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The Constituents of *Schizonepeta tenuifolia* BRIQ. II.[1] Structure of a New Monoterpene Glucoside, Schizonepetoside C[2]

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Besides three known flavonoid glycosides, a new monoterpene glucoside named schizonepetoside C (**3**) was isolated from the spikes of *Schizonepeta tenuifolia* BRIQ. (Labiatae), as an amorphous powder. The structure of **3** was established as (1*S*,4*E*)-9-*O*-*β*-D-glucopyranosyl-*p*-menth-4(8)-en-3-one by chemical methods and spectral analyses.

Keywords——*Schizonepeta tenuifolia*; Labiatae; monoterpene glucoside; schizonepetoside C; (1*S*,4*E*)-9-*O*-*β*-D-glucopyranosyl-*p*-menth-4(8)-en-3-one; apigenin-7-*O*-*β*-D-glucoside; luteolin-7-*O*-*β*-D-glucoside; hesperidin; ^{13}C-NMR

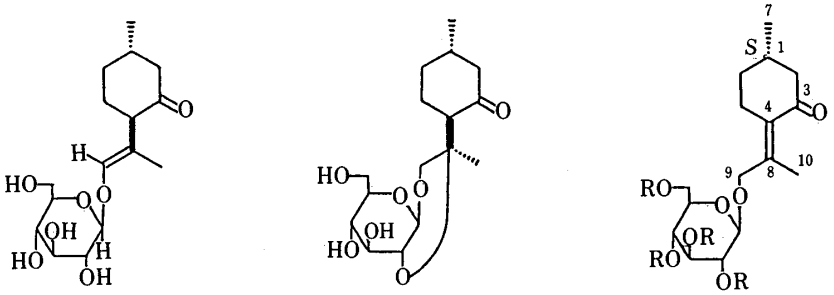
Previously we reported the isolation and characterization of two new glucosides designated as schizonepetosides A (**1**) and B (**2**) from the spikes of *Schizonepeta tenuifolia* BRIQ. (syn., *Nepeta japonica* MAXIM.) (Labiatae).[1,3] Schizonepetosides A and B are rare *p*-menthone-type glucosides; **1** is an aldehyde enol glucoside and **2** is a glucoside possessing a dioxane ring formed by double linkage between glucose and the aglycone. This paper deals with the isolation and structure determination of a new monoterpene glucoside named schizonepetoside C (**3**) and the isolation of three known flavonoid glycosides from the same source.

The spikes of the plant were extracted with methanol. The methanolic extract was dissolved in water, and extracted with butanol. The butanolic extract was successively subjected to polyamide column chromatography to give a mixture of **1** and **3** from the water eluate and three known flavonoid glycosides, **4**, **5** and **6**, from the methanol eluate.

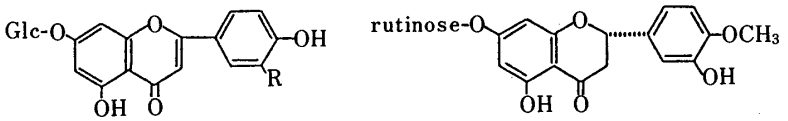
Field desorption-mass spectra (FD-MS) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **4** and **5** suggested them to be flavonoid monoglucosides. Physico-chemical data of **4** were in good agreement with those reported for apigenin-7-*O*-*β*-D-glucoside.[4] Compound **5** was found to be identical with luteolin-7-*O*-*β*-D-glucoside by comparison of infrared (IR) spectra and mixed fusion. The result of ^{13}C-NMR analysis indicated that **6** is the rutinoside of a flavanone. Finally its octaacetate (**7**), mp 171—181 °C, was found to be identical with hesperidin octaacetate on the basis of mixed mp and IR spectral comparison.

By preparative high performance liquid chromatographic (HPLC) separation of the mixture of **1** and **3**, **3** was isolated as a very hygroscopic white amorphous powder, $[\alpha]_D^{28}$ −48.9° (EtOH), (yield, 0.007%), from the more polar fraction. Compound **3** afforded a crystalline tetraacetate (**8**), $C_{24}H_{34}O_{11}$, mp 75.5—76 °C, $[\alpha]_D^{27}$ −43.9° (EtOH), on acetylation with acetic anhydride and pyridine. The molecular formula of **8** was the same as that of schizonepetoside A tetraacetate.

