Sesquiterpene lactones from feverfew, *Tanacetum parthenium*: isolation, structural revision, activity against human blood platelet function and implications for migraine therapy

PERKIN 1

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The structures of two series of sesquiterpene lactones (the 'α'-series 11, 12 and 16 and the 'β'-series 15, 17 and 18) present in the herb feverfew have been revised in the light of both X-ray analysis and chemical correlations. A biosynthetic pathway is proposed, involving a Diels–Alder type addition of oxygen followed by two contrasting rearrangements, starting from a common intermediate, the cyclopentadiene 19, which has not been identified in feverfew extracts but which is related to a recently isolated structure. The activity of some of these metabolites as well as of the major sesquiterpene lactone present in feverfew, parthenolide 2, and some simpler synthetic models as inhibitors of human blood platelet function has been determined. The possible relevance of this effect to migraine prophylaxis by feverfew is briefly discussed.

Introduction

The herb feverfew, *Tanacetum parthenium* (L.) Schulz Bip., has been widely valued since ancient times for an array of beneficial medicinal properties which have led some authors to refer to the plant as the 'mediaeval aspirin'.[1–3] During the past 15 years or so, the plant has come to prominence by reason of its claimed prophylactic properties with respect to the frequency and severity of migraine attacks. These claims have been supported by statistical analyses of the results from two placebo-controlled 'double-blind' studies, one involving some 17 migraine patients carried out in London[4] and a later, larger survey of 59 migraine sufferers undertaken in Nottingham.[5] Although these data supported the contention that feverfew contains components which are of benefit to migraine sufferers, a similar test of the claimed prophylactic effects of the herb on the symptoms of rheumatic arthritis was much less encouraging.[6]

Our chemical interest in feverfew was stimulated by these reports and especially by the positive results of the 'double-blind' migraine studies.[4,5] We reasoned that there should be metabolites present in the plant which could in some way modify or interfere with basic blood chemistry and hence give rise to migraine prophylaxis. This view was supported by the observations of Makheja and Bailey who reported that crude extracts of feverfew leaves were capable of inhibiting human blood platelet function, induced *in vitro* by adenosine diphosphate (ADP);[7] other experiments reported by these authors indicated that the extracts were able to interfere with prostaglandin synthesis, possibly at the phospholipase A_2 stage. Our own initial experiments clearly showed that a crude chloroform extract of fresh feverfew leaves was capable of completely inhibiting human blood platelet function, apparently at rather low concentrations. Subsequent rough separation by HPLC of a small sample of the crude extract revealed that only those fractions which contained an α-methylenebutyrolactone function displayed this activity.[8] At this stage, both single compounds and mixtures were assayed; the presence of such lactone units in a particular fraction was determined predominantly from ^{1}H NMR data, which showed a characteristic pair of doublets (J 2.0–3.5 Hz) at $\delta_H(CDCl_3)$ 5.4–5.8 and 6.1–6.4, due to the α-methylene group in lactones **1**. Significantly, those fractions which did not display these resonances were devoid of activity. We therefore separated a much larger sample of the leaf extract, in order to isolate sufficient quantities of the pure lactones to allow for further bioassay as well as full structural characterization. Bohlmann and Zdero had previously reported the presence of a complex series of sesquiterpene α-methylenebutyrolactones in their seminal study of feverfew metabolites, along with a variety of simpler monoterpenes (camphor, various α-pinene derivatives), the sesquiterpenes costic acid methyl ester, germacrene D and β-farnesene together with examples of spiroketal enol ethers having penta-1,3-diynyl side chains.[9] Our interest was focused on the more polar sesquiterpene α-methylenebutyrolactones in view of the preliminary bioassay results[8] and therefore these latter metabolites were not examined. However, when we obtained pure samples of the lactones, some inconsistencies between the literature data[9] and our own led us to investigate further the structures of these metabolites.

Isolation and structural revision

The predominant sesquiterpene lactone present in feverfew is parthenolide **2**. This compound appears to have been first isolated from *Tanacetum parthenium* by Sorm and his colleagues,[10] who incorrectly assigned the structure as a Δ^2 germacrene. Subsequently, the same compound, but initially named 'champakin', was isolated from the roots of *Michelia champaca* and its gross structure correctly defined;[11] later studies revealed most of the stereochemical features,[12] which were confirmed by an X-ray determination.[13] Our samples of feverfew also contained parthenolide **2** as by far the major sesquiterpene lactone. Guided by ^{1}H NMR spectroscopy, we also isolated samples of costunolide **3a**,[9] 3-hydroxycostunolide **3b**,[9,14] epoxyartemorin **4**,[9] (but not the closely related artemorin **5** reported to be present by Bohlmann and Zdero[9])

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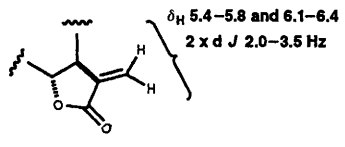

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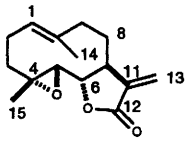

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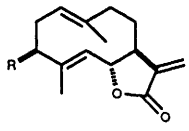
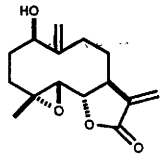


3 a; R = H
b; R = OH



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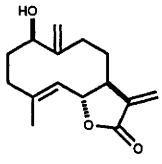

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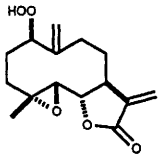

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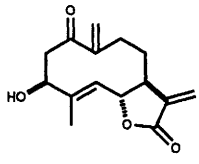

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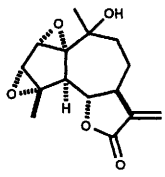

