Communications to the Editor

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SWEET AND BITTER GLYCOSIDES OF THE CHINESE PLANT DRUG, BAI-YUN-SHEN (ROOTS OF SALVIA DIGITALOIDES)

Takashi Tanaka,[a] Osamu Tanaka,*,[a] Zhong-Wen Lin,[b] Jun Zhou[b] and Hiroyuki Ageta[c]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] Kasumi, Minami-ku, Hiroshima, 734 Japan, Kunming Institute of Botany, Academia Sinica,[b] Kunming, Yunnan, China and Showa College of Pharmaceutical Sciences,[c] 5-1-8 Tsurumaki, Setagaya-ku, Tokyo 154, Japan

From a Chinese plant drug, Bai-Yun-Shen (roots of Salvia digitaloides), a new sweet glycoside named baiyunoside (**1**) was isolated. The structure of the acid-unstable aglycone of **1** named baiyunol (**2**) was elucidated by MS and NMR spectroscopy especially by comparison with the ^{13}C chemical shifts of β-onocerin (**3**). The structure of **1** was determined to be β-D-xylopyranosyl(1→2)-β-D-glucopyranoside of **2** by MS and NMR spectroscopy. Besides this sweet principle (**1**), several bitter iridoid glucosides were also isolated from this plant. Two of these glucosides were identified as shanzhiside methyl ester (**13**) and its 8-O-acetate (=acetylbarlerin, **10**), respectively. A new glucoside (**9**) was formulated as 6-O-syringyl-8-O-acetyl shanzhiside methyl ester by the correlation with **13**.

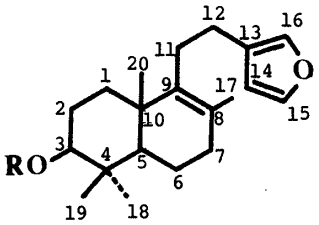
KEYWORDS —— Bai-Yun-Shen; Salvia digitaloides Diels; Labiatae; sweet glycoside; baiyunoside; labdane-type diterpene; ^{13}C-NMR; β-onocerin; iridoid glucoside; acylated shanzhiside methyl ester

We have been engaged in the investigation of the sweet principles of Chinese plants, i.e., a sweet steviol-glycoside, rubusoside from Chinese Rubus chingii Hu (Rosaceae),[1] dihydrochalcone-glycosides, phlorizin and trilobatin from Lithocarpus litseifolius (Hance) Rehd. (Fagaceae)[2] and trilobatin from Vitis piasezkii Maxim. and V. saccharifera Makino (Vitaceae)[3]. The present communication deals with the chemical study of sweet and bitter glycosides of the Chinese plant drug "Bai-Yun-Shen", roots of Salvia digitaloides Diels (Labiatae) which has been used for women's diseases.

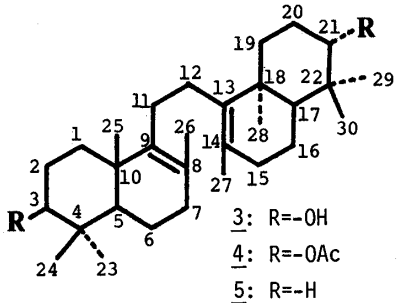
An aqueous suspension of the methanolic extract of the dried roots collected in Li-jiang, Yunnan, China, was washed with ether and then extracted with 1-butanol saturated with water. The butanolic fraction was chromatographed to give a new sweet principle named baiyunoside (**1**), white powder, $C_{31}H_{48}O_{11}$, EI-MS as its hexa-acetate: M+ m/z 890, $[\alpha]_D^{26}$ +13.8° (c=2.50, MeOH), UV λ_{max}^{EtOH} 213nm (logε=3.8), yield: 0.11%. This glycoside (**1**) is about 500-fold sweeter than sucrose and its sweetness lasts more than one h.

On hydrolysis with crude hesperidinase,[4] an acid unstable aglycone, named baiyunol (**2**), was obtained from **1** as colorless needles mp 85.5-86°C (from n-hexane), $C_{20}H_{30}O_2$, EI-MS: M+ 302, $[\alpha]_D^{21}$ +64.0° (c=1.47, $CHCl_3$). It was not sweet. The UV

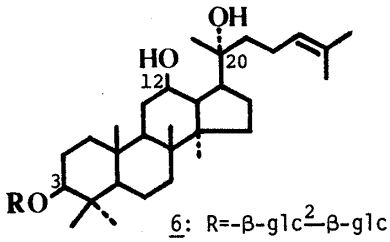
absorption maximum at 212nm (in MeOH) ($\log\varepsilon$=3.9), strong ions at m/z 81 and 95 in its EI-MS, ^{1}H-NMR signals at δ 7.35(1H, t, J=1.8Hz), 7.23(1H, broadened dd) and 6.30(1H, broadened dd) in $CDCl_3$ as well as ^{13}C signals at δ 125.5 (or 126.7)(s, 13-C), 110.8(d, 14-C), 142.6(d, 15-C) and 138.4(d, 16-C) in $CDCl_3$ revealed the presence of a β-substituted furan ring[5] in **2**. Further, multiplicities of the ^{13}C-NMR signals of **2** demonstrated the presence of one carbinyl carbon, one tetra-substituted double bond, four methyls, six methylenes, two methines and two quarternary carbons. Its proton signals in $CDCl_3$ at δ 0.81(3H, s), 0.97(3H, s), 1.02(3H, s), 1.61(3H, s) and 3.25(1H, dd, J=6.0 and 10.8Hz) revealed the presence



