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A new steroidal glycoside from the fruits of *Solanum myriacanthum*

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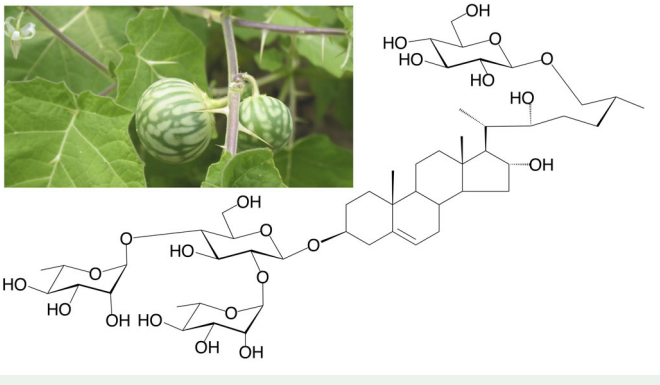
ABSTRACT

A new cholestane-type steroidal glycoside, solamyriaside A (**1**), was isolated from the fruits of *Solanum myriacanthum* Dunal (Solanaceae), along with two known steroidal glycosides, namely, solaviaside A (**2**) and aculeatiside A (**3**), and three known steroidal alkaloid glycosides, namely, solamargine (**4**), khasianine (**5**) and solasonine (**6**), which were isolated for the first time from this plant. Based on spectroscopic data as well as chemical evidence, **1** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl-22*R*,25*R*-cholest-5-ene-3β,16α,22,26-tetraol 26-*O*-β-D-glucopyranoside. The cytotoxic activity of **1**–**6** against HL-60 human promyelocytic leukaemia cells was examined. Compounds **4**–**6** showed cytotoxic activity. Among them, **4** exhibited the strongest activity with an IC_{50} value of 4.64 ± 0.17 μM, similar to the activity of cisplatin, a positive control.



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1. Introduction

Solanum myriacanthum Dunal is a solanaceous plant native to Central and South America. The fruits of this plant are used in traditional folk medicines in India for treating stomach ache and intestinal worms (Yadav and Tangpu 2012). Recently, the anthelmintic activity of the methanol (MeOH) extract of the ripe fruits of *S. myriacanthum* was reported (Yadav and Tangpu 2012). As a part of an ongoing study of the constituents of solanaceous plants (Ono et al. 2015), we focussed on the fruits of *S. myriacanthum* in this study. This report describes the isolation and structural elucidation of a new cholestane-type steroidal glycoside, two known steroidal glycosides and three known steroidal alkaloid glycosides obtained from the fruits of this plant. In addition, the cytotoxic activity of the isolated compounds against HL-60 human promyelocytic leukaemia cells was examined.

2. Results and discussion

The fruits of *S. myriacanthum* were extracted with MeOH. The MeOH extract was subjected to Diaion HP20, silica gel and Chromatorex ODS column chromatography as well as HPLC on an ODS column to yield three steroidal glycosides (**1**–**3**) and three steroidal alkaloid glycosides (**4**–**6**).

Compounds **2**–**6** were identified as solaviaside A (**2**) (Ono et al. 2009), aculeatiside A (**3**) (Saijo et al. 1983; Ono et al. 2009), solamargine (**4**) (Mahato et al. 1980; Ono et al. 2006), khasianine (**5**) (Mahato et al. 1980) and solasonine (**6**) (Usubillaga et al. 1997), based on the comparison of their physical and spectral data with those of authentic samples or those already reported (Figure 1).

