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Steroidal saponins and pseudoalkaloid oligoglycoside from Brazilian natural medicine, "fruta do lobo" (fruit of *Solanum lycocarpum*) ☆

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Abstract

Steroidal saponins, lyconosides Ia, Ib, II, III, and IV and a steroidal pseudoalkaloid oligoglycoside, lobofrutoside, were isolated from a Brazilian natural medicine, "fruta do lobo" (the fruit of *Solanum lycocarpum* St. Hil.). The chemical structures of these compounds were elucidated on the basis of analysis of chemical and physicochemical evidence.

Keywords: *Solanum lycocarpum*; Solanaceae; Brazilian natural medicine; Steroidal saponin; Steroidal pseudoalkaloid oligoglycoside; Wolf-fruit; Lyconoside; Lobofrutoside

1. Introduction

The Brazilian Solanaceae plant, *Solanum* (*S.*) *lycocarpum* St. Hil. is distributed in the Southeast and West-Central provinces of Brazil. The fruit of this plant is popularly called "fruta do lobo" (wolf-fruit) and is used as a sedative, diuretic, antiepileptic, and antispasmodic in Brazilian folk medicine. Recently, the fruit is widely used as hypoglycemic and hypocholesterolemic agents as well as for the control of obesity (Vieira et al., 2003; Dall'Agnol and Lino von Poser, 2000; Perez et al., 2006). On the other hand, it was reported that many steroidal glycosides were isolated from *Solanum* species. For example, spirostane and pregnane steroidal oligoglycosides were isolated from the solanaceous plants, *S. sodomaeum* and *S. nigrum*, recently (Ono et al., 2006; Zhou et al., 2006).

In the course of our studies on the bioactive oligoglycoside constituents (Yoshikawa et al., 2007a–d; Nakamura et al., 2007), the methanolic extract from the fruit of *S. lycocarpum* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats. By bioassay-guided separation, two steroidal pseudoalkaloid oligoglycosides, robeneosides A (**8**) and B (**10**), were isolated together with solamargine (**7**) (Yoshida et al., 1987; Puri et al., 1994; Wanyonyi et al., 2002), solasonine (**9**) (Yoshida et al., 1987; Puri et al., 1994), and 12-hydroxysolasonine (**11**) (Yoshida et al., 1987), which showed hypoglycemic and gastric emptying inhibitory activities (Yoshikawa et al., 2007e). As a continuing study on the fruit of *S. lycocarpum*, five steroidal saponins named lyconosides Ia (**1**), Ib (**2**), II (**3**), III (**4**), and IV (**5**) and a steroidal pseudoalkaloid oligoglycoside termed lobofrutoside (**6**) was isolated. In this paper, the isolation and structure elucidation of lyconosides Ia-IV (**1**–**5**) and lobofrutoside (**6**) are described.

2. Results and discussion

2.1. Isolation

The fruits of *S. lycocarpum* (cultivated in Minas Gerais state, Brazil) were extracted with methanol to give a

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methanolic extract (15.3%). After partition of the methanolic extract with an EtOAc and water mixture, the aqueous phase was subjected to HP-20 cc to give an acetone-eluted fraction (0.08%), methanol-eluted fraction (2.5%), and a water-eluted fraction (2.3%). The methanol-eluted fraction was further subjected to normal and reversed-phase column chromatographies and finally HPLC to provide lyconosides Ia (**1**, 0.0059% from the dried fruit), Ib (**2**, 0.0005%), II (**3**, 0.0012%), III (**4**, 0.0004%), and IV (**5**, 0.0014%), lobofrutoside (**6**, 0.0047%), solamargine (**7**, 0.2827%), robeneoside A (**8**, 0.0268%), and solasonine (**9**, 0.4456%) together with kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (0.0018%) (Markham et al., 1978) and 5-*O*-caffeoyl-D-quinic acid (0.0198%) (Fuchs and Spiteller, 1996).

2.2. Structures

Lyconoside Ia (**1**) was isolated as white powder and exhibited a negative optical rotation ($[\alpha]_D^{23}$ −70.0 in MeOH). The IR spectrum of **1** showed strong broad absorption bands at 3423 and 1039 cm^{-1} suggestive of an oligoglycoside structure together with an absorption band at 1655 cm^{-1} assignable to an olefin function. In the positive- and negative-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 937 $[M+Na]^+$ and *m/z* 913 $[M-H]^-$ and high-resolution (HR) positive-ion FAB-MS analysis indicated a molecular formula of **1** to be $C_{45}H_{82}O_{22}$. Furthermore, the negative-ion FAB-MS showed a fragment ion peak at *m/z* 767 $[M-C_6H_{11}O_4]^-$, which was formed by the cleavage at the terminal deoxyhexose (the 2- or 4-*O*-α-L-rhamnopyranosyl moiety, *vide intra*) unit. Acid hydrolysis of **1** with 5% aqueous sulfuric acid (H_2SO_4)-1,4-dioxane (1:1, v/v) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector (Morikawa et al., 2007; Yoshikawa et al., 2007f,g).

