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Identification and Ecdysteroid Antagonist Activity of Three Resveratrol Trimers (Suffruticosols A, B and C) from *Paeonia suffruticosa*

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Abstract: Bioassay-guided HPLC analysis of the seeds of *Paeonia suffruticosa* has afforded three novel resveratrol trimers (suffruticosol A, suffruticosol B and suffruticosol C), together with *cis*-resveratrol and paeoniflorin. The structures of these new compounds have been elucidated mainly by comprehensive 1D- and 2D-NMR experiments. Resveratrol and its oligomers are active as ecdysteroid antagonists (ED_{50} values = 10 to 50 μM *vs.* 5 x 10^{-8}M 20-hydroxyecdysone) in the *Drosophila melanogaster* B_{II} bioassay. The activities of other "pseudo-oestrogens" in this bioassay have also been assessed.

INTRODUCTION

Paeonia suffruticosa Andrews (Family: Paeoniaceae), commonly known as "moutan peony", is found extensively in the western part of China and is also naturalized in some parts of Bhutan. It is an important Chinese medicinal plant from the section *Moutan* of the genus *Paeonia* L. This genus consists of *ca.* 35 species placed in three sections: *Moutan*, *Oneapia* and *Paeonia*.[1-4] The root cortex of *P. suffruticosa* (Chinese name: mudanpi; Japanese name: Botanpi) is a Chinese traditional medicine. Numerous studies on the chemistry and pharmacology of this species have been performed.[5] We report on the isolation, structure elucidation and biological activities of three novel resveratrol trimers, together with two known compounds, *cis*-resveratrol and paeoniflorin, as part of our search for ecdysteroid antagonists from plant sources.[6-14] Ecdysteroids are the steroid hormones of insects, crustaceans and probably of other invertebrates too.[15] Antagonists of ecdysteroid

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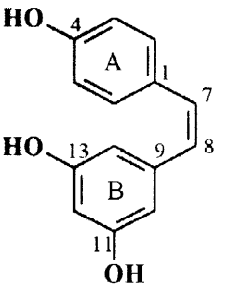
action in invertebrate systems would be useful as biochemical probes for the investigation of the control of gene expression by ecdysteroids and, possibly, as lead compounds for new insect pest control agents.[16,17]



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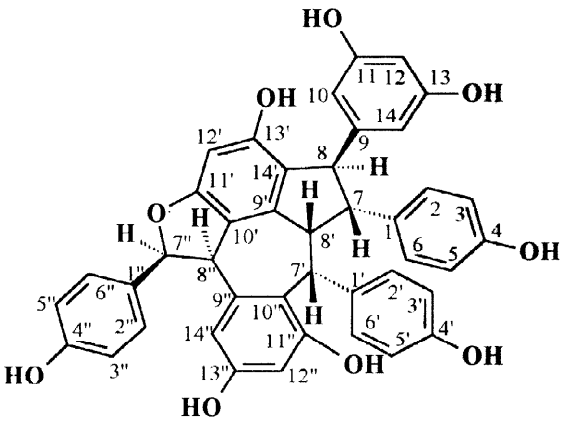

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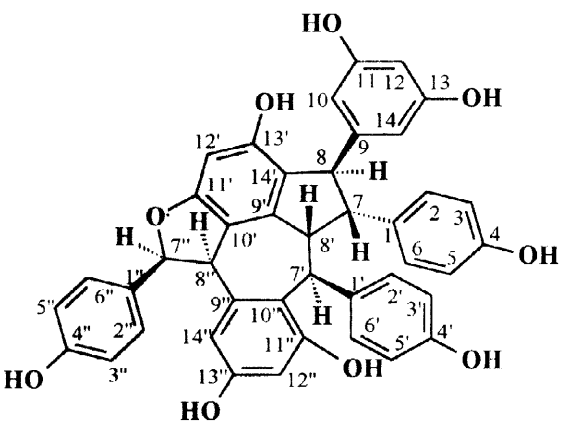

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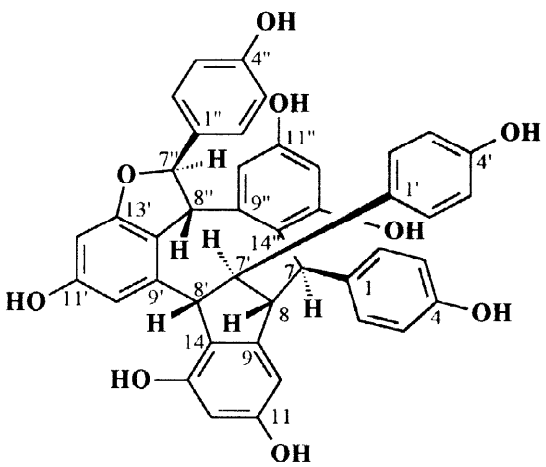

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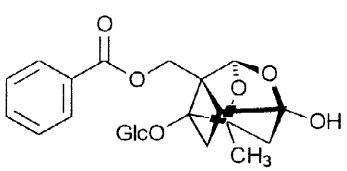