Solamyriaside A (**1**) was obtained as an amorphous powder and exhibited an $[M + Na]^+$ ion peak at *m/z* 1073 in the positive-ion FAB-MS (Figure S1). The molecular formula of **1** was determined to be $C_{51}H_{86}O_{22}$ using HR-positive-ion FAB-MS (Figure S1). The ^{1}H NMR spectrum of **1** showed signals corresponding to two tertiary methyl groups (δ 1.07, 0.74), four secondary methyl groups [δ 1.77 (d, $J = 6.5$ Hz), 1.63 (d, $J = 6.0$ Hz), 1.16 (br d, $J = 6.0$ Hz), 1.05 (d, $J = 7.0$ Hz)], one olefinic proton [δ 5.33 (d, $J = 3.5$ Hz)] and some monosaccharide units (Figure S2). The ^{13}C NMR spectrum of **1** showed a total of 51 carbon signals, including those of two olefinic carbons (δ 140.7, 121.8) and four anomeric carbons (δ 104.7, 102.7, 101.9, 100.1) (Figure S3). These NMR signals were assigned with the aid of ^{1}H–^{1}H COSY, HMQC and HMBC spectra (Table S1, Figures S4–S6). The NMR data were similar to those of **2**, except for the appearance of signals corresponding to one additional oxymethine group and the absence of signals arising from one methylene group. In particular, the ^{13}C NMR data from the sugar moiety and C-1–C-12 and C-24–C-27 of the aglycone moiety (Agl) were almost superimposable. Further, the correlations observed in the ^{1}H–^{1}H COSY and HMBC spectra indicated that the planar structure in **1** was a tetraglycoside of cholest-5-ene-3,16,22,26-tetraol (Figure S7). On acidic hydrolysis, **1** afforded **1a**, along with L-rhamnose and D-glucose. The latter two monosaccharides were confirmed by optical rotation using chiral detection in HPLC. The coupling constants of the signals of the anomeric and methine protons of the sugar moiety in the ^{1}H NMR spectrum as well as the chemical shifts of ^{13}C NMR signals (Seo et al. 1978; Ono et al. 2009)

