J. Pharm. Pharmacol. 1986, 38: 709–712
Communicated May 8, 1986

Compounds extracted from feverfew that have anti-secretory activity contain an α-methylene butyrolactone unit

W. A. GROENEWEGEN*, D. W. KNIGHT, S. HEPTINSTALL, *Departments of Chemistry and *Medicine, University of Nottingham, Nottingham NG7 2UH, UK*

Extracts of feverfew inhibit secretion of granular contents from platelets and neutrophils and this may be relevant to the therapeutic value of feverfew in migraine and other conditions. In this investigation we fractionated an extract of feverfew and obtained eleven fractions with anti-secretory activity. The active fractions, together with two fractions that were devoid of anti-secretory activity, were examined using ^{1}H NMR and infrared spectroscopy. All the active fractions (but neither of the inactive fractions) contained compounds with an α-methylene butyrolactone unit. Five compounds that contain this unit were identified as parthenolide, 3-β-hydroxyparthenolide, secotanapartholide A, canin and artecanin, all of which are sesquiterpene lactones. It is very likely that these and other sesquiterpene lactones that contain an α-methylene butyrolactone unit are responsible for the anti-secretory activity in extracts of feverfew.

There is evidence that the herb feverfew (*Tanacetum parthenium*), taken orally, reduces the frequency and severity of migraine (Johnson et al 1985); the herb is also reputed to be of value in conditions such as arthritis and psoriasis (Editorial 1985). Crude extracts of feverfew have been shown to inhibit agonist-induced secretion of the contents of intracellular storage granules from blood platelets and polymorphonuclear leucocytes, and it has been suggested that inhibition of secretory activity may relate to its medicinal properties (Heptinstall et al 1985). We have now analysed an extract of feverfew and identified several compounds with anti-secretory activity. The compounds are sesquiterpene lactones possessing an α-methylene butyrolactone unit.

Materials and methods

Materials. Feverfew (*Tanacetum parthenium* (L.) Sch. Bip., formerly *Chrysanthemum parthenium*) was grown in the Department of Botany, University of Nottingham; leaves taken from plants were dried in air. Blood for the determination of anti-secretory activity was obtained from healthy volunteers who took no medication for at least 14 days before donation. [^{14}C]5-Hydroxytryptamine (spec. act. 57·4 mCi mmol^{-1}; 50 μCi ml^{-1}) was from Amersham International. Adrenaline was from the Sigma Chemical Co., and was dissolved in 150 mM NaCl before use. Phosphate-buffered saline (PBS), pH 7·4, was prepared using buffer tablets from Oxoid Ltd. Acetylsalicylic acid was from the Sigma Chemical Co. and was dissolved in 150 mM NaCl. All solvents were from M & B Chemicals and were redistilled before use. The column used for the initial fractionation of feverfew extract contained Merck Kieselgel 60, 0·040–0·063 mm (100 g). The HPLC column contained microporasil and had the dimensions 7·8 mm × 30 cm. Fractions from this column were detected using a Waters 440 u.v. detector or a Waters R401 refractive index detector. Plates for thin layer chromatography (TLC) were polygram SilG/UV254 from CamLab; 4% methanol in chloroform (v/v) was used as eluent and 10% sulphuric acid (v/v) was used to detect the spots. ^{1}H Nuclear magnetic resonance (NMR) data were obtained using a Bruker WM-250 spectrometer (250 MHz); deuteriochloroform was used as solvent throughout with tetramethylsilane as internal standard. Infrared (IR) spectra were obtained using a Perkin-Elmer 710B spectrophotometer for chloroform solutions. Radioactivity was determined using Optiphase scintillant (from Fisons Ltd.) and a Beckman Scintillation Counter.

Extraction procedure. Powdered leaves (11·4 g) were steeped in chloroform at room temperature (20 °C) for 1 h, and then removed by filtration. The extraction procedure was repeated four times. The combined chloroform extracts were evaporated to dryness at reduced pressure at room temperature, and the residue (ca 1 g) was subjected to column chromatography (Kieselgel 60) and eluted with 40% hexane–chloroform (v/v) up to 4% methanol–chloroform (v/v) to give fractions 1–7. These were tested for anti-secretory activity (see below) and fractions 5–7 were found to be active. These fractions were subjected to HPLC and they were separated using 40% hexane–chloroform (v/v) as solvent for fraction 5, 100% chloroform for fraction 6 and 2% methanol–chloroform (v/v) for fraction 7. The detection system found to be useful for fractions 5 and 7 was UV-detection at 254 nm. Refractive index-detection was used for fraction 6. The operating pressure used for all separations was 3 ml min^{-1}. 29 different subfractions were tested further for anti-secretory activity. Activity was found in 11 of these fractions and these (together with two fractions devoid of activity) were analysed using TLC and NMR and infrared spectroscopy.

