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# Phytochemicals of the Chinese Herbal Medicine

## *Tacca chantrieri* Rhizomes

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Additional information is available at the end of the chapter

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### 1. Introduction

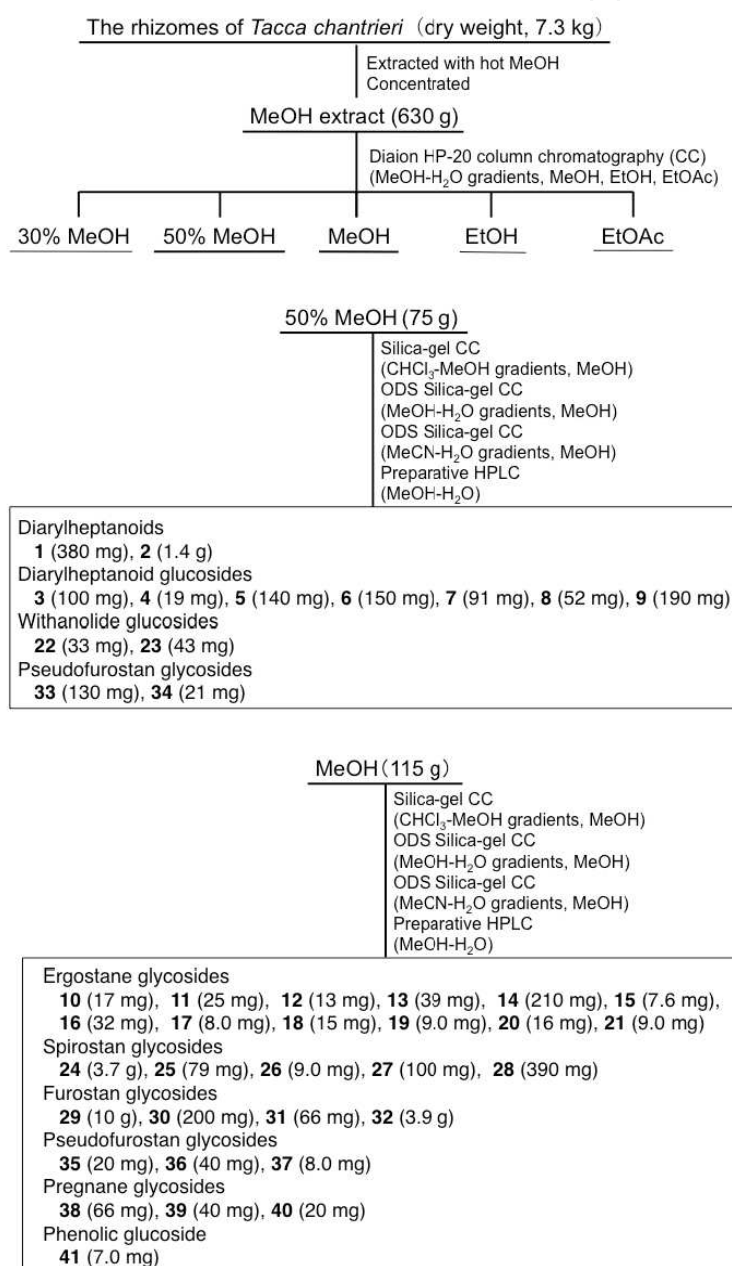
The family Taccaceae is composed of two genera, *Tacca* and *Schizocapsa*, and about 10 species, with most distributed in tropical regions of Asia, the Pacific Islands, and Australia [1]. *Tacca chantrieri* André is a perennial plant that occurs in the southeast region of mainland China, and its rhizomes have been used for the treatment of gastric ulcers, enteritis, and hepatitis in Chinese folk medicine. According to a Chinese herbal dictionary, *T. plantaginea* has also been used for the same purposes as *T. chantrieri* [2]. The chemical constituents of *T. plantaginea* have been extensively examined and a series of highly oxygenated pentacyclic steroids named taccalonolids, which have a  $\gamma$ -enol lactone, have been isolated as characteristic components of the herb [3], but there has been only one report of the secondary metabolites of *T. chantrieri*, in which a few trivial sterols such as stigmasterol and daucosterol, and a diosgenin glycoside were found [4]. Therefore, we focused our attention on the constituents of *T. chantrieri* rhizomes, and a detailed phytochemical investigation of this herbal medicine has been carried out.

In this chapter, we describe the phytochemicals isolated from *T. chantrieri* rhizomes and their biological activities with a focus on cytotoxicity against human cancer cells.

### 2. Isolation and structural determination

*T. chantrieri* specimens were collected in Yunnan Province, People's Republic of China. The rhizomes of *T. chantrieri* (fresh weight, 7.3 kg) were extracted with hot MeOH (3 L  $\times$  2). The MeOH extract was concentrated under reduced pressure, and the extract was passed through a polystyrene resin (Diaion HP-20) column eluted with MeOH/H<sub>2</sub>O gradients,