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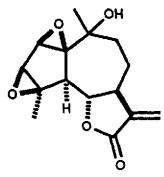

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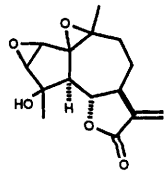

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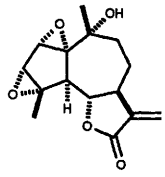

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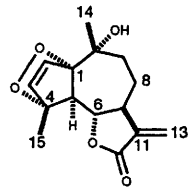

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and peroxyparthenolide **6**;[9] traces of the related ketone **7**[9] were also detected. The hydroperoxide **6** has not previously been reported as being present in feverfew, but has been isolated from *Magnolia grandiflora*;[15] our sample showed spectral data identical to those previously recorded. However, all of these compounds were present at very low levels ($\leqslant$*ca.* 3 mg kg^{-1}) in the fresh leaves, later confirmed by our new assay procedure (see below). Traces of other sesquiterpene lactones were also observed by ^{1}H NMR spectroscopy in various mixtures, some of which displayed resonances corresponding to those reported by Bohlmann and Zdero for additional metabolites,[9] but these were not isolated in either sufficient quantities or purities to allow for a definitive structural determination. The remaining bulk of the sesquiterpene lactones present in our feverfew sample turned out to be two closely related series of highly oxygenated metabolites, the most abundant of which was a highly crystalline *endo*-peroxide.[9] The remainder consisted of three bis-epoxides, one of which was present in much greater quantities than the other two, and small but similar amounts of two cyclopentenones, all of which were separated by repeated silica gel column chromatography using chloroform and up to 5% methanol in chloroform as eluents. The structures were tentatively assigned by comparison with previously reported spectral data.[9] Although it was plain which set of data exhibited by these compounds corresponded to the Bohlmann and Zdero data,[9] it was far from clear what the exact stereochemistry was.

Our starting point was the major bis-epoxide which, from literature comparisons, appeared to be identical with 'canin', a material which has been the subject of a highly confusing series of structural assignments and reassignments during the past two decades.[16] Originally designated as the bis-α-epoxide **8**,[17] the compound was later said to have a β-epoxide stereochemistry **9**[18] and has also been confused with the isomeric 'chrysartemin' structures **10**.[19] X-Ray analysis has been used to firmly establish that 'canin' (identical with 'chrysartemin A') present in various *Artemisia* species indeed possesses the bis-α-epoxide stereochemistry **11**,[16] supporting the original Geissman structural assignment.[17] However, Bohlmann and Zdero have assigned the alternative bis-β-epoxide stereochemistry (*cf.* **9**) to the major bis-epoxide present in feverfew, but designated it as 'canin'.[9] It was clear that our major bis-epoxide corresponded exactly to this compound, particularly from ^{1}H NMR data. What was far from clear was the exact stereochemistry of this, and by implication, the remainder of this series of sesquiterpene lactones. This was compounded by the fact that solid samples show differing melting points (both literature and our own data), possibly due to decomposition close to the melting point and hence a dependence between the value reported and the rate of heating. Optical rotation measurements were similarly beset by literature variations and are in any event renowned for being an inaccurate guide. Even the X-ray data for canin[16] could not be directly related to our sample due to a lack of suitable comparative NMR data. We therefore carried out our own X-ray analysis of the major bis-epoxide present in feverfew and found it to be identical to canin present in the *Artemisia* species[16] and to have the bis-α-epoxide structure **11**, or its enantiomer; attempts to define the absolute configuration were not successful and the representation of the structure follows an unproven convention.[16] In the light of this finding, the remaining structures proposed by Bohlmann and Zdero for this series of metabolites must be revised.[20]

Similarly, we used X-ray analysis to determine the relative configuration of the major component of the mixture, an *endo*-peroxide, the ^{1}H NMR data of which corresponded to that reported by Bohlmann and Zdero[9] and which they had assigned as having a β-orientated dioxygen bridge (*cf.* **18**), related to the bis-β-epoxide structure **9**. The *endo*-peroxide, mp 95–96 °C (lit.,[9] mp 117 °C, $[\alpha]_D^{20}$ −32.1 (*c* 0.11, $CHCl_3$) {lit.,[9] $[\alpha]_D^{24}$ −24 (*c* 0.22, $CHCl_3$)}, $C_{15}H_{18}O_5$, crystallized from dichloromethane–hexane as a monohydrate in monoclinic crystals, space group $P2_1$, with cell dimensions a = 9.248(5), b = 6.835(2), c = 11.756(5) Å and β = 94.82(4)°. There were two molecules in the unit cell. A crystal of approximate dimensions 0.75 × 0.2 × 0.02 mm was used to collect intensity data on a CAD4 diffractometer. Of the 1,209 independent reflections measured, 880 had $I > 2\sigma(I)$ and were considered and used in the subsequent refinement. The structure was solved by direct methods[21,22] and refined to a final R value of 6.52%.‡ The resulting structure is shown in Fig. 1. For clarity, the water molecule is shown. In the overall structure, the individual molecules were linked with water (W) *via* O(3)H · · · O(W), O(W)H · · · O(3) and O(W)H · · · O(4) hydrogen bonds (O · · · O 2.82, 2.91 and 2.85 Å respectively) and formed layers parallel to the (110) plane. This corresponds to the structure **12** or its enantiomer; attempts to define the absolute configuration were again unsuccessful. Therefore, this metabolite **12** belongs to the same 'α-series' as canin **11** and not the corresponding 'β-

‡ Atomic coordinates, thermal parameters and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1996, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 207/34.

