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Phenylbenzofuran-type stilbenoids from *Stemona* species

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ABSTRACT

Five new phenylbenzofuran-type stilbenoids, stemofurans S-W (**1**–**5**), were isolated from underground parts of four *Stemona* species collected in Thailand and Australia together with the known stemofurans A–C, G, E, J, M, the stilbostemins A–D, F, the stemanthrenes A–D, dihydropinosylvin, pinosylvin, and 4′-methylpinosylvin. The chemical structures of the new compounds were elucidated by ^{1}H NMR and ^{13}C NMR spectroscopy and mass spectrometry. In accordance with previous reports all compounds were characterized by C-methylations of the aromatic rings. Stemofurans S (**1**) and U (**3**) showed an unusual hydroxylation at position C-4′, a hitherto unknown substitution of *Stemona* stilbenoids.

1. Introduction

In the course of a broad-based phytochemical screening for bioactive compounds of the family Stemonaceae various types of stilbenoids have been isolated from *Stemona* species (Pacher et al., 2002; Greger, 2012). Together with family specific alkaloids (Greger, 2006) they represent the characteristic chemical make-up of the tuberous roots which are widely used in China and SE Asia as an anti-cough remedy and anti-parasitic drug. Bioautographic tests on TLC plates and germ-tube inhibition assays in microwells against different fungi exhibited antifungal activities for almost all stilbenoids (Pacher et al., 2002). Some derivatives were shown to possess antibacterial properties (Yang et al., 2006; Lin et al., 2008; Sastraruji et al., 2011). The accumulation of several stilbenoids after fungal infection suggested their protective role as phytoalexins (Pacher, 2005; Greger, 2012). Moreover, Adams et al. (2005) reported on the antiinflammatory activity of *Stemona* stilbenoids related to inhibition of leukotriene biosynthesis, and Khamko et al. (2013) on their cytotoxic effects against cancer cell lines. In the present paper we report on the isolation and identification of stilbenoids of four *Stemona* species collected in Thailand and Australia. In addition to 18 known compounds the structures of five hitherto unknown phenylbenzofurans, the stemofurans S-W (**1**–**5**), were elucidated by spectroscopic methods.

2. Results and discussion

The methanolic crude extracts of air dried roots and rhizomes from *Stemona involuta* Inthachub, *Stemona lucida* (R. Br.) Duyfjes, *Stemona burkillii* Prain and *Stemona collinsiae* Craib were evaporated to dryness and partitioned between water and $CHCl_3$. Characteristic UV-spectra of HPLC/DAD analyses of the organic phases, together with TLC/anisaldehyde detection suggest the presence of a series of stilbenoids. All major compounds were isolated by preparative MPLC and TLC, and partly by crystallization as described previously (Pacher et al., 2002). Most of them turned out to be known stilbenoids consisting of seven phenylbenzofurans, the stemofurans A–C, G, E, J, M, five dihydrostilbenes, the stilbostemins A–D, F, four dihydrophenanthrenes, the stemanthrenes A–D, and the two stilbenes pinosylvin and 4′-methylpinosylvin. They were identified by co-chromatography with authentic samples and comparison with previously published data (Zhao et al., 1995; Pacher et al., 2002; Hartl, 2003; Wiboonpun et al., 2004; Chaiyong et al., 2010; Sastraruji et al., 2011). Moreover, five unknown derivatives were isolated and identified. All of them were shown to belong to phenylbenzofuran-type stilbenoids and were named stemofurans S-W (**1**–**5**).

