *albiflora* by two of the authors (G.H. and E.S.). Voucher specimens (91D 066) are deposited in the Herbaria of the Faculty of Pharmaceutical Sciences, Kyoto University, and the Faculty of Pharmacy, Gazi University.

EXTRACTION AND ISOLATION.—The dried aerial parts (1.93 kg) of *N. nuda* ssp. *albiflora* were extracted with MeOH (16 liters) at room temperature for 2 weeks. The MeOH extract was concentrated *in vacuo*. The residue was dissolved in 90% MeOH (700 ml) and the solution was washed with *n*-hexane (700 ml×3). The 90% MeOH layer was concentrated *in vacuo*, the resultant residue was suspended in H_2O (700 ml), and the suspension was extracted with EtOAc (700 ml×3). The aqueous layer was extracted with *n*-BuOH (700 ml×3). The *n*-BuOH extract was evaporated *in vacuo*. The residue (30 g) was suspended in MeOH (200 ml) and the insoluble material was filtered and washed with MeOH (100 ml) to give nepetanudoside [**1**] (5.07 g). Mp 248–250° (dec), $[\alpha]^{28.5}_D$ +83.7° (c=0.37, H_2O); uv λ max nm (ε) 239 (8525); ir ν max (KBr) 3400 (br), 1690, 1630, 1440, 1310, 1285, 1190, 1070, 1030, 900, 845 cm^{-1}; ^{1}H nmr (C_5D_5N) δ 1.66 (1H, m, H_a-6), 1.70 (3H, s, H_3-10), 1.87 (1H, m, H_a-7), 2.01 (1H, m, H_b-7), 2.52 (1H, m, H_b-6), 2.95 (1H, dd, J=3.9 and 9.5 Hz, H-9), 3.53 (1H, m, H-5), 3.59 (3H, s, COOMe), 3.98 (1H, m, H-5′), 4.08 (1H, m, H-2′), 4.25 (2H, H-3′ and H-4′), 4.36 (1H, dd, J=11.7 and 4.9 Hz, H_a-6′), 4.51 (1H, dd, J=11.7 and 2.2 Hz, H_b-6′), 5.27 (1H, d, J=7.8 Hz, H-1′), 5.96 (1H, d, J=3.9 Hz, H-1), 7.70 (1H, s, H-3); ^{13}C-nmr data, see Table 1; negative-ion hrfabms m/z [M−H]$^-$ 389.1450 ($C_{17}H_{25}O_{10}$ requires 389.1448).

ACETYLATION OF NEPETANUDOSIDE [**1**].—Nepetanudoside [**1**] (447 mg) was suspended in a mixture of pyridine (2.5 ml) and Ac_2O (2.5 ml) and the suspension was stirred overnight at room temperature. After addition of excess MeOH, the solvent was removed *in vacuo*. The residue was separated by Si gel (80 g) chromatography using Et_2O as eluent. The faster eluate gave the pentaacetate [**2**] (53.9 mg) and the slower eluate gave the tetraacetate [**3**] (555.2 mg) as amorphous powders.

Pentaacetate [**2**].—Ir ν max ($CHCl_3$) 1750, 1705, 1640, 1370, 1220, 1070, 1035 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.46 (3H, s, H_3-10), 1.63 (1H, m), 1.97, 2.01, 2.03, 2.09, 2.10 (3H each, s, OCOMe), 2.59 (1H, br d, J=8.8 Hz, H-9), 3.09 (1H, m, H-5), 3.71 (3H, s, COOMe), 3.75 (1H, m, H-5′), 4.11 (1H, dd, J=12.2 and 2.4 Hz, H_a-6′), 4.25 (1H, dd, J=12.2 and 5.4 Hz, H_b-6′), 4.79 (1H, d, J=8.3 Hz, H-1′), 4.99 (1H, dd, J=8.3 and 9.5 Hz, H-2′), 5.06 (1H, dd, J=9.5 and 9.5 Hz, H-4′), 5.22 (1H, dd, J=9.5 and 9.5 Hz,

TABLE 1. ^{13}C-Nmr Data (δ) of Nepetanudoside [**1**] (measured in C_5D_5N).

Carbon	δ (ppm)	Carbon	δ (ppm)
C-1	99.47	C-1′	104.11
C-3	151.70	C-2′	75.37
C-4	112.44	C-3′	78.58
C-5	31.33	C-4′	71.23
C-6	30.41	C-5′	78.99
C-7	41.11	C-6′	62.51
C-8	78.93		
C-9	52.29		
C-10	25.13		
C-11	167.54		
OMe	50.86		

H-3′), 5.60 (1H, d, J=1.5 Hz, H-1), 7.34 (1H, s, H-3); negative-ion hrfabms *m/z* $[M-H]^-$ 599.1983, calcd for $C_{27}H_{35}O_{15}$, 599.1976.