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RESULTS AND DISCUSSION

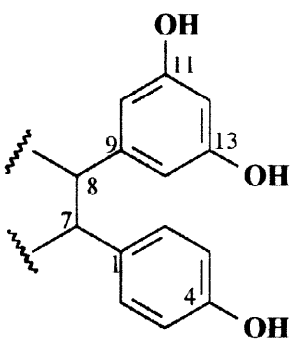
Preliminary studies revealed that methanolic seed extracts of several species in the genus *Paeonia* (*P. anomala*, *P. daurica*, *P. obovata* and *P. suffruticosa*) antagonized the action of 20-hydroxyecdysone on *Drosophila melanogaster* B_{II} cells, while seed extracts of several other species (*P. cambedesii*, *P. lutea* var. *ludlowii* and *P. officinalis*) were not active. Bioassay-guided HPLC analysis of the active Sep-Pak fractions of a

MeOH extract (defatted with *n*-hexane) of the seeds of *Paeonia suffruticosa* resulted in the isolation of *cis*-resveratrol (**1**) (as a mixture with a small amount of its *trans*-isomer), paeoniflorin and three novel resveratrol trimers (suffruticosol A [**2**], suffruticosol B [**3**] and suffruticosol C [**4**]) and paeoniflorin (**5**). The two known compounds (**1** and **5**) were readily identified as *cis*-resveratrol[18-20] and paeoniflorin[21] by direct comparison of their UV, MS, ^{1}H- and ^{13}C-NMR data with published data. The structures of the three novel compounds (**2-4**) were unambiguously determined by extensive 1D- and 2D-NMR experiments.

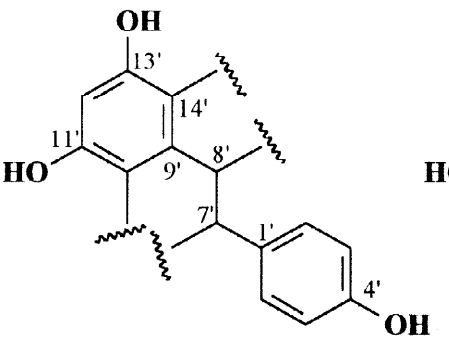
LSIMS spectra of compounds **2-4**, showed $[M-H]^-$ ions at *m/z* 679 [- ve ion mode] and $[M+H]^+$ ions at 681 [+ ve ion mode] compatible with the molecular formula $C_{42}H_{32}O_9$, expected for resveratrol trimers.[22] UV absorption maxima (λ_{max}) at 283 and 226 indicated the presence of phenolic chromophores, which are chracteristic for such oligostilbenes.[23] In the ^{1}H- and ^{13}C-NMR spectra (Table 1) of these compounds, some common features were observed, which formed the basis of the structure elucidation process for these compounds. ^{1}H-NMR spectra (Table 1) revealed the presence of six sets of *ortho*-coupled aromatic hydrogens assignable to three 4-hydroxyphenyl groups ("A" ring of **1**) and signals from three other 3,5-dihydroxyphenyl systems ("B" ring of **1**) characteristic for three resveratrol units. Instead of the signals for olefinic protons (*cis* or *trans*) of resveratrol units, the presence of six methine hydrogens strongly suggested reduction of these olefinic bonds and trimerisation involving these carbons of the three resveratrol units. In ^{13}C PENDANT NMR[24] of these compounds (Table 1), signals for six phenyl ring systems, including 9 oxygenated aromatic quarternary carbons (δ_C 150.0-160.0), five methine carbons (δ_C 35.0 – 66.0), and a very deshielded oxymethine (δ_C 84.0 – 90.0), supported the hypothesis that these compounds are resveratrol trimers.[25,26] The presence of the highly deshielded oxymethine (δ_H ~ 6.00, δ_C ~90.0) in the spectrum of each of these three trimers was indicative of a dihydrofuran ring system as found in the resveratrol dimers balanocarpol[27] and viniferin,[28] in the resveratrol trimer distichol[25,29] and in the resveratrol tetramer, vaticaffinol.[28] However, there were also some distinct differences in ^{1}H- and ^{13}C-NMR signals of these three trimers and this suggested these trimers are structurally significantly different. Extensive use of 2D-NMR techniques, notably ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H NOESY, in conjunction with 1D ^{1}H- and ^{13}C-NMR, enabled us to deduce unequivocally the structures of these new trimers, which are discussed in detail in the following paragraphs.