As shown in Table I, the ^{13}C-NMR spectrum of **3** revealed that the aglycone and sugar moiety were extremely similar to those of **1** except for the olefinic carbon signals. The sugar moiety gave signals characteristic of a *β*-D-glucopyranosyl group, comparable with the signals



1 2 3 : R=H 8 : R=Ac



4 : R=H
5 : R=OH

6

Chart 1

TABLE I. The ^{13}C and ^{1}H Chemical Shifts[a] of **1** and **3**

	1	3	1	3
1	35.0 d	32.3 d		
2	50.5 t	51.5 t		
3	209.7 s	204.0 s		
4	54.1 d	136.5 s		
5	31.3 t	28.8 t		
6	33.9 t	33.5 t		
7	22.3 q	21.7 q	0.87 (3H, d, J=5 Hz)	0.82 (3H, d, J=5 Hz)
8	113.6 s	137.8 s		
9	141.9 d	68.6 t	6.53 (1H, br s)	
10	12.1 q	18.7 q	1.83 (3H, br s)	2.23 (3H, br s)
1′	104.7 d	103.3 d	5.07 (1H, d, J=7 Hz)	4.84 (1H, d, J=7 Hz)
2′	74.7 d	75.1 d		
3′	78.8 d	78.7 d		
4′	71.2 d	71.8 d		
5′	78.2 d	78.6 d		
6′	62.4 t	62.9 t		

a) δ ppm from internal tetramethylsilane (TMS) in C_5D_5N.

of **1**. This finding led us to assume that **3** is a glucopyranoside isomeric with **1** only in the position of the double bond of the aglycone.

The IR and ultraviolet (UV) spectra of **3** suggested the presence of an α,β-unsaturated ketone; the former showed a strong absorption at 1680 cm^{-1}, while the latter showed an absorption maximum at 247 nm ($\log\varepsilon$=3.61). In the proton nuclear magnetic resonance (^{1}H-NMR) spectra (Table I), **1** showed a broad singlet at δ_H 6.53 ppm due to an olefinic proton, while the corresponding signal was not observed for **3**. These findings show that the double bond of **3** is at C-4(8), α to the carbonyl function.

Enzymatic hydrolysis of **3** using β-glucosidase afforded its aglycone (**9**), colorless oil, $[\alpha]_D^{28}$ −31.4° (EtOH). The chemical shifts of the ^{1}H-NMR signals of **9** are summarized in Chart 2, and a broad singlet at δ_H 1.98 ppm was readily assignable to the olefinic methyl protons.