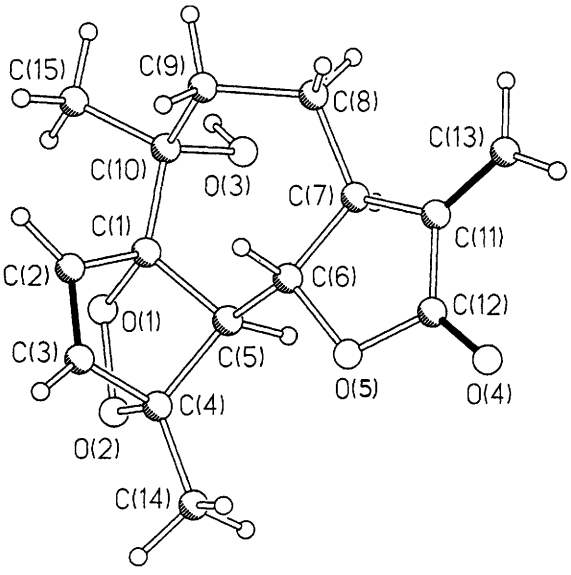
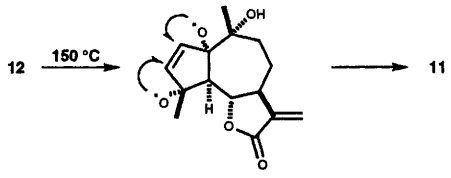


Fig. 1 X-Ray structure of tanaparthin-α-peroxide **12**

series' as proposed by Bohlmann and Zdero, and should be renamed tanaparthin-α-peroxide.§[9]

The *endo*-peroxide **12** was a surprisingly stable material. Consistent with this, the X-ray analysis showed it to have no unusual bond lengths or angles; a search of the Cambridge X-ray data base revealed that typical O–O bond lengths in similar structures are in the region 1.44–1.50 Å. In the *endo*-peroxide **12**,



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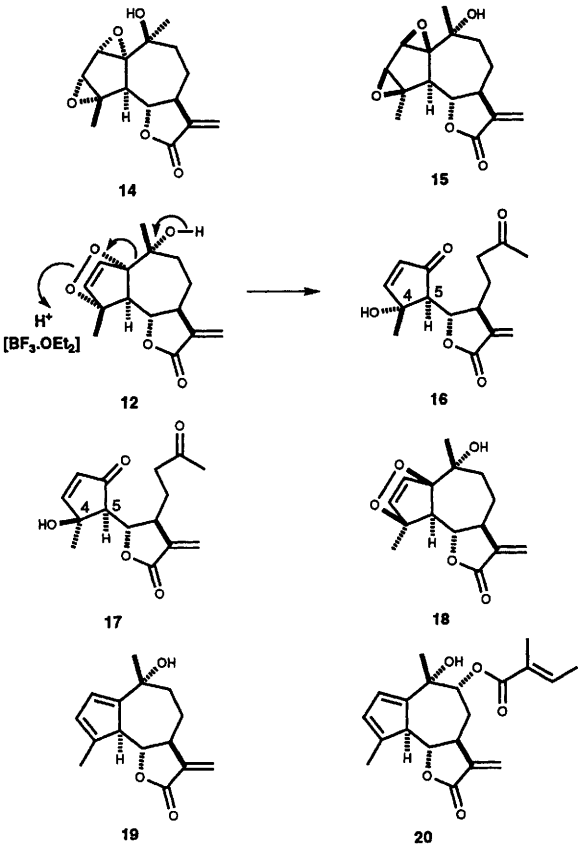
the O–O bond length is 1.48 Å. The interrelationship between canin **11** and tanaparthin-α-peroxide **12** was further proven by a thermally-induced stereospecific cyclopentene *endo*-peroxide–bis-epoxide rearrangement,[9] the progress of which was followed by ^{1}H NMR spectroscopy in deuteriobenzene (sealed tube), and which required 150 °C (oil bath temperature) to proceed at a reasonable rate; after 1.5 h, complete and quantitative conversion to canin **11** was observed, presumably *via* the diradical species **13**.[23]

We next deduced the structures of the two other, isomeric bis-epoxides, both of which were isolated in much smaller quantities (see below). One showed essentially identical ^{1}H NMR data to that reported by Bohlmann and Zdero[9] for '10-*epi*-canin'. The rationale for this assignment was the very close similarity between these data and the corresponding data exhibited by canin **11**, with the exception of the resonance for the 14-CH_3 group. We therefore propose, in the light of the structural revision of canin, that this compound has structure **14**, and is epimeric with canin at C-10. The very small quantities isolated did not allow any further meaningful data, either spectroscopic or chemical, to be obtained. The third bis-epoxide was also present in small quantities in our feverfew samples and was not isolated in a completely pure state. However, the spectroscopic data corresponded to those reported by Bohlmann and Zdero[9] and others[18,19,24] for artecanin, for which structure **11** (canin) was assigned.[9] On the basis of the foregoing results, we propose that artecanin is in fact the isomeric β-bis-epoxide **15**; this assignment is also consistent with the structural assignments of the two cyclopentenones which were the final components of the mixture.