In compound **2**, two of the three "B" rings of the resveratrol units are further substituted, as was evident from the ^{1}H NMR signals: a singlet at δ_H 6.24 (H-12') from one "B" ring, two *meta* doublets at δ_H 6.29 (H-12'') and δ_H 5.95 (H-14'') from another "B" ring, and a 2H *meta* doublet at δ_H 6.00 (H-10, H-14) and a triplet at δ_H 6.10 (H-12) from the third "B" ring. ^{13}C PENDANT NMR, in addition to the signals for aromatic methines of three "A" rings (δ_C 112.9, 114.2, 115.1, 129.1, 129.3 and 129.4), also showed signals for six (not nine) aromatic methine carbons (δ_C 95.0, 100.2, 100.8, 104.7 and 105.7 for two carbons). This additional substitution on two "B" rings suggested their involvement in the formation of this trimer. A ^{1}H-^{1}H COSY45 spectrum revealed all the possible ^{1}H-^{1}H correlations within this molecule, the most important of which were: H-7'↔H-8'↔H-7 and

H-7''↔H-8''. A ^{1}H-^{13}C HMQC spectrum (Table 2) identified all ^{1}H-^{13}C direct ^{1}J correlations and thus confirmed the assignment of all methine carbons. A ^{1}H-^{13}C HMBC spectrum (Table 2) played a very crucial role by revealing ^{2}J and ^{3}J ^{1}H-^{13}C correlations and thus helped in joining different fragments leading to the elucidation of this structure. In the HMBC spectrum (Table 2), ^{2}J ^{1}H-^{13}C correlations from H-3 (δ_H 6.39) to C-4 (δ_C 154.8), H-5 (δ_H 6.39) to C-4, from H-7 (δ_H 3.69) to C-1 (δ_C 134.2) and C-8 (δ_C 53.1), from H-8 (δ_H 4.75) to C-7 (δ_C 59.5) and C-9 (δ_C 147.0), from H-10 (δ_H 6.00) to C-11 (δ_C 157.7), from H-12 (δ_H 6.10) to C-11 and C-13 (δ_C 157.7), from H-14 (δ_H 6.00) to C-13, and ^{3}J correlations from H-2 (δ_H 6.96) to C-4, C-6 (δ_C 129.3) and C-7, from H-3 to C-1 and C-5 (δ_C 114.2), from H-5 to C-1 and C-3 (δ_C 114.2), from H-6 (δ_H 6.96) to C-2 (δ_C 129.3), C-4 and C-7, from H-7 to C-2, C-6 and C-9, H-8 to C-1, C-10 (δ_C 105.7) and C-14 (δ_C 105.7), from H-10 to C-8, C-12 (δ_C 100.2) and C-14, from H-12 to C-10 and C-14, and from H-14 to C-8, C-10 and C-12 confirmed the structure of fragment **2a**. Similar reasoning based on HMBC correlations (Table 2) confirmed other two fragments: **2b** and **2c**.



2a



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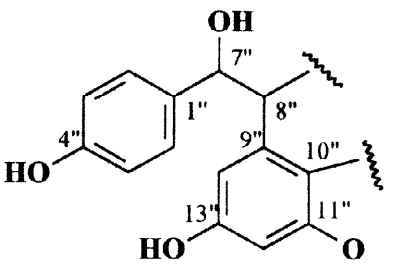

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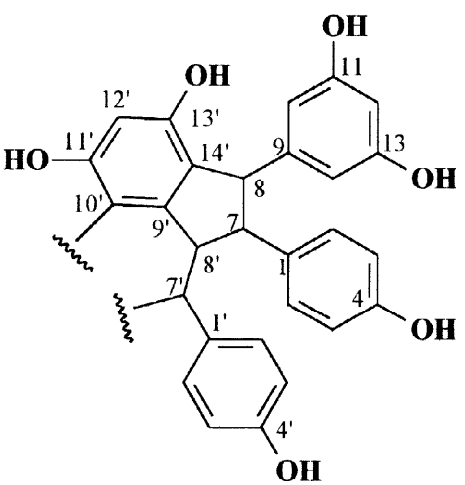

2d

Table 1: ^{1}H-NMR (400 MHz) and ^{13}C PENDANT NMR (100 MHz) data (δ in ppm, *J* in Hz) for **2-4**