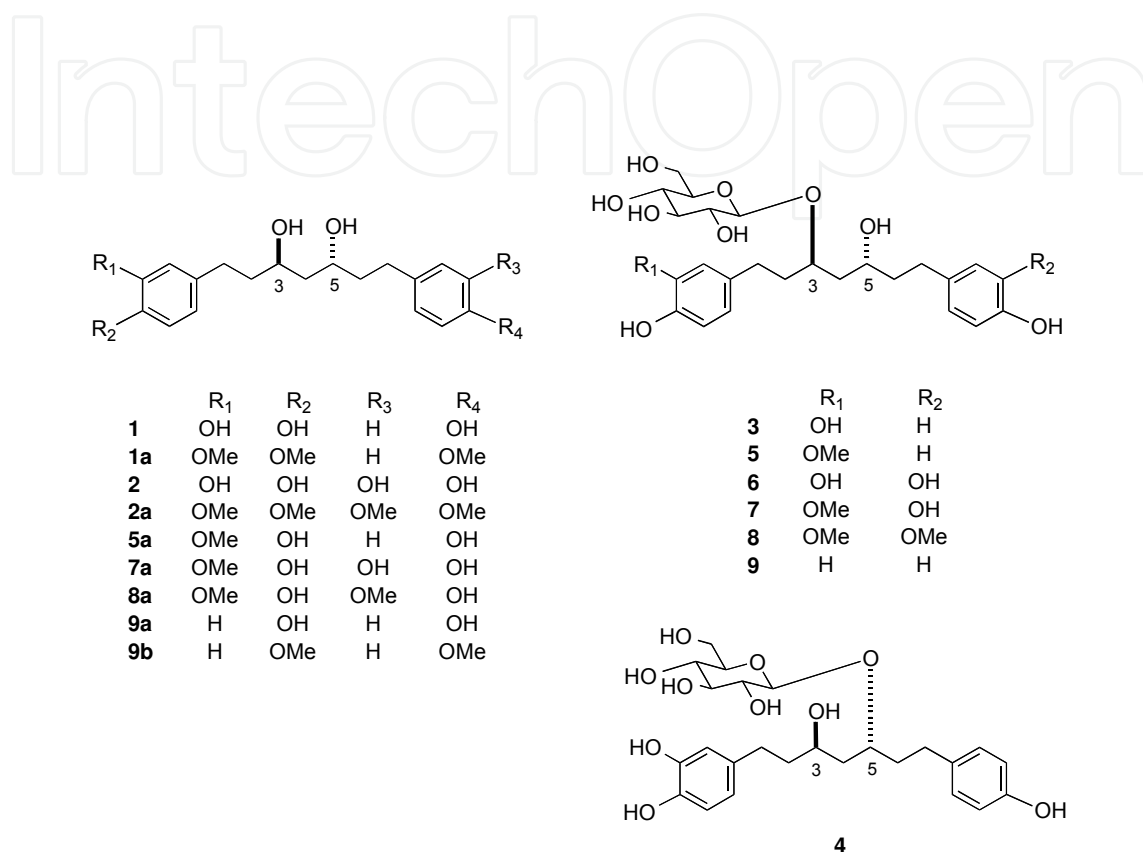
EtOH, and EtOAc. The 50% MeOH and MeOH eluate portion was subjected to silica gel and octadecylsilanized silica gel column chromatography to afford a total of 41 compounds, classified into diarylheptanoids (**1** and **2**), diarylheptanoid glucosides (**3–9**), ergostane glucosides (**10–21**), withanolide glucosides (**22** and **23**), spirostan glycosides (**24–28**), furostan glycosides (**29–32**), pseudofurostan glycosides (**33–37**), pregnane glycosides (**38–40**), and a phenolic glucoside (**41**) (Fig.1). Their structures were determined through extensive spectroscopic studies and through chemical transformations followed by chromatographic and spectroscopic analysis.



**Figure 1.** Extraction, partition, and purification procedures

### 3. Diarylheptanoids and diarylheptanoid glucosides

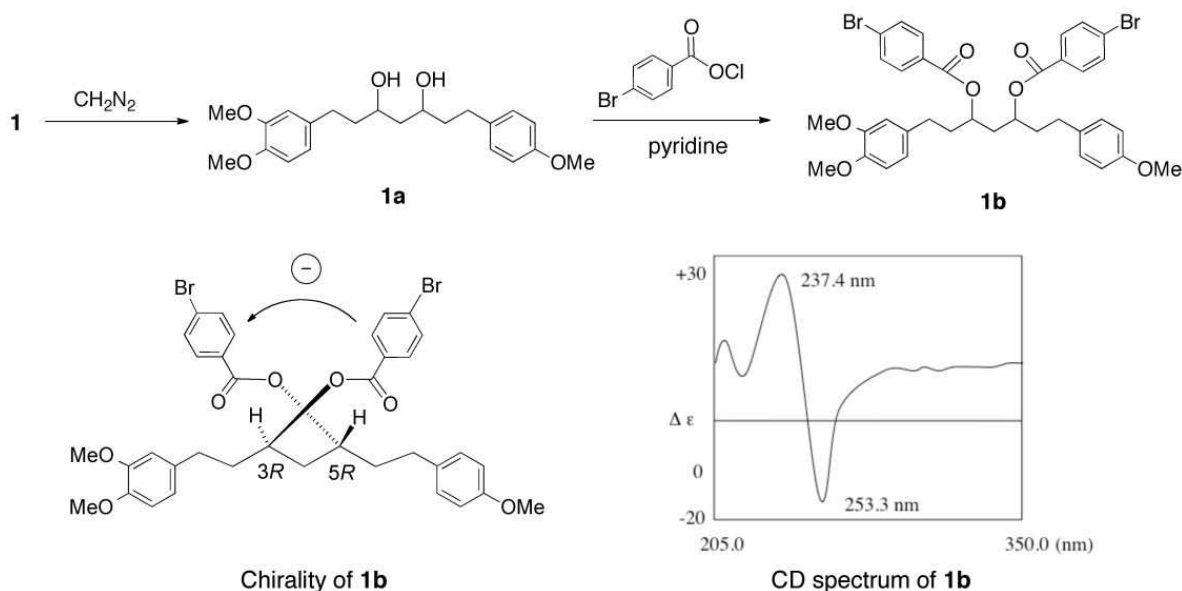
Diarylheptanoids consist of two phenyl groups linked by a linear seven-carbon aliphatic chain. Compounds **1** and **2** are diarylheptanoids and **3–9** are diarylheptanoid monoglucosides (Fig. 2) [5].



