Parthenolide Content of Feverfew (Tanacetum parthenium) Assessed by Hplc and H-nmr Spectroscopy

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PARTHENOLIDE CONTENT OF FEVERFEW (*TANACETUM PARTHENIUM*) ASSESSED BY HPLC AND ^{1}H-NMR SPECTROSCOPY

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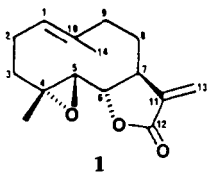
ABSTRACT.—Parthenolide [**1**], the main sesquiterpene lactone in some feverfew plants, has been quantified by a straightforward hplc procedure. ^{1}H-nmr analysis provides assay results in good general agreement and confirms parthenolide identity. Both authenticated *Tanacetum parthenium* and purported feverfew products on the UK and North American markets have been examined. A number of UK products and authentic feverfew from the UK satisfy the minimum level of 0.2% parthenolide proposed by Canada for commercial leaf products. However, no North American commercial product has yet been found to contain as much as 0.1% parthenolide. Leaves of a plant of *T. parthenium* f. *flosculosum* purchased and grown locally were found to have the highest level of parthenolide (1.27%) ever recorded in *T. parthenium* leaves, post-flowering roughly four times higher than pre-flowering, and higher than in its flowering tops.

Feverfew, *Tanacetum parthenium* (L.) Schultz-Bip. (Asteraceae), has been used for centuries as an antipyretic or febrifuge. Europeans have also long used the leaf of the plant as a treatment for migraine, asthma, rheumatism, and gynecological problems (1). Interest in feverfew, however, has dramatically increased since the publication of two double-blind placebo-controlled clinical trials in England, the first at the City of London Migraine Clinic (2) and the latter, also of randomized and crossover design, at the University of Nottingham (3). These orthodox studies clearly established the potential of feverfew in the prophylaxis of migraine, a statistically significant proportion of subjects reporting a reduction in the incidence and severity of headache and nausea.

A number of hypotheses have been advanced and tested to account for feverfew's activity in relieving migraine symptoms, but the actual mechanism is not unequivocally established. However, it is thought that the ability of extracts of feverfew to inhibit release of serotonin (5-hydroxytryptamine) from blood platelets may be relevant to its effects in migraine (4). The component of feverfew regarded as responsible for this activity is the germacranolide sesquiterpene lactone parthenolide [**1**] (5), and, in the clinical trials that have been performed, only feverfew that contained defined amounts of parthenolide was used. Parthenolide is the predominant sesquiterpene lactone found in British- (6) and German-grown feverfew (7), but no parthenolide was found in either Mexican-grown feverfew [in which were identified only two eudesmanolides, santamarin [**2**] and reynosin [**3**], and the guaianolides canin [**4**] and artecanin [**5**] (8)] or in Yugoslavian-grown feverfew, which yielded only five eudesmanolides, including santamarin and reynosin (9).

The effectiveness of extracts of various preparations of feverfew in inhibiting, in vitro, the release of serotonin from human blood platelets has been found to correlate well with levels of parthenolide (10). In view of the biological effects of parthenolide-containing feverfew in migraine prophylaxis it is surmised that the effectiveness of feverfew leaf preparations, either freeze-dried or air-dried whole leaf, may be ensured by the requirement that they contain a minimum concentration of parthenolide. A mini-



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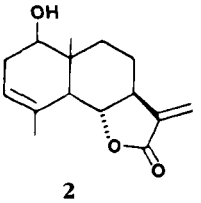

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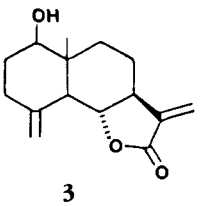

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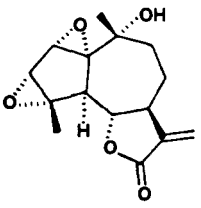

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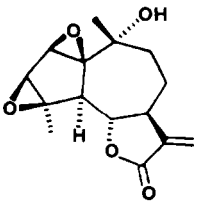

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mum level of 0.2% has been proposed by the Health Protection Branch of Health and Welfare Canada; this is roughly half the average content of all feverfew leaf samples (0.42%) found in material grown at the Chelsea Physic Gardens and used in the earlier clinical study (11). A proposal of 0.1% has been made in a document under consideration by the French Ministry of Health and the Family (12), which accepts the marketing of feverfew herbage (leaf plus stalk).