The two cyclopentenones (the secotanapartholides[9]) isolated in the present work showed similar but not identical spectroscopic data to the two components of an inseparable mixture reported by Bohlmann and Zdero.[9] Careful chromatography allowed the isolation of pure samples of each, although the spectroscopic data did not allow unambiguous structural assignments to be made. We therefore examined a chemical correlation method based upon the unusual biosynthetic pathway which could lead to these compounds[9] and which consists of a proton-induced fragmentation of an endoperoxide. Thus, protonation of tanaparthin-α-peroxide **12** would be expected to lead to the secotanapartholide **16**. We have found that when a sample of peroxide **12** is exposed to a solution of boron trifluoride–diethyl ether in chloroform, the only product formed (*ca.* 30%) which contained a cyclopentenone function was identical to the minor of the two secotanapartholides we isolated from feverfew. We therefore propose that this compound is secotanapartholide A **16** and that the major cyclopentenone is secotanapartholide B **17**, the C-4 epimer.[25,26] There is, however, still some uncertainty regarding these structures: a recent report[26] has suggested that secotanapartholide B, isolated from *Artemesia xerophytica*, is in fact epimeric with secotanapartholide A **16** at the 5- rather than the 4-position. Our spectral data for the major feverfew secotanapartholide ('B', **17**) correspond very closely to that reported for 'secotanapartholide A' from *A. xerophytica*, while our data for the minor feverfew secotanapartholide ('A', **16**) are clearly very different from that reported for the C-5 epimer from *A. xerophytica*, referred to by the Chinese workers as secotanapartholide B.[26] It may simply be that the two plant species produce different epimers of these structures. We failed to isolate the putative precursor to this latter compound, tanaparthin-β-peroxide **18**, although traces were detected in mixtures by ^{1}H NMR analysis. Bohlmann and Zdero[9] report the isolation of a small, pure sample (1 mg) from an extract which produced *ca.* 10 mg of the α-peroxide **12**. In principle, a similar, (Lewis)-acid catalysed transformation of the corresponding bis-epoxide, canin **11**, could also lead to secotanapartholide A **16**; however, attempts to achieve this transformation were not successful.

In the light of these findings, the revision necessary to the Bohlmann–Zdero assignments[9] essentially consists of a reversal of the α- and β-series structures (**11**, **12**, **16** and **15**, **17**, **18** respectively). It appears that all of these sesquiterpene lactones could be derived from a common precursor, the cyclopentadiene **19**. Evidence for the presence of this compound in feverfew has not been obtained as yet[9,20] although this proposal is supported by the recent isolation of the closely related sesquiterpene lactone isoachifolidiene **20** from *Achillea millefolium* L., thus at least showing that such a structure is capable of discrete existence in a plant source.[27] Furthermore, the derived peroxides have also been isolated from the same plant.[28] These structural revisions are also perhaps more consistent with the likely biosynthetic pathways involved. Given that the cyclopentadiene **19** is the precursor and that the first formed metabolites are the *endo*-peroxides **12** and **18**, *via* a Diels–Alder reaction with dioxygen, tanaparthin-α-peroxide **12** would be expected to be the major isomer formed as addition of the dienophile is to the more exposed, convex α-face with possibly assistance from the 10-(α)-hydroxy group. Subsequent rearrangement would lead to the bis-α-epoxide canin **11**. In line with this, these were the two most abundant members of these series found in this work. The same *endo*-peroxide **12** would also be the precursor to secotanapartholide A **16** (see above). The minor 'β-series' (**15**, **17**, **18**), arising initially from addition of dioxygen to the less exposed β-face of the cyclopentadiene **19** were all present in much smaller quantities, with the exception

§ IUPAC name: 1α,4α-epidioxytanaparthin.



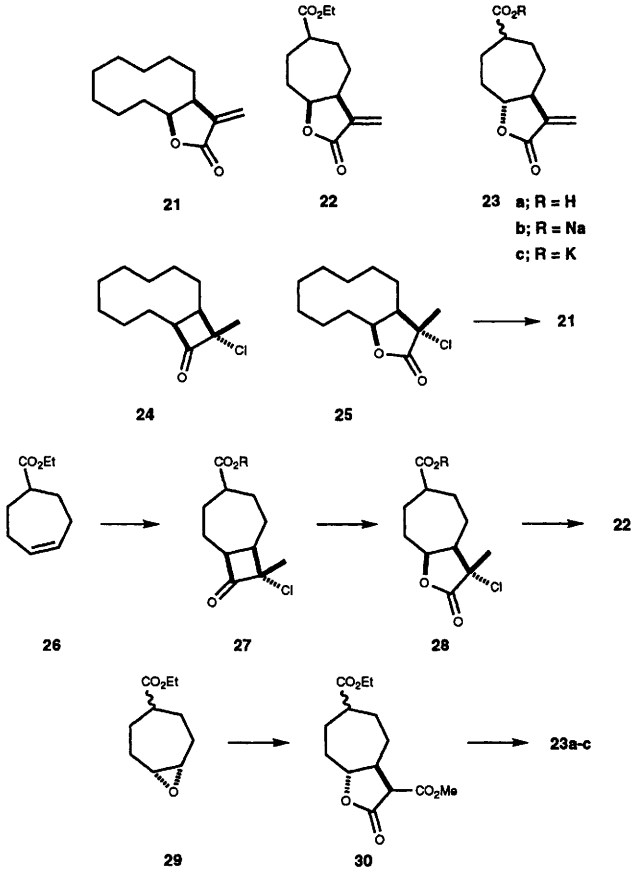
of the ring-opened secotanapartholide B **17**; possibly this is a consequence of the *anti* disposition of the 10-hydroxy function and the peroxide bridge in the likely precursor **18**, which should render the fragmentation process more facile.