**Figure 2.** Structures of **1–9** and their derivatives

Compound **1** was isolated as a viscous syrup,  $[\alpha]_D +1.7^\circ$  (MeOH). HREIMS of **1** showed an  $[M]^+$  peak at  $m/z$  332.1623, corresponding the empirical molecular formula of  $C_{19}H_{24}O_5$ , which was also deduced by analysis of its  $^{13}C$  NMR and DEPT spectral data. The IR spectrum suggested the presence of hydroxy groups ( $3347\text{ cm}^{-1}$ ) and aromatic rings ( $1611$  and  $1515\text{ cm}^{-1}$ ). The UV spectrum showed an absorption maximum due to substituted aromatic rings ( $281.4\text{ nm}$ ). The planar structure of **1** was assigned as 3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane by analysis of the 1D ( $^1H$  and  $^{13}C$ ) and 2D ( $^1H$ - $^1H$  COSY, HMQC, and HMBC) spectra. The absolute configuration of the 3,5-dihydroxy moieties of the new diarylheptanoids were determined by applying the CD exciton chirality method to acyclic 1,3-dibenzoates [6]. The trimethyl derivative (**1a**) was converted to the corresponding 3,5-bis(*p*-bromobenzoate) (**1b**) and its CD spectrum exhibited positive ( $237.4\text{ nm}$ ,  $\Delta\epsilon +29.9$ ) and negative ( $253.3\text{ nm}$ ,  $\Delta\epsilon -20.0$ ) Cotton effects, which were consistent with a negative chirality. Thus, the absolute configurations were determined as  $3R$  and  $5R$  (Fig. 3). The

structure of **1** was shown to be (3*R*,5*R*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane. In the same way, the structure of **2** was elucidated as (3*R*,5*R*)-3,5-dihydroxy-1,7-bis(3,4-dihydroxyphenyl)heptane.



**Figure 3.** Determination of the absolute configurations at C-3 and C-5 of **1**

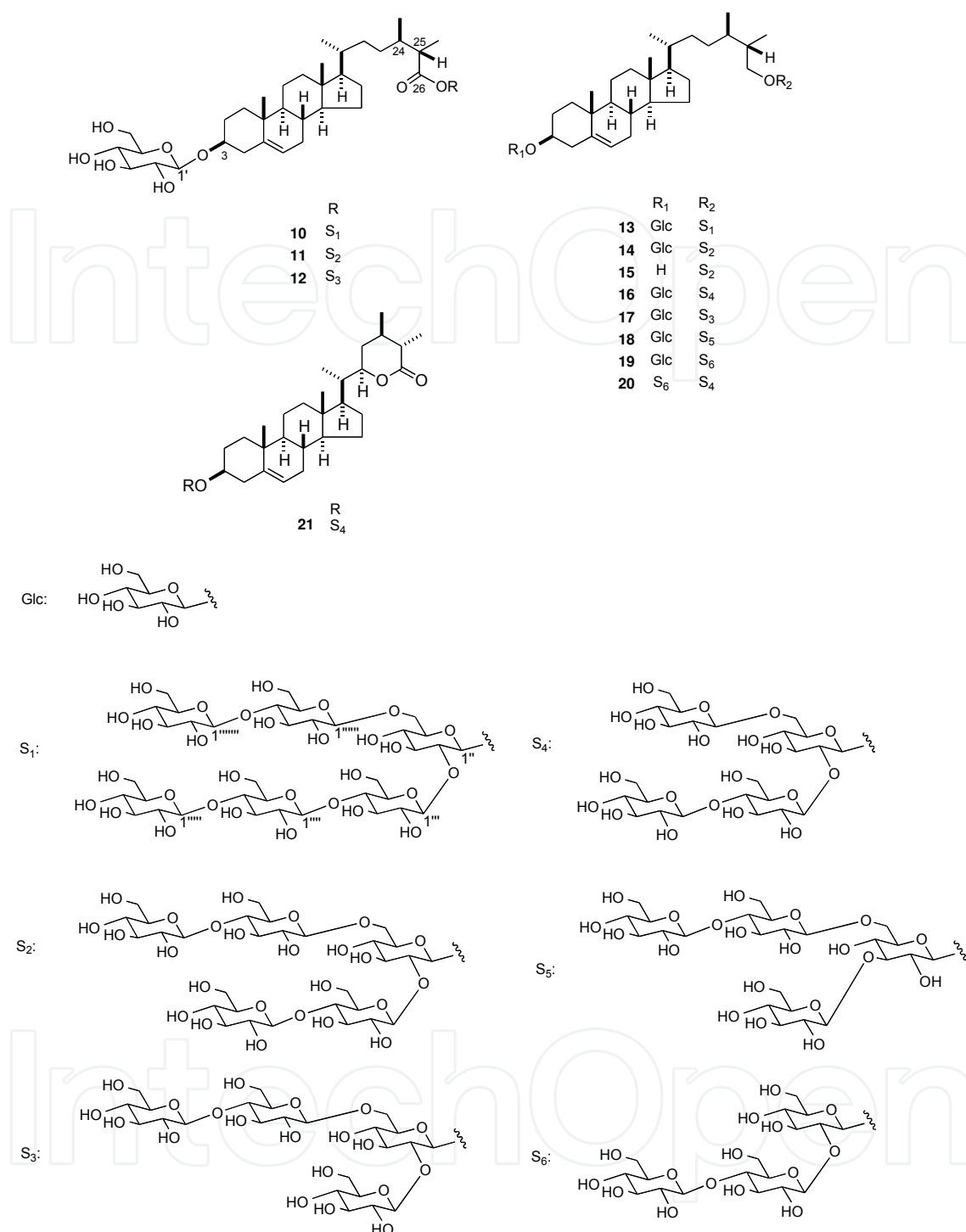
Compounds **3–9** are diarylheptanoid monoglucosides. Enzymatic hydrolysis of **3–9** with naringinase gave the diarylheptanoid derivatives and D-glucose. Identification of D-glucose, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysates. In the HMBC spectra, a long-range correlation was observed from each anomeric proton to the C-3 carbon in **3** and **5–9**, and to the C-5 carbon in **4**.

Diarylheptanoids are known to occur in only a limited number of species of higher plants belonging to the families Zingiberaceae [7–10], Betulaceae [11], and Aceraceae [12]. This is the first isolation of diarylheptanoids from a plant of the family Taccaceae.

