PERGAMON

Phytochemistry 50 (1999) 781–785

PHYTOCHEMISTRY

Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*

Lai-King Sy, Geoffrey D. Brown *

Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, People's Republic of China

Revised 10 June 1998

Abstract

Plant tissue cultures of both *Artemisia annua* and *Tanacetum parthenium* were found to produce the novel compound (2-glyceryl)-*O*-coniferaldehyde as the major constituent. Cultures of *A. annua* produced several other coniferaldehyde derivatives, including the novel (2-propenal)-*O*-coniferaldehyde and the unusual neolignan balanophonin. None of the cinnamaldehyde derivatives have been reported previously from the parent plants. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Artemisia annua*; *Tanacetum parthenium*; Compositae; Coniferaldehyde derivatives; 2-D NMR; Plant tissue culture; Chemotaxonomy

1. Introduction

Artemisia annua L. (sweet wormwood) and *Tanacetum parthenium* (L.) Schultz Bip. (feverfew) are two very important medicinal plants which have much in common: both belong to the subtribe Anthemidae of the Compositae family and both produce highly oxygenated sesquiterpenes (artemisinin and parthenolide, respectively) which are responsible for their respective antimalarial and antimigraine properties. The phytochemistry for both species has been extensively investigated (Hewlett et al., 1996; Milbrodt, Schroder, & Konig, 1997; Sy, Brown, & Haynes, 1998).

Production of chemically complex drugs, such as artemisinin and parthenolide, in plant tissue culture is an attractive goal and has been the subject of intensive efforts over the past two decades (Ellis, 1988; Banthorpe, 1994). Tissue culture of *A. annua* has received a good deal of attention (He, Zeng, Li, & Zheng, 1983; Nair et al., 1986; Park, Hu, & Staba, 1989; Shetty, Bothra, Crawford, & Korus, 1990; Whipkey, Simon, Charles, & Janick, 1992; Elhag, El-Domiaty, El-Feraly, Mossa, & El-Olemy, 1992; Kim, Kim, Lim, Hahn, & Kim, 1992; Basile, Akhtari, Durand, & Nair, 1993; Delabays, Benakis, & Collet, 1993; Paniego, Maligne, & Giulietti, 1993; Haigh, 1993; Martinez Isaza; Chen, Hua, & Yim, 1993; Brown, 1993a; Brown, 1994a; Weathers, Cheatham, & Dilocino), and it seems that organ cultures are more successful in producing artemisinin than undifferentiated cultures, although it is noteworthy that a commercially viable process has yet to be achieved. Less work has been undertaken with *T. parthenium* cultures (Banthorpe & Brown, 1989, 1990, 1993; Brown, 1993a).

