TRIFOLIOSIDE II, AN ACYCLIC SESQUITERPENE OLIGOGLYCOSIDE FROM PERICARPS OF *SAPINDUS TRIFOLIATUS*

Ryoji Kasai, Masatoshi Nishi,* Kenji Mizutani, Izumi Miyahara, Toshie Moriya, Kazumoto Miyahara*
and Osamu Tanaka†

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi, Minami-Ku, Hiroshima 734, Japan;
*Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge-cho, Hirakata 573-01, Japan

Abstract—New acyclic sesquiterpene oligoglycoside named trifolioside II was isolated from pericarps of *Sapindus trifoliatus* along with two known saponins, and the structure of trifolioside II was elucidated.

INTRODUCTION

Pericarps of *Sapindus mukurossi* Gaertn. (Sapindaceae) have been used as a folk medicine as well as a source of a natural surfactant. From this folk medicine, a number of saponins of hederagenin were isolated [1]. Of these saponins, monodesmosides, saponin A, sapindoside B and saponin C are hardly soluble in water in the pure state. It was found that the water solubilities of these monodesmosides were greatly increased in the presence of the bisdesmosides, mukurozi-saponins Y_1, Y_2 or X [2]. Acyclic sesquiterpene oligoglycosides, mukuroziosides Ia, Ib, IIa and IIb which also increased the water solubilities of these monodesmosides, were also isolated from pericarps of this plant [3]. It is noteworthy that solutions of these monodesmosides solubilized with the aid of the bisdesmosides produced a remarkable enhancement of the absorption of antibiotics from rat intestine and rectum [4].

In continuing the studies on surface active oligoglycosides from *Sapindus* species, isolation and structural investigation of saponins of pericarps of *S. delavayi* (Franch.) Radlk. collected in Yunnan, China, were reported [5]. The present paper deals with chemical study on pericarps of *S. trifoliatus* L. which grows in Maharashtra, Mysore, Punjab in India and other tropical districts of south-east Asia.

RESULTS AND DISCUSSION

A methanolic extract of *Sapindus trifoliatus* was subjected to column chromatography on highly porous polymer to yield a saponin fraction and a non-saponin glycoside fraction. The saponin fraction was repeatedly chromatographed on silica gel affording two saponins in yields of 0.5 and 1.6%, respectively, which were identified as saponin A and sapindoside B already isolated from pericarps of *S. mukurossi* [1].

The thin layer chromatogram (TLC) of the non-saponin glycoside fraction indicated the presence of a number of compounds. Treatment of the fraction with weak alkali led to the disappearance of the relatively less polar compounds seen by TLC, resulting in the increase of the more polar compound named trifolioside II(1). This indicated that these less polar compounds would be acyl derivatives of compound 1. It was difficult to separate these acylated glycosides from each other probably because of the facile acyl migration and partial deacylation during the process of purification. Therefore, in this study, the above non-saponin glycoside fraction was treated with alkali under the mild conditions and the resulting products was chromatographed on columns of silica gel and silanized silica gel to give compound 1.

The ^{13}C NMR spectrum of compound 1 showed the homogeniety of this compound and the presence of six monosaccharide units (Table 1). Mineral acid hydrolysis of compound 1 gave D-glucose, L-rhamnose and D-xylose [6]. Comparison of the ^{13}C NMR spectra of mukurozioside IIb and 1 showed that the signals due to an olefinic carbon and a methyl carbon on an olefinic carbon, were displaced downfiled in the spectrum of 1 and the signal due to –CH$_2$O– was shifted upfield, while other signals due to the aglycone moiety were almost unchanged (Table 1). This indicated that the aglycone of compound 1 must be the Z-isomer of 12-hydroxyfarnesol with respect to the double bond between C-11 and C-12, leading to the formulation of aglycone of 1 as 15-hydroxyfarnesol (2). On enzymatic hydrolysis with crude cellulase, compound 1 afforded partially hydrolysed compounds 3 and 4. Inspection of the ^{13}C NMR spectra (Table 1) indicated that compounds 3 and 4 are represented by 15-O-and 1-O-glycosides of 2 with the same trisaccharide unit, respectively, suggesting that 1 is the 1,15-bis-O-trisaccharide of 2. The sequencing analysis of a permethylated compound 1 showed the presence of 2,3-linked glucopyranoside, terminal rhamnopyranoside and terminal xylopyranoside [7], leading to the formulation of both the trisaccharide moieties of 1 as Xyl(1→3) [Rha(1→2)] Glc or Xyl(1→2)[Rha(1→3)]Glc.

†Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR chemical shifts of compounds 1, 3, 4 and mukurozioside IIb (MIIb) in pyridine-d_5

C	MIIb[a]	1[a]	1	3	4	C	1	3	4
1	66.3	66.3	66.9	58.9	65.9	Glc-1	102.8 / 102.9	102.5	103.0
2	121.4	121.3	120.9	126.3	121.1	-2	78.3[c] / 78.5[c]	78.2[e]	78.2[d]
3	141.8	142.0	140.4	136.6	140.4	-3	88.0 (2C)	88.1	88.2
4	40.4[b]	40.9[b]	40.1[b]	40.1[b]	40.4[b]	-4	69.5 (2C)[f]	69.5[f]	69.6[e]
5	27.4	27.3	26.7[d]	26.7[d]	26.7	-5	77.9 (2C)[e]	77.9[e]	78.0[d]
6	125.3	125.4	124.5	124.7	124.7	-6	62.1 / 62.3	62.2	62.4
7	136.0	135.9	135.1	135.0	135.3	Rha-1	100.5 / 101.5	100.5	101.6
8	40.5[b]	40.6[b]	39.8[b]	39.8[b]	39.9[b]	-2	72.8 (2C)[h]	72.7[h]	73.0[g]
9	27.4	27.3	26.6[d]	26.6[d]	26.7	-3	72.3 (2C)[h]	72.3[h]	72.5[g]
10	130.6	131.2	131.4	131.4	126.5	-4	74.0 (2C)[g]	73.9[g]	74.1[f]
11	132.6	132.6	130.8	130.8	136.6	-5	69.6 (2C)[f]	69.6[f]	69.9[e]
12	76.0	22.6	22.4	22.4	21.9	-6	18.6 (2C)	18.6	18.7
13	16.2[c]	16.6[e]	16.6[c]	16.3[c]	16.6[c]	Xyl-1	105.4 (2C)	105.4	105.6
14	16.1[c]	16.3[c]	16.1[c]	16.1[c]	16.1[c]	-2	74.6 (2C)[g]	74.6[g]	74.8[f]
15	14.8	67.8	67.4	67.2	67.2	-3	77.6 (2C)[e]	77.5[h]	78.0[d]
						-4	70.6 (2C)	70.6	70.8
						-5	67.1 (2C)	66.9	67.3

[a] in CD_3OD.

[b,c,d,e,f,g,h] These assignments may be interchanged in each column.

	R^1	R^2
1	—Glc$\frac{2}{3}$—Rha \ Xyl	—Glc$\frac{2}{3}$—Rha \ Xyl
2	—H	—H
3	—Glc$\frac{2}{3}$—Rha \ Xyl	—H
4	—H	—Glc$\frac{2}{3}$—Rha \ Xyl
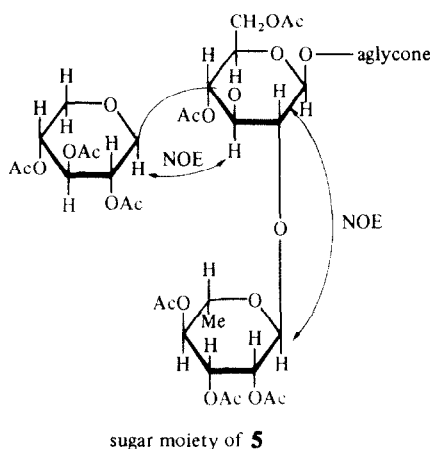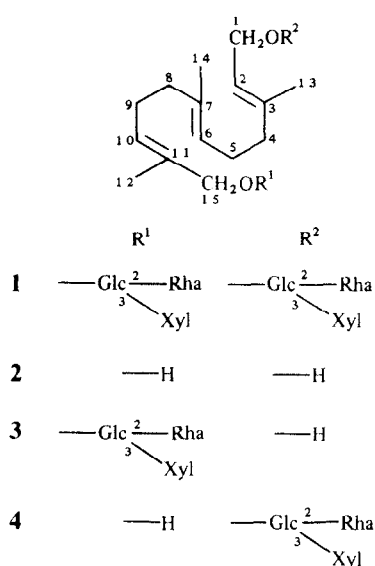
sugar moiety of 5

Glc: β - D - glucopyranosyl
Rha: α - L - rhamnopyranosyl
Xyl: β - D - xylopyranosyl

Fig. 1.

The location of xylosyl and rhamnosyl linkages on the glucose moiety are confirmed as follows. The assignment of the proton signals due to the sugar moiety of the acetylated compound 5 was performed by means of ^{13}C-^{1}H and ^{1}H-^{1}H two dimensional correlation spectroscopy (2D COSY). In the two dimensional NOE correlation spectroscopy (2D NOESY) spectrum of 5, the cross peaks were observed between the H-1 of the rhamnoside moiety and the H-2 of the glucoside moiety as well as between the H-1 of the xyloside moiety and the H-3 of the glucoside moiety. The anomeric configuration of each sugar unit was elucidated by ^{1}H and ^{13}C NMR spectroscopy [8]. Based on these results, the structure of 1 was formulated as shown in Fig. 1.

As mentioned above, we reported the solubilizing effect of mukurozi-saponins Y1, Y2 and X, and mukuroziosides Ia, Ib, IIa and IIb on co-occuring monodesmosides, saponin A, sapindoside B and saponin C[2, 3]. The solubilizing effect of compound 1 on saponin A was also investigated. As shown in Table 2, the more potent

Table 2. Solubilizing effect of compound **1** and mukurozioside
IIb (**MIIb**) on saponin A in water at 37°

Compound	A (mg/ml)	B (mg/ml)	C (mg/ml)
None	0	1.6	0.017
1	1.0	8.0	8.0
1	1.0	15.0	10.8
MIIb	1.0	7.5	4.7

A: Amount of **1** or **MIIb**, B: amount of saponin A, C: solubility
of saponin A.

solubilizing effect on saponin A was observed with compound **1** than that of mukurozioside IIb.