#### 4. Ergostane glucosides

Compounds **10–21** are new ergostane glucosides (Fig. 4) [13–15]. Taccasterosides A–C (**10–12**) are novel bisdesmosideic oligoglucosides of (24*R*,25*S*)-3β-hydroxyergost-5-ene-26-oic acid (**10a**), whereas **13–20** are those of (24*S*,25*R*)-ergost-5-ene-3β,26-diol (**10b**). Compound **21** is an ergostane glucoside with the six-membered lactone on the side chain of the aglycone.

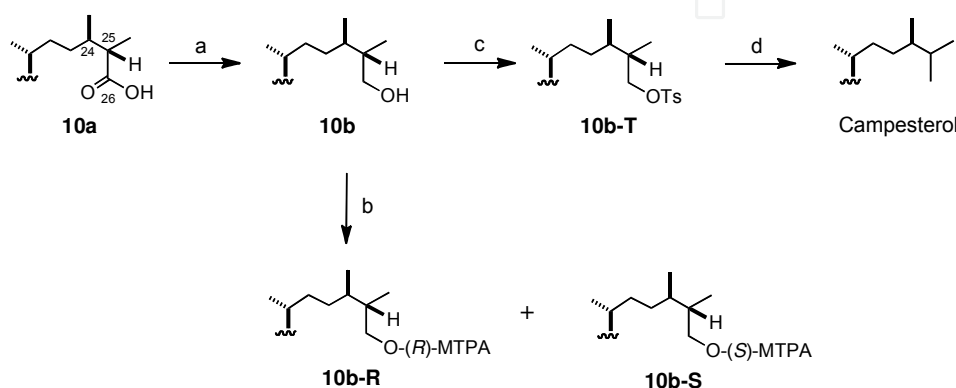
Taccasteroside A (**10**) was obtained as an amorphous solid. Acid hydrolysis of **10** with 1 M HCl in dioxane/H<sub>2</sub>O gave D-glucose and a C<sub>28</sub>-sterol as the aglycone (**10a**). The structure of **10a**, except for the absolute configurations at C-24 and C-25, was identified as 3β-hydroxyergost-5-en-26-oic acid by analysis of its <sup>1</sup>H, <sup>13</sup>C, and 2D NMR spectra. In order to determine



**Figure 4.** Structures of **10–21**

the absolute configuration at C-25, **10a** was reduced with  $\text{LiAlH}_4$  to (24*R*,25*S*)-ergost-5-ene-3 $\beta$ ,26-diol (**10b**). Then, **10b** was converted to the diastereomeric pairs of (*R*)-MTPA (**10a-R**) and (*S*)-MTPA (**10a-S**) esters with respect to the C-26 primary hydroxy group next to the C-25 chiral center and the differences in the  $^1\text{H}$  NMR coupling patterns of the  $\text{H}_2$ -26 protons

were inspected. The H<sub>2</sub>-26 protons of **10a-R** were observed as a doublet-like signal at  $\delta$  4.20 ( $J$  = 6.3 Hz), whereas those of **10a-S** were observed as a doublet of doublets at  $\delta$  4.30 ( $J$  = 10.8, 6.6 Hz) and 4.09 ( $J$  = 10.8, 7.2 Hz). Application of these spectral data to the empirical rule reported by Yasuhara et al. [17] allowed us to confirm that the C-25 configuration was exclusively *S*. The configuration of C-24 position and other steroidal skeleton were established by the following chemical transformations. Compound **10b** was treated with *p*-toluenesulfonyl chloride to give the 26-*O*-tosylate of **10b** (**10b-T**), which was then reduced with LiAlH<sub>4</sub>, affording (24*R*)-ergost-5-ene-3 $\beta$ -ol, that is, campesterol. The structure of **10a** was determined as (24*R*,25*S*)-3 $\beta$ -hydroxyergost-5-en-26-oic acid (Fig. 5).



Reagents and conditions: a, LiAlH<sub>4</sub>, THF, 0 °C, 5 h; b, (*R*)-MTPA or (*S*)-MTPA, EDC·HCl, 4-DMAP, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 12 h; c, *p*-TsCl, pyridine, r.t., 6 h; d, LiAlH<sub>4</sub>, THF, 0 °C, 5 h

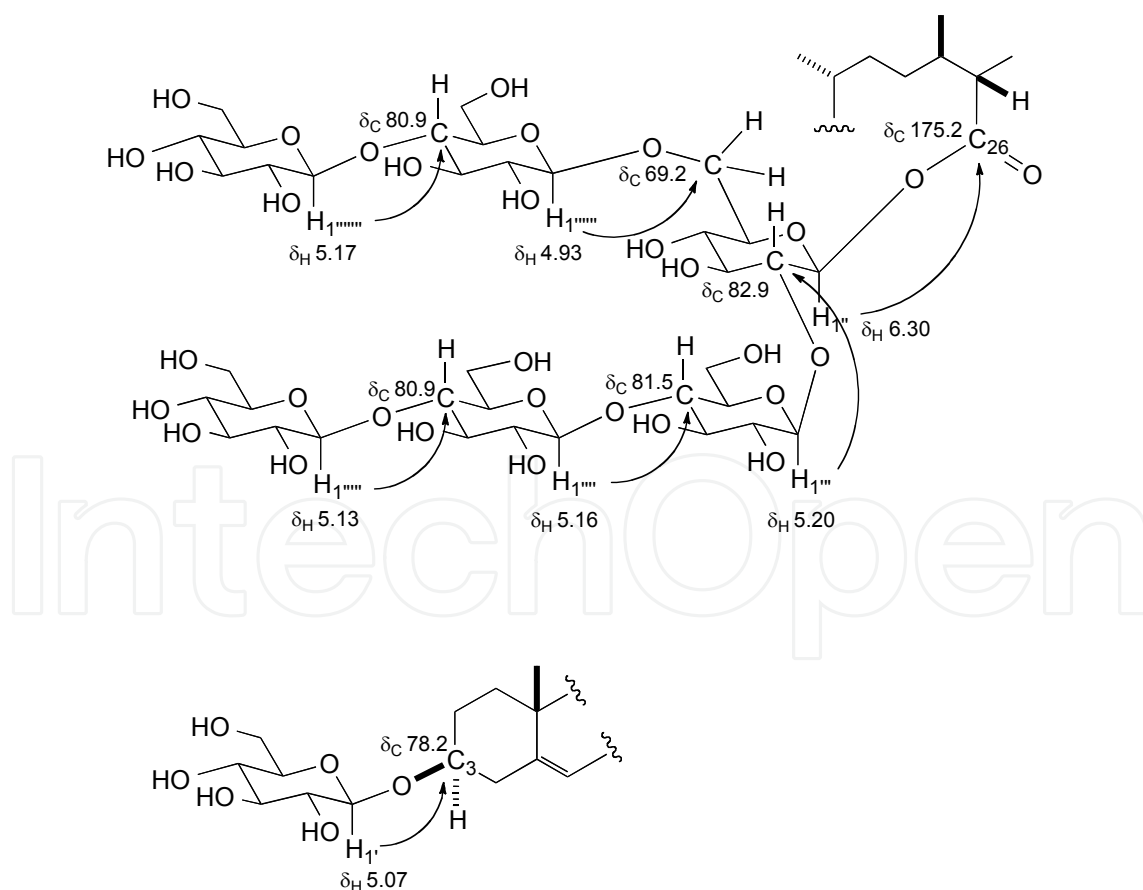
**Figure 5.** Chemical transformations of **10a**