A final aspect of this section concerns the discovery of a relatively facile method for the isolation of parthenolide **2** from feverfew leaves, which relies on the perhaps surprising level of water solubility, which we observed during the preliminary bioassays, of this apparently hydrophobic compound. It is known that the bulk of the sesquiterpene lactones in feverfew occur in glands on the underside of the leaves.[29] Thus, simply washing or steeping the aerial parts of the plant in chloroform or dichloromethane extracts the vast majority of the sesquiterpene lactones present, together with the other metabolites described above; subsequent separations require extensive chromatography to obtain pure samples. However, when fresh leaves are steeped in water and the decanted aqueous solution back-extracted with chloroform, the product consists essentially of parthenolide **2**, camphor and unidentified fatty acid material. One careful column chromatogram is then sufficient to separate pure parthenolide. Studies using our assay procedure[30] for α-methylenebutyrolactones confirmed that >95% of the parthenolide had been extracted in this way. Although we have not determined the precise level of solubility of parthenolide in water, our extractions suggest a level of >1 mg ml^{-1}.

Activity against human blood platelet function

A link between blood platelet chemistry and migraine has been suggested in a number of reports in the literature; hence, our initial studies focused on the effects of feverfew extracts on blood platelets (see above), which led to the conclusion that α-methylenebutyrolactone groups were responsible for the inhibitory effect of feverfew extracts on blood platelet function.[3,8] Upon agonist-induced aggregation, blood platelets release 5-hydroxytryptamine (serotonin) which has been linked to the onset of migraine[31] and which further augments the aggregation induced by the original agonist.[3] In addition, it has been observed that platelets taken from patients who suffer from 'classical' migraine during a headache-free period display significantly higher spontaneous platelet aggregation and adhesion properties relative to platelets from controls.[31,32]

In order to further probe the preliminary bioassay results, we chose to compare the effects of four of the feverfew metabolites (**2**, **11**, **12** and **16**) together with the five, model, synthetic lactones **21**–**23**. The latter compounds were obtained following literature methods. The cyclodecane derivative **21** was prepared starting from (*Z*)-cyclodecene by cycloaddition of chloro(methyl)ketene followed by Baeyer–Villiger oxidation of the resulting cyclobutanone **24** to the corresponding lactone **25** and dehydrochlorination.[33] The starting material for the other four trial compounds **22** and **23a**–**c** was the cycloheptenecarboxylate **26**;[34] a sequence similar to the foregoing[33] was used to obtain



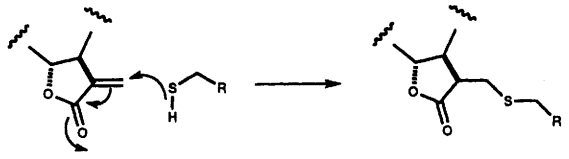
the *cis*-fused lactone ester **22**, *via* the cyclobutanone **27** and the chloro(methyl)lactone **28**. Unexpectedly, the lactone ester **22** appeared to be a single diastereoisomer according to NMR data. While no effort was made to determine the stereochemistry, we presume that the lactone ring and ester function are positioned *trans* as a consequence of the approach of chloro(methyl)ketene to the less hindered face of the cycloheptenecarboxylate **26**. The corresponding *trans*-fused lactone carboxylic acid **23a** was obtained as a mixture of diastereoisomers from the epoxide **29** following ring opening by malonate, lactonization and α-methenylation.[35] The derived sodium and potassium salts **23b**,**c** were then obtained by neutralization using one equivalent of the appropriate hydroxide.

When blood platelets aggregate, 5-hydroxytryptamine (serotonin; 5-HT) is released. The degree of platelet aggregation, when induced by an external agent such as adrenaline or arachidonic acid, in platelet-rich plasma (PRP) can be estimated by using platelets labelled with [^{14}C]-5-hydroxytryptamine;[36] the less 5-HT released, the less

Table 1 Effect of α-methylenebutyrolactones on 5-hydroxytryptamine release in human PRP

Compound	Parthenolide equivalents
Parthenolide **2**	1.00
Canin **11**	0.48
Tanaparthin-α-peroxide **12**	0.45
cis-Cycloheptane lactone ester **22**	0.33
Secotanapartholide A **16**	0.31
trans-Cycloheptane lactone acid **23a**	0.12
trans-Cycloheptane lactone Na salt **23b**	0.10
cis-Cycloheptane lactone K salt **23c**	0.02

aggregation has occurred. The test compounds were added at levels lower than those required to cause complete inhibition of aggregation and the results are summarized in Table 1. These are expressed relative to parthenolide **2** and are derived from the averages of three separate runs using different platelet agonists and are relative to a blank run using saline in place of the test compound. Clearly, parthenolide **2** is the most active compound of the series as an inhibitor in PRP aggregation. The much lower levels of activity displayed by the synthetic compounds suggest that an α-methylenebutyrolactone function is a necessary but not sufficient requirement. That the highly water-soluble salts **23b,c** were the least active may be due in part



31 **32** **33**

to their reduced electrophilicity. Water solubility was a limitation with the cyclodecane derivative **21**; very low levels of activity were recorded for this compound, which appeared to be due to a lack of solubility in the aqueous test medium.

A classical reaction of an α-methylenebutyrolactone function **31**, of relevance to many biological effects displayed by this class of compounds, is as a Michael acceptor of thiols **32** leading to the adducts **33**, as pointed out by Kupchan's group.[3,37] It is tempting to assume that exactly such a reaction is responsible for the inhibitory effect on platelet function. Three pieces of evidence support this. Firstly, the addition of cysteine or 2-mercaptoethanol to crude feverfew extracts or to pure parthenolide completely suppresses their ability to inhibit platelet aggregation. Secondly, the inhibitory effects are dose and time-dependent and thirdly, treatment of platelets with feverfew extracts or parthenolide causes a dramatic reduction in the number of acid-soluble thiol groups present.[38] While this may well be a viable explanation for the observed biological activity on PRP *in vitro*, a direct chemical link between feverfew and its prophylactic effect upon the symptoms of migraine remains to be established.