The ^1H NMR (pyridine-d_5) and ^{13}C NMR (Table 1) spectra of **1**, which were assigned by various NMR spectroscopic analyses, showed signals assignable to two tertiary methyls [δ 1.00 (6H, *s*, H_3-18, 19)], two secondary methyls [δ 0.88, 1.33 (3H each, both *d*, $J = 6.2$ Hz, H_3-27, 21), a methoxy [δ 3.44 (3H, *s*, CH_3O-26)], three methines bearing an oxygen function [δ 3.50, 3.78 (1H each, both *m*, H-12, 3), 4.65 (1H, *dd* like, $J = 6.8$, 13.7 Hz, H-16)], an acetal proton [δ 4.43 (1H, *d*, $J = 9.6$ Hz, H_3-26)], and an olefinic proton [δ 5.26 (1H, *br d*, H-6)] in the aglycone part together with a β-D-glucopyranosyl [δ 4.83 (1H, *d*, $J = 6.2$ Hz, H-1')] and two α-L-rhamnopyranosyl [5.74, 6.28 (1H each, both *s*, H-1‴, H-1″)] parts. The proton and carbon signals due to the triglycoside moiety in the ^1H and ^{13}C NMR spectra of **1** were superimposable on those of solamargine (**7**) and robeneoside A (**8**), whereas the resonances assignable to the aglycone part of **1** were similar to those of 12-hydroxysolasonine (**11**), except for the signals due to the F-ring moiety (the 22–26 positions), which very closely

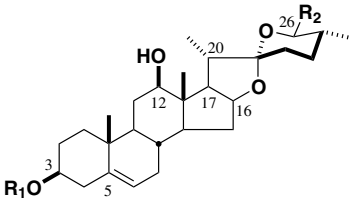
Table 1
^{13}C NMR Spectroscopic data for **1**–**6** (pyridine-d_5)

	1	2	3	4	5	6		1	2	3	4	5	6
C-1	37.3	37.5	37.3	37.5	37.4	37.5	C-1′	100.1	100.3	100.1	100.4	100.3	100.3
C-2	29.9	30.1	29.9	30.1	30.0	30.1	C-2′	77.7	77.7	77.6	75.1	75.1	78.1
C-3	78.3	77.9	77.9	77.5	77.4	77.9	C-3′	77.6	78.1	77.8	84.8	84.7	77.7
C-4	38.7	38.9	38.7	38.7	38.7	38.9	C-4′	77.8	78.5	78.4	70.5	70.4	78.5
C-5	140.7	140.8	140.6	140.9	140.9	140.8	C-5′	76.7	76.7	76.8	76.6	76.5	76.9
C-6	121.7	121.8	122.0	121.8	121.8	121.9	C-6′	61.1	61.3	61.1	62.6	62.5	61.3
C-7	31.4	31.4	31.9	32.2	31.4	32.3	C-1″	101.8	102.0	101.8	102.3	102.2	102.0
C-8	30.7	30.8	30.7	30.9	30.9	32.2	C-2″	72.3	72.5	72.4	72.6	72.5	72.5
C-9	49.9	50.0	49.9	50.1	50.0	50.1	C-3″	72.6	72.7	72.5	72.9	72.8	72.8
C-10	37.1	37.1	37.1	37.3	37.2	37.3	C-4″	73.7	73.9	73.7	74.2	74.1	74.1
C-11	32.0	31.8	31.3	31.5	31.9	30.9	C-5″	69.3	69.5	69.3	69.5	69.4	69.5
C-12	77.8	79.0	78.8	79.0	78.4	79.0	C-6″	18.4	18.5	18.5	18.6	18.6	18.6
C-13	46.1	46.3	46.1	46.3	46.2	46.5	C-1‴	102.7	102.9	102.7	105.9	105.8	102.9
C-14	55.3	55.4	55.2	55.4	55.4	53.4	C-2‴	72.3	72.5	72.3	75.0	74.9	72.6
C-15	31.8	31.9	31.8	31.9	32.2	31.5	C-3‴	72.5	72.8	72.6	78.4	78.3	72.7
C-16	81.3	81.2	81.0	81.2	81.4	79.8	C-4‴	73.9	74.1	73.9	71.6	71.5	73.9
C-17	62.5	62.8	62.6	62.8	62.6	63.2	C-5‴	70.2	70.4	70.2	78.5	78.9	70.4
C-18	10.9	11.1	10.9	11.1	11.0	11.2	C-6‴	18.3	18.6	18.3	62.6	62.5	18.5
C-19	19.2	19.3	19.2	19.4	19.3	19.4							
C-20	42.9	43.2	42.8	43.0	43.0	42.8							
C-21	14.1	14.4	14.2	14.3	14.3	14.9							
C-22	111.9	112.3	109.3	109.5	112.1	98.6							
C-23	31.3	32.1	31.7	31.9	31.4	34.5							
C-24	28.2	28.7	29.2	29.3	28.4	31.0							
C-25	35.3	37.8	30.5	30.6	35.5	30.7							
C-26	102.9	96.6	66.7	66.9	103.1	47.6							
C-27	16.6	17.5	17.2	17.4	16.7	19.6							
OCH_3	55.4				55.5								