Anti-secretory activity. To test for anti-secretory activity in the seven fractions obtained from the Kieselgel column, PBS (500 μl) was added to tubes which

* Correspondence.

contained 2 mg of each fraction; the tubes were vortexed vigorously, any insoluble material was allowed to settle, and 100 μl of clear supernatant was then added to platelet-rich plasma (460 μl) and stirred at 37 °C. The platelet-rich plasma had been prepared from citrated human blood in which the platelets had been labelled with [^{14}C]5-HT (Heptinstall & Fox 1983). After 2 mins a solution of adrenaline (40 μl to give a final concentration of 100 μM) was added and stirring was continued for a further 6 mins. At this point a solution (50 μl) of 14 mM acetylsalicylic acid was added and the sample transferred to ice. The amount of [^{14}C]5-HT that had been released from the platelets in response to adrenaline was determined by centrifuging the sample and measuring the amount of radioactivity in aliquots (50 μl) of the supernatant. A sample of platelet-rich plasma that contained PBS in place of extract was taken through the same procedure to quantify secretion in the absence of inhibitory material. In this sample the amount of [^{14}C]5-HT released from the platelets was 78% of the amount that had been intracellular. In the three samples in which inhibitory activity was detected, the amount of [^{14}C]5-HT released from the platelets was reduced to 0%. In the four other samples 77, 65, 77 and 66% of the [^{14}C]5-HT was released in response to adrenaline.

To test for anti-secretory activity in the 29 subfractions obtained after subjecting the three active fractions to HPLC, PBS (200 μl) was added to tubes which contained 0·5 mg of each subfraction and 50 μl of clear supernatant (diluted to 100 μl with PBS) were tested as described above. In the absence of any extract, between 61 and 79% of the [^{14}C]5-HT was released from platelets (five experiments using different preparations of platelet-rich plasma).

Results and discussion

The amounts of [^{14}C]5-HT released in the presence of samples of each of the 29 subfractions of the feverfew extract obtained by HPLC are given in Table 1. The Table also contains the information we obtained on the identity of the compounds found in the active fractions. We were able to identify five of the active compounds that were present and these are given in Fig. 1. All are sesquiterpene lactones that possess an α-methylene butyrolactone unit. Although we were unable to identify the other active compounds completely, it is clear from the NMR data that all these compounds also contain an α-methylene butyrolactone unit. The two fractions we investigated which were completely inactive in our assay of platelet secretion showed no evidence of the presence of this unit. The data strongly suggest that the α-methylene butyrolactone unit is relevant to the mode of action of the compounds, particularly in view of the other structural diversities within the compounds identified.

Consideration of the structures of the compounds we have isolated (Fig. 1) suggests that they would be no more than sparingly soluble in aqueous media. Despite

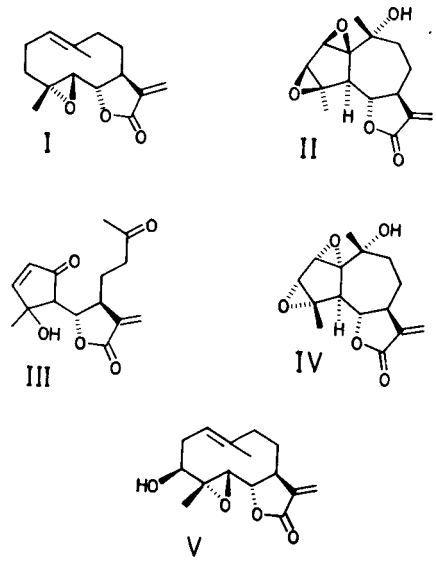

FIG. 1. Compounds with anti-secretory activity isolated from feverfew.
Parthenolide (I); Canin (II); seco-Tanapartholide A (III); Artecanin (IV); 3-β-Hydroxyparthenolide (V).

this, it proved easy to demonstrate their anti-secretory activity in an aqueous medium. This implies that only small amounts of the compounds may be required for their biological activity. We have found that aqueous as well as chloroform extracts of dried leaves of feverfew possess anti-secretory activity (Heptinstall et al 1985), although the degree of activity is lower in aqueous media than in chloroform. Similarly biological activities have also been found in aqueous extracts of feverfew by others (Makheja & Bailey 1982).