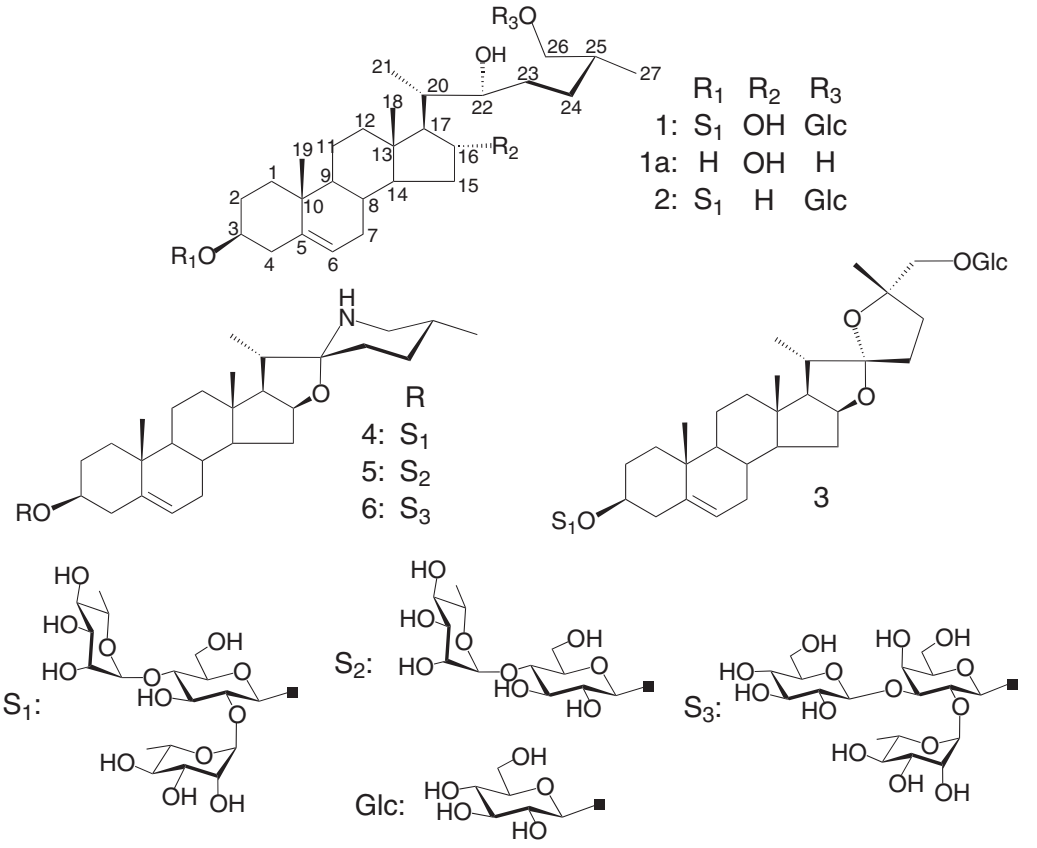

Figure 1. Structures of **1**–**6** and **1a**.

corresponding to the sugar moiety suggested that all monosaccharide units were in the pyranose form. Furthermore, the glycosidic linkages of the glucopyranosyl unit was β in $^4C_1$ conformation and that of the rhamnopyranosyl unit was α in $^1C_4$ conformation (Table S1). The molecular formula of **1a** was determined to be $C_{27}H_{46}O_6$ using HR-positive FAB-MS (Figure S8). The ^{1}H NMR spectrum of **1a** showed signals corresponding to two tertiary methyl groups (δ 0.77, 1.06), two secondary methyl groups [δ 1.20 (d, $J = 6.0$ Hz), 1.15 (d, $J = 6.5$ Hz)], one olefinic proton [δ 5.40 (d, $J = 4.0$ Hz)], one oxygenated methylene group [δ 3.82 (m), 3.60 (m)] and three oxygenated methine protons [δ 4.51 (m), 4.31 (m), 3.83 (m)] (Figure S10). Thus, **1a** was identified as a genuine aglycone of **1** and was named solamyriagenin. In the ^{13}C NMR spectrum of the sugar moiety, glycosylation shifts ($\Delta\delta_C = \delta_C\mathbf{1} - \delta_C\mathbf{1a}$) (Kasai et al. 1977; Tori et al. 1977) were observed at C-3 ($\Delta\delta_C$ +6.7 ppm) and C-26 ($\Delta\delta_C$ +7.5 ppm) of Agl. Furthermore, the HMBC spectrum of **1** showed key correlations between H-1 of the first glucosyl unit (Glc) and C-3 of Agl, H-1 of the first rhamnosyl unit (Rha) and C-2 of Glc, H-1 of the second rhamnosyl unit (Rha′) and C-4 of Glc, and H-1 of the second glucosyl unit (Glc′) and C-26 of Agl (Figure S7). Based on these data, **1** was proposed to be a derivative of **2**, in which a hydroxyl group was attached to C-16 of the Agl. The configurations at C-16, C-17 and C-20 of Agl were identified to be *R*, *R* and *S*,

respectively, based on the NOESY spectrum of **1**, in which key NOEs were observed between H-12a and H_3-21, H-16 and H_3-18, H-16 and H-22, H_3-18 and H_3-19, and H_3-18 and H-20 (Figures S13 and S14). The α-configuration of the hydroxyl group at C-16 was supported by the ^{1}H NMR chemical shift for H_3-18, because the signal of **1** resonated at δ 0.74, which was similar to the chemical shift (δ 0.71) of abutiloside H (26-acetylamino-3β,16α-dihydroxy-cholest-5-en-22-one 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside) (Yoshimitsu et al. 2002). On the other hand, the ^{1}H NMR signal for bethoside B [3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-22*R*,25*R*-cholest-5-en-3β,16β,22,26-tetraol 26-*O*-β-D-glucopyranoside] was de-shielded owing to the β-hydroxyl group at C-16 and appeared at δ 1.15 (Hayes et al. 2009).