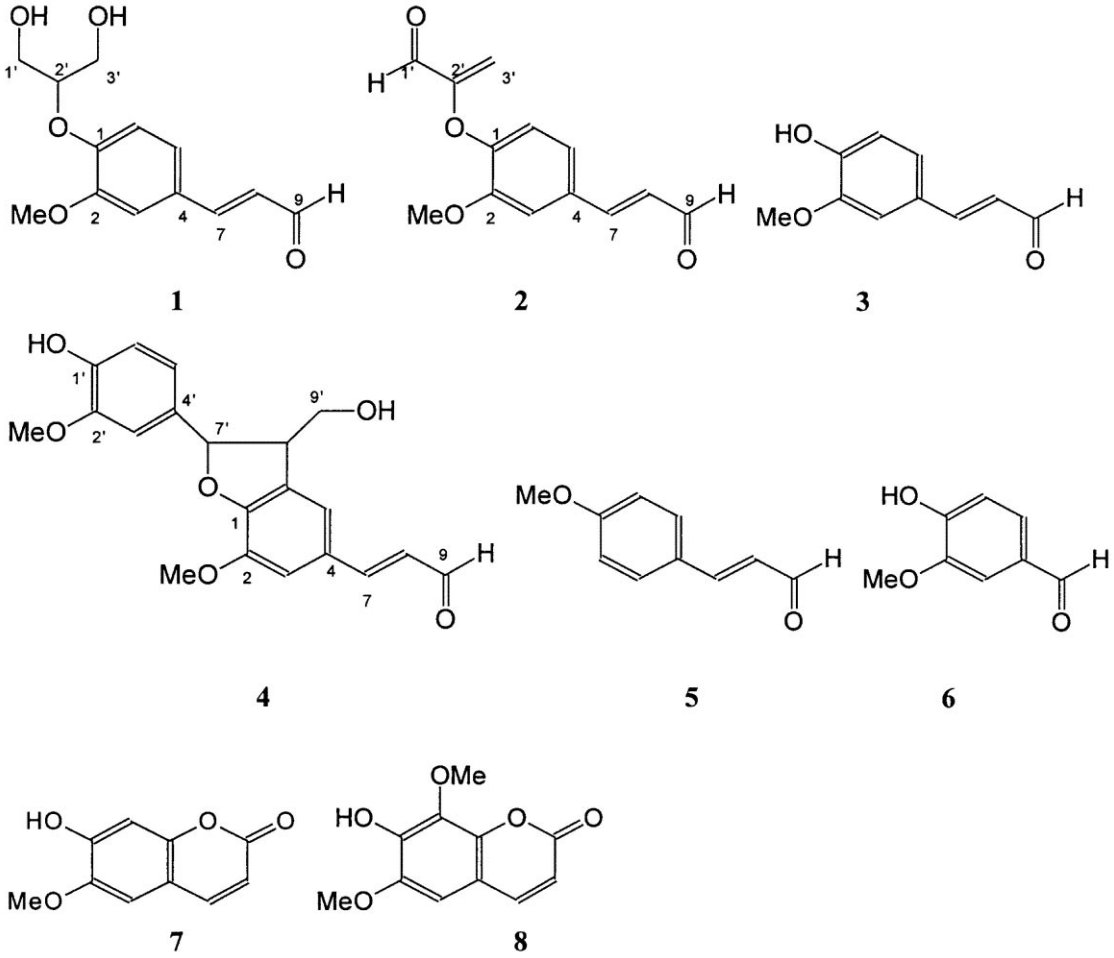
In this study we have attempted to define the secondary metabolism of undifferentiated callus culture for both species. It is hoped that this will serve as a basis for future investigation in which culture conditions that will allow cost-effective production of both drugs from plant tissue culture will be defined.

2. Results and discussion

Callus cultures of *A. annua* and *T. parthenium* were derived from seedlings and were subcultured for periods in excess of 18 months prior to extraction with dichloromethane and chemical characterization of the extracts. The parent plants from which the seeds for both species were taken have themselves been the subject of previous detailed chemical investigations, which are reported in the literature. Thus, the chemistry of the *A. annua* parent plants used in this study is domi-

* Corresponding author. Tel.: +86-852-2859-7915; Fax: +86-852-2857-1586; E-mail: gdbrown@hkucc.hku.hk.

0031-9422/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(98)00607-4



nated by the production of artemisinin and related sesquiterpenes (Brown, 1992, 1993b, 1994b), whilst *T. parthenium* parent plants produced large amounts of parthenolide and other terpenoids (Banthorpe, Brown, Janes, & Marr, 1990).

Extraction of callus culture of *A. annua* and *T. parthenium* followed by CC and HPLC revealed that one of the major components present in both species was (2-glyceryl)-*O*-coniferaldehyde (**1**). The structure of **1** was established by 2-D NMR, leading to assignments for all carbons and protons (Table 1) as determined by HSQC, HMBC (Fig. 1) and ^{1}H–^{1}H COSY (Fig. 1).

Smaller quantities of (2-propenal)-*O*-coniferaldehyde (**2**) were also obtained from *A. annua* tissue culture, together with coniferaldehyde (**3**) itself. In addition to novel derivatives of coniferaldehyde **1** and **2**, tissue cultures of *A. annua* also contained large quantities of the unusual neolignan balanophonin (**4**) which can be viewed biogenetically as a dimer of coniferaldehyde. Balanophonin has been reported previously as a constituent of *Balanophora japonica* (Haruna, Koube, Ito, & Murata, 1982) and *Chrysolaena verbascifolia* (Bardon, Montanaro, Catalan, Diaz, & Herz, 1993). Structure elucidation and NMR assignments for **4** were made by 2-D NMR methods and previous erroneous assignments have been corrected in Table 1.