Tetraacetate [**3**].—Ir ν max ($CHCl_3$) 3450, 1750, 1700, 1640, 1370, 1220, 1070 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.30 (3H, s, H_3-10), 1.43 (1H, m, H_a-6), 1.71 (2H, m, H_2-7), 2.01, 2.03, 2.05, 2.10 (3H each, s, OCOMe), 2.25 (1H, dd, J=9.8 and 3.9 Hz, H-9), 2.32 (1H, m, H_b-6), 3.15 (1H, m, H-5), 3.71 (3H, s, COOMe), 3.76 (1H, m, H-5′), 4.17 (1H, dd, J=12.2 and 2.9 Hz, H_a-6′), 4.23 (1H, dd, J=12.2 and 5.1 Hz, H_b-6′), 4.83 (1H, d, J=7.8 Hz, H-1′), 5.03 (1H, dd, J=7.8 and 9.3 Hz, H-2′), 5.07 (1H, dd, J=9.3 and 9.3 Hz, H-4′), 5.14 (1H, d, J=3.9 Hz, H-1), 5.21 (1H, dd, J=9.3 and 9.3 Hz, H-3′), 7.31 (1H, s, H-3); negative-ion hrfabms *m/z* $[M-H]^-$ 557.1881, calcd for $C_{25}H_{33}O_{14}$, 557.1870.

DEHYDRATION OF NEPETANUDOSIDE TETRAACETATE [**3**].—To a solution of **3** (462.6 mg) in dry pyridine (3 ml) was added $POCl_3$ (3 ml) at 0° and the mixture left to stand overnight in a refrigerator. The reaction mixture was poured dropwise into ice H_2O and the resulting precipitates were extracted with $CHCl_3$ (200 ml×2). After washing with 1 N HCl, 5% $NaHCO_3$ and then H_2O, successively, the $CHCl_3$ extract was dried ($MgSO_4$) and evaporated *in vacuo* to give a residue (377.8 mg) which was purified on a Si gel (25 g) column with Et_2O as eluent to give **7** (314.8 mg) as an amorphous powder. Ir ν max ($CHCl_3$) 1750, 1705, 1640, 1370, 1220, 1095, 1070 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.75 (3H, d, J=1.5 Hz, H_3-10), 2.007, 2.014, 2.03, 2.09 (3H each, s, OCOMe), 2.53 (1H, br t, J=7.6 Hz, H-9), 2.78 (1H, m, H_a-6), 3.15 (1H, br q, J=7.8 Hz, H-5), 3.72 (3H, s, COOMe), 3.78 (1H, m, H-5′), 4.15 (1H, dd, J=12.2 and 2.4 Hz, H_a-6′), 4.29 (1H, dd, J=12.2 and 4.9 Hz, H_b-6′), 4.82 (1H, d, J=7.3 Hz, H-1), 4.88 (1H, d, J=7.8 Hz, H-1′), 5.04–5.22 (3H, H-2′, H-3′, and H-4′), 5.53 (1H, br s, H-7), 7.44 (1H, d, J=1.0 Hz); negative-ion hrfabms *m/z* $[M-H]^-$ 539.1748, calcd for $C_{25}H_{31}O_{13}$, 539.1764.

DEACETYLATION OF THE DEHYDRATED PRODUCT [**7**].—To a solution of **7** (288.8 mg) in MeOH (3 ml) was added 0.2 N NaOH aqueous solution (0.14 ml) and the solution was stirred at room temperature for 2 h. The reaction mixture was neutralized with an ion exchange resin, Amberlite IR-120B (H^+ form). After removing the resin by filtration, the filtrate was concentrated *in vacuo* to give the deacetylated product [**8**] (190.9 mg) as an amorphous powder: ir ν max (KBr) 3350, 1690, 1660, 1070 cm^{-1}; ^{1}H nmr (CD_3OD) δ 1.84 (3H, br s, H_3-10), 2.07 (1H, m, H_a-6), 2.72 (2H, m, H-9 and H_b-6), 3.14 (1H, br q, J=7.1 Hz, H-5), 3.70 (3H, s, COOMe), 3.84 (1H, dd, J=11.7 and 2.0 Hz, H_a-6′), 4.58 (1H, d, J=7.8 Hz, H-1′), 5.15 (1H, d, J=5.9 Hz, H-1), 5.48 (1H, br s, H-7), 7.45 (1H, d, J=2.4 Hz); negative-ion hrfabms *m/z* $[M-H]^-$ 371.1343, calcd for $C_{17}H_{23}O_9$, 371.1342.