C/H	δ_H			δ_C		
	2	**3**	**4**	**2**	**3**	**4**
1				134.2	134.1	136.6
2, 6	6.96 d (2H, 8.6)	6.28 d (2H, 8.6)	6.93 d (2H, 8.4)	129.3	128.1	128.4
3, 5	6.39 d (2H, 8.6)	6.30 d (2H, 8.6)	6.52 d (2H, 8.4)	114.2	113.8	114.1
4				154.8	154.7	154.4
7	3.69 d (1H, 7.6)	3.82 d (1H, 6.0)	5.09 s (1H)	59.5	61.7	35.4
8	4.75*	4.11 s (1H)	4.14 d (1H, 11.7)	53.1	55.5	50.9
9				147.0	146.0	142.5
10	6.00 d (1H, 2.2)	6.24 s (1H)	5.87 s (1H)	105.7	106.0	106.2**
11				157.7	158.0	157.3
12	6.10 t (1H, 2.2)	6.16 t (1H, 2.2)	6.00 s (1H)	100.2	100.1	100.0
13				157.7	158.0	157.3
14	6.00 d (1H, 2.2)	6.23 s (1H)		105.7	106.0	121.7
1'				132.6	132.4	131.6
2', 6'	6.47 d (2H, 8.5)	6.92 br d (2H)	7.20 d (2H, 8.6)	129.4	131.7	129.5
3', 5'	6.14 d (2H, 8.5)	6.52 d (2H, 8.6)	6.69 d (2H, 8.6)	112.9	113.4	114.5
4'				152.9	154.8	155.5
7'	5.40 d (1H, 3.2)	4.23 d (1H, 11.7)	3.00 dd (1H, 9.8, 11.7)	38.4	45.1	65.5
8'	3.90 m (1H)	4.12 m	4.26 d (1H, 9.8)	47.6	46.4	56.2
9'				143.2	146.1	145.6
10'			5.87 s (1H)	115.9	117.1	106.2**
11'				158.6	158.8	158.2
12'	6.24 s (1H, 0.8)	6.20 s (1H)	6.00 s (1H)	95.0	94.9	94.0
13'				153.7	154.3	153.5
14'				121.8	122.2	118.1
1''				129.8	129.5	133.4
2'', 6''	7.12 d (2H, 8.7)	7.58 d (2H, 8.6)	7.09 d (2H, 8.6)	129.1	129.1	126.6
3'', 5''	6.73 d (2H, 8.7)	6.91 d (2H, 8.6)	6.70 d (2H, 8.6)	115.1	115.1	114.8
4''				157.3	157.7	156.8
7''	5.71 d (1H, 11.7)	5.86 d (1H, 7.6)	6.00 s (1H)	90.2	89.7	84.9
8''	4.34 d (1H, 11.7)	5.09 br d (1H, 11.4)	4.24 d (1H, 2.5)	47.9	48.3	49.6
9''				140.3	141.0	146.2
10''			6.39 d (1H, 2.2)	125.6	121.5	102.1
11''				153.4	155.8	155.6
12''	6.29 d (1H, 2.4)	6.19 d (1H, 2.2)	6.24 d (1H, 2.2)	100.8	103.6	100.0
13''				155.0	156.9	157.3
14''	5.95 d (1H, 1.7)	5.96 d (1H, 2.4)		104.7	102.3	117.2

* masked by the broad water peak, obtained from ^{1}H-^{1}H COSY correlation; **Was not found in ^{13}C PENDANT NMR spectrum, but was found in a broad-band decoupled ^{13}C NMR spectrum and also from HMQC/HMBC correlation

Combination of **2a** and **2b** formed **2d** which was confirmed from the following ^{1}H-^{13}C correlations- ^{2}J: from H-7 to C-8'(δ_C 47.6) and from H-8 to C-14'(δ_C 121.8), and ^{3}J: from H-7 to C-9' (δ_C 143.2) and C-14', from H-8 to C-8' (δ_C 47.6) and C-9', and from H-8' (δ_H 3.90) to C-1. Now, combination of **2c** and **2d**, forming a seven membered ring and a dihydrofuran ring, completed the structure of compound **2** which was confirmed from HMBC correlations- ^{2}J: from H-7' (δ_H 5.40) to C-10'' (δ_C 125.6), from H-8'' (δ_H 4.34) to C-10' (δ_C 115.9) and ^{3}J: from H-7' to C-9'' (δ_C 140.3) and C-11'' (δ_C 153.4), from H-8'' to C-9' and C-11' and from H-7''(δ_H 5.71) to C-11' (δ_C 158.6). The relative stereochemistry of **2** was determined by a ^{1}H-^{1}H NOESY experiment which is summarised in Table 3. Thus, the structure of suffruticosol A was determined unequivocally as **2**.

While the ^{13}C PENDANT NMR spectrum of **3** (Table 1) was similar to that of **2**, significant differences in ^{1}H-NMR spectra (Table 1) of **2** and **3** were observed, the most notable being the extraordinary broadness of the signal for H-2' (δ_H 6.92) which suggested a semi-restricted rotation of that ring. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC spectra of **3** showed exactly identical correlations to those found for **2** (Table 2), suggesting that the structure of **3** was same as **2**, excepting in their stereochemistry. From analysis of the ^{1}H-^{1}H NOESY spectrum (Table 3), it was found that, in **3**, H-7' was in the α-orientation, and thus the 4-hydroxyphenyl substituent at C-7' encountered more restriction in its rotation compared to that found in **2**. Thus, the structure of suffruticosol B was determined as **3**, which is a stereo-isomer of **2**.

