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Chapter 4

Phytochemicals of the Chinese Herbal Medicine
*Tacca chantrieri* Rhizomes

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53668

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 γ-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 × 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/H₂O 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 glycosides (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 monogluco‐sides (Fig. 2) [5].

Figure 2. Structures of 1–9 and their derivatives

Compound 1 was isolated as a viscous syrup, [α]D +1.7˚ (MeOH). HREIMS of 1 showed an [M+] peak at m/z 332.1623, corresponding the empirical molecular formula of C19H24O5, which was also deduced by analysis of its 13C NMR and DEPT spectral data. The IR spectrum suggested the presence of hydroxy groups (3347 cm⁻¹) and aromatic rings (1611 and 1515 cm⁻¹). The UV spectrum showed an absorption maximum due to substituted aromatic rings (281.4 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 13C) 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 nm, Δε +29.9) and negative (253.3 nm, Δε -20.0) Cotton effects, which were consistent with a negative chirality. Thus, the absolute configurations were determined as 3R and 5R (Fig. 3).
structure of 1 was shown to be (3R,5R)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane. In the same way, the structure of 2 was elucidated as (3R,5R)-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 (24R,25S)-3β-hydroxyergost-5-ene-26-oic acid (10a), whereas 13–20 are those of (24S,25R)-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/H2O gave D-glucose and a C28-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 1H, 13C, and 2D NMR spectra. In order to determine
the absolute configuration at C-25, 10a was reduced with LiAlH₄ to (24R,25S)-ergost-5-ene-3β,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 ¹H NMR coupling patterns of the H₂-26 protons.
were inspected. The H-26 protons of 10a-R were observed as a doublet-like signal at δ 4.20 (J = 6.3 Hz), whereas those of 10a-S were observed as a doublet of doublets at δ 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 LiAlH₄, affording (24R)-ergost-5-ene-3β-ol, that is, campesterol. The structure of 10a was determined as (24R,25S)-3β-hydroxyergost-5-en-26-oic acid (Fig. 5).

![Chemical transformations of 10a](image)

**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 1H-1H 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 1H 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 1H-1H 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 α-D- and β-D-glucosides [20], taking into account the known effects of O-glycosylation shifts. The comparison indicated that 10 contained three terminal β-D-glucopyranosyl moieties (Glc′, Glc′″, Glc′″″), three C-4 substituted β-D-glucopyranosyl moieties (Glc′″, Glc′″″″, Glp′″″″), and a C-2 and C-6 disubstituted β-D-glucopyranosyl moiety (Glc″). The β-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 δ 5.07 exhibited a long-range correlation with C-3 of the aglycone at δ 78.2, indicating that one glucosyl unit was attached to the C-3 hydroxy group of the aglycone. Consequently, an oligo-glucoside 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 δ 6.30 to C-26 of the aglycone at δ 175.2, H-1 of Glc’’’ at δ 5.20 to C-2 of Glc’’ at δ 82.9, H-1 of Glc’’’’ at δ 5.17 to C-4 of Glc’’’’’ at δ 80.9, H-1 of Glc’’’’’ at δ 5.16 to C-4 of Glc’’’ at δ 81.5, H-1 of Glc’’’’ at δ 5.13 to C-4 of Glc’’’’’ at δ 80.9, and H-1 of Glc’’’’’ at δ 4.93 to C-6 of Glc’’’ at δ 69.2 confirmed the hexaglucoside sequence as Glc-(1→4)-Glc-(1→4)-Glc-(1→2)-[Glc-(1→4)-Glc-(1→6)]-Glc, which was attached to C-26 of the aglycone (Fig. 6). Accordingly, the structure of 10 was elucidated as (24R,25S)-3β-[(β-D-glucopyranosyl)oxy]-ergost-5-en-26-oic acid O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-O-[O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl ester.

In the same way, the structures of 11–20 were elucidated as shown in Fig. 4.
Phytosterols and their monoglucosides such as campesterol, stigmasterol, and β-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](image-url)

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 β-d-glucopyranose, except for one furostan glycoside from _Dracaena afromontana_, which has an α-l-rhamnopyranosyl group at C-26 [28]. Compound 31 is distinctive in carrying a diglucosyl group, O-glucosyl-(1→6)-glucosyl, in place of a monoglucosyl unit at C-26.
Compounds 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 Ac$_2$O in pyridine at 110 °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 CrO$_3$ in 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
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.
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 (IC$_{50}$ 1.8–6.4 μg/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 μg/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 CH$_3$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 IC$_{50}$ values ranging 1.8 to 6.4 μg/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 μg/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.

Table 1. Cytotoxic activities of compounds 1-9 and their derivates (1a, 4a, 5a, 7a-9a, and 9b), and etoposide against HL-60 cells, HSC-2 cells, and HGF.

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

*Key: HL-60 (human promyelocytic leukemia cells); HSC-2 (human oral squamous carcinoma cells); and HGF (normal human gingival fibroblasts). ^not determined.

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

Spirostan glycosides (24 and 28) showed moderate cytotoxicity (IC₅₀ 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 GI50 levels against the panel of cells were 87 μM and 1.8 μM, respectively. Although 2 and 24 exhibited no significant differential cellular sensitivity, some cell lines such as colon cancer HCT-116 (GI50 25 μM), ovarian cancer OVCAR-3 (GI50 36 μM), OVCAR-4 (GI50 39 μM), and stomach MKN-7 (GI50 34 μM) 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], antiviral [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.

Acknowledgements

We are grateful to Dr. Hiroshi Sakagami for evaluating the cytotoxic activities against HSC-2 cells and HGF.

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