The need for a simple assay for determination of parthenolide in commercial feverfew preparations, which would be easily accessible to the herbal industry, led to development of a straightforward reversed-phase hplc procedure. An assay based on derivatization with an alkylthiol, followed by hplc of the Michael addition product, promises to be useful for analysis of complex mixtures of sesquiterpene lactones containing an α-methylenebutyrolactone moiety (D.M. Dolman, D.W. Knight, U. Salan, and D. Toplis, personal communication, 1991).

Parthenolide extracted from British-grown feverfew leaf was purified by recrystallization and used as an analytical standard. Authenticated and purported feverfew samples were extracted with organic solvent and subjected to chromatographic and ^{1}H-nmr spectroscopic analyses. The latter examination was conducted in order to test the reliability of chromatographic identification of parthenolide, because of the wide variety of reported feverfew constituents and the difficulty of acquiring reference samples of them. In the case of two samples, one of authentic feverfew and the other a commercial product, the chromatographic fraction corresponding to parthenolide was collected and submitted to ^{1}H-nmr analysis.

RESULTS AND DISCUSSION

In 1986, Groenewegen and Heptinstall (13) published an assessment of "feverfew" preparations commercially available in the United Kingdom. These authors judged

that such preparations generally either contained relatively low amounts of feverfew leaf or had deteriorated, based on the capacity of extracts to inhibit release of serotonin from platelets in response to an agonist. However, because the bioassay used does not discriminate between parthenolide and other active sesquiterpene lactones, the activity of extracts so determined cannot be a reliable indicator of either parthenolide content or feverfew identity. The assay values for parthenolide content of commercial purported feverfew preparations reported here likewise do not imply anything about botanical species identity. A survey of the literature (14) reveals that parthenolide has been identified in twenty-five species of the Asteraceae, including *Tanacetum vulgare* L. (common tansy), in which levels comparable to those in clinically tested British feverfew have been found (15); parthenolide has also been detected in nine species of the Magnoliaceae.

Authentic feverfew (leaf and seed) acquired from the Department of Botany, University of Nottingham, was extracted with petroleum ether and analyzed by hplc and ^{1}H-nmr spectroscopy, along with six purported feverfew products commercially available in the UK and four formulations on the North American market, as well as a U.S.-grown feverfew variety and a commercial variety grown locally.

Parthenolide [**1**] has a retention time of roughly 6 min under the hplc conditions used in this study. In a representative series of runs, artecanin (1.7 min), santamarin [**2**] (3.3 min), and reynosin [**3**] (2.8 min), as well as all the other available eudesmanolides, guaianolides, and pseudoguaianolides (ambrosanolides) tested in this hplc system had retention times (roughly 2 to 4 min) considerably less than that of parthenolide (5.7 min).

As can be seen from the results presented in Table 1, there is generally good agreement between the values for parthenolide content derived from both chromatographic and spectrometric assay procedures. In no instance was coelution of other secondary plant metabolites detected. The ^{1}H-nmr assay was based on determination of peak heights of the pair of characteristic doublets due to the exocyclic C-13 protons of parthenolide, roughly centered at δ 6.3 and 5.6 ppm (17). In those instances where there was significant background contribution, this generally occurred with the lower field resonance, allowing a satisfactory estimate based on the 5.6 ppm peak height. Peak height was preferred over peak area because of usual broad background contribution, and a correction factor of 40% was applied to compensate for the reduced peak height of the parthenolide proton, as compared with that of the standard (See Experimental). Significant discrepancies between the parthenolide assay results from hplc and nmr analyses are attributed to enhancement of the doublet resonances by unidentified closely related sesquiterpene lactones, such as 3β-hydroxyparthenolide which has almost identical C-13 proton chemical shifts (7); this indicates that the nmr method should be used mainly for confirmatory purposes.

It is notable that no North American purported feverfew product has yet been found to contain as much as 0.1% parthenolide [**1**], whereas a number of British products comfortably satisfy the proposed 0.2% minimum. The gross mixture of flowering tops and leaves from Trout Lake Farm contained 0.18% parthenolide, but consisted of almost half flowering tops, which have roughly three times the level of parthenolide found in the leaves; flowering tops of feverfew have not been clinically tested. Most notable is the very high percentage of parthenolide (almost 1%) in the bulk purported feverfew leaf powder provided by Herbal Laboratories Ltd. in the UK, as well in the leaves and seeds of plants grown at the Department of Botany, University of Nottingham; the latter are of the variety with one row of white ray flowers surrounding the yellow inflorescence of disc flowers. This variety, which has yellow-green leaves, is promoted in the popular literature as the truly efficacious feverfew (18); a description con-

TABLE 1. Parthenolide [**1**] Content of Extracts by Hplc and ^{1}H-nmr Assay Procedures.