Compound **4** displayed all the ^{1}H and ^{13}C NMR spectral features for a similar trimer. In the ^{1}H-^{1}H COSY spectrum, a chain of correlations was observed, amongst which the most important were: H-7 (δ_H 5.09)↔H-8 (δ_H 4.14)↔H-7'(δ_H 3.00)↔H-8'(δ_H 4.26) and H-7''(δ_H 6.00)↔H-8''(δ_H 4.24). The ^{1}H-^{13}C HMBC spectrum (Table 2) of **4**, in addition to the correlations confirming the three resveratrol-derived structural units similar to those described for **2**, showed a series of ^{2}J and ^{3}J ^{1}H-^{13}C correlations which led to the unambiguous determination of the structure of suffruticosol C as **4**. Some of these key correlations were from the six non-aromatic methine protons: from H-7, ^{2}J to C-14''(δ_C 117.2) and ^{3}J to C-7'(δ_C 65.5), C-9''(δ_C 146.2) and C-13''(δ_C 157.3); from H-8, ^{2}J to C-7' and ^{3}J to C-1'(δ_C 131.6) and C-14''; from H-7', ^{2}J to C-8 (δ_C 50.9) and ^{3}J to C-7 (δ_C 35.4) and C-9 (δ_C 142.5); from H-8', ^{2}J to C-14 (δ_C 121.7); from H-7'', ^{3}J to C-13' (δ_C 153.5) and C-14' (δ_C 118.1); from H-8'', ^{2}J to C-14'. The relative stereochemistry of this molecule was determined from a series of nOe interactions (Table 3).

Compounds **1-4** were found to be active as ecdysteroid antagonists (Table 4), but inactive as agonists in the *Drosophila melanogaster* B_{II} cell bioassay for ecdysteroid agonists/antagonists.[30] Paeoniflorin (**5**)was also found to be a weak antagonist, but the very low activity (ED_{50} = 1.5 x 10^{-3}M) may result from a slight contamination (*ca.* 1%) by one or more of the other compounds. However, the antagonistic potencies of resveratrol and its oligomers are similar to those of the other plant-derived ecdysteroid antagonists - cucurbitacins,[9] withanolides[6] and limonoids[11] - previously investigated in our laboratory. This is the first report on the occurrence of

Table 2: ^{1}H-^{13}C correlations in **2** and **4** obtained from HMQC (^{1}J) and HMBC (^{2}J and ^{3}J)

H	C					
	2			**4**		
	^{1}J	^{2}J	^{3}J	^{1}J	^{2}J	^{3}J
H-2	C-2		C-4, C-6, C-7	C-2	C-3	C-4, C-6, C-7
H-3	C-3	C-4	C-1, C-5	C-3	C-4	C-1, C-5
H-5	C-5	C-4	C-1, C-3	C-5	C-4	C-1, C-3
H-6	C-6		C-2, C-4, C-7	C-6	C-5	C-2, C-4, C-7
H-7	C-7	C-1, C-8, C-8'	C-2, C-6, C-9, C-9', C-14'	C-7	C-1, C-8, C-14''	C-2, C-6, C-9, C-9'', C-7'', C-13''
H-8	C-8	C-7, C-9, C-14'	C-1, C-10, C-14, C-8', C-9'	C-8	C-7, C-9, C-7'	C-1, C-10, C-1', C-14''
H-10	C-10	C-11	C-8, C-12, C-14	C-10 (w)		
H-12	C-12	C-11, C-13	C-10, C-14	C-12	C-11, C-13	C-10, C-14
H-14	C-14	C-13	C-8, C-10, C-12	-		
H-2'	C-2'		C-4', C-6', C-7'	C-2'	C-3'	C-4', C-6', C-7'
H-3'	C-3'	C-4'	C-1', C-5'	C-3'	C-2', C-4'	C-1', C-5'
H-5'	C-5'	C-4'	C-1', C-3'	C-5'	C-4', C-6'	C-1', C-3'
H-6'	C-6'		C-2', C-4', C-7'	C-6'	C-5'	C-2', C-4', C-7'
H-7'	C-7'	C-1', C-8', C-10''	C-2', C-9', C-9'', C-11''	C-7'	C-8, C-1', C-8'	C-7, C-9, C-2', C-6', C-9'
H-8'	C-8'	C-9'	C-1, C-1'	C-8'	C-7', C-9', C-14	C-1', C-10', C-14'
H-10'	-			C-10' (w)		
H-12'	C-12'	C-11', C-13'	C-10', C-14'	C-12'	C-11', C-13'	C-10', C-14'
H-2''	C-2''		C-4'', C-6'', C-7''	C-2''	C-3''	C-4'', C-6'', C-7''
H-3''	C-3''	C-4''	C-1'', C-5''	C-3''	C-2'', C-4''	C-1'', C-5''
H-5''	C-5''	C-4''	C-1'', C-3''	C-5''	C-4'' , C-6''	C-1'', C-3''
H-6''	C-6''		C-2'', C-4'', C-7''	C-6''	C-5''	C-2'', C-4'', C-7''
H-7''	C-7''	C-1'', C-8''	C-2'', C-9'', C-11'	C-7''	C-1'', C-8''	C-2'', C-6'', C-9'', C-13', C-14'
H-8''	C-8''	C-7'', C-9'', C-10'	C-9', C-11', C-1'', C-10''	C-8''	C-14', C-9''	C-1'', C-14''
H-10''	-			C-10''	C-11''	C-8'', C-12'', C-14''
H-12''	C-12''	C-11'', C-13''	C-10'', C-14''	C-12''	C-11'', C-13''	C-10'', C-14''
H-14''	C-14''	C-9'', C-13''	C-10'', C-8''	-		