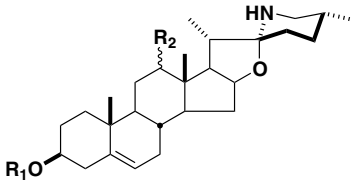
resembled those of solasodoside A (Ono et al., 2006) having the (25*R*,26*R*)-26-methoxyspirostan structure. The structure of the aglycone part with the 26-methoxyl group in **1** was determined by a detail double quantum filter correlation spectroscopy (DQF COSY) and heteronuclear multiple-bond correlations (HMBC) experiments as shown in Fig. 2. Thus, the DQF COSY experiments on **1** indicated the presence of partial structures written in bold lines. The connectivities of the quaternary carbons and the 3-*O*-triglycoside structure were characterized by an HMBC experiment, which showed long-range correlations between the following protons and carbons: H-1′ and C-3; H-1″ and C-2′; H-1‴ and C-4″. The stereostructure of the A–D ring part in the aglycon part was clarified by nuclear Overhauser enhancement spectroscopy (NOESY) experiment (Fig. 2) and the stereostructure of the F-ring part was characterized by comparison of the coupling constant value ($J = 9.6$ Hz) of the 26-proton and the chemical shift values (δ 31.3, 28.2) of the 23- and 24-carbons (Yoshikawa et al., 2007b) with those of solasodoside A, SNF-3 (Ando et al., 1999), and SNF-4 (Ando et al., 1999). On the basis of this evidence, the chemical structure of lyconoside Ia (**1**) was determined as (25*R*,26*R*)-26-methoxyspirost-5-en-3β,12β-diol-3-*O*-{α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→4)]}-β-D-glucopyranoside (Chart 1).

Lyconoside Ib (**2**) was also isolated as a white powder with a negative optical rotation ($[\alpha]_D^{23}$ −80.9 in MeOH) and its IR spectrum showed absorption bands at 3568, 1655 and 1041 cm^{-1} assignable to hydroxyl, olefin, and ether functions. The molecular formula $C_{46}H_{74}O_{19}$ of **2** was determined by the quasimolecular ion peaks at *m/z* 923 $[M+Na]^+$ and *m/z* 899 $[M-H]^-$ in the positive-and negative-ion FAB-MS, respectively and by HRMS measurement. In addition, a fragment ion peak (*m/z* 753 $[M-C_6H_{11}O_4]^-$) was observed in the negative-ion FAB-MS of **2**. The acid hydrolysis of **2** provided D-glucose and L-rhamnose. The proton and carbon signals in the ^1H and ^{13}C NMR (Table 1) spectra of **2** were superimposable on those of **1**, except for the resonances due to the F-ring part of **2**, which were similar to those of SNF-10 (Ando et al., 1999) having the 26-hydroxyl group. The detail DQF COSY, HMBC and NOESY data on **2** led us to elucidate the structure of **2** to be the 26-hydroxyl analog of **1**. On the basis of this evidence, the chemical structure of lyconoside Ib (**2**) was determined as (25*R*,26*R*)-26-hydroxyspirost-5-en-3β,12β-diol-3-*O*-{α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→4)]}-β-D-glucopyranoside.

Lyconoside II (**3**), obtained as a white powder with a negative optical rotation ($[\alpha]_D^{27}$ −75.7 in MeOH), showed absorption bands at 3452, 1655 and 1055 cm^{-1} in the IR spectrum. The molecular formula $C_{45}H_{72}O_{17}$ was determined by positive-and negative-ion FAB-MS (*m/z* 907 $[M+Na]^+$, *m/z* 883 $[M-H]^-$ and *m/z* 737 $[M-C_6H_{11}O_4]^-$) and by HRMS analysis. The acid hydrolysis of **3** liberated D-glucose and L-rhamnose. The ^1H and ^{13}C NMR (Table 1) spectra of **3** indicated the presence of two tertiary methyls [δ 1.00, 1.02 (3H each, both *s*, H_3-19, 18)], two secondary methyls [δ 0.63, 1.36 (1H each, both *br s*, $J = 6.2$ Hz, H_3-27, 21)], three methines bearing an oxygen function [δ 3.49, 3.78, 4.56 (1H each, all *m*, H-12, 3, 16)], an oxy-

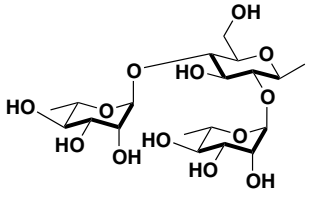


	R_1	R_2
lyconoside Ia (**1**):	S	OCH_3
lyconoside Ib (**2**):	S	OH
lyconoside II (**3**):	S	H
lyconoside III (**4**):	S'	H
lyconoside IV (**5**):	S'	OCH_3



	R_1	R_2
lobofrutoside (**6**):	S	β-OH
solamargine (**7**):	S	H
robeneoside A (**8**):	S	α-OH
solasonine (**9**):	S'	H
robeneoside B (**10**):	S'	α-OH
12-hydroxysolasonine (**11**):	S'	β-OH

S =



S' =

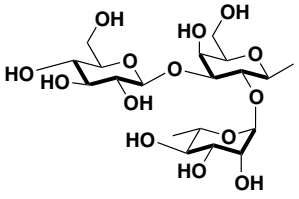


Chart 1. Structures of new compounds from the fruit of *Solanum lycocarpum*.