Sample	Percentage of Dry Wt[a]	
	Hplc	^{1}H-nmr
United Kingdom		
Univ. of Nottingham		
Leaf	0.83	0.85
Seed	1.52	1.82
Herbal Laboratories: bulk feverfew leaf[b]	0.92	0.70
Herbal Laboratories 125: tablets	0.45	0.62
Dooley: herbage	0.30	0.32
Potter's: tablets	0.17	0.08
Bio-Health: capsules	0.25	0.25
Power Health: capsules	0.18	0.56
Arkopharma[c]: capsules	ND	ND
Canada		
Abco Laboratories (USA); bulk powder	0.01	+
Christmas Natural Foods (source Abco); capsules	0.08	+
Eclectic Institute (USA)[b]; capsules	0.08	+
Yerba Prima (USA)[d]; tablets	0.06	0.06
Trout Lake Farm (USA)[e]		
Mixed leaf and flowering top	0.18	0.21
Leaf	0.09	0.13
Flowering top	0.27	0.35
Locally grown feverfew[f]		
Leaf, preflowering	0.33	0.64
Leaf, post flowering	1.27	1.62
Flowering top	0.46	1.01

[a]ND Not detected; + chromatographic fractions not quantitated.

[b]Hplc fraction corresponding to parthenolide collected and analyzed by ^{1}H nmr.

[c]Imported from France. Arkopharma, in correspondence (23/7/90) to the Health Protection Branch of Health and Welfare Canada, has claimed that the absence of parthenolide here noted is due to this particular batch of product being *Matricaria maritima*, rather than *Tanacetum parthenium*. It should be recalled also that British importers of feverfew were provided *Tanacetum vulgare* as well as *Matricaria recutita* (German chamomile) by Eastern European growers (16).

[d]Claimed by the manufacturer to be from wild *T. parthenium* plants grown in Northern California.

[e]55% leaves, 45% flowering tops, based on weight of manually separated parts. Microscopic examination of dried leaf revealed characteristic glandular and covering trichomes.

[f]From Ritchie Seed and Feed in Ottawa, grown in Chelsea, Quebec.

sistent with this variety appears in the feverfew monograph of the 1990 British Herbal Pharmacopoiea. However, Johnson (20) believes that the cultivated varieties "are probably similarly effective when used medicinally, although this has to be verified." Herbal Laboratories has indicated that the plants from which this material was derived were certified by M.I. Berry (16) of the School of Health Sciences, Liverpool Polytechnic. Also remarkable is the high percentage of parthenolide found in the commercial cultivated form of feverfew grown locally, termed *Tanacetum parthenium* f. *flosculosum* (D.C.) Beck., with flowering top devoid of ray flowers. It had a higher level (1.27%) of parthenolide in its leaves than so far reported for feverfew leaf, and comparable to levels reported in feverfew seeds (15,19); the high level of parthenolide in the leaf extract allowed clear discernment in the nmr spectrum of the characteristic apparent triplet (doublet of doublets, $J = 8.6, 8.6$ Hz), centered at δ 3.84 ppm, due to the C-6 proton. The post-flowering leaves of this plant contained roughly four times the level of parthenolide (0.33%) found in its leaves picked just before flowering, the time at which

the sesquiterpene lactone level in feverfew leaves is claimed to be highest (19). A complete survey of British and North American products, also submitted to the antisecretory bioassay (20), will be published later.

In summary, estimation of parthenolide content in feverfew products is conveniently made by the hplc proceudre reported in this study. No interference from other plant constituents has been encountered in analysis of both British and North American purported feverfew products. The method is readily accessible to the herbal industry and, coupled with certification of botanical identity, should ensure efficacy of feverfew products in migraine prophylaxis.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—*^{1}H-nmr quantitation.*—Nmr spectra were recorded at 296K on a Bruker AM 400 spectrometer equipped with an Aspect 3000 computer and process controller. The signal from residual $CHCl_3$ in the $CDCl_3$ solvent was used for chemical shift reference (δ 7.26 ppm). Either 128 or 256 scans were acquired with a relaxation delay of 10 sec to allow quantitation.