EXPERIMENTAL

NMR: TMS as int. standard. CC: silica gel (Kieselgel 60, 70–230 mesh, Merck), silanized silica gel (LiChroprep RP-8, 40–63 μm, Merck) and highly porous polymer [Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd,)] were used. All solvent systems for chromatography were homogeneous. Acid hydrolysis of glycosides followed by identification of the resulting monosaccharides including absolute configuration [6] and the sequencing analysis of the sugar moiety by GC-MS were carried out as described in a previous paper [7]. The solubilizing effect of compound **1** was determined by the method described in previous paper [2, 3].

Extraction and separation. Pericarps of *Sapindus trifoliatus* imported from India were extracted with hot MeOH. After removal of the solvent by evapn, the MeOH extract was suspended in H_2O and chromatographed on a column of highly porous polymer (solvent: 10%, 50%, 75% aq. MeOH, MeOH and finally Me_2CO). The combined fractions, eluted with 75% MeOH and MeOH, was concd and sepd by repeated CC on silica gel [solvent: EtOAc–EtOH–H_2O (25:5:2) and then $CHCl_3$–MeOH–H_2O (40:10:1)], affording saponin A and sapindoside B in yields of 0.5 and 1.6%, respectively.

The fraction eluted with 50% MeOH was concd and dissolved in 100 ml of 2% KOH in 50% MeOH, and the soln was allowed to stand for 2.5 hr at room temp. The reaction mixture was neutralized with Amberlite MB-3, and the ion-exchange resin was filtered off. The filtrate was concd to dryness and the residue was purified by CC on silica gel [solvent:

AcOEt–EtOH–H_2O (5:2:1)] to give compound **1** in a yield of 5.3%. Compound **1**: A white powder, $[\alpha]_D^{16}$ −58.8° (MeOH; *c* 0.98). (Found: C, 50.53; H, 7.30. $C_{49}H_{82}O_{28} \cdot 5/2H_2O$ requires: C, 50.55; H, 7.53%). The ^{13}C NMR data is given in Table 1.

Enzymatic hydrolysis of **1**. A soln of **1** (500 mg) and celluase (500 mg, Sigma, Type II, Lot. No. C-7502) in acetate buffer (50 ml, pH 5.0) was incubated at 37° for 7 days. The reaction mixture was chromatographed on a column of highly porous polymer eluting with H_2O and then MeOH. The MeOH eluate was concd to dryness and the residue was purified by silica gel CC [(AcOEt–EtOH–H_2O (5:2:1)] and HPLC on TSK gel ODS-120T [(30% aq. MeCN, 21.5 mm × 30 cm, detection, UV(210 nm)] to yield **3** (46 mg) and **4** (14 mg). Compound **3**: A white powder, $[\alpha]_D^{24}$ −79.3° (pyridine; *c* 0.87). FD-MS: *m/z* 701 [M + Na]$^+$. Compound **4**: A white powder, $[\alpha]_D^{20}$ −36.3° (pyridine; *c* 0.8). FD-MS: *m/z* 701 [M + Na]$^+$. ^{13}C NMR data of **3** and **4** are given in Table 1.

REFERENCES

1. Kimata, H., Nakashima, T., Kokubun, S., Nakayama, K., Mitoma, Y., Kitahara, T., Yata, N. and Tanaka, O. (1983) *Chem. Pharm. Bull.* **31**, 1998.
2. Nakayama, K., Fujino (neé Kimata), H., Kasai, R., Mitoma, Y., Yata, N. and Tanaka, O. (1986) *Chem. Pharm. Bull.* **34**, 3279.
3. Kasai, R., Fujino, H., Kuzuki, T., Wong, W., Goto, C., Yata, N., Tanaka, O., Yasuhara, F. and Yamaguchi, S. (1986) *Phytochemistry* **25**, 871.
4. Yata, N., Sugihara, N., Yamajo, R., Murakami, T., Higashi, Y., Kimata, H., Nakayama, K., Kuzuki, T. and Tanaka, O. (1985) *J. Pharm. Dyn.* **8**, 1042.
5. Nakayama, K., Fujino (neé Kimata), H., Kasai, R., Tanaka, O. and Zhou, J. (1986) *Chem. Pharm. Bull.* **34**, 2209.
6. Oshima, R., Kumanotani, J. and Watanabe, C. (1983) *J. Chromatogr.* **259**, 159.
7. Bjoendal, H., Lindberg, B., Pilotti, A. and Svensson, S. (1970) *Carbohydr. Res.* **15**, 339.
8. Kasai, R., Okihara, M., Asakawa, J., Mizutani, K. and Tanaka, O. (1979) *Tetrahedron* **35**, 1427.