methylenes [3.45, 3.53 (1H each, both *m*, H_2-26)], an olefin proton [δ 5.24 (1H, *br d*, H-6)] together with one β-D-glucopyranosyl [δ 4.86 (1H, *d*, $J = 6.2$ Hz, H-1′)] and two α-L-rhamnopyranosyl [5.79, 6.33 (1H each, both *s*, H-1‴, H-1″)] parts. The proton and carbon signals due to the 3-*O*-triglycoside moiety in the ^{1}H and ^{13}C NMR spectroscopic data of **3** were very similar to those of **7**, **8**, and **1**, while the signals due to the aglycone part of **3** resembled those of isochiagenin (Agrawal et al., 1985). On the basis of the detail DQF COSY, HMBC, and NOESY spectroscopic data of **3** (Figs. 1 and 2), the chemical structure of lyconoside II (**3**) was characterized as shown.

Lyconoside III (**4**) and IV (**5**) were obtained as a white powders with negative optical rotations (**4**: $[\alpha]_D^{27}$ −67.9 in MeOH; **5**: $[\alpha]_D^{27}$ −94.6, both in MeOH) and their IR spectrum showed absorption bands suggestive of an oligoglycoside structure. The positive- and negative-ion FAB-MS of **4** and **5** showed pseudomolecular ion peaks (**4**: *m/z* 753 $[M-C_6H_{11}O_4]^-$, *m/z* 737 $[M-C_6H_{11}O_5]^-$; **5**: *m/z* 767 $[M-C_6H_{11}O_5]^-$) and their molecular formulas (**4**: $C_{42}H_{72}O_{18}$, **5**: $C_{46}H_{74}O_{19}$) were determined by HRMS analysis. Acid hydrolysis of **4** and **5** individualy liberated D-galactose, D-glucose and L-rhamnose. The proton and carbon signals due to the triglycoside moiety in the ^{1}H and ^{13}C NMR spectroscopic data of **4** and **5** were superimposable on those of solasonine (**9**) and robenoside B (**10**) (Yoshikawa et al., 2007e). The proton and carbon signals due to the steroidal aglycone part of **4** were very similar to those of **3**, whereas the aglycone signals of **5** resembled those of **1**. On the basis of those findings and the detailed ^{1}H and ^{13}C NMR spectroscopic data (Table 1) analysis including the DQF COSY, HMBC and NOESY experiments on **4** and **5** (Figs. 1 and 2), the chemical structures of lyconoside III (**4**) and IV (**5**) were elucidated as shown.

Lobofrutoside (**6**) was isolated as a white powder with a negative optical rotation ($[\alpha]_D^{27}$ −92.3 in MeOH). The molecular formula $C_{45}H_{72}NO_{16}$ of **6** was determined from the positive-and negative-ion FAB-MS (*m/z* 906 $[M+Na]^+$, *m/z* 882 $[M-H]^-$ and *m/z* 736 $[M–C_6H_{11}O_4]^-$) and by HRMS measurement. The acid hydrolysis of **6** liberated D-glucose and L-rhamnose. The proton and carbon signals due to the triglycoside moiety in the ^{1}H and ^{13}C NMR data of **6** were superimposable to those of **7**, **8** and **1**, while the signals due to the aglycone part were similar to those of 12-hydroxysolasonine (**11**). The detail ^{1}H and ^{13}C NMR data (Table 1) analysis including the DQF COSY, HMBC and NOESY experiments (Figs. 1 and 2) led us to formula the structure of lobofrutoside (**6**) as shown.

2.3. Concluding remarks

In conclusion, five steroidal saponins, lyconosides Ia (**1**), Ib (**2**), II (**3**), III (**4**) and IV (**5**), and a steroidal pseudoalkaloid oligoglycoside, lobofrutoside (**6**), were isolated from the fruits of *S. lycocarpum* and their structures were determined on the basis of chemical and physicochemical evi-