The severe overlap of the proton signals for the sugar moieties in **10** excluded the possibility of complete assignment in a straightforward way by conventional 2D NMR methods such as the <sup>1</sup>H-<sup>1</sup>H COSY, 2D TOCSY, and HSQC spectroscopy. The exact structures of the sugar moieties and their linkage positions of the aglycone were resolved by detailed analysis of the 1D TOCSY and 2D NMR spectra. The <sup>1</sup>H NMR subspectra of individual monosaccharide units were obtained by using selective irradiation of easily identifiable anomeric proton signals, as well as irradiation of other nonoverlapping proton signals in a series of 1D TOCSY experiments [17–19]. Subsequent analysis of the <sup>1</sup>H-<sup>1</sup>H COSY spectrum resulted in the sequential assignment of all the proton resonances due to the seven glucosyl units, including identification of their multiplet patterns and coupling constants. The HSQC and HSQC-TOCSY spectra correlated the proton resonances to those of the corresponding one-bond coupled carbons, leading to unambiguous assignments of the carbon shifts. The carbon chemical shifts thus assigned were compared with those of the reference methyl  $\alpha$ -D- and  $\beta$ -D-glucosides [20], taking into account the known effects of *O*-glycosylation shifts. The comparison indicated that **10** contained three terminal  $\beta$ -D-glucopyranosyl moieties (Glc', Glc''', Glc'''''), three C-4 substituted  $\beta$ -D-glucopyranosyl moieties (Glc'', Glc''', Glp'''''), and a C-2 and C-6 disubstituted  $\beta$ -D-glucopyranosyl moiety (Glc''). The  $\beta$ -orientations of the anomeric centers of all the glucosyl moieties were supported by the relatively large  $J$  values of their anomeric protons (7.7–8.4 Hz).



In the HMBC spectrum, the anomeric proton of the terminal glucosyl unit (Glc') at  $\delta$  5.07 exhibited a long-range correlation with C-3 of the aglycone at  $\delta$  78.2, indicating that one glucosyl unit was attached to the C-3 hydroxy group of the aglycone. Consequently, an oligoglucoside composed of six glucosyl units was presumed to be linkage with the C-26 carboxy group of the aglycone. Further HMBC correlations from H-1 of Glc'' at  $\delta$  6.30 to C-26 of the aglycone at  $\delta$  175.2, H-1 of Glc''' at  $\delta$  5.20 to C-2 of Glc'' at  $\delta$  82.9, H-1 of Glc'''' at  $\delta$  5.17 to C-4 of Glc'''' at  $\delta$  80.9, H-1 of Glc'''' at  $\delta$  5.16 to C-4 of Glc''' at  $\delta$  81.5, H-1 of Glc'''' at  $\delta$  5.13 to C-4 of Glc''' at  $\delta$  80.9, and H-1 of Glc'''' at  $\delta$  4.93 to C-6 of Glc'' at  $\delta$  69.2 confirmed the hexaglycoside sequence as Glc-(1 $\rightarrow$ 4)-Glc-(1 $\rightarrow$ 4)-Glc-(1 $\rightarrow$ 2)-[Glc-(1 $\rightarrow$ 4)-Glc-(1 $\rightarrow$ 6)]-Glc, which was attached to C-26 of the aglycone (Fig. 6). Accordingly, the structure of **10** was elucidated as (24*R*,25*S*)-3 $\beta$ -[( $\beta$ -D-glucopyranosyl)oxy]-ergost-5-en-26-oic acid *O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranosyl ester.

In the same way, the structures of **11–20** were elucidated as shown in Fig. 4.



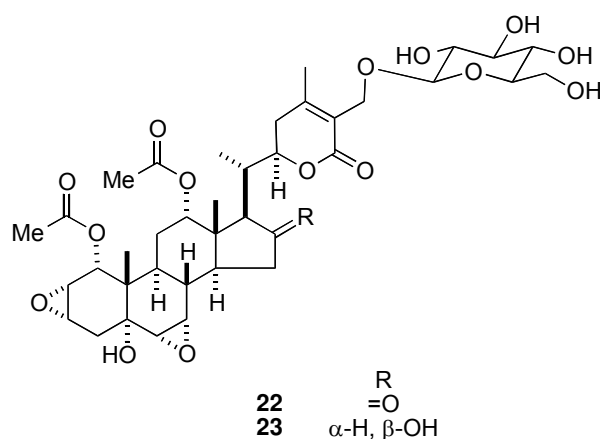
**Figure 6.** HMBC correlations of the sugar moieties of **10**



Phytosterols and their monoglucosides such as campesterol, stigmasterol, and  $\beta$ -sitosterol, and their 3-O-glucoside, widely occur in the plant kingdom. However, **10–20** are the first representatives of oligoglucosides of a phytosterol derivative to have sugar moieties with a total of four to seven glucose units. The bisdesmosidic nature of these structures, except for **15**, is also notable.