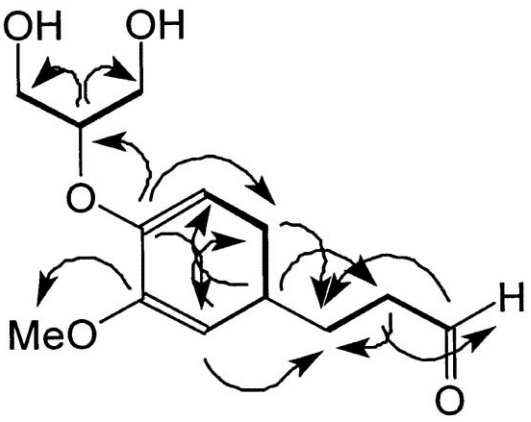


Fig. 1. HMBC correlations used in establishing the structure of **1**, are indicated by arrows from ^{13}C to ^{1}H. ^{1}H–^{1}H COSY correlations are indicated by heavy lines in the structure.

Table 1
^{13}C and ^{1}H assignments for compounds **1–4**

Assignment	δ $^{13}C^{a,b}$				δ $^{1}H^{a,c}$			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1	149.1 (C)	145.7 (C)	148.9 (C)	151.6 (C)	–	–	–	–
2	150.1 (C)	151.3 (C)	146.9 (C)	144.8 (C)	–	–	–	–
3	110.0 (CH)	112.0 (CH)	109.4 (CH)	112.3 (CH)	7.11 (d 1.8)	7.16 (br d)	7.07 (d 1.8)	7.01 (d 1.2)
4	128.2 (C)	131.0 (C)	126.7 (C)	128.1 (C)	–	–	–	–
5	122.2 (CH)	122.4 (CH)	124.1 (CH)	118.3 (CH)	7.15 (dd 8.3, 1.8)	7.17 (d 8.3, 2.0)	7.13 (dd 8.1, 1.8)	7.13 (d 1.2)
6	117.7 (CH)	121.7 (CH)	114.9 (CH)	129.3 (C)	7.13 (d 8.3)	7.06 (d 8.3)	6.96 (d 8.1)	–
7	151.3 (CH)	151.7 (CH)	153.1 (CH)	153.4 (CH)	7.41 (d 15.9)	7.44 (d 15.9)	7.40 (d 15.8)	7.40 (d 15.8)
8	126.6 (CH)	128.6 (CH)	126.5 (CH)	126.3 (CH)	6.62 (dd 15.9, 7.7)	6.67 (dd 15.9, 7.6)	6.60 (dd 15.8, 7.7)	6.58 (dd 15.8 7.8)
9	192.5 (CH)	193.4 (CH)	193.6 (CH)	193.8 (CH)	9.66 (d 7.7)	9.71 (d 7.6)	9.66 (d 7.7)	9.58 (d 7.8)
1′	61.0 (CH_2)	186.5 (CH)		146.0 (C)	3.88 (2H m)	9.46 (s)		–
2′	81.8 (CH)	157.4 (C)		146.8 (C)	4.33 (quin 4.8)	–		–
3′	61.0 (CH_2)	109.5 (CH_2)		108.8 (CH)	3.88 (2H m)	5.39 (d 2.9) 5.26 (d 2.9)		6.90 (s)
4′				132.3 (C)				–
5′				119.4 (CH)				6.89 (d 8.0)
6′				114.5 (CH)				6.87 (d 8.0)
7′				89.0 (CH)				5.62 (d 7.0)
8′				53.0 (CH)				3.67 (m)
9′				63.9 (CH_2)				3.95 (2H m)
1-OH							5.98 (s)	
2-OMe	55.0 (CH_3)	56.0 (CH_3)	56.0 (CH_3)	56.1 (CH_3)	3.91 (3H s)	3.88 (3H s)	3.95 (3H s)	3.93 (3H s)
2′-OMe				56.0 (CH_3)				3.85 (3H s)

[a]^{13}C directly attached to ^{1}H determined by HSQC.[b]Multiplicity determined by DEPT, indicated in parentheses.[c]Integral (if not [1H]), multiplicity and coupling constant(s) (in Hz), determined from 1-D NMR, indicated in parentheses.