Agrawal previously reported the difference ($\Delta\delta = \delta$H-26a $- \delta$H-26b) between the ^{1}H NMR chemical shifts of the geminal protons of the glycosyloxy methylene group of furostane-type glycosides; this difference reflected the orientation of the C-27-methyl group. The $\Delta\delta$ value is usually greater than 0.57 ppm in 25*S* compounds and less than 0.48 ppm in 25*R* compounds (Agrawal 2005). Although **1** is a cholestane-type glycoside, its side chain moiety (C-23–C-27) is same as that of furostane-type glycosides. The $\Delta\delta$ observed in the ^{1}H NMR spectrum of **1** was 0.31 ppm. Based on this, the configuration at C-25 was deduced to be *R*. The remaining absolute configuration at C-22 was determined to be *R*, because the ^{13}C NMR signals of C-21 (δ 13.4) and C-22 (δ 73.7) of **1a** were in better agreement with those of 22*R*,25*R*-cholest-5-ene-3β,16β,22,26-tetraol (C-21, δ 13.7; C-22, δ 73.4) rather than those of its 22-epimer (C-21, δ 15.3; C-22, δ 75.5) (Challinor et al. 2011).

Accordingly, **1** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl-22*R*,25*R*-cholest-5-ene-3β,16α,22,26-tetraol 26-*O*-β-D-glucopyranoside, which is a stereoisomer at C-16 of anguivioside A (Zhu et al. 2001). However, the ^{1}H NMR chemical shift of H_3-18 in anguvioside A was in better agreement with that in **1** rather than that in bethoside B. Therefore, further analyses of the NMR data of anguvioside A and its structure may be needed. Compound **1** is considered to be a key precursor of the spirostanol and furostanol on the biogenetic route (Nohara et al. 2006).

Compounds **1**–**6** were evaluated for their cytotoxic activities against HL-60 cells (Table S2) because the MeOH extract of the fruits of *S. myriacanthum* was found to be active with an IC_{50} value of 15.5 μg/mL. Additionally, some steroidal glycosides possessing cytotoxic activity against HL-60 cells have been previously reported (Yoshioka and Mimaki 2007; Yokosuka and Mimaki 2008; Li et al. 2013). Of the six compounds, three steroidal alkaloid glycosides (**4**–**6**) exhibited activity. Among the active compounds (**5**, IC_{50} 143 ± 5 μM; **6**, IC_{50} 17.1 ± 0.3 μM), **4** exhibited the strongest activity with an IC_{50} value of 4.64 ± 0.17 μM, which was almost the same activity as that of the positive control, cisplatin (IC_{50} 6.23 ± 0.71). In contrast, **1**–**3** (IC_{50} >300 μM) were inactive (Table S2). The cytotoxic activities of steroidal alkaloid glycosides including **4**–**6** against cancer cell lines other than HL-60 cells have been reported (Bhutani et al. 2010; Ding et al. 2013; Gu et al. 2018; Tai et al. 2018). Furthermore, these results suggested that the bonding of the rhamnosyl unit to C-2 of the glucosyl unit increased the cytotoxic activity (specifically compare **4** vs. **5**). A similar structure–activity

relationship and the cytotoxic activities of **4** and **6** against HL-60 cells have been previously reported (Yokosuka and Mimaki 2008). A detailed elucidation of the structure–activity relationship requires further sample collection.

3. Experimental

3.1. General experimental procedures

Optical rotations were performed with a JASCO P-1020 polarimeter (JASCO, Tokyo, Japan). MS were recorded on a JEOL JMS-700 (JEOL, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded with JEOL alpha 500 spectrometer and ECA-500 spectrometer (JEOL, Tokyo, Japan), and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. Silica gel 60 (Merck, Art. 1.09385 and 1.07734; Merck, Darmstadt, Germany), Chromatorex ODS (Fuji Silysia Chemical, Ltd., Aichi, Japan) and Diaion HP20 (Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan) were used for column chromatography. HPLC separation was run on a Shimadzu LC-10AS micro pump (Shimadzu, Kyoto, Japan) with Shimadzu RID-10A RI-detector (Shimadzu, Kyoto, Japan). For HPLC column chromatography, Cosmosil 5C18-AR-II [Nacalai Tesque, Inc., Kyoto, Japan, 20 mm i.d. $\times$ 250 mm (column 1), 4.6 mm i.d. $\times$ 250 mm (column 2)] was used. Cisplatin (*cis*-diammineplatinum(II)dichloride) was a product from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