## 5. Withanolide glucosides

Compounds **22** and **23** are withanolide glucosides, named chantriolides A and B (Fig. 7) [21]. Chantriolides A and B were found to be minor components relative to the other secondary metabolites concomitantly isolated from *T. chantrieri*. However, it is notable that withanolides, which have been isolated almost exclusively from plants of the family Solanaceae previously [22, 23], have now been found in a species of the family Taccaceae in the study.



**Figure 7.** Structures of **22** and **23**

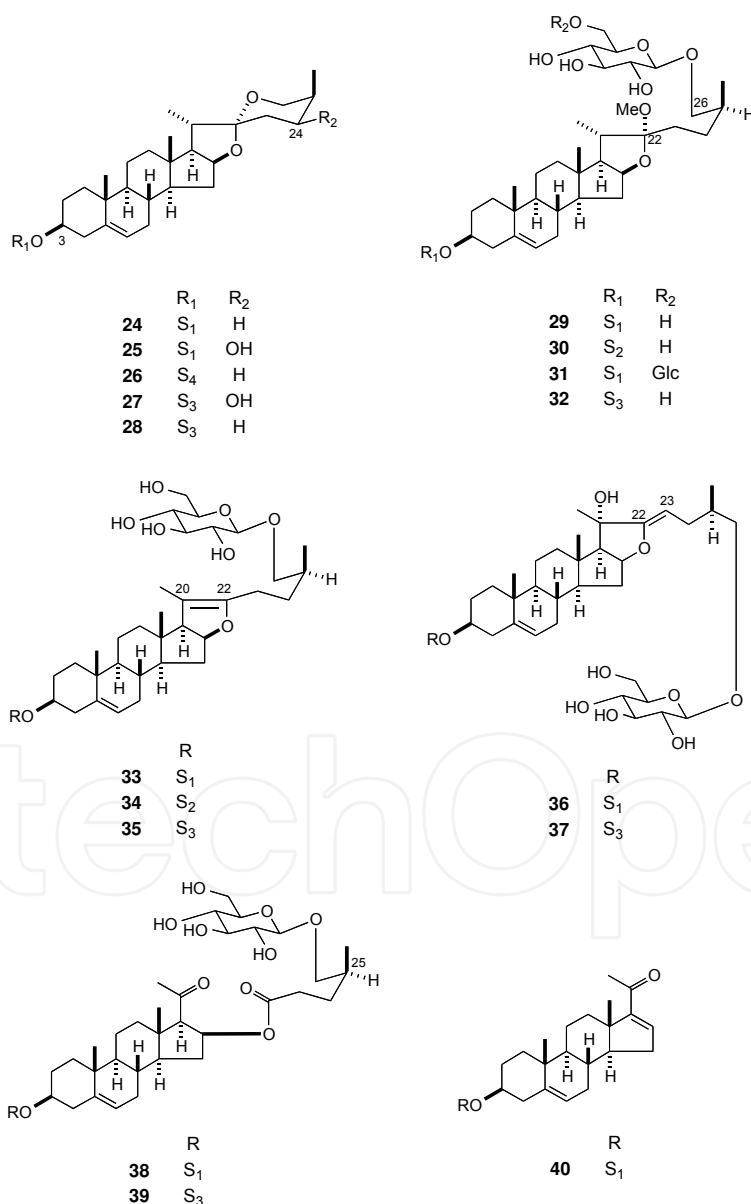
## 6. Other glycosides

Spirostan glucosides (**24–28**), furostan glycosides (**29–32**), pseudofurostan glycosides (**33–37**), pregnane glycosides (**38–40**), and a phenolic glucoside (**41**) were also isolated from *T. chantrieri* rhizomes (Fig. 8) [15, 24–26].

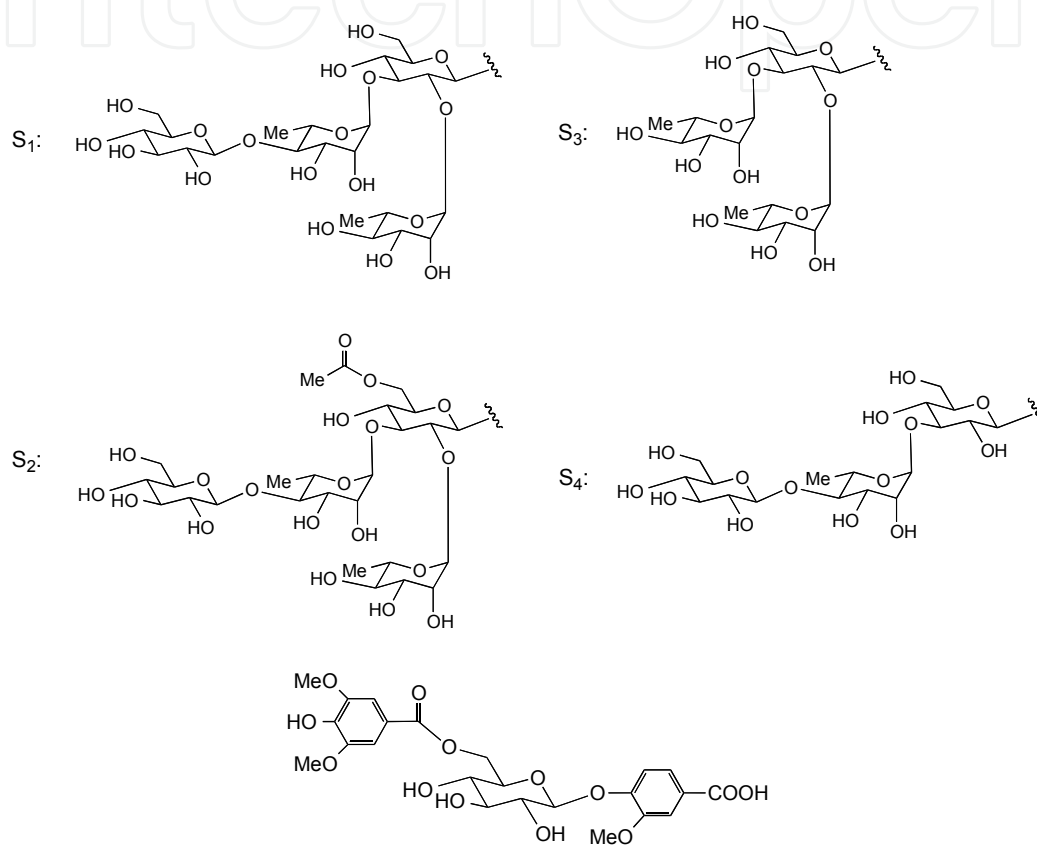
The known naturally occurring 22,26-hydroxyfurostan glycosides exclusively exist in the form of glycoside, bearing a monosaccharide at C-26 [27]. The monosaccharide among the furostan glycosides reported thus far is limited to  $\beta$ -D-glucopyranose, except for one furostan glycoside from *Dracaena afromontana*, which has an  $\alpha$ -L-rhamnopyranosyl group at C-26 [28]. Compound **31** is distinctive in carrying a diglucosyl group, O-glucosyl-(1 $\rightarrow$ 6)-glucosyl, in place of a monoglucosyl unit at C-26.