It is known that the α-methylene butyrolactone unit of sesquiterpene lactones interacts with biological nucleophiles such as sulphydryl groups via Michael addition (Kupchan et al 1970), and it is possible that the anti-secretory activity of such compounds is consequent to sulphydryl group blockade. Our preliminary experiments indicate such a mode of action in that cysteine can block the effects of extracts of feverfew on platelet secretion (W. A. Groenewegen and S. Heptinstall, unpublished observation) and extracts of feverfew with anti-secretory activity reduce the number of soluble and protein sulphydryl groups in platelets (P. Spangenberg, W. Lösche and S. Heptinstall, unpublished observations). It is important that the α-methylene butyrolactone unit in the compounds remains intact in aqueous media for such a mode of action.

The sesquiterpene lactones in feverfew could be responsible for any anti-inflammatory effects of the herb (Johnson et al 1985; Editorial 1985). Indeed, other sesquiterpene lactones that possess an α-methylene-butyrolactone unit have already been shown to be

Table 1. Results for anti-secretory activity screening in blood platelets of each subfraction after separation by HPLC. Between 61 and 79% of the [^{14}C]5-HT was released in the absence of any extract. Details of subsequent analysis of the active fractions in terms of NMR, TLC and IR data are listed. R_F-values were obtained by TLC using 4% methanol in chloroform as eluent and 10% sulphuric acid to detect the spots. NMR data splittings (J) are given in Hz. Percentages of the active material represented by each fraction and names of identified compounds are also given. Subfractions 1–10 were separated from fraction 5, 11–20 from fraction 6 and 21–29 from fraction 7.

Fraction no.	[^{14}C]5-HT released (%)	Comments
1	60	
2	62	
3	61	
4	49	
5	0	Contained one compound, R_F 0·73 (purple) identified as structure I (parthenolide, Fig. 1) by comparing the NMR data with that described by Govindachari et al (1965) and Quick & Rogers (1976) and with that obtained for authentic parthenolide (supplied by P. Hylands). Co-chromatographed with authentic parthenolide. About 22% of active material.
6	57	
7	59	Contained a compound R_F 0·55 (blue/purple) NMR analysis revealed no trace of a structure with an α-methylene butyrolactone unit.
8	61	
9	51	Contained a compound R_F 0·40 (blue) NMR analysis revealed no trace of a structure with an α-methylene butyrolactone unit.
10	0	NMR analysis revealed two compounds (in the proportion ca 2 : 1), R_F 0·46 (blue/brown) both α-methylenebutyrolactones. Major—δ 5·55 (d, J3·3, 13-H), 6·29 (d, J3·6, 13′-H), and 4·62 (dd, J8·6 and 8·4, 6-H); minor—δ 5·69 (d, J2·0, 13-H), 6·34 (d, J2·25, 13′-H) and 4·29 (dd, J10·3 and 5·4, 6-H); IR absorbance at 1780 cm^{-1}. Full identification of these compounds was not possible. About 7% of active material.
11	75	
12	72	
13	69	
14	72	
15	0	One compound, R_F 0·29 (purple) identified as structure II (canin, Fig. 1) by comparing the NMR data with that given by Bohlmann & Zdero (1982), Bhadane & Shafizadeh (1975), Osawa et al (1977) and Lee et al (1969). About 3% of active material.
16	0	Mixture of ca 20% canin and two other closely related α-methylene butyrolactones, R_F 0·27 (purple) IR 1775 cm^{-1}. About 8% of active material. The minor component is identical with structure III (Fig. 1)—see fraction 17.
17	0	One compound, R_F 0·27 (purple) identified as structure III (seco-tanapartholide A, Fig. 1) by comparing the NMR data with that described by Bohlmann & Zdero (1982). About 6% of active material.
18	0	One compound, R_F 0·24 (purple) identified as structure IV (artecanin, Fig. 1) by comparing the NMR data with that described by Bohlmann & Zdero (1982), Bhadane & Shafizadeh (1975), Osawa et al (1977) and Lee et al (1969). About 2% of active material.
19	72	
20	71	
21	65	
22	65	
23	70	
24	0	Contained a mixture of the compounds found in fractions 16 and 17. About 6% of active material.
25	0	Identical to fraction 17 (structure III). About 7% of active material.
26	0	One compound, R_F 0·29 (purple) identified as structure V (3-β-hydroxyparthenolide, Fig. 1) by comparing the NMR data with that described by Bohlmann & Zdero (1982), Bhadane & Shafizadeh (1975), Osawa et al (1977) and Lee et al (1969). About 30% of active material.
27	1	Contained a mixture of a non-lactonic component and an α-methylene butyrolactone, R_F 0·32 (purple): δ 6·35 (d, J2·7) and 5·67 (d, J2·4); IR absorbance at 1780 cm^{-1}. About 7% of the active material.
28	2	Contained an α-methylene butyrolactone, R_F 0·27 (purple), but this remains unidentified due to impurities obscuring the signals in the NMR spectrum: δ 6·35 (d, J2·7) and 5·65 (d, J2·45); IR absorbance at 1778 cm^{-1}. About 3% active material.
29	58	