1: R=-β-glc^2—β-xyl
2: R=-H



3: R=-OH
4: R=-OAc
5: R=-H

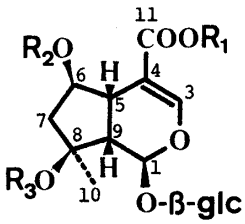


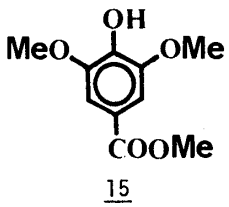
6: R=-β-glc^2—β-glc
7: R=-H

Table I. ^{13}C-NMR Spectra of Aglycone Moieties (in Pyridine-d_5 at 25.15 MHz)

	1	2	3		1	2	3
C-1	35.3	35.5	36.3	C-11	29.0	29.2	28.7
2	27.2	29.2	29.1	12	26.1	26.1	
3	89.3	78.1	78.0	13	126.1†	126.1†	
4	38.7*	39.1*	39.2	14	111.5	111.4	
5	51.6	51.4	51.4	15	143.3	143.3	
6	18.9	19.5	19.4	16	139.0	139.1	
7	34.0	34.1	34.6	17(26)	19.0	19.2	20.7
8	126.5†	126.6†	126.5	18(23)	28.0	28.7	28.8
9	139.9	140.1	141.1	19(24)	16.4	16.5	16.4
10	39.6*	39.5*	39.5	20(25)	20.2	20.3	21.0

( ); For 3. *,†; These signals may be reversed.





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9: R_1=-Me, R_2=-OOC-(OMe, OH, OMe), R_3=-Ac
10: R_1=-Me, R_2=-H, R_3=-Ac
13: R_1=-Me, R_2=R_3=-H
14: R_1=R_2=R_3=-H
16: R_1=-Me, R_2=-OOC-(OMe, OH, OMe), R_3=-H

Table II. ^{13}C-NMR Spectra of Sugar Moieties (in Pyridine-d_5 at 25.15 MHz)

	1	(inner)	6
glc-1	105.0	glc-1	105.1
2	83.8	2	83.5
3	78.3*	3	78.2*
4	71.4†	4	71.7
5	78.1*	5	78.0*
6	62.6	6	62.7
xyl-1	106.8		
2	76.5		
3	77.9		
4	71.0†		
5	67.4		

*,†; These signals may be reversed.

Table III. ^{13}C-NMR Spectra of Iridoids (in Pyridine-d_5 at 25.15 MHz)

	9	16	10	13		9	16	10	13
C-1	95.1	94.9	95.8	94.9	11-COOMe	51.1	51.3	51.1	52.0
3	153.6	152.6	152.9	152.3	OCOMe	170.5		170.9	
4	107.7	109.3	108.9	110.6	OCOMe	21.9*		22.0	
5	39.2	38.2	42.0	41.5					
6	78.1	78.3	74.7	76.6	1'	120.4	120.9		
7	44.7	48.1	47.4	48.8	2',6'	108.4	108.4		
8	88.3	77.7	88.6	78.3	3',5'	148.5	148.6		
9	49.7	51.3	49.6	51.1	4'	143.0	142.7		
10	21.5*	25.7	22.0	24.9	φ-OMe	56.4	56.4		
11	166.6†	167.1†	167.5	168.0	φ-COO	165.9†	166.4†		

*,†; These signals may be reversed.

glc; -D-glucopyranosyl, xyl; -D-xylopyranosyl.

of three methyl groups on quarternary carbons, one allylic methyl group and one equatorial secondary hydroxyl group, respectively. This evidence suggestes that **2** is a monohydroxy-labdane-type diterpene having a β-substituted furan ring. One of the present authors, Ageta, has investigated the ^{13}C-NMR spectra of onocerane-type triterpenes in connection with his chemical studies of fern triterpenes, assigning the carbon signals of β-onocerin (**3**)[6] derived from α-onocerin[7] by comparing them with those of its acetate (**4**) and β-onoceradiene (**5**)[8] with consideration for the substitution and acetylation effects. As shown in Table I, it was found that the carbon signals of **2** other than those associated with its β-substituted furan moiety appeared at almost the same positions as those of **3** except for the slight differences in the chemical shift of the allylic methyl signal (C-17 for **2** and C-26 and -27 for **3**), leading to the formulation of **2** as illustrated in Chart 1. The ^{1}H-NMR of **2** was consistent with this formulation. The chirality of the 3-equatorial-hydroxyl group of **2** was established as S-configuration by the modified Horeau's method.[9] Accordingly, the absolute configuration of the skeleton of **2** should be the normal type.