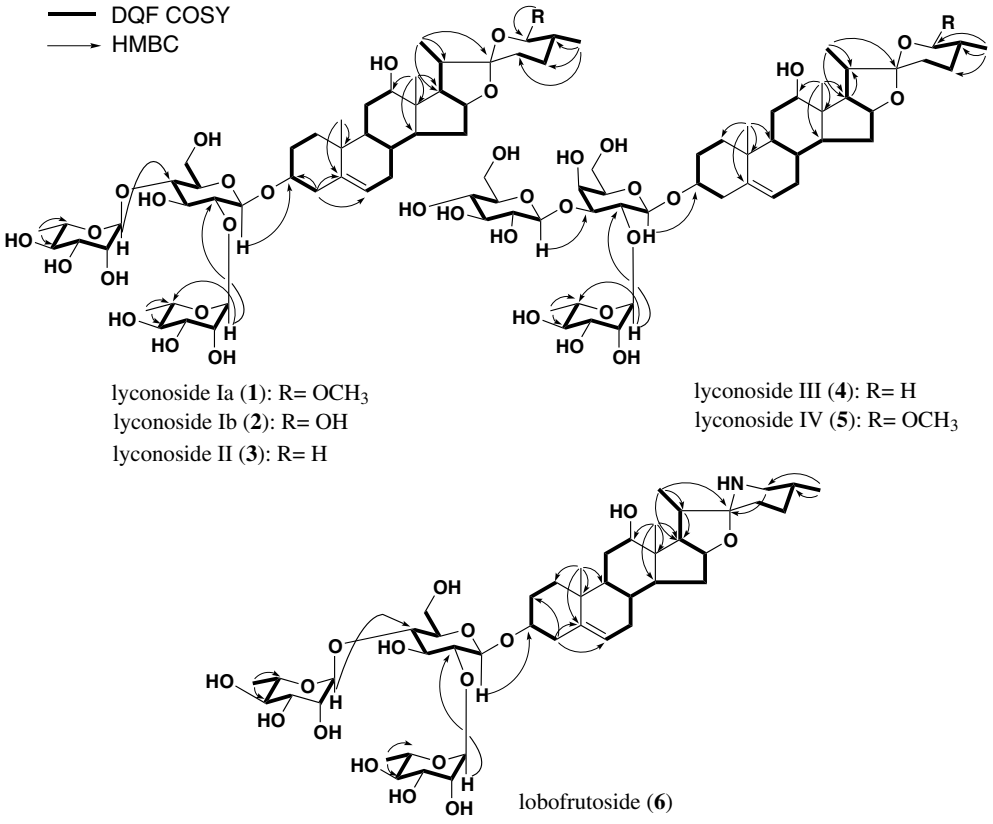
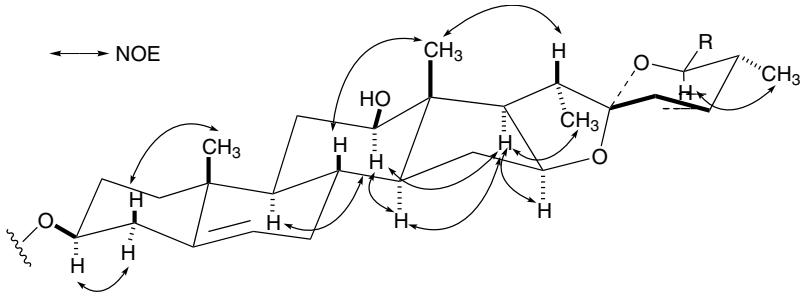
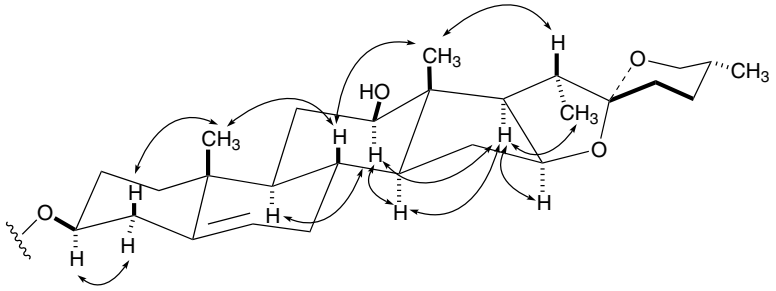


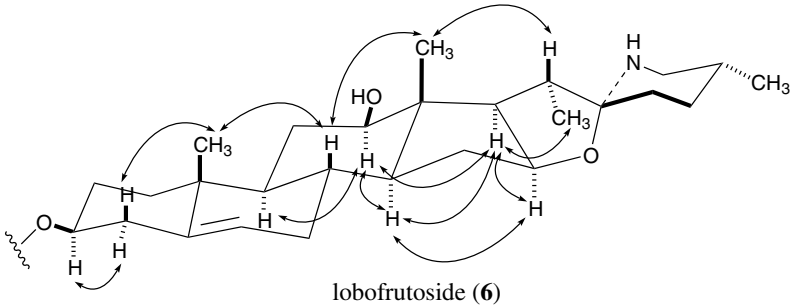
Fig. 1. Significant DQF COSY and HMBC correlations for new compounds from *Solanum lycocarpum*.



lyconosides Ia (**1**), Ib (**2**) and IV(**5**) : R= OCH_3 or OH



lyconosides II (**3**) and III (**4**)



lobofrutoside (**6**)

Fig. 2. Significant NOESY for new compounds from *Solanum lycocarpum*.

dence. Since spirostane steroidal oligoglycosides, lyconosides Ia (**1**), Ib (**2**), and IV (**5**), with an acetal moiety at the 26-position were obtained together with steroidal pseudoalkaloid oligoglycosides (**6**–**11**), those pseudoalkaloids (**6**–**11**) were thought to be derived from acetal derivatives such as **1**, **2**, and **5** *via* their imine derivatives. This knowledge is interesting from the perspective of biosynthesis of steroidal pseudoalkaloid oligoglycosides.