3.2. Plant material

The fruits of *S. myriacanthum* were collected in the Medical Plant Garden of Kumamoto University, Kumamoto prefecture, Japan, in October 2003, and identified by one of authors (emeritus professor T. Nohara). A voucher specimen (SM 2003) has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

3.3. Cell

The HL-60 human promyelocytic leukaemia cells (JCRB0085) was obtained from Japanese Collection of Research Bioresources (Tokyo, Japan).

3.4. Extraction and isolation

The fruits (3.95 g) of *S. myriacanthum* were extracted with MeOH at room temperature. The solvent was then removed under reduced pressure to yield a syrup (183.3 g). The MeOH extract was chromatographed over Diaion HP20 column (H_2O, MeOH, acetone) to afford MeOH–eluate (fr.) (55.10 g) and acetone–eluate (162 mg). A part (49.9 g) of the MeOH–eluate was subjected to silica gel column chromatography [Merck Art. 1.07734, $CHCl_3$–MeOH–H_2O (10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1)] to yield fractions 1–6. Chromatography of a part (1.74 g) of fraction 4 (2.07 g) on a Chromatorex ODS column (60% MeOH, 70% MeOH, 100% MeOH) furnished fractions 4-1–4-4. HPLC (column 1, 60% MeOH) of a part (61 mg) of fraction 4-2 (289 mg) afforded **3** (23 mg). Fraction 5 (4.83 g) was subjected to

silica gel column chromatography [Merck Art. 1.09385, $CHCl_3$–MeOH–H_2O (10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1)] to yield fractions 5-1–5-8. Fraction 5-5 (1.46 g) was chromatographyed over Chromatorex ODS column (50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, 100% MeOH) to yield fractions 5-5-1–5-5-4. Fractions 5-5-2 (107 mg) and 5-5-3 (155 mg) were each subjected to HPLC (column 1, 65% MeOH) to yield **1** (74 mg) from fraction 5-5-2 and **2** (31 mg) from fraction 5-5-3, respectively. Fraction 6 (17.33 g) was subjected to silica gel column chromatography [Merck Art. 1.07734, $CHCl_3$–MeOH–H_2O (14:2:0.1, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1)] to yield fractions 6-1–6-4. Fraction 6-2 (1.71 g) was successively subjected to HPLC (column 1, 80% MeOH) and silica gel column chromatography [Merck Art. 1.09385, $CHCl_3$–MeOH–H_2O (8:2:0.2, 7:3:0.5, 6:4:1)] to yield **4** (48 mg) and **6** (118 mg). The successive chromatography of fraction 6-3 (5.20 g) over Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, 100% MeOH) and silica gel column [Merck Art. 1.09385, $CHCl_3$–MeOH–H_2O (10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1)] furnished **5** (9 mg).

3.4.1. Solamyriaside A (1)

Amorphous powder. $[\alpha]_D^{17}$–86.5° ($c = 1.1$, MeOH). Positive-ion FAB-MS *m/z*: 1073 $[M + Na]^+$. HR-positive-ion FAB-MS *m/z*: 1073.5470 (Calcd for $C_{51}H_{86}O_{22}Na$: 1073.5508). ^{1}H NMR (in pyridine-d_5, 500 MHz) and ^{13}C NMR (in pyridine-d_5, 125 MHz), see Table S1.