w = weak correlation

oligostilbenes in the family Paeoniaceae. It can be noted here that the distribution of oligostilbenes was reported to be restricted to only five plant families: Dipterocarpaceae, Vitaceae, Cyperaceae, Gnetaceae and Leguminosae.[31] Since resveratrol possesses oestrogenic activity[32] and all steroid hormone receptors belong to one family of related proteins, it was of interest to see if other environmental and pseudo-oestrogens[33] also possess activity in the B_{II} bioassay (*i.e.* possibly interact with the ecdysteroid receptor). None of the tested compounds (Table 4) possessed agonistic activity, but several (γ-BHC, bisphenol A, daidzein, *p,p'*-DDT, diethylphthalate, zearalenone) possessed antagonistic activity, although only γ-BHC was as active as resveratrol

and its trimers. Interestingly, the synthetic oestrogenic stilbene, diethylstilboestrol, is not active, nor are steroidal oestrogens.[30] Most of the compounds tested here were cytotoxic at high concentrations.

Table 3: Key nOe interactions found in ^{1}H-^{1}H NOESY spectra of **2 - 4**

From:	To:		
	2	**3**	**4**
H-2/H-6	H-2'/H-6', H-7'	H-8, H-7, H-7'	H-7, H-8, H-8''
H-7	H-10/H-14, H-8'	H-2/H-6, H-8', H-2'/H-6'	H-2/H-6, H-8 (w), H-2'/H-6', H-2''/H-6'', H-7'
H-8		H-2/H-6, H-7'	H-7 (w), H-2/H-6, H-2'/H-6', H-8', H-7' (w)
H-2'/H-6'	H-2/H-6, H-7', H-8''	H-7, H-7'	H-7, H-8, H-7', H-8'
H-3'/H-5'		H-14''	
H-7'	H-8', H-2/H-6, H-2'/H-6'	H-2/H-6, H-8'', H-8, H-2'/H-6'	H-7, H-8 (w), H-2'/H-6', H-8' (w)
H-8'	H-10/H-14, H-7	H-7	H-2'/H-6', H-8, H-8''
H-2''/H-6''	H-7'', H-8'', H-14''	H-7'', H-8'', H-14''	H-7, H-7'', H-8'', H-10'
H-7''	H-2''/H-6'', H-8'', H-14''	H-2''/H-6'', H-8''	H-10'', H-8'' (w), H-2''/H-6''
H-8''	H-2'/H-6', H-2''/H-6''	H-2''/H-6'', H-7'	H-2/H-6, H-7''(w), H-2''/H-6'',
H-10''	-	-	H-7''

w = weak, COSY-type correlation

MATERIALS AND METHODS

General experimental procedures. UV spectra were obtained in EtOH. NMR spectra were performed in CD_3OD, on a Bruker AVANCE DRX400 instrument using Bruker microprograms. ^{1}H-NMR and ^{13}C-NMR spectra were referenced to CH_3OH at δ 3.31 and δ 49.15, respectively. LSIMS (+ve and -ve ion modes); glycerol matrix using a Cs^{+} primary ion beam on a VG Quattro triple quadrupole mass spectrometer (VG Biotech, Altrincham, U.K.); Sep-Pak Vac 35cc (10g) C_{18} cartridge (Waters) was used for pre-HPLC fractionation; HPLC: a) preparative/semipreparative - Gilson model 806 HPLC coupled with Gilson UV-Visible detector, b) analytical- Gilson model 811 HPLC coupled with Gilson 160 diode array detector and using Gilson Unipoint computer program; RP, RP-prep., RP-semiprep. and RP-anal. stand respectively for reversed-phase, Technoprep 10C_8 preparative C_8 column, Spherisorb semipreparative C_{18} column and Spherisorb 5 ODS-2 analytical C_{18} column throughout this text. Chromatographic separations were monitored at 230 and 280 nm.

Table 4: Activities of the compounds isolated from seed of *Paeonia suffruticosa* and various pseudo-oestrogens in the B_{II} bioassay for ecdysteroid antagonists.