inhibitory in a general screen for anti-inflammatory agents (Hall et al 1979). It is interesting that some other agents that are known to interact with sulphydryl groups also have anti-inflammatory properties (Oronsky et al 1969). It seems possible that the anti-inflammatory effects of sesquiterpene lactones, or of other agents, that interact with sulphydryl groups may be consequent on inhibition of cellular secretory activity.

As well as having anti-secretory and anti-inflammatory activity, sesquiterpene lactones are spasmolytic and reduce the contractility of preparations of smooth muscle (Johnson et al 1985). They are also cytotoxic for tumour cells (Lee et al 1971) and have antimicrobial properties (Blakeman & Atkinson 1979). A crude extract of feverfew has been shown to reduce the phagocytic activity of polymorphonuclear leucocytes (L. Williamson, personal communication) but whether this is a property of the sesquiterpene lactones is not yet known. This last property of feverfew might be considered disadvantageous rather than advantageous. Another disadvantageous property of feverfew is its capacity to induce contact dermatitis in some individuals (Vickers 1950). This also appears to be brought about by sesquiterpene lactones such as parthenolide in the plant (Evans & Schmidt 1980). Certainly further work is required to assess the benefits and safety of feverfew in man.

We are grateful to Dr. P. J. Hylands for providing an authentic sample of parthenolide. We are also grateful to Dr J. B. Power, Department of Botany, University of Nottingham, for providing the facilities and expertise to grow *Tanacetum parthenium*. We thank the National Westminster Bank and the R. P. Scherer Corporation for financial support.

REFERENCES

Bhadane, N. R., Shafizadeh, F. (1975) Phytochemistry 14: 2651–2653

Blakeman, J. P., Atkinson, P. (1979) Physiol. Plant Path. 15: 183–192

Bohlmann, F., Zdero, C. (1982) Phytochemistry 21: 2543–2549

Editorial (1985) Lancet 1: 1084

Evans, F. J., Schmidt, R. J. (1980) Planta Medica 38: 289–316

Govindachari, T. R., Joshi, B. S., Kamat, V. N. (1965) Tetrahedron 21: 1509–1519

Hall, I. H., Lee, K. H., Starnes, C. O., Samida, Y., Wu, R. Y., Waddell, T. G., Cochran, J. W., Gerhart, K. G. (1979) J. Pharm. Sci. 68: 537–541

Heptinstall, S., Fox, S. C. (1983) Br. J. Clin. Pharmacol. 15: 31S–37S

Heptinstall, S., Williamson, L., White, A., Mitchell, J. R. A. (1985) Lancet i: 1071–1074

Johnson, E. S., Kadam, N. P., Hylands, D. M., Hylands, P. J. (1985) Br. Med. J. 291: 569–573

Kupchan, S. M., Fessler, D. C., Eakin, M. A., Giacobbe, T. J. (1970) Science 168: 376–377

Lee, K. H., Simpson, R. F., Geissman, T. A. (1969) Phytochemistry 8: 1515–1521

Lee, K. H., Huang, E. S., Piantadosi, C., Pagano, J. S., Geissman, T. A. (1971) Cancer Res. 31: 1649–1654

Makheja, A. N. Bailey, J. M. (1982) Prostaglandins, Leukotrienes and Med. 8: 653–660

Oronsky, A. L., Triner, L., Steinsland, O. S., Nahas, G. G. (1969) Nature 223: 619–621

Osawa, T., Taylor, D., Suzuki, A., Tamura, S. (1977) Tetrahedron Lett.: 1169–1172

Quick, A., Rogers, D. (1976) Chem. Soc. Perkin Trans. II: 465–469

Vickers, H. R. (1950) Practitioner 164: 226–233