3. Experimental

3.1. General experimental procedures

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l = 5$ cm); IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JEOL EX-270 (270 MHz), JEOL JNM-LA500 (500 MHz), ECA-600K (600 MHz) spectrometer; ^{13}C NMR spectra, JEOL EX-270 (68 MHz), JEOL JNM-LA500 (125 MHz), ECA-600K (150 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–VIS detectors. HPLC column, GL-Science ODS-3 (Nacalai Tesque Inc., 250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel cc, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica gel cc, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); Diaion HP-20 cc (Nippon Rensui); TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

3.2. Plant material

The fruits of *S. lycocarpum*, was commercial product, were purchased at Minas Gerais state, Brazil in 2005 and exported *via* Target Exportacao E Importacao Ltda, Sao

Paulo, Brazil and Fukuda Ryu, Co., Ltd. Osaka, Japan. The plant was identified by one of the authors (M. Y.). A voucher specimen of the plant is maintained in our laboratory (2005.01. Brazil-01).

3.3. Extraction and isolation

The dried fruits of *S. lycocarpum* (5.0 kg) were extracted with MeOH (3×25L) under conditions when the solvent was heated until refluxing. Evaporation of the combined extracts under reduced pressure gave the MeOH extract (746.1 g, 15.3%). After partition of the methanolic extract (263.8 g) with an EtOAc–H_2O mixture (3:1, 20L), the aqueous layer was subjected to Diaion HP-20 column chromatography [3.0 kg, H_2O → MeOH → acetone] to give H_2O^-, $MeOH^-$, and acetone-eluted fractions (113.4, 126.4 and 4.2 g, respectively). The MeOH-eluted fraction was subjected to normal-phase silica gel cc [3.5 kg, $CHCl_3$–MeOH–H_2O (50:10:1 → 10:3:1 → 7:3:1 → 65:35:10 → 6:4:1, v/v/v) → MeOH] to give seven fractions [Fr. 1 (1.0 g), Fr. 2 (1.3 g), Fr. 3 (2.8 g), Fr. 4 (8.7 g), Fr. 5 (80.2 g), Fr. 6 (17.9 g), Fr. 7 (9.0 g)]. Fraction 3 (2.8 g) was subjected to reversed-phase silica gel cc [90 g, MeOH–H_2O (35:65 → 45:55 → 55:45 → 60:40 → 65:35 → 75:25 → 85:15, v/v) → MeOH] to afford ten fractions [Fr. 3–1 (172 mg), Fr. 3–2 (108 mg), Fr. 3–3 (102 mg), Fr. 3–4 (99 mg), Fr. 3–5 (32 mg), Fr. 3–6 (188 mg), Fr. 3–7 (369 mg), Fr. 3–8 (157 mg), Fr. 3–9 (643 mg), Fr. 3–10 (363 mg)]. Fr. 3–7 (369 mg) was purified by HPLC [MeOH–H_2O (60:40, v/v)] to afford two fractions [Fr. 3–7–1 (271 mg), Fr. 3–7–2 (lyconoside Ia, **1**, 71.9 mg, 0.0042%)]. Fr. 3–7–1 (271 mg) was purified by HPLC [MeOH–H_2O (70: 30, v/v)] to afford lyconosides Ia (**1**, 31.8 mg, 0.0018%), II (**3**, 21.5 mg, 0.0012%), III (4, 6.5 mg, 0.0004%), and IV (5, 25.1 mg, 0.0014%). Fr. 3–9 (643 mg) was purified by HPLC [MeOH–H_2O (80:20, v/v)] to afford solamargine (**7**, 133.6 mg, 0.0077%). Fr. 3–10 (363 mg) was purified by HPLC [MeOH–H_2O (80:20, v/v)] to furnish solamargine (**7**, 34.0 mg, 0.0020%). Fraction 4 (8.7 g) was subjected to reversed-phase silica gel cc [270 g, MeOH–H_2O (40:60 → 50:50 → 60:40 → 70:30 → 80:20, v/v) → MeOH] to afford nine fractions [Fr. 4–1 (327 mg), Fr. 4–2 (64 mg), Fr. 4–3 (79 mg), Fr. 4–4 (148 mg), Fr. 4–5 (275 mg), Fr. 4–6 (588 mg), Fr. 4–7 (3.6 g), Fr. 4–8 (780 mg), Fr. 4–9 (764 mg)]. Fr. 4–2 (64 mg) was purified by HPLC [MeOH–H_2O (50:50, v/v)] to furnish kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (30.7 mg, 0.0018%). Fr. 4–4 (148 mg) was purified by HPLC [MeOH–H_2O (60:40, v/v)] to furnish lyconoside Ib (**2**, 9.4 mg, 0.0005%). Fr. 4–7 (171 mg) was purified by HPLC [MeOH–H_2O (75:25, v/v)] to afford solamargine (7, 24.9 mg, 0.0305%). Fraction 5 (60.0 g) was subjected to normal-phase silica gel cc to yield solamargine (**7**, 3.44 g, 0.2656%) and solasonine (**9**, 5.72 g, 0.4412%). Fraction 6 (17.9 g) was subjected to reversed-phase silica gel cc [80 g, MeOH–H_2O (10:90 → 30:70 → 50:50 → 60:40 → 70:30, v/v) → MeOH] to afford eight fractions [Fr. 6–1 (190 mg), Fr. 6–2 (1.6 g), Fr. 6–3 (2.6 g), Fr. 6–4 (3.4 g), Fr. 6–5 (2.0 g), Fr. 6–6 (2.0 g), Fr. 6–7 (1.6 g), Fr. 6–8 (2.3 g)]. Fr. 6–3 (450 mg) was purified by HPLC [MeOH–H_2O (25:75, v/v)] to furnish 5-*O*-caffeoyl-D-quinic acid (60.2 mg, 0.0198%). Fr. 6–4 (800.0 mg) was purified by HPLC [MeOH–H_2O (40:60, v/v)] to afford six fractions [Fr. 6–4–1 (103 mg), Fr. 6–4–2 (77 mg), Fr. 6–4–3 (30 mg), Fr. 6–4–4 (63 mg), Fr. 6–4–5 (robenoside A, **8**, 111.6 mg, 0.0268%), Fr. 6–4–6 (409 mg)]. Fr. 6–4–2 (77 mg) was subjected to reversed-phase silica gel cc [3 g, MeOH–H_2O (40:60 → 60:40, v/v) → MeOH] to furnish lobofrutoside (**6**, 19.9 mg, 0.0047%). Fr. 6–7 (300 mg) was purified by HPLC [MeOH–H_2O (75: 25, v/v)] to afford two fractions [Fr. 6–7–1 (168 mg), Fr. 6–7–2 (135 mg)]. Fr. 6–7–1 (168 mg) was subjected to normal-phase silica gel cc to furnish solamargine (**7**, 9.9 mg, 0.0074%) and solasonine (**9**, 5.9 mg, 0.0044%).