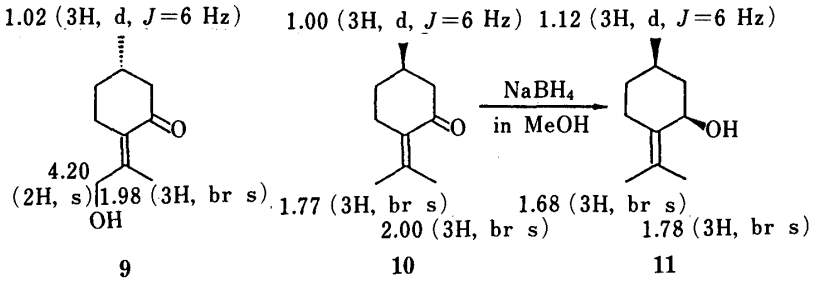


Chart 2. The ^1H Chemical Shifts (δ ppm) of **9**, **10** and **11** in $CDCl_3$

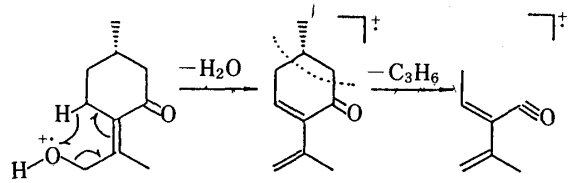


Chart 3. MS Fragmentation of **9**

In order to determine the stereochemistry of the double bond of **9**, ^1H-NMR experiments on (−)-pulegone (**10**) and pulegol (**11**) were carried out. The chemical shift data of **10** and **11** are shown in Chart 2. Two broad singlets at δ_H 1.77 and 2.00 ppm due to olefinic methyl protons in **10** were shifted to δ_H 1.68 and 1.78 ppm in **11**. This observation shows that the olefinic methyl group sterically close to the ketone group appears at around δ_H 2.00 ppm. On the other hand, the methyl signal of **9** appears at δ_H 1.98 ppm, indicating that the olefinic methyl group is close to the ketone group and thus that the stereochemistry of the double bond C-4(8) is *E* form. This was further supported by MS analysis based on the presence of the dehydrated fragment ion at *m/z* 150 in **9** (Chart 3).[5]

In the circular dichroism (CD) spectrum, **3** exhibited a positive Cotton effect $[\theta]_{241}$ $+6.99 \times 10^5$ and a negative Cotton effect $[\theta]_{320}$ -2.03×10^5, while (1*R*)-pulegone has a reverse Cotton effect, $[\theta]_{246}$ -1.10×10^6, $[\theta]_{320}$ $+2.38 \times 10^5$.[6] These findings show that the absolute configuration of **3** (C-1) is *S* form.

On the basis of all the above results and discussion, the structure of schizonepetoside C (**3**) was established as (1*S*,4*E*)-9-*O*-*β*-D-glucopyranosyl-*p*-menth-4(8)-en-3-one (Chart 1). Schizonepetoside C (**3**) is a novel menthone-type glucoside resembling schizonepetosides A (**1**) and B (**2**). Michael reaction of **3** may be involved in the biosynthesis of **2**. The existence of a series of menthone-type glucosides including **1**, **2** and **3**, would be interesting from the biosynthetic viewpoint.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (a hot stage type) and are uncorrected. IR spectra were recorded with a Hitachi EPI-G2 unit, and UV spectra on a Hitachi 624 digital spectrophotometer. NMR spectra were measured with a Varian T-60 and a Varian FT-80A using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were obtained with a JEOL JMS DX-300. The specific rotations were measured with a JASCO DIP-SL. CD spectra were taken with a JASCO J-40A. Preparative HPLC was performed on a JASCO Trirotar (column: semiprep. μ-Bondapak C_{18}, Waters Assoc.) and a Waters system 500A (column: Prep PAK 500/C_{18}, Waters Assoc.) with a refractive index monitor. Thin-layer chromatography (TLC) was carried out on Merck plates precoated with Kieselgel 60 F_{254}.

Materials——The BuOH extract described in the experimental section of the previous paper[1] was used as the source material.

Isolation of 3, 4, 5 and 6——The BuOH extract (51.5 g) was chromatographed on a polyamide column. The column was washed successively with water and MeOH. The H_2O eluate was concentrated to afford a brown syrup (15.5 g), which was rechromatographed on silica gel, and elution with $CHCl_3$–MeOH (12 : 1) afforded a mixture of **1** and **3** (2.2 g). The mixture was subjected to preparative HPLC (System 500A), developing with a CH_3CN–MeOH–H_2O (1 : 1 : 6) solvent system, to give crude **3** (0.55 g), which was purified by semiprep. HPLC (Trirotar, CH_3CN–MeOH–H_2O (1 : 1 : 10)) to give pure **3** (0.22 g) (yield, 0.007%). Compound **6** (yield, 0.42%) and a mixture of **4** and **5** were obtained from the MeOH eluate from the polyamide chromatography. The latter was purified by prep. HPLC (System 500A), developing with a CH_3CN–MeOH–1% AcOH (1 : 1 : 5) solvent system, to give **4** and **5** (yield, each 0.08%).