Cultures of *A. annua* contained also small amounts of the simple cinnamaldehyde-related compounds, 4-methoxycinnamaldehyde (**5**) and vanillin (**6**), which were identified from their 1-D NMR spectra (Bellassoued & Majidi, 1993; Pouchert & Behnke). Finally, scopoletin (**7**) was obtained as a minor constituent of the extract and identified from its 1-D NMR spectra (Banthorpe & Brown, 1989), while scopoletin and another coumarin, isofraxidin (**8**), were obtained as minor constituents of the *T. parthenium* callus culture.

Both scopoletin (**7**) and isofraxidin (**8**) have been reported previously as the major constituents of undifferentiated tissue culture of *T. parthenium* (Banthorpe & Brown, 1989, 1990, 1993; Brown, 1993a) and *A. annua* (Brown, 1993a, 1994a). These workers found (in agreement with the current study) that no trace of the sesquiterpenes present in the parent plants could be detected in the undifferentiated tissue cultures and suggested that these coumarins might constitute chemotaxonomic markers for the Compositae (Banthorpe & Brown, 1989). In the present study, the novel cinnamaldehyde derivative **1** was found in tissue culture of both *A. annua* and *T. parthenium*. It is surprising that cinnamaldehyde derivatives (**1–6**) should dominate the chemistry of *A. annua* cultures in this investigation at the expense of the previously reported scopoletin. Similarly, cultures of *T. parthenium* consisted primarily of **1**, accompanied by only small amounts of **7** and **8**, which were previously reported as the major metabolites. None of these cinnamaldehyde derivatives have been reported previously as constituents of *A. annua* or *T. parthenium* plants.

However, the cinnamaldehydes **1–6** and the coumarins **7–8** are closely related biogenetically: oxidation of the 9-aldehyde group in coniferaldehyde accompanied by *trans*/*cis* isomerization of the double bond would lead directly to coumarins such as scopoletin. Therefore, we suggest that the final chemical composition of these tissue cultures (i.e. the presence of either coumarins or cinnamaldehydes) is determined to some extent by variables in culture conditions, although the essential biochemical pathways operating in undifferentiated tissue culture remains the same. The results of this study are therefore consistent with the original suggestion that individual compounds in undifferentiated tissue cultures be considered as chemotaxonomic markers, although obviously far more wide-ranging studies are still required in order for this hypothesis to be validated.

3. Experimental

3.1. Methods

Chemical shifts are expressed in ppm (δ) relative to TMS as int. standard. All NMR experiments were run on a Bruker DRX 500 instrument. Two-dimensional spectra were recorded with 1024 data points in F_2 and 256 data points in F_1. HREIMS were recorded at 70 eV on a Finnigan-MAT 95 MS spectrometer. IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8201 PC spectrometer. TLC plates were developed using *p*-anisaldehyde. CC was performed using silica gel 60–200 μm (Merck). HPLC separations were performed using a Varian chromatograph equipped with RI star 9040 and UV 9050 detectors and a Intersil PREP-SIL 20 mm × 25 cm column, operating isocratically with EtOAc/hexane mixtures at a flow rate 8 ml/min.

3.2. Plant material

Seeds of *A. annua* were provided from the Artemisia NCCPG collection (Cambridge, UK) and taxonomically verified by Dr. J. Twibell. Seeds of *T. parthenium* were obtained from plants growing in a private garden in Oxford (plant material was taxonomically verified by Dr. Braithwaite of Nottingham University and a voucher specimen is deposited at the herbarium of the Botany Department, Nottingham University).

3.3. Initiation of Callus

Seedlings of *A. annua* were germinated on agar in an incubator (see Section 3.5) for 15 days until they were 2–3 cm in length, then surface sterilized with 2% $Ca(OCl)_2$ soln. (10 min) and rinsed ×3 in sterile H_2O before transfer to growth media. Seedlings of *T. parthenium* were obtained and treated in a similar manner.