Compound **33** is the corresponding  $\Delta^{20(22)}$ -furostan glycoside of **29**. This was confirmed by the fact that the peracetate (**33a**) of **33** agreed with the product (**29a**) obtained by treatment of **29** with  $\text{Ac}_2\text{O}$  in pyridine at  $110^\circ\text{C}$  for 2.5 h, during which dehydration at C-20 and C-22, as well as the introduction of an acetyl group to all the hydroxy groups of the sugar moieties, occurred (Fig. 9).

The structure of **38**, including the absolute configuration at C-25, was found by the following chemical conversion. When the C-20 and C-22 bond of **33a** was oxidatively cleaved by treating it with  $\text{CrO}_3$  in  $\text{AcOH}$  at room temperature for 2 h, the resultant product was completely consistent with the peracetyl derivative of **38** (**38a**) (Fig. 9).



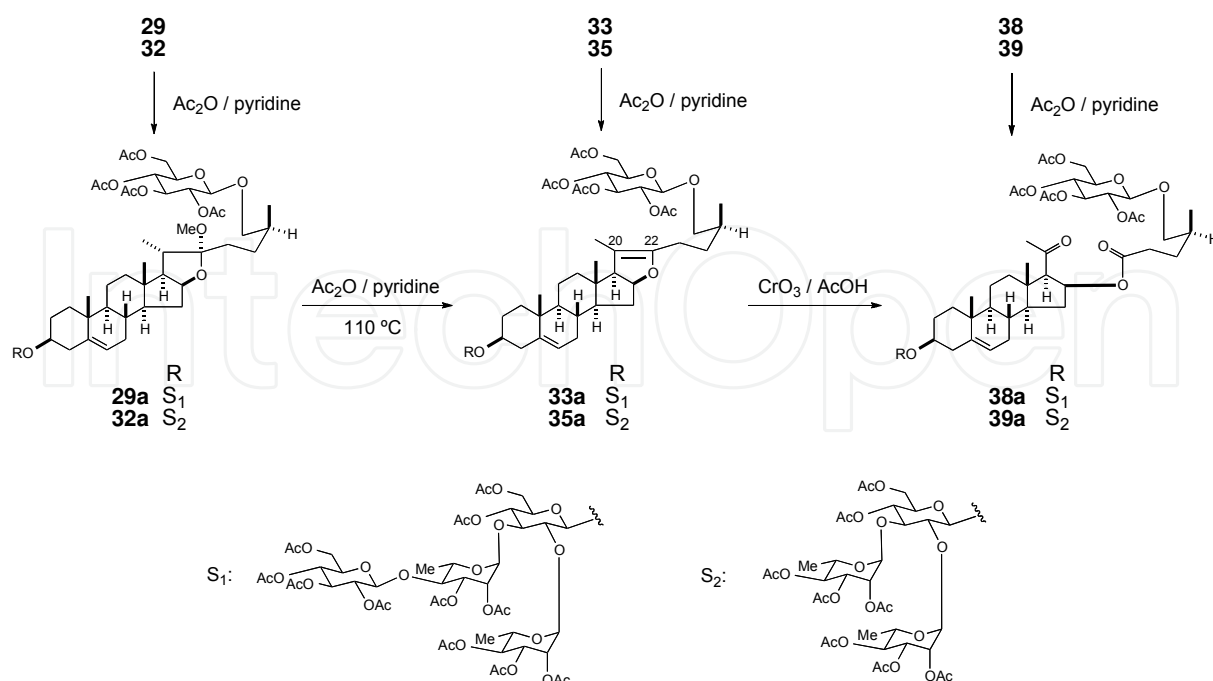
**Figure 8.** Structures of **24–41**



41

**Figure 8.** Continued.

A few compounds related to **38** and **39** have been isolated [29-31]; however, their C-25 configuration is not clearly presented in all the reports. In this investigation, we unequivocally determined the C-25 configuration of **38** to be *S* by a chemical correlation method. Compounds **38** and **39** could be defined as pregnane glycosides rather than furostan glycosides.



**Figure 9.** Chemical correlations of the furostan glycosides

## 7. Biological activity

### 7.1. Cytotoxic activity against HL-60 cells

The isolated compounds were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells by a modified MTT assay method [32]. Diarylheptanoids (**1** and **2**), diarylheptanoid glucosides (**3**, **4**, **6**, and **7**), and spirostan glycosides (**24** and **28**) showed moderate cytotoxicity ( $\text{IC}_{50}$  1.8–6.4  $\mu\text{g/mL}$ ) against HL-60 cells. Compounds **5**, **8–23**, **25–27**, and **29–41** did not show apparent cytotoxic activity against HL-60 cells at a sample concentration of 10  $\mu\text{g/mL}$ .