Compound	Max. concentration tested	Antagonist Activity	Cytotoxicity
cis-resveratrol (**1**)	10^{-2}M	active: ED_{50} = 1.2 x 10^{-5}M	≥2.5 x 10^{-4}M
suffruticosol A (**2**)	5.0 x 10^{-3}M	active: ED_{50} = 5.3 x 10^{-5}M	≥10^{-3}M
suffruticosol B (**3**)	10^{-2}M	active: ED_{50} = 1.4 x 10^{-5}M	≥2.5 x 10^{-3}M
suffruticosol C (**4**)	5.0 x 10^{-3}M	active: ED_{50} = 2.2 x 10^{-5}M	≥10^{-3}M
paeoniflorin (**5**)	5.0 x 10^{-3}M	active?: ED_{50} = 1.5 x 10^{-3}M	≥5.0 x 10^{-3}M
apigenin	10^{-3}M	inactive	-
γ-BHC (lindane)	10^{-3}M	active: ED_{50} = 3.0 x 10^{-5}M	slightly at 10^{-3}M
biochanin A	10^{-3}M	inactive	at 10^{-3}M
bisphenol A	10^{-3}M	active: ED_{50} = 1.0 x 10^{-4}M	≥2.5 x 10^{-4}M
daidzein	10^{-3}M	weak activity at ≥10^{-4}M	-
diethylphthalate	10^{-2}M	active: ED_{50} = 2.0 x 10^{-3}M	≥5.0 x 10^{-3}M
diethylstilboestrol	10^{-3}M	inactive	≥2.5 x 10^{-3}M
o,p'-DDT	10^{-3}M	inactive	≥10^{-4}M
p,p'-DDT	10^{-3}M	weak antagonist at ≥2.5 x 10^{-5}M	≥10^{-4}M
genistein	10^{-3}M	inactive	at 10^{-3}M
methoxychlor	10^{-3}M	inactive	≥10^{-4}M
octylphenol	10^{-3}M	inactive	≥2.5 x 10^{-5}M
quercetin	10^{-3}M	inactive	at 10^{-3}M
zearalenone	10^{-3}M	weak activity at ≥10^{-5}M	≥2.5 x 10^{-4}M

Bioassay. Ecdysteroid agonist/antagonist activities of the extract, Sep-Pak fractions, HPLC fractions and the isolated compounds were assessed with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line[30]. Pure compounds were tested at concentrations ranging from 10^{-8} M to 10^{-2} M. For the antagonist assay, a concentration of 20-hydroxyecdysone of 5 x 10^{-8} M was used. Pseudo-oestrogens were purchased from Sigma, Aldrich or Lancaster and stock solutions were prepared in methanol.

Plant material. Seeds of *P. suffruticosa* Andrews were a gift of Ness Botanical Gardens, University of Liverpool, U.K. and also purchased from B & T World Seeds, Olonzac, France. A voucher specimen has been retained at the Department of Biological Sciences, University of Exeter.

Extraction and isolation. Ground seeds (4.8 g) were extracted four times (4 x 24 h) with 4 x 300 mL of MeOH at 55 ° C with constant stirring using a magnetic stirrer. Extracts were pooled and made to a 70% aq. methanolic solution. After being defatted with *n*-hexane, the extract was concentrated using a rotary evaporator at a maximum temperature of 45 °C. The defatted extract was subjected to Sep-Pak fractionation (using MeOH-H_2O step-gradient) which afforded six fractions. Two fractions (eluted with 35% and 50% aq. MeOH, hereafter termed as SP35 and SP50, respectively) showed ecdysteroid antagonistic activity. SP50 was subjected to RP-prep. HPLC, eluting with 55% MeOH in water, at a flow rate of 5 mL/min to yield active fractions with retention times 21-35 min. Further HPLC analysis of these fraction on RP-semiprep., eluting with the same solvent mixture, at a flow rate of 2 mL/min afforded **1** (retention time: 39 min). Similar RP-prep. HPLC on SP35 resulted in a mixture of active principles in the fractions eluted between 14-30 minutes. These fractions were combined and subjected to RP-semiprep. HPLC (40% MeOH in Water, 2 mL/min) to isolate paeoniflorin (**5**; ret. time: 15.4 min), and three novel compounds (**2** – **4**) (ret. times: 17.2, 26.2, and 41.5 min, respectively).

Cis-Resveratrol (**1**) (8.1 mg): Brown amorphous. LSIMS: *m/z* 227 $[M - H]^-$ (-ve ion mode). UV, ^{1}H-NMR and ^{13}C-NMR as published data.[18-20]

Suffruticosol A (**2**) (56.0 mg): Brownish-white amorphous, UV λ_{max} nm (log ε) = 283 (3.43), 226 (4.12). HRMS: $C_{42}H_{33}O_9$ $[M+H]^+$ requires 681.21246 (found 681.21273), LSIMS: *m/z* 679 $[M - H]^-$ (-ve ion mode). ^{1}H-NMR and ^{13}C-NMR (Table 1).

Suffruticosol B (**3**) (74.0 mg): Brownish-white amorphous, UV λ_{max} nm (log ε) = 283 (3.46), 226 (4.10). HRMS: $C_{42}H_{33}O_9$ $[M+H]^+$ requires 681.21246 (found 681.21205), LSIMS: *m/z* 679 $[M - H]^-$ (-ve ion mode). ^{1}H-NMR and ^{13}C-NMR (Table 1).

Suffruticosol C (**4**) (6.2 mg): Brownish-white amorphous, UV λ_{max} nm (log ε) = 283 (3.42), 226 (4.08). HRMS: $C_{42}H_{33}O_9$ $[M+H]^+$ requires 681.21246 (found 681.21273), LSIMS: *m/z* 679 $[M - H]^-$ (-ve ion mode). ^{1}H-NMR and ^{13}C-NMR (Table 1).