A correction factor of 1.4 was applied to the calculated parthenolide percentages as noted earlier to compensate for differences in peak height between the internal standard, 1,2,4,5-tetrachlorobenzene (TCB), and the parthenolide methylene protons: ^{1}H-nmr spectra were recorded for a series of $CDCl_3$ solutions containing approximately equimolar amounts of TCB and parthenolide, over a range of concentration from 10 mg to 100 μg/ml; the ratio of the sum of the peak heights for the doublet at δ 5.6 ppm to the TCB peak height was calculated for each concentration (Table 1), using the equation:

$$\%\,\text{Parthenolide} = \frac{248.32 \times \dfrac{\text{Peak Height (13-H)}}{(0.5)\,\text{Peak Height TCB}} \times \dfrac{\text{Weight of TCB (mg)}}{215.89} \times \chi}{\text{Weight of Extracted Material (mg)}} \times 100 \times 1.4,$$

where $\chi = 5$ (20% fraction), 10 (10% fraction), or 20 (5% fraction), and 248.32 and 215.89 are the mol wt's of parthenolide and TCB, respectively. In the range of concentrations relevant to this study, the average ratio was found to be about 0.7; when a plant extract containing no detectable level of parthenolide was spiked with TCB and parthenolide for this range of concentrations, no departure from the ratio was seen.

Hplc assay.—A Spectra-Physics SP8700 solvent delivery system and a Valco C6W injector equipped with a 20 μl loop were used for all determinations; uv detection was with an LKB 2140 Rapid Spectral Detector operating over a range of 190–370 nm. A Brownlee Laboratories Spheri-10 RP-18 column (250 × 4.6 mm, 10 μm) was used with a mobile phase of H_2O-MeCN (55:45) at a flow rate of 2 ml/min. Quantitation was done at 210 nm, with reference to a standard curve derived from five concentrations of parthenolide ranging from 30 to 400 μg/ml. Seven consecutive injections of parthenolide at a concentration of 200 μg/ml gave a deviation in area of 1.26% and in retention time of 0.45% (5.93–6.02 min); the lower limit of detection is 0.1 μg on column.

PLANT MATERIAL.—Authentic feverfew was obtained from the Department of Botany, University of Nottingham; a voucher specimen has been deposited in the Herbarium of the Department of Life Science (formerly Department of Botany). Herbal Laboratories, Lancashire, UK, provided bulk purported powdered feverfew leaf, the source of our parthenolide standard. Commercial purported feverfew products were either purchased in Nottingham, UK, or were acquired following submission to the Bureau of Nonprescription Drugs, Health and Welfare Canada. Abco brand bulk feverfew powder was purchased directly from the manufacturer. The Trout Lake Farm sample was submitted by the Herb Research Foundation, Boulder, Colorado. Another feverfew plant was purchased from Ritchie Feed and Seed Co., Ottawa, and grown outdoors in Chelsea, Quebec by David D. Pyke. A voucher specimen (CC40) has been deposited with the Department of Agriculture Herbarium (DAO), Ottawa. This plant and plant material acquired from Trout Lake Farm, Washington State, were examined and compared with specimens in the DAO, with the benefit of the recent taxonomic treatment of Wagenitz (21). The plant from Ritchie Feed and Seed Co. had the diagnostic features common to *T. parthenium* f. *flosculosum* and had yellow inflorescences without ray flowers; the sample from Trout Lake Farm had ray and disc flowers common to the typical species. Examination with a 30X microscope clearly revealed the presence of the green glands of f. *typical*, but the glands of f. *flosculosum* only became discernable under 60X magnification; however, electron microscopic examination of the specimens' leaf surfaces showed that the glandular trichomes on f. *flosculosum* were not surrounded by septated aglandular hairs, having only occasional irregularly dispersed very fine hairs; leaf

surface of f. *typical*, on the other hand, was extremely pubescent, having glandular trichomes located adjacent to the aglandular ones. Further biochemical and taxonomic studies of additional samples of both taxa are required to determine if the parthenolide yields are constant and linked to these diverse morphological features. Fresh plant material was dried in an oven at 38° for 3 days.

SAMPLE PREPARATION.—Dried leaves, seeds, and tablets were powdered in a Tecator Cyclotec 1093 Sample Mill, while the contents of gelatin capsules were used directly. Varying amounts (600 mg to 30 g) of sample were extracted in a Soxhlet apparatus for roughly 24 h using 450 ml petroleum ether (30–60°). Appropriately sized aliquots (5, 10, or 20%) of the resulting solution were removed for hplc and ^{1}H-nmr analyses and reduced to dryness using a rotary evaporator with bath temperature 40°, followed by vacuum pumping for approximately 2 h. The residues for hplc analysis were treated with MeCN, passed through a Millex-HV (0.45 μm) filter, and injected directly onto the column. Residues for nmr analysis were spiked with an appropriate amount of TCB (δ 7.56 ppm) in $CHCl_3$, generally 1 mg/ml, and the solution again reduced to dryness; quantitation was performed following redissolution in $CDCl_3$.

PARTHENOLIDE.—Parthenolide [**1**] was isolated from powdered feverfew leaf following established procedures (22), and exhibited physical and spectroscopic characteristics consistent with published data (17).

ACKNOWLEDGMENTS

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