3.4. Media

Media for growth of undifferentiated callus cultures of *A. annua* comprised the basal medium mixture of Murashige and Skoog (1962) (Sigma, 4.43 g/l), sucrose (20 g/l), agar (Sigma, 0.24 g/20 ml) and phytohormones 1-naphthaleneacetic acid (NAA) (0.5 mg/l) and 6-benzyladenine purine (BAP) (0.5 mg/l). Filtered coconut milk (10 vol%) was included in the first subculture only to help initiate callus production. *T. parthenium* media were prepared in the same manner, except that 2,4-dichlorophenoxyacetic acid (2,4-D) (1 mg/l) replaced the phytohormones NAA and BAP.

3.5. Maintenance of Callus

Callus of *A. annua* appeared within 4 weeks when seedlings were maintained on growth media in an incubator with an artificial daylight cycle (16 h light–330 ft candles, 25°C; 8 h darkness, 15°C). Callus could then be subcultured at 4 week intervals. Subcultures were made over an 18-month period prior to extraction. Callus of *T. parthenium* appeared after 8 weeks and required subculturing every 6 weeks (culture conditions as above).

3.6. Extraction and isolation

Callus material of *A. annua* (2.27 kg) was pulverized to a fine powder under liq. N_2, repeatedly extracted with CH_2Cl_2, dried and solvent removed under red. pres. to yield a yellow gum (1.05 g; 0.05 wt%).The extract was subjected to gradient CC (developing solvents 100% hexane → 100% ethyl acetate → 100% methanol). Some column fractions were further purified by HPLC. **1** (25 mg, R_f 0.27 in 90% EtOAc/hexane), **2** (6 mg, R_t 49.6 mins in 25% EtOAc/hexane), **3** (4 mg, R_t 37.8 mins in 25% EtOAc/hexane), **4** (62 mg, R_f 0.53, 90% EtOAc/hexane), **5** (2 mg, R_t 31.0 mins, 25% EtOAc/hexane). Callus material of *T. parthenium* (133 g) was pulverized to a fine powder under liq. N_2, repetitively extracted with CH_2Cl_2, dried and solvent removed under red. pres. to yield a yellow oil (0.035 g). The extract was purified by gradient CC (developing solvents 100% hexane to 100% ethyl acetate) and HPLC as above.

3.7. (2-Glyceryl)-O-coniferaldehyde (*1*)

Oil. ^{13}C and ^{1}H NMR — see Table 1. HREIMS *m/z* (rel. int): 252.1002 (80) [M^+, $C_{13}H_{16}O_5$ required 252.0998], 222 (10), 178 (100), 163 (22), 161 (25).

3.8. (2-Propenal)-O-coniferaldehyde (*2*)

Oil. ^{13}C and ^{1}H NMR — see Table 1. HREIMS *m/z* (rel. int): 232.0736 (100) [M^+, $C_{13}H_{12}O_4$ required 232.0736], 201 (15), 177 (20), 161 (60).

3.9. Coniferaldehyde (*3*)

Oil. ^{13}C and ^{1}H NMR — see Table 1. HREIMS *m/z* (rel. int): 178.0637 (100) [M^+, $C_{10}H_{10}O_3$ required 178.0630], 161 (15), 135 (18), 107 (22).

3.10. Balanophonin (**4**)

Oil. $[\alpha]_D$ −10.7° (*c* 2.37, $CHCl_3$). ^{13}C and ^{1}H NMR see Table 1. HREIMS *m/z* (rel. int) 356.1266 (70)

[M^+, $C_{20}H_{20}O_6$ required 356.1259], 338 (100), 326 (41), 323 (40), 306 (20), 295 (15).

Acknowledgements

The authors thank the Biotechnology Research Institute for funding this research.