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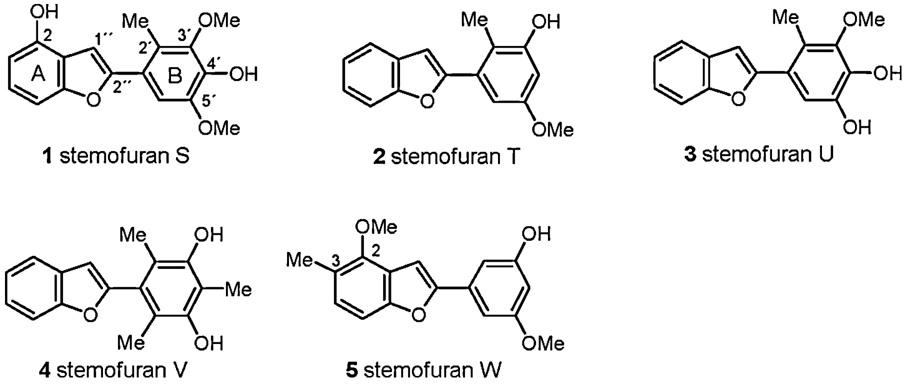

1 stemofuran S **2** stemofuran T **3** stemofuran U **4** stemofuran V **5** stemofuran W

Fig. 1. Chemical structures of stemofurans S-W.

The HPLC-UV profile from the recently newly described *S. involuta* (Inthachub et al., 2010) was characterized by dominating phenylbenzofurans, from which stemofuran C (Pacher et al., 2002) and the hitherto unknown stemofuran U (**3**) represent the major compounds. Moreover, small amounts of the new stemofuran V (**4**) and the known stemofurans A and B (Pacher et al., 2002) together with the widespread dihydrostilbene stilbostemin B (Zhao et al., 1995) were detected. The Australian *S. lucida* exhibited a HPLC pattern characterized by four main peaks representing the two known dihydrophenanthrenes stemanthrenes B and C (Kostecki et al., 2004), and the two phenylbenzofurans stemofurans G (Pacher et al., 2002) and W (**5**). The latter was shown to be a hitherto unknown compound. In addition, small amounts of the two stemanthrenes A and D and the dihydrostilbenes dihydropinosylvin, stilbostemins B, D and F were detected. Due to the instability of dihydrophenanthrenes and scarce amounts of plant material, the stemanthrenes A–D (Kostecki et al., 2004) could only be identified by co-chromatography with authentic samples.

A reinvestigation of *S. burkillii* afforded in addition to the already reported stemofurans B, E, and J (Hartl, 2003; Pacher, 2005), the new stemofurans U (**3**) and V (**4**) as major compounds together with the known stemofuran M (Sastraruji et al., 2011), and small amounts of the new stemofuran T (**2**). From *S. collinsiae* a number of phenylbenzofurans have already been described previously (Pacher et al., 2002; Greger, 2012). In the present study we additionally isolated the new stemofuran S (**1**) in small amounts by pooling together the root extracts from four collection sites.

All isolated compounds were analyzed by NMR spectroscopy and mass spectrometry. The carbon skeletons and substitution patterns were determined in particular by $^{2}J_{C-H}$ and $^{3}J_{C-H}$ long range couplings gained from HMBC and dipolar ^{1}H–^{1}H interactions measured by NOESY. Proton H-1″ shows $^{2}J_{C-H}$ to C-2″ and C-1 as well as $^{3}J_{C-H}$ to C-2, C-6 and C-1′ in all five samples, indicating the central annulated heterocyclic moiety of the 2″-phenyl benzofurane skeleton. Aromatic proton H-5 and H-6′ were used to identify C-1, C-3, C-4 and C-6 ($^{2}J_{C-H}$) as well as C-1′, C-2′, C-4′, C-5′ and C-2″ ($^{3}J_{C-H}$), respectively. Long range couplings of the protons of the methyl groups and methoxy groups were also helpful to identify the connected aromatic moieties. Further NOEs of the H-1″ to H-2 as well as to H-2′, H-6′ or the methyl groups bound to these positions proofed the special closeness between these nuclei. Such proximities are significant for the stemofuran skeletons. Also closeness between aromatic protons, methyl groups and methoxy groups were determined by NOEs for each compound, to proof their neighboured positions on the aromatic moieties. The resulting structures and concomitant measured ^{13}C NMR shifts and multiplicities were verified using the combined CSEARCH and SPECINFO database system for spectral similarity searches by application of the SAHO-protocol (Schütz et al., 1997; Robien, 2009). Furthermore, all determined structures were in good agreement with mass spectrometric measurements.