3.3.1. Lyconoside Ia (*1*)

White powder, ($[\alpha]_D^{23}$ −70.0 (*c* 3.60, MeOH); IR (KBr) ν_{max} cm^{-1}: 3423, 2936, 1655, 1039; ^1H NMR (600 MHz, pyridine-d_5) δ: 0.88 (3H, *d*, $J = 6.2$ Hz, H_3-27), 1.00 (6H, *s*, H_3-18,19), 1.33 (3H, *d*, $J = 6.2$ Hz, H_3-21), 3.44 (3H, *s*, CH_3O-26), 3.50 (1H, *m*, H-12), 3.78 (1H, *m*, H-3), 4.43 (1H, *d*, $J = 9.6$ Hz, H-26), 4.65 (1H, *dd* like, $J = 6.8$, 13.7 Hz, H-16), 4.83 (1H, *d*, $J = 6.2$ Hz, H-1′), 5.26 (1H, *br d*, H-6), 5.74 (1H, *s*, H-1‴), 6.28 (1H, *s*, H-1″); for ^{13}C NMR spectroscopic data, see Table 1. Positive-ion FABMS *m/z*: 937 $[M+Na]^+$; Negative-ion FABMS *m/z*: 913 $[M-H]^-$ and 767 $[M-C_6H_{11}O_4]^-$; HRFABMS *m/z*: 906.4779 $[M+Na]^+$ (calc. for $C_{45}H_{82}O_{22}Na$, 906.4773).

3.3.2. Lyconoside Ib (*2*)

White powder, ($[\alpha]_D^{23}$ −80.9 (*c* 0.80, MeOH); IR (KBr) ν_{max} cm^{-1}: 3568, 2934, 1655, 1041, 980; ^1H NMR (500 MHz, pyridine-d_5) δ: 1.09 (3H, *s*, H_3-19), 1.11 (3H, *s*, H_3-18), 1.18 (3H, *d*, $J = 6.3$ Hz, H_3-27), 1.52 (3H, *d*, $J = 6.4$ Hz, H_3-21), 3.57 (1H, *m*, H-12), 3.87 (1H, *m*, H-3), 4.67 (1H, *m*, H-16), 4.95 (1H, *d*, $J = 6.1$ Hz, H-1′), 5.32 (1H, *brd*, H-6), 5.87 (1H, *s*, H-1‴), 6.41 (1H, *s*, H-1″); for ^{13}C NMR spectroscopic data, see Table 1. Positive-ion FABMS *m/z*: 923 $[M + Na]^+$; Negative-ion FABMS *m/z*: 899 $[M - H]^-$ and 753 $[M - C_6H_{11}O_4]^-$; HRFABMS *m/z*: 923.4612 $[M + Na]^+$ (calc. for $C_{46}H_{74}O_{19}Na$, 923.4616).

3.3.3. Lyconoside II (*3*)

White powder, ($[\alpha]_D^{27}$ −75.7 (*c* 1.10, MeOH); IR (KBr) ν_{max} cm^{-1}: 3452, 2934, 1655, 1055, 981; ^1H NMR (600 MHz, pyridine-d_5) δ: 0.63 (3H, *br s*, H_3-27), 1.00 (3H, *s*, H_3-19), 1.02 (3H, *s*, H_3-18), 1.36 (3H, *br s*, H_3-21), 3.49 (1H, *m*, H-12), 3.78 (1H, *m*, H-3), 4.56 (1H, *m*, H-16), 4.86 (1H, *d*, $J = 6.2$ Hz, H-1′), 5.24 (1H, *br d*, H-6), 5.79 (1H, *s*, H-1‴), 6.33 (1H, *s*, H-1″); for ^{13}C NMR spectroscopic data, see Table 1; Positive-ion FABMS *m/z* 907 $[M+Na]^+$; Negative-ion FABMS *m/z*: 883 $[M-H]^-$

and 737 $[M–C_6H_{11}O_4]^-$; HRFABMS *m/z*: 907.4659 $[M+Na]^+$ (calc. for $C_{45}H_{72}O_{17}Na$, 907.4667).