References

Banthorpe, D. V. (1994). *Nat. Prod. Rep., 11,* 303.
Banthorpe, D. V., & Brown, G. D. (1989). *Phytochemistry, 28,* 3003.
Banthorpe, D. V., & Brown, G. D. (1990). *Plant Sci., 67,* 107.
Banthorpe, D. V., & Brown, G. D. (1993). In: Y. P. S. Bajaj, *Biotechnology in agriculture and forestry, vol. 24*, (pp. 361–372. *Medicinal and aromatic plants V*. Berlin, Heidelberg: Springer-Verlag.
Banthorpe, D. V., Brown, G. D., Janes, J. F., & Marr, I. M. (1990). *Flav. Frag. J., 5,* 183.
Bardon, A., Montanaro, S., Catalan, C. A. N., Diaz, J. G., & Herz, W. (1993). *Phytochemistry, 34,* 253.
Basile, D. V., Akhtari, N., Durand, Y., & Nair, M. S. R. (1993). *In Vivo Cell. Dev. Biol., 29 P,* 143.
Bellassoued, M., & Majidi, A. (1993). *J. Org. Chem., 58,* 2517.
Brown, G. D. (1992). *J. Nat. Prod., 55,* 1756.
Brown, G. D. (1993a). *Acta Hortic., 330,* 269.
Brown, G. D. (1993b). *Phytochemistry, 32,* 391.
Brown, G. D. (1994a). *J. Nat. Prod., 57,* 975.
Brown, G. D. (1994b). *Phytochemistry, 36,* 637.
Chen, P. K., Hua, H., & Yim, J. W. (1993). *PGRSA Q., 21,* 151.
Delabays, N., Benakis, A., & Collet, G. (1993). *Acta Hortic., 330,* 203.
Elhag, H. M., El-Domiaty, M. M., El-Feraly, F. S., Mossa, J. S., & El-Olemy, M. M. (1992). *Phytother. Res., 6,* 20.
Ellis, B. E. (1988). *Nat. Prod. Rep., 5,* 581.
Haigh, J. R. (1993). Ph.D. thesis, Colorado State University (diss. abs. 9331377).
Haruna, M., Koube, T., Ito, K., & Murata, H. (1982). *Chem. Pharm. Bull., 30,* 1525.
He, X.-C., Zeng, M.-Y., Li, G.-F., & Zheng, L. (1983). *Acta Bot. Sin., 25,* 87.
Hewlett, M. J., Begley, M. J., Groenewgen, W. A., Heptinstall, S., Knight, D. W., May, J., Salan, U., & Toplis, D. (1996). *J. Chem. Soc. Perkin Trans., I,* 1979.
Kim, N.-C., Kim, J.-G., Lim, H.-J., Hahn, T.-R., & Kim, S.-U. (1992). *J. Korean. Agric. Chem. Soc., 35,* 99.
Martinez Isaza, B. C. Ph.D. thesis, University of Minnesota (diss. abs. 8907392).
Milbrodt, M., Schroder, F., & Konig, W. A. (1997). *Phytochemistry, 44,* 471.
Murashige, T., & Skoog, F. (1962). *Physiol. Plant, 15,* 473.
Nair, M. S. R., Acton, N., Klayman, D. L., Kendrick, K., Basile, D. V., & Mante, S. (1986). *J. Nat. Prod., 49,* 504.
Paniego, N. B., Maligne, A. E., & Giulietti, A. M. (1993). In: Y. P. S. Bajaj, *Biotechnology in agriculture and forestry, vol. 24*, (pp. 64–78). *Medicinal and aromatic plants V*. Berlin, Heidelberg: Springer-Verlag.
Park, J. M., Hu, W.-S., & Staba, E. J. (1989). *Biotech. Bioeng., 34,* 1209.
Pouchert, C. J., & Behnke, J. *Aldrich Library of ^{13}C and ^{1}H FT-NMR spectra, vol. 2*, (p. 959 A).
Shetty, K., Bothra, D., Crawford, D. L., & Korus, R. A. (1990). *Appl. Biochem. Biotech., 24/25,* 213.
Sy, L.-K., Brown, G. D., & Haynes, R. (1998). *Tetrahedron, 54,* 4345.
Weathers, P. J., Cheatham, R. D., & Dilocino, A., *PCT Int. Appl.* WO 94 00, p. 584.
Whipkey, A., Simon, J. E., Charles, D. J., & Janick, J. (1992). *J. Herbs Spices, 1,* 15.