Common to the new stemofurans is a phenylbenzofuran core structure with varying substitution patterns on the two benzene ring systems A and B. Position 1″ of the furan ring did not carry a substituent in any of the investigated compounds. Compounds **2**, **3** and **4** show four protons on ring A, indicating that no substituent is present on this moiety in all three structures. However, stemofuran S (**1**) has a hydroxyl group on position 2, and stemofuran W (**5**) carries a methoxy group and a methyl group on positions 2 and 3, respectively. On ring B all five compounds carry hydroxyl or methoxy groups on positions 3′ and 5′. Position 4′ is, however, variably substituted. Stemofurans S (**1**) and U (**3**) possess a hydroxyl group, while stemofuran V (**4**) carries a methyl group. Stemofurans T (**2**) and W (**5**) have no substituent in this position. On positions 2′ and 6′ there are no substituents or methyl groups. All structures are shown in Fig. 1 and the analytical NMR spectroscopic data are merged in Tables 1 and 2. The numbering is shown for stemofuran S (**1**) (Fig. 1) and is in accordance with those used earlier for stemofurans (Pacher et al., 2002).

In accordance with previous reports on *Stemona* species (Greger, 2012), all newly described compounds were characterized

Table 1
^{1}H NMR data for stemofurans S-W (**1**–**5**) (δ, ppm).

Pos.	**1**[a,b,c]	**2**	**3**	**4**	**5**
2		7.61 dd	7.50 dd	7.51 dd	
3	6.68 dd	7.25 ddd	7.28 ddd	7.30 ddd	
4	7.07 dd	7.30 ddd	7.23 ddd	7.26 ddd	7.07 d
5	7.04 ddd	7.62 ddd	7.58 ddd	7.61 ddd	7.14 dd
2′					6.91 dd
4′		6.51 d			6.39 dd
6′	7.17 s	6.91 d	7.23 s		6.97 dd
1″	6.99 d	6.86 d	6.78 d	6.63 d	7.09 d
2-O–CH_3					4.05 s
3-CH_3					2.33 s
2′-CH_3	2.39 s	2.34 s	2.42 s	2.06 s	
3′-O–CH_3	3.81 s		3.85 s		
4′-CH_3				2.24 s	
5′-O–CH_3	3.90 s	3.86 s			3.86 s
6′-CH_3				2.06 s	

[a] Coupling constants: **1**: J(3,4) = J(4,5) = 8.0 Hz, J(3,5) = 0.9 Hz, J(5,1″) = 0.8 Hz; **2**: J(2,3) = 7.8, J(3,4) = J(4,5) = 8.0 Hz, J(2,4) = J(3,5) = 0.9 Hz, J(5,1″) = 0.9 Hz, J(4′,6′) = 0.7 Hz; **3**: J(2,3) = J(3,4) = J(4,5) = 7.9 Hz, J(2,4) = J(3,5) = 0.8 Hz, J(5,1″) = 0.7 Hz; **4**: J(2,3) = J(3,4) = J(4,5) = 7.7 Hz, J(2,4) = J(3,5) = 0.8 Hz, J(5,1″) = 0.8 Hz; **5**: J(4,5) = 7.8 Hz, J(5,1″) = 0.8 Hz, J(2′,4′) = J(4′,6′) = 2.0 Hz, J(2′,6′) = 1.2 Hz.

[b] Solvents: **1**: acetone-d_6, **2**, **3**, **4**, **5**: $CDCl_3$.

[c] OH-resonances, if identified: **1**: 8.82 br, s; 7.78 br, s; **3**: 5.21 br, s; 5.62 br, s; **4**: 4.68 s (2H).