Experimental

General details

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were measured using either a Pye-Unicam SP3-100 or a Philips PU 9706 spectrometer for liquid films, unless otherwise stated. ^{1}H NMR spectra were determined using dilute solutions in deuteriochloroform with tetramethylsilane as internal standard and a Perkin-Elmer R32 CW spectrometer operating at 90 MHz; other instruments employed included Bruker WP80SY (80 MHz) and Bruker AM400 (400 MHz) PFT machines. Couplings (J) are quoted in Hz and standard abbreviations are used to describe multiplicities. ^{13}C NMR spectra were obtained using similar solutions and the Bruker AM400 instrument operating at 100 MHz and were assigned using ^{1}H–^{13}C correlation spectra. Mass spectra were recorded using either a VG 7070E or AEI MS902 instrument operating in the EI mode at 70 eV. Optical rotations were measured using an Optical Activity Ltd. Type AA-10 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. All organic solvents were distilled before use; petrol refers to the fraction of light petroleum with bp 40–60 °C. Ether refers to diethyl ether. All organic solutions were dried using anhydrous magnesium sulfate. Column chromatography (CC) was carried out using either Fluka AG Kieselgel HF_{254} or Kieselgel 60 (Art. 9383) silica gel and the eluents specified in parentheses.

Isolation of feverfew metabolites

Fresh leaves (3.6 kg), taken from flowering plants grown in Sutton Bonington and Castle Donington, North West Leicestershire, were covered with chloroform and the mixture gently agitated for 0.5 h. The chloroform was decanted and the extraction repeated with fresh chloroform. The combined chloroform solutions were dried and evaporated (below 30 °C) to leave a viscous green oil (*ca.* 43 g). A portion (10 g) was roughly separated by CC (gradient elution from 1:1 $CHCl_3$–petrol to 9:1 $CHCl_3$–MeOH). Repeated CC using Kieselgel HF_{254} generally at *ca.* 100:1 loading and pure chloroform or $CHCl_3$–MeOH mixtures then gave pure samples of the various metabolites. The progress of the separations was monitored by TLC and infrared [ν_{max} 1760–1790 cm^{-1} (C=O)] and ^{1}H NMR [δ_H 5.4–5.8 and 6.1–6.4 (both sharp doublets, J *ca.* 3)] spectroscopy. The following metabolites were isolated in a pure state, unless otherwise stated.

Parthenolide 2. Colourless crystals (from ether–petrol), mp 115–116 °C (lit.,[11] mp 115 °C); $[\alpha]_D$ −80.2 (*c* 0.4, CH_2Cl_2) {lit.,[11] $[\alpha]_D$ −78 (*c* 0.25, CH_2Cl_2)}; δ_H(400 MHz) 1.31 (3 H, s, 15-Me), 1.72 (3 H, s, 14-Me), 2.11–2.53 (8 H, m), 2.77–2.83 (1 H, m, 7-H), 2.79 (1 H, d, J 8.5, 5-H), 3.87 (1 H, dd, J 8.5 and 8.5, 6-H), 5.20 (1 H, br dd, J *ca.* 12 and 2.5, 1-H), 5.63 (1 H, d, J 3.5, 13-H$_a$) and 6.34 (1 H, d, J 3.5, 13-H$_b$); δ_C 17.0 (Me), 17.3 (Me), 24.1, 30.7, 36.4, 41.2 (2-, 3-, 8- and 9-CH_2), 47.7 (7-CH), 61.6 (4-C), 66.4 (5-CH), 82.5 (6-CH), 121.3 (13-CH_2), 125.3 (1-CH), 134.6, 139.3 (10- and 11-C) and 169.3 (12-CO).

Epoxyartemorin 4. An oil, ν_{max}($CHCl_3$)/cm^{-1} 3450 and 1770; δ_H(400 MHz) 1.46 (3 H, s, 15-Me), 1.65–2.48 (8 H, m), 2.87 (1 H, d, J 9, 5-H), 3.30 (1 H, m, 7-H), 3.78 (1 H, dd, J 9 and 9, 6-H), 4.37 (1 H, dd, J 11.5 and 4.5, 1-H), 5.25 (1 H, apparent br s, 14-H$_a$), 5.47 (1 H, apparent br s, 14-H$_b$), 5.55 (1 H, d, J 3, 13-H$_a$) and 6.24 (1 H, d, J 3.5, 13-H$_b$).

Canin 11. Colourless crystals, mp 240–242 °C (lit.,[9] mp 246 °C; $[\alpha]_D^{20}$ −13.4 (*c* 0.35, $CHCl_3$) {lit.,[17] $[\alpha]_D^{20}$ −30.5 (*c* 0.67, EtOH)} (Found: C, 64.7; H, 6.6. Calc. for $C_{15}H_{18}O_5$: C, 64.7; H, 6.5%); ν_{max}(KBr)/cm^{-1} 3510, 1755 and 1655; δ_H(400 MHz) 1.16 (3 H, s, 14-Me), 1.45–1.58 (1 H, m), 3 H, s, 15-Me), 1.77–1.91 (1 H, m), 1.96–2.09 (1 H, m), 2.11 (1 H, br s, OH), 2.32–2.43 (1 H, m), 2.55 (1 H, d, J 12, 5-H), 3.30 (1 H, d, J 1, 3-H), 3.42 (1 H, m, 7-H), 3.49 (1 H, d, J 1, 2-H), 4.24 (1 H, dd, J 12 and 9.5, 6-H), 5.51 (1 H, d, J 3.5, 13-H$_a$) and 6.22 (1 H, d, J 3.5, 13-H$_b$); δ_C 19.5 (15-Me), 23.8 (8-CH_2), 27.3 (14-Me), 34.1 (9-CH_2), 44.8 (7-CH), 50.4 (5-CH), 58.4 (3-CH), 58.8 (2-CH), 72.5, 73.8, 79.9 (1-, 4- and 10-C), 78.4 (6-CH), 120.3 (13-CH_2), 139.7 (11-C), and 169.5 (12-CO); m/z 278 (M$^+$, 15%), 151 (33), 112 (53), 111 (100) and 53 (32) (Found: M$^+$, 278.1143. Calc. for $C_{15}H_{18}O_5$: 278.1154).