3.3.4. Lyconoside III (**4**)

White powder, ($[\alpha]_D^{27}$ −67.9 (*c* 0.28, MeOH); IR (KBr) ν_{max} cm^{-1}: 3433, 2932, 1655, 1055, 981; ^1H NMR (500 MHz, pyridine-d_5) δ: 0.69 (3H, *br s*, H_3-27), 1.15 (6H, *s*, H_3-18, 19), 1.43 (3H, *br s*, H_3-21), 3.56 (1H, *m*, H-12), 3.78 (1H, *m*, H-3), 4.60 (1H, *m*, H-16), 4.92 (1H, *d*, $J = 7.9$ Hz, H-1′), 5.20 (1H, *s*, H-1‴), 5.32 (1H, *br d*, H-6), 6.29 (1H, *s*, H-1″); for ^{13}C NMR spectroscopic data, see Table 1; Positive-ion FABMS *m/z* 923 $[M+Na]^+$; Negative-ion FABMS *m/z*: 899 $[M-H]^-$, 753 $[M–C_6H_{11}O_4]^-$ and 737 $[M–C_6H_{11}O_5]^-$; HRFABMS *m/z*: 923.4612 $[M+Na]^+$ (calc. for $C_{45}H_{72}O_{18}Na$, 923.4616).

3.3.5. Lyconoside IV (**5**)

White powder, ($[\alpha]_D^{27}$ −94.6 (*c* 0.80, MeOH); IR (KBr) ν_{max} cm^{-1}: 3452, 2934, 1655, 1056, 980; ^1H NMR (600 MHz, pyridine-d_5) δ: 0.94 (3H, *d*, $J = 6.2$ Hz, H_3-27), 1.06 (6H, *s*, H_3-18, 19), 1.40 (3H, *d*, $J = 6.2$ Hz, H_3-21), 3.51 (3H, *s*, CH_3O-26), 3.90 (1H, *m*, H-12), 3.62 (1H, *m*, H-3), 4.72 (1H, *dd*, $J = 6.9$, 18.5 Hz, H-16), 4.91 (1H, *d*-like, H-1′), 5.18 (1H, *d*, $J = 8.2$ Hz, H-1‴), 5.33 (1H, *br d*, H-6), 6.27 (1H, *s*, H-1″); for ^{13}C NMR spectroscopic data, see Table 1; Positive-ion FABMS *m/z*: 953 $[M+Na]^+$; Negative-ion FABMS *m/z* 929 $[M-H]^-$ and 767 $[M–C_6H_{11}O_5]^-$; HRFABMS *m/z*: 953.4725 $[M+Na]^+$ (calc. for $C_{46}H_{74}O_{19}Na$, 953.4722).

3.3.6. Lobofrutoside (**6**)

White powder, ($[\alpha]_D^{27}$ –92.3 (*c* 1.00, MeOH); IR (KBr) ν_{max} cm^{-1}: 3433, 2934, 1655, 1043, 912; ^1H NMR (600 MHz, pyridine-d_5) δ: 0.82 (3H, *d*, $J = 6.2$ Hz, H_3-27), 1.10 (3H, *s*, H_3-19), 1.13 (3H, *s*, H_3-18), 1.46 (3H, *d*, $J = 6.4$ Hz, H_3-21), 3.59 (1H, *m*, H-12), 3.86 (1H, *m*, H-3), 4.69 (1H, *m*, H-16), 4.93 (1H, *d*, $J = 6.8$ Hz, H-1′), 5.33 (1H, *d*, $J = 4.8$ Hz, H-6), 5.89 (1H, *s*, H-1‴), 6.41 (1H, *s*, H-1″); for ^{13}C NMR spectroscopic data, see Table 1; Positive-ion FABMS *m/z* 906 $[M+Na]^+$; Negative-ion FABMS *m/z* 882 $[M–H]^-$ and 736 $[M–C_6H_{11}O_4]^-$; HRFABMS *m/z*: 906.4824 $[M+Na]^+$ (calc. for $C_{45}H_{72}NO_{16}Na$, 906.4827).

3.4. Acid hydrolysis of lyconosides (**1**–**5**) and lobofrutoside (**6**)

Solutions of **1**–**6** (13.0, 1.7, 1.5, 1.3, 2.7, 2.4 mg) in 5.0%-H_2SO_4-1,4-dioxane (11: 1,v/v) (1m1) were each heated under conditions of reflux for 3 h. After cooling, each reaction mixture was neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. The filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis [column: Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection: optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase: MeCN–H_2O (85:15, v/v); flow rate: 0.80 ml/min; column temperature: room temperature]. Identification of (i) L-ramnose from **1**–**6**, (ii) D-glucose from **1**–**6** and (iii) D-galactose from **4** and **5**, present in the aqueous layer, were carried out by comparison of their retention times and optical rotation with those of authentic samples; t_R: (i) 9.5 min (negative optical rotation), (ii) 13.7 min (positive optical rotation), (iii) 14.1 min (positive optical rotation).

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