On mineral acid hydrolysis, **1** yielded glucose and xylose. The EI-MS of the acetate of **1** exhibited fragment ions at m/z 547 ((glucose-xylose)Ac_6) and 259 ((xylose)Ac_3). The coupling constants of two anomeric proton signals of **1** (δ 4.93 and 5.25 (1H d each, J=6.0Hz) in C_5D_5N) indicated the β-configuration of both glycosyl linkages. Inspection of the ^{13}C-NMR spectrum of **1** in comparison with those of known glycosides revealed that signals due to the sugar moiety of **1** consisted of those due to a terminal β-xylopyranosyl unit and a 2-O-glycosylated β-glucopyranoside unit, the latter of which appeared at almost the same positions as those of the 2-O-substituted β-D-glucopyranosyl unit of the 3-O-β-sophorosyl moiety of the prosapogenin (**6**) of Ginseng saponins[10] as shown in Table II. It was revealed that the glycosylation shifts are significantly related to the combination of absolute configurations of an aglycone alcohol[11] and a sugar moiety. On going from **2** to **1**, the glycosylation shift values of C-2, -3 and -4 as well as that of the anomeric carbon signal of the inner glucosyl unit were found to be almost identical with those for **6** from 20(R)-protopanaxadiol (**7**).[11] This evidence led to the formulation of **1** including absolute configuration of the sugar moiety as β-D-xylopyranosyl(1→2)-β-D-glucopyranoside of **2**.

Besides this sweet principle (**1**), six bitter iridoid-glucosides, **8-13**, were isolated from this plant in yields of 0.02, 0.19, 0.28, 0.11, 0.17 and 0.03%, respectively. Glucoside (**13**), white powder, $[\alpha]_D^{22}$ -123.3°(c=3.8, MeOH), UV λ_{max}^{MeOH} 236nm (log ε =3.9) was identified as shanzhiside methyl ester which was previously isolated from Mussaenda parviflora Miquel.[12] Glucoside (**10**), white powder, $[\alpha]_D^{26}$ -84.8°(c=1.28, MeOH), UV λ_{max}^{MeOH} 234nm (logε=4.0), ^{1}H-NMR in C_5D_5N: δ 1.65(3H, s, 10-CH_3), 1.88(3H, s, $OCOCH_3$), 3.60(3H, s, $COOCH_3$), 5.32(1H, d, J=7.2Hz, anomeric H), 6.43(1H, d, J=1.0Hz, 1-H) and 7.68(1H, s, 3-H) afforded **13** on mild alkaline hydrolysis, being identical with 8-O-acetate of **13** named acetylbarlerin which was recently isolated from Barleria prionitis L..[13]

On alkaline hydrolysis with 1N NaOH in methanol at room temperature, a new glucoside **9**, white powder, $[\alpha]_D^{24}$ -71.0°(c=1.01, MeOH), UV λ_{max}^{MeOH} 221 and 276nm (logε =4.2 and 3.7) yielded shanzhiside (**14**) and methyl syringate (**15**). The ^{1}H-NMR spectrum of **9** in C_5D_5N exhibited a signal at δ 1.93(3H, s, $OCOCH_3$) along with

signals at δ 1.71(3H, s, 10-CH_3), 3.60(3H, s, $COOCH_3$), 3.83(6H, s, OCH_3), 5.36(1H, d, J=7.2Hz, anomeric H), 6.31(1H, d, J=3.6Hz, 1-H), 7.69(2H, s, aromatic H) and 7.74(1H, d, J=1.2Hz, 3-H) indicating the presence of an acetoxyl group which was supported by its ^{13}C-NMR spectrum (Table III). Mild hydrolysis of **9** with 0.1N NaOH in methanol at room temperature resulted in deacetylation, yielding **16** (a syringate of **13**), white powder, $[\alpha]_D^{22}$ -117.3°(c=1.05, MeOH), UV λ_{max}^{MeOH} 221 and 275nm(log ε =4.3 and 3.9), ^1H-NMR in C_5D_5N: δ 1.43(3H, s, 10-CH_3), 3.55(3H, s, $COOCH_3$), 3.80(6H, s, OCH_3), 5.37(1H, d, J=7.2Hz, anomeric H), 6.07(1H, d, J=4.5Hz, 1-H), 7.74(1H, s, 3-H) and 7.78(2H, s, aromatic H). As shown in Table III, on going from 13 to **16**, the signal due to 6-C was deshielded by 1.7 ppm and that due to 4-, 5- and 11-C were shielded, leading to the formulation of **16** as 6-O-syringate of **13**. On going from **13** to **9**, signals due to both 6- and 8-C were displaced downfield and those due to 4-, 5-, 7-, 9-, 10- and 11-C were shielded. It follows that **9** can be formulated as 6-O-syringyl-8-O-acetyl shanzhiside methyl esters. The structures elucidation of **8**, **11** and **12** are being studied.

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