Tanaparthin-α-peroxide 12. Colourless needles, mp 95–96 °C (decomp.) (lit.,[9] mp 117 °C), $[\alpha]_D^{20}$ −32.1 (*c* 0.11, $CHCl_3$) {lit.,[9] $[\alpha]_D^{24}$ −24 (*c* 0.22, $CHCl_3$)} (Found: C, 64.8; H, 6.6%), ν_{max}(KBr)/cm^{-1} 3580 and 1750; δ_H(400 MHz) 1.40 (3 H, s, 14-Me), 1.72 (3 H, s, 15-Me), 2.66 (1 H, d, J 10, 5-H), 3.37 (1 H, m, 7-H), 3.76 (1 H, dd, J 10 and 10, 6-H), 5.43 (1 H, d, J 3.5, 13-H$_a$), 6.16 (1 H, d, J 3.5, 13-H$_b$), 6.29 (1 H, d, J 6, 3-H) and 6.34 (1 H,

d, *J* 6, 2-H); δ_C 13.7 (15-Me), 22.9 (8-CH_2), 27.7 (14-Me), 33.2 (9-CH_2), 42.9 (7-CH), 69.6 (5-CH), 71.1 (10-C), 79.6 (6-CH), 93.5, 99.9 (1- and 4-C), 119.6 (13-CH_2), 134.0, 137.3 (2- and 3-CH), 140.0 (11-C) and 170.0 (12-CO); *m/z* 278 (M^+, 3%), 260 (9), 246 (20) and 111 (100) (Found: M^+, 278.1150).

Secotanapartholide A 16. A colourless oil, $[\alpha]_D^{20}$ −11.2 (*c* 1.05, EtOH); ν_{max}/cm^{-1} 3510, 1770 and 1715; δ_H(400 MHz) 1.58 (3 H, s, 15-Me), 1.89–1.99 (2 H, m, 8-CH_2), 2.21 (3 H, s, 14-Me), 2.55–2.68 (2 H, m, 9-CH_2), 2.71 (1 H, d, *J* 10.5, 5-H), 3.47 (1 H, m, 7-H), 4.47 (1 H, dd, *J* 10.5 and 2, 6-H), 5.78 (1 H, d, *J* 1.5, 13-H_a), 6.07 (1 H, d, *J* 6, 2-H), 6.35 (1 H, d, *J* 2, 13-H_b) and 7.49 (1 H, d, *J* 6, 3-H); δ_C 25.2 (15-Me), 28.7 (8-CH_2), 30.0 (14-Me), 39.7 (9-CH_2), 42.1 (7-CH), 62.7 (5-CH), 78.7, (4-C), 80.3 (6-CH), 124.8 (13-CH_2), 131.0 (2-CH), 137.6 (11-C), 166.8 (3-CH), 169.8 (12-CO), 202.6 (CO) and 207.7 (CO); *m/z* 278 (M^+, 2%), 260 (8), 163 (15), 112 (19), 98 (11), 94 (22) and 43 (100) (Found: M^+, 278.1155).

Artecanin 15. A colourless oil, ν_{max}/cm^{-1} 3520, 1755 and 1655; δ_H(400 MHz) 1.14 (3 H, s, 14-Me), 1.56 (3 H, s, 15-Me), 2.87 (1 H, d, *J* 12, 5-H), 3.31 (1 H, d, *J* 1, 3-H), 3.31 (1 H, m, 7-H), 3.56 (1 H, d, *J* 1, 2-H), 4.10 (1 H, dd, *J* 10.5 and 10.5, 6-H), 5.44 (1 H, d, *J* 3, 13-H_a) and 6.20 (1 H, d, *J* 3.5, 13-H_b); δ_C 19.7 (15-Me), 22.5 (8-CH_2), 27.8 (14-Me), 37.9 (9-CH_2), 43.0 (5-CH), 44.3 (7-CH), 56.2 (2-CH), 57.5 (3-CH), 71.0, 72.2 (1-, 4- and/or 10-C; one resonance obscured), 82.3 (6-CH), 118.8 (13-CH_2), 139.4 (11-C) and 170.5 (12-CO); *m/z* 278 (M^+, 11%), 151 (23), 112 (61), 111 (100) and 53 (18) (Found: M^+, 278.1151).

Secotanapartholide B 17. A colourless oil, ν_{max}/cm^{-1} 3510, 1775 and 1715; δ_H(400 MHz) 1.60 (3 H, s, 15-Me), 1.89 (2 H, apparent dq, *J ca.* 7 and 1.5, 8-CH_2), 2.19 (3 H, s, 14-Me), 2.33 (1 H, d, *J* 9, 5-CH), 2.45–2.62 (2 H, m, 9-CH_2), 3.54 (1 H, ddddd, *J ca.* 7, 2.7, 2.2, 1.9 and 1.9, 7-H), 4.58 (1 H, dd, *J* 9 and 2.7, 6-H), 5.75 (1 H, d, *J* 1.9, 13-H_a), 6.17 (1 H, d, *J* 5.5, 2-H), 6.35 (1 H, d, *J* 2.2, 13-H_b) and 7.47 (1 H, d, *J* 5.5, 3-H); δ_C 28.4 (8-CH_2), 28.9 (15-Me), 30.0 (14-Me), 39.5 (9-CH_2), 40.9 (7-CH), 58.2 (5-CH), 78.1 (4-C), 80.6 (6-CH), 124.3 (13-CH_2), 133.1 (2-CH), 137.9 (11-C), 165.8 (3-CH), 169.5 (12-CO), 204.7 (CO) and 207.7 (CO); *m/z* 278 (M^+, 6%), 260 (19), 163 (53), 124 (33), 98 (44) and 94 (100) (Found: M^+, 278.1153). A small amount of impurity meant that a meaningful optical rotation value could not be obtained.