Compound 4——Yellow needles (from pyridine–H_2O), mp 218—220 °C. $FeCl_3$ reagent: positive (brown). FD-MS *m/z*: 433 (MH^+). IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1660 (C=O), 1603, 1498 (arom.). UV λ_{max}^{MeOH} nm (log ε): 269 (4.22), 335 (4.28). ^{1}H-NMR (in DMSO-d_6) δ: 3.00—4.00 (6H, m), 5.04 (1H, d, J=7.5 Hz, anomeric H), 6.43 (1H, d, J=3 Hz, H-6), 6.68 (1H, s, H-3), 6.75 (1H, d, J=3 Hz, H-8), 6.92 (2H, d, J=9 Hz, H-3′, 5′), 7.86 (2H, d, J=9 Hz, H-2′, 6′), 12.79 (1H, br s, 5-OH). ^{13}C-NMR (in DMSO-d_6) δ: 60.8 (t, C-Glc-6′), 69.9 (d, C-Glc-4′), 73.1 (d, C-Glc-2′), 76.4 (d, C-Glc-5′), 77.0 (d, C-Glc-3′), 94.9 (d, C-8), 99.6 (d, C-6), 100.3 (d, C-Glc-1′), 103.0 (d, C-3), 105.3 (s, C-4a), 115.8 (2C, d, C-3′, 5′), 121.1 (s, C-1′), 128.1 (2C, d, C-2′, 6′), 156.8 (s, C-8a), 160.9 (s, C-4′), 161.0 (s, C-5), 162.8 (s, C-7), 164.2 (s, C-2), 181.6 (s, C-4). **4** was identified as apigenin-7-*O*-β-D-glucoside by comparison of the spectral data with the reported values.[4]

Compound 5——Yellow needles (from H_2O–EtOH), mp 257—260 °C. FD-MS *m/z*: 449 (MH^+). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1658 (C=O), 1605, 1498 (arom.). **5** was found to be identical with an authentic sample of luteolin-7-*O*-β-D-glucoside by mixed mp determination and IR spectral comparison.

Compound 6——Needles (by precipitation from MeOH), mp 253—259 °C, $[\alpha]_D^{25}$ −68.7° (c=0.63, pyridine). Treatment of **6** (100 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temperature for 24 h gave a crude product, which was purified by recrystallization from MeOH to give a crystalline octaacetate (**7**) as colorless needles (96 mg), mp 179—181 °C. IR ν_{max}^{KBr} cm^{-1}: 1755 (acetyl), 1692 (C=O), 1618, 1515 (arom.). **7** was identified by mixed mp and IR comparison with an authentic sample of hesperidin octaacetate.

Schizonepetoside C (3)——White hygroscopic amorphous powder, $[\alpha]_D^{28}$ −48.9° (c=0.7, EtOH). FD-MS *m/z*: 331 (MH^+). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1680 (C=O). UV λ_{max}^{EtOH} nm (log ε): 247 (3.61). CD ($c=7.87\times10^{-4}$, MeOH) $[\theta]^{25}$ (nm): $+6.99\times10^5$ (241) (positive maximum), -2.03×10^5 (320) (negative maximum). See Table I for ^{1}H-NMR and ^{13}C-NMR spectral data.

Acetylation of 3, Giving the Tetraacetate (8)——Ac_2O (0.5 ml) was added to a solution of **3** (55.8 mg) in pyridine (0.5 ml), and the solution was allowed to stand for a day at room temperature. The reaction mixture was worked up as usual and the crude acetate was recrystallized from ether–hexane to give **8** as colorless needles (49.5 mg), mp 75.5—76.0 °C, $[\alpha]_D^{27}$ −43.9° (c=0.3, EtOH). *Anal.* Calcd for $C_{24}H_{34}O_{11}$: C, 57.82; H, 6.87. Found: C, 57.50; H, 6.70. High MS *m/z*: Calcd for $C_{24}H_{34}O_{11}$ (M^+), 498.2101. Found: 498.2130. MS *m/z*: 498 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1760 (acetyl), 1685 (C=O). UV λ_{max}^{EtOH} nm (log ε): 246 (3.81). CD ($c=4.49\times10^{-4}$, MeOH) $[\theta]^{25}$ (nm): $+1.38\times10^6$ (240) (positive maximum), -3.21×10^5 (320) (negative maximum). ^{1}H-NMR (in $CDCl_3$) δ: 1.02 (3H, d, J=6 Hz, H-7), 1.88 (3H, br s, H-10), 2.02 (9H, s, AcO- × 3), 2.07 (3H, s, AcO-), 4.23 (2H, br s, H-9). ^{13}C-NMR (in $CDCl_3$) δ: 17.9 (q, C-10), 20.6, 20.7 (each q, $-COCH_3$ × 4), 21.7 (q, C-7), 28.6 (t, C-5), 32.3 (d, C-1), 33.5 (t, C-6), 51.4 (t, C-2), 62.1 (t, C-6′), 68.1 (t, C-9), 68.6 (d, C-4′), 71.3 (d, C-2′), 72.0 (d, C-5′), 72.8 (d, C-3′), 98.8 (d, C-1′), 135.9 (s, C-4), 137.2 (s, C-8), 169.2, 169.4, 170.3, 170.5 (each s, $-COCH_3$ × 4).