3.5. Acidic hydrolysis of 1

Compound **1** (6 mg) was heated in 2 M HCl–dioxane (1:1, 1 mL) at a temperature of 95 °C for 1 h. The reaction mixture was neutralised with 1 M NaOH and then extracted with ethyl acetate (EtOAc) (3 mL × 3). The aqueous layer was evaporated under reduced pressure to give a residue. The residue was extracted with MeOH and MeOH extract was analysed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (Showa Denko K.K., Tokyo, Japan, 6.0 mm i.d. × 150 mm), solvent, CH_3CN–H_2O (3:1); flow rate, 1.0 mL/min; column temperature, 70 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; column oven, JASCO CO-2060. The retention time (t_R) and optical activity of each of the monosaccharides were detected as follows. L-rhamnose [t_R (min) 4.0; optical activity, negative] and D-glucose [t_R (min) 6.6; optical activity, positive]. The EtOAc extract was subjected to silica gel column chromatography [Merck Art. 1.09385, hexane–acetone (5:1, 3:1, 2:1, 1:1, 1:2, 1:5, 1:9, 0:1)] to yield **1a** (1 mg).

3.5.1. Solamyriagenin A (1a)

Amorphous powder. $[\alpha]_D^{17}$–27.7° ($c = 0.09$, MeOH). Positive-ion FAB-MS *m/z*: 457 $[M + Na]^+$. Negative-ion FAB-MS *m/z*: 433 $[M - H]^-$. HR-positive FAB-MS *m/z*: 457.3303 (Calcd for $C_{27}H_{46}O_4Na^+$: 457.3288).^{1}H NMR spectral data (pyridine-d_5, 500 MHz) δ: 5.40 (1H, d, $J = 4.0$ Hz, H-6), 4.51 (1H, m, H-16), 4.31 (1H, m, H-22), 3.82 (1H, m, H-26a), 3.60 (1H, m, H-26b), 2.63 (2H, H_2-4), 1.20 (3H, d, $J = 6.0$ Hz, H_3-21), 1.15 (3H, d, $J = 6.5$ Hz, H_3-27), 1.06 (3H, s, H_3-19), 0.77 (3H, s, H_3-18). ^{13}C NMR spectral data (pyridine-d_5, 125 MHz), δ: 142.0 (C-5), 121.1 (C-6), 75.1 (C-16), 73.7 (C-22), 71.3 (C-3), 67.7 (C-26), 63.7 (C-17), 54.3 (C-14), 50.5 (C-9), 44.0 (C-13), 43.5 (C-4), 40.5 (C-12), 38.6 (C-20), 37.7 (C-1), 37.0 (C-25)[a], 36.9 (C-10)[a], 36.8 (C-15)[a], 32.6 (C-2)[b], 32.4 (C-23)[b], 32.2 (C-7), 31.9 (C-8),

31.3 (C-24), 21.2 (C-11), 19.6 (C-19), 17.4 (C-27), 14.1 (C-18), 13.4 (C-21). (a, b) Assignments may be interchangeable.

3.6. Cytotoxic assay

HL-60 human leukaemia cells seeded in individual wells of a 96-well culture plate at a density of 5×10^4 cells/100 μL per well were incubated in the presence of test samples. A cell counting kit-8 (CCK-8) containing 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2*H*-tetrazolium monosodium salt (WST-8) was used to measure the activities of dehydrogenase enzyme(s) in viable cells according to the manufacturer's instruction (Dojindo Labs, Kumamoto, Japan). After incubation for 24 h, CCK-8 solution was added to each well, followed by another 3 h of incubation. To determine cytotoxic activity, the reduction of WST-8 was determined colorimetrically at 450 nm using a grating microplate reader (SH-1000Lab, Corona Electric, Ibaraki, Japan). Cisplatin was used as a positive control.

4. Conclusion

In this study, we isolated and elucidated the structure of a new cholestane-type steroidal glycoside, named solamyriaside A from the fruits of *S. myriacanthum*, along with two known steroidal glycosides, and three known steroidal alkaloid glycosides, whose isolation from *S. myriacanthum* has been described here for the first time. In addition, one known compound, solamargine, indicated the clear cytotoxic activity towards HL-60 cells similar to that of cisplatin.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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