Paeoniflorin (**5**) (3.8 mg): Amorphous: UV, ^{1}H-NMR and ^{13}C-NMR as published data.[21]

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REFERENCES

1. Tzanoudakis, D. Nord. J. Bot. 1983, 3, 307-318.
2. Sang, T.; Donoghue, M. J.; Zhang, D. Mol. Biol. Evol. 1997, 14, 994-1007.
3. Pan, K. –Y.: *Paeonia*. In Flora Republica Sinicae, Vol. 27, Science Press, Beijing, 1979; pp. 37-59.
4. USDA-ARS GRIN database, GRIN/NPGS Taxonomy information, 1998, available at: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?26332
5. Lin, H. –C.; Ding, H. –Y.; Wu, T.-S.; Wu, P.-L. Phytochemistry 1996, 41, 237-242.
6. Dinan, L.; Whiting, P.; Alfonso, D.; Kapetanidis, I. Entomol. Exp. Appl. 1996, 80, 415-420.
7. Dinan, L.; Sarker, S. D.; Šik, V. Phytochemistry 1997, 44, 509-512.
8. Dinan, L.; Whiting, P.; Sarker, S. D.; Kasai, R.; Yamasaki, K. Molec. Life Sci.1997, 53, 271-274.
9. Dinan, L.; Whiting, P.; Girault, J. P.; Lafont, R., Dhadialla, T. S.; Cress, D. E.; Mugat, B.; Antoniewski, C.; Lepesant, J.-A. Biochem. J. 1997, 327, 643-650.
10. Sarker, S. D.; Whiting, P.; Lafont, R.; Girault, J. P.; Dinan, L. Biochem. Syst. Ecol. 1997, 25, 79-80.
11. Sarker, S. D.; Savchenko, T.; Whiting, P.; Šik, V.; Dinan, L. Arch. Insect Biochem. Physiol. 1997, 35, 211-217.
12. Sarker, S. D.; Lafont, R.; Šik, V.; Dinan, L. Biochem. Syst. Ecol. 1997, 25, 365-366.
13. Sarker, S. D.; Savchenko, T.; Whiting, P.; Šik, V.; Underwood, E.; Dinan, L. Biochem. Syst. Ecol. 1997, 25, 473-474.
14. Savchenko, T.; Whiting, P.; Sarker, S. D.; Dinan, L. Biochem. Syst. Ecol. 1998, 26, 131-134.
15. Koolman, J. Ecdysone, Thieme Verlag, Stuttgart, 1989.
16. Dinan, L. Eur. J. Entomol. 1995, 92, 271-283.
17. Dinan,L.; Savchenko, T., Whiting, P.; Sarker, S.D. Pestic. Sci. 1999, in press.
18. Fulvio, M.; Fabiano, R.; Siegfried, K. J.Agric. Food Chem. 1995, 43, 1820-1823.
19. Ingham, J. L. Phytochemistry 1976, 15, 1791-1793.
20. MacRae, W. D.; Towers, G. H. N. Phytochemistry 1985, 24, 561-566.
21. Cai, Y.; Philipson, J. D.; Harper, J. I.; Corne, S. J. Phytochemical Analysis 1994, 5, 183-189.
22. Ohyama, M.; Tanaka, T.; Iinuma, M.; and Goto, K. Chem. Pharm. Bull. 1994, 42, 2117-2120.
23. Kawabata, J.; Mishima, M.; Kurihara, H.; Mizutani, J. Phytochemistry 1991, 30, 645-647
24. Homer, J.; Perry, M. C. J. Chem. Soc. Chem. Commun. 1994, 373-375.
25. Bokel, M.; Diyasena, M. N. C.; Gunatilaka, A. A. L.; Kraus, W.; Sotheeswaran, S. Phytochemistry 1988, 27, 377-380.
26. Samaraweera, U.; Sotheeswaran, S.; Sultanbawa, M. U. S. Phytochemistry, 1982, 21, 2585-2587.

27. Diyasena, M. N.C.; Sotheeswaran, S.; Surendarkumar, S.; Balasubramaniam, S.; Bokel, M.; Kraus, W. H. J. Chem. Soc. Perkin Trans I 1985, 1807-1810.
28. Sotheeswaran, S.; Sultanbawa, M. U. S.; Surendarkumar, S.; Balasubramaniam, S.; Bladon, P. H.; J. Chem. Soc. Perkin Trans I 1985, 159-161.
29. Surendarkumar, S. PhD Thesis 1980, University of Peradeniya, Sri Lanka
30. Clément, C.Y.; Bradbrook, D. A.; Lafont, R.; Dinan, L. Insect. Biochem. Mol. Biol. 1993, 23, 187-190.
31. Kurihara, H.; Kawabata, J.; Ichikawa, S.; Mishima, M.; Mizutani, J. Phytochemistry 1991, 30, 649-653.
32. Williams, R.L., Rutledge, T., Chemistry & Industry, 1998, 14-16
33. Kardinaal, A.F.M., Waalkens-Berendsen, D.H. and Arts, C.J.M., Trends Food Sci. Technol. 1997, 8, 327-333