Enzymatic Hydrolysis of 3——A solution of **3** (30.1 mg) in H_2O was treated with β-glucosidase (Miles Laboratories (PTY) Ltd., 23.0 mg) and the mixture was kept for a day with gentle stirring at 38 °C. The incubation mixture was concentrated, and extracted with ether. The ether layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated. The resulting product was purified by prep. TLC using $CHCl_3$–MeOH (9 : 1) to give **9** as a colorless oil (9.1 mg), $[\alpha]_D^{28}$ −31.4° (c=0.5, EtOH). MS *m/z*: 168 (M^+). ^{1}H-NMR spectral data are given in Chart 2.

Acetylation of 9——**9** (19.3 mg) was acetylated overnight with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temperature. After work-up in the usual manner, the products was passed through a silica gel column. The fraction eluted with benzene–EtOAc (20 : 1) afforded a monoacetate as a colorless oil (13.7 mg), $[\alpha]_D^{28}$ −21.4° (c=0.3, EtOH). MS *m/z*: 210 (M^+). UV λ_{max}^{EtOH} nm (log ε): 240 (3.78). ^{1}H-NMR (in $CDCl_3$) δ: 1.00 (3H, d, J=6 Hz, H-7), 1.91 (3H, br s, H-10), 2.05 (3H, s, AcO-), 4.60 (2H, br s, H-9).

(−)-Pulegone (10)——**10** (Tokyo Chemical Industry Co., Ltd.) was purified by prep. TLC using benzene–EtOAc (24 : 1) to give a colorless oil. MS *m/z*: 152 (M^+). CD ($c=1.64\times10^{-3}$, MeOH) $[\theta]^{25}$ (nm): -1.10×10^6 (246) (negative maximum), $+2.38\times10^5$ (320) (positive maximum). See the text for ^{1}H-NMR spectral data (Chart 2). ^{13}C-NMR (in $CDCl_3$) δ: 21.8 (q, C-7), 22.1 (q, C-9), 23.0 (q, C-10), 28.6 (t, C-5), 31.6 (d, C-1), 32.8 (t, C-6), 50.9 (t, C-2), 131.8 (s, C-4), 141.8 (s, C-8), 204.0 (s, C-3).

Reduction of 10 with $NaBH_4$, Giving 11——$NaBH_4$ (15 mg) was added to a solution of **10** (20 mg) in MeOH (2 ml) and the reaction mixture was allowed to stand at room temperature for 2.5 h, then passed through an Amberlite IR-120B (H^+) column and evaporated to dryness. The residue was purified by prep. TLC (benzene–EtOAc (24 : 1)) to give pulegol (**11**) (12 mg) as a colorless oil. ^{1}H-NMR spectral data are given in Chart 2. ^{13}C-NMR (in $CDCl_3$) δ: 19.9

(q, C-10), 20.6 (q, C-9), 21.7 (q, C-7), 22.5 (t, C-5), 27.0 (d, C-1), 32.1 (t, C-6), 39.7 (t, C-2), 68.4 (d, C-3), 126.5 (s, C-4), 132.9 (s, C-8).

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References and Notes

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