### 7.2. Cytotoxic activity and structure–activity relationships of diarylheptanoids and diarylheptanoid glucosides against HL-60 cells, HSC-2 cells, and HGF

The diarylheptanoids and some derivatives, including **9b** prepared by treatment of **9** with  $\text{CH}_2\text{N}_2$ , were evaluated for their cytotoxic activities against HL-60 cells, HSC-2 human oral squamous carcinoma cells, and normal human gingival fibroblasts (HGF) (Table 1). The diarylheptanoids **1**, **2**, and **7a**, and the diarylheptanoid glucosides **3**, **4**, **6**, and **7**, each of which has three or four phenolic hydroxy groups, showed moderate cytotoxic activity against HL-60 cells with  $\text{IC}_{50}$  values ranging 1.8 to 6.4  $\mu\text{g/mL}$ , while those possessing two phenolic hydroxy groups (**5**, **5a**, **8**, **8a**, **9**, and **9a**) did not exhibit apparent cytotoxic activity even at a sample concentration of 10  $\mu\text{g/mL}$ . Notably, the diarylheptanoids whose phenolic hydroxy

groups were all masked with methyl groups (**1a**, **2a**, and **9b**) were also cytotoxic. These observations suggest that the number of phenolic hydroxy groups contributes to the resultant cytotoxicity. Compounds **1a**, **2a**, and **9b** showed considerable cytotoxic activity against HSC-2 cells, whereas they had little effect on normal HGF.

compound	IC <sub>50</sub> (μg/mL)		
	HL-60	HSC-2	HGF
<b>1</b>	2.1	54.0	162
<b>1a</b>	5.5	3.9	176
<b>2</b>	1.8	54.0	>250
<b>2a</b>	4.9	6.6	174
<b>3</b>	6.2	158	220
<b>4</b>	5.5	155	>250
<b>5</b>	>10	160	>250
<b>5a</b>	>10	— <sup>b</sup>	— <sup>b</sup>
<b>6</b>	3.0	92.0	189
<b>7</b>	4.5	209	>250
<b>7a</b>	4.1	— <sup>b</sup>	— <sup>b</sup>
<b>8</b>	>10	198	>250
<b>8a</b>	>10	— <sup>b</sup>	— <sup>b</sup>
<b>9</b>	>10	157	213
<b>9a</b>	>10	231	177
<b>9b</b>	6.4	23.0	173
etoposide	0.2	24.0	>200

<sup>a</sup>Key: HL-60 (human promyelocytic leukemia cells); HSC-2 (human oral squamous carcinoma cells); and HGF (normal human gingival fibroblasts). <sup>b</sup>not determined.

**Table 1.** Cytotoxic activities of compounds **1-9** and their derivatives (**1a**, **4a**, **5a**, **7a-9a**, and **9b**), and etoposide against HL-60 cells, HSC-2 cells, and HGF<sup>a</sup>

**7.3. Cytotoxic activity and structure–activity relationships of steroidal glycosides against HL-60 cells**

Spirostan glycosides (**24** and **28**) showed moderate cytotoxicity (IC<sub>50</sub> 1.9 and 1.8 μg/mL) against HL-60 cells. Compounds **25** and **27**, the corresponding C-24 hydroxy derivatives of **24** and **28**, and **26**, the analogue of **24** without the terminal rhamnosyl group linked to C-2 of the inner glucosyl residue, did not show any cytotoxic activity at a sample concentration of 10 μg/mL. Furostan glycosides (**29–32**), pseudofurostan glycosides (**33–37**), and pregnane glycosides (**38–40**) also did not show cytotoxic activity. These data suggest that the structures of both the aglycone and sugar moieties contribute to the cytotoxicity.

#### 7.4. Panel screening in the Japanese Foundation for Cancer Research 39 cell line assay

Diarylheptanoid **2** and spirostan glycosides **24**, which showed significant cytotoxic activity against HL-60 cells, were subjected to the Japanese Foundation for Cancer Research 39 cell line assay [33]. Subsequent evaluation of **2** and **24** showed that the mean concentration required for achieving  $GI_{50}$  levels against the panel of cells were 87  $\mu M$  and 1.8  $\mu M$ , respectively. Although **2** and **24** exhibited no significant differential cellular sensitivity, some cell lines such as colon cancer HCT-116 ( $GI_{50}$  25  $\mu M$ ), ovarian cancer OVCAR-3 ( $GI_{50}$  36  $\mu M$ ), OVCAR-4 ( $GI_{50}$  39  $\mu M$ ), and stomach MKN-7 ( $GI_{50}$  34  $\mu M$ ) were relatively sensitive to **2**.

### 8. Conclusion

Our systematic chemical investigations of *T. chantrieri* rhizomes revealed that this plant contains a variety of secondary metabolites, namely, diarylheptanoids, diarylheptanoid glucosides, steroidal glycosides with the aglycone structures of ergostane, withanolide, spirostan, furostan, pseudofurostan, and pregnane, as well as a phenolic glucoside. Some diarylheptanoids and steroidal glycosides showed cytotoxicity against human cancer cells. These compounds may be possible leads for new anticancer drugs.

On the other hand, a number of researchers have reported biological activities of diarylheptanoids and steroidal glycosides other than cytotoxicity. It has been reported that curcuminoids, well-known diarylheptanoid derivatives, showed antioxidant [34, 35], anti-inflammatory [35, 36], estrogenic [37, 38], and anticancer [39] effects. Steroidal glycosides have been shown to have antidiabetic [40, 41], antitumor [42], antitussive [43], antiherpes virus [44], and platelet aggregation inhibitory [45] activities. *T. chantrieri* rhizomes could be applied to treating a wide variety of ailments as an alternative herbal medicine.

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### Author details

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