ENZYMATIC HYDROLYSIS OF COMPOUND [**8**].—Compound **8** (169.7 mg) was dissolved in H_2O (10 ml) and β-glucosidase (from almond, Toyobo, 100 mg) was added to the solution. The mixture was incubated overnight at 37° and then extracted with Et_2O (50 ml×3). After washing with saturated aqueous NaCl solution, the Et_2O extract was dried and evaporated *in vacuo* to give an aglucone [**9**] (59.1 mg) as a syrup: ir ν max ($CHCl_3$) 3600, 3400, 1690, 1630, 1440, 1285, 1100 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.86 (3H, d, J=1.5 Hz, H_3-10), 1.99 (1H, m, H_a-6), 2.83 (1H, br t, J=7.8 Hz, H-9), 2.78 (1H, m, H_b-6), 3.15 (1H, br q, J=8.3 Hz, H-5), 3.73 (3H, s, COOMe), 4.86 (1H, d, J=7.8 Hz, H-1), 5.53 (1H, br s, H-7), 7.49 (1H, s, H-3); negative-ion hrfabms *m/z* $[M-H]^-$ 209.0847, calcd for $C_{11}H_{13}O_4$, 209.0814.

ACETYLATION OF *ENT*-10-DEOXYGENIPIN [**9**].—*ent*-10-Deoxygenipin [**9**] (55.8 mg) was acetylated with a mixture of Ac_2O (0.5 ml) and pyridine (0.5 ml) overnight. After addition of excess MeOH, the solvent was removed *in vacuo*. The residue was purified by prep. tlc (*n*-hexane-Et_2O, 7:3) to give the monoacetate

[**10**] (41.9 mg) as a syrup: $[\alpha]^{23}D$ −55.7° (*c*=1.99, $CHCl_3$), ir ν max ($CHCl_3$) 1750, 1700, 1630, 1440, 1280, 1180, 1080, 960 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.77 (3H, s, H_3-10), 2.15 (3H, s, OCOMe), 2.68 (1H, m, H-9), 3.22 (1H, m, H-5), 3.73 (3H, s, COOMe), 5.53 (1H, br s, H-7), 5.93 (1H, d, *J*=6.8 Hz, H-1), 7.44 (1H, d, *J*=1.0 Hz); hreims *m/z* $[M]^+$ 252.0994, calcd for $C_{13}H_{16}O_5$, 252.0997.

ACETYLATION OF 10-DEOXYGENIPIN [**13**].—10-Deoxygenipin [**10**] (5) (32.8 mg) was acetylated and purified as above to give the monoacetate **11** (33.5 mg) as a syrup. This compound showed identical ir and nmr (^1H and ^{13}C) spectra, and opposite specific rotation, $[\alpha]^{23}D$ +56.0° (*c*=1.12, $CHCl_3$) to those of **10**. Hreims *m/z* $[M]^+$ 252.1005, calcd for $C_{13}H_{16}O_5$, 252.0997.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. A.T. Bottini, V. Dev, D.J. Garfagnoli, H. Lahani, C.S. Mathela, and A.K. Pant, *Phytochemistry*, **26**, 1200 (1987), and references cited therein.
2. Y. Takeda, H. Nishimura, and H. Inouye, *Phytochemistry*, **16**, 1401 (1977).
3. D.R. Gardner, J. Narum, D. Zook, and F.R. Stermitz, *J. Nat. Prod.*, **50**, 485 (1987).
4. S. Damtoft, S.R. Jensen, and B.J. Nielsen, *Phytochemistry*, **20**, 2717 (1981).
5. F. Murai, M. Tagawa, S. Damtoft, S.R. Jensen, and B.J. Nielsen, *Chem. Pharm. Bull.*, **32**, 2809 (1984).
6. G.G. Zapesochnaya, V.A. Kurkin, L.N. Pervykh, and B.S. Karasatov, *Khim. Prir. Soedin.*, 771 (1991).
7. K. Inoue, M. Ono, H. Nakajima, I. Fujie, H. Inouye, and T. Fujita, *Heterocycles*, **33**, 673 (1992).
8. R. Touyama, K. Inoue, Y. Takeda, M. Yatsuzuka, T. Ikumoto, N. Moritome, T. Shingu, T. Yokoi, and H. Inouye, *Chem. Pharm. Bull.*, **42**, 1571 (1994).
9. C.A. Boros and F.R. Stermitz, *J. Nat. Prod.*, **53**, 1055 (1990).

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