Thermal rearrangement of tanaparthin-α-peroxide 12

A solution of tanaparthin-α-peroxide **12** (1 mg) in hexadeuteriobenzene (1 ml) was sealed under nitrogen in an NMR tube which was then heated to 150 °C. Monitoring by ^1H NMR spectroscopy showed complete conversion to canin **11** after 1.5 h at this temperature. The material was pure according to the spectrum which was identical to that displayed by an authentic sample, δ_H(400 MHz, C_6D_6) 0.68 (3 H, s, 14-Me), 1.41 (3 H, s, 15-Me), 2.30 (1 H, d, *J* 12, 5-H), 2.51 (1 H, d, *J* 1, 3-H), 2.67 (1 H, d, *J* 1, 2-H), 3.41 (1 H, dd, *J* 12 and 9, 6-H), 4.81 (1 H, d, *J* 3.5, 13-H_a) and 6.03 (1 H, d, *J* 3.5, 13-H_b).

Lewis-acid catalysed rearrangement of tanaparthin-α-peroxide 12

A stirred solution of tanaparthin-α-peroxide (7 mg) in deuteriochloroform (3 ml) was treated with boron trifluoride–ether (1 drop) and the resulting solution stirred at ambient temperature for 15 h. ^1H NMR analysis indicated the formation of a single secotanapartholide; the solution was washed with (3 ml), dried and evaporated. CC ($CHCl_3$) of the residue separated secotanapartholide A **16** (*ca.* 3 mg) as the sole product which displayed identical spectroscopic data to those given above.

3aβ,11aβ-Decahydro-3-methylenecyclodeca[*b*]furan-2(3*H*)-one 21

(*Z*)-Cyclodecene (7.9 ml, 50 mmol) in refluxing hexane (50 ml) was treated successively with anhydrous triethylamine (7.7 ml, 55 mmol) and a solution of 2-chloropropanoyl chloride (4.4 ml, 45 mmol) in hexane (20 ml). The resulting mixture was vigorously stirred and refluxed for 18 h then cooled, filtered and concentrated by rotary evaporation. The residue was taken up in ether (100 ml) and the solution washed with saturated aqueous sodium hydrogen carbonate (2 × 100 ml) followed by water (100 ml), dried and evaporated to leave the chlorocyclobutanone **24** (12.9 g) as a brown oil. Without further purification, the sample (12.9 g) was dissolved in glacial acetic acid (100 ml), cooled to 0 °C and the solution treated with 30% aqueous hydrogen peroxide (200 mmol). After stirring at this temperature for 24 h, the solution was poured into water (100 ml) and extracted with petrol (3 × 100 ml). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate (2 × 150 ml) and water (100 ml) then dried and evaporated. The residue was purified by CC (gradient from hexane to 1:1 hexane–ether) to give the chlorolactone **25** (7.59 g, 69%) as a pale yellow oil which solidified upon cooling to 0 °C and which showed R_F 0.32 (4% ether–petrol), ν_{max}/cm^{-1} 1780; δ_H 1.70 (3 H, s, Me) and 4.55 (1 H, m); δ_C 21.4 (CH_2), 22.0 (coincident CH_2 and CH_3), 22.4, 24.2, 25.0, 26.3, 27.2 (all CH_2), 51.3 (3a-CH), 68.3 (3-C), 82.3 (11a-CH) and 173.6 (CO); *m/z* 209 ([M^+ − Cl]; 19%), 102 (39), 95 (60), 81 (76), 67 (73), 55 (100) and 41 (78).

The chlorolactone **25** (1.95 g, 8 mmol) in dry dimethyl sulfoxide (50 ml) was treated with diazabicyclo[2.2.0]octane (1.35 g, 12 mmol) and sodium iodide (1.86 g, 12 mmol). The resulting mixture was heated at 80 °C for 48 h, then cooled and poured into a 1:1 mixture of 2 M hydrochloric acid and petrol (200 ml). The organic phase was separated and washed with water (3 × 50 ml), dried and evaporated. CC of the residue (10% ether in petrol) gave the lactone **21** (1.37 g, 82%) as a pale yellow oil which solidified on cooling to 0 °C and which showed R_F 0.13 (10% ether–petrol); ν_{max}/cm^{-1} 1755 and 1660; δ_H 3.10 (1 H, m, 3a-H), 4.72 (1 H, ddd, *J* 7.0, 3.0 and 3.0, 11a-H), 5.51 (1 H, d, *J* 3.0, =CH_a), and 6.15 (1 H, d, *J* 3.0, =CH_b); δ_C 23.3, 23.5, 23.8, 24.7, 25.4, 25.7, 27.1 (all CH_2), 43.1 (3a-CH), 81.0 (11a-CH), 119.6 (12-CH_2), 140.3 (3-C) and 169.8 (CO); *m/z* 208 (M^+, 23%), 179 (28), 124 (62), 109 (25), 82 (70) and 54 (100).[33]

Ethyl (3aβ,8aβ)-octahydro-3-methylene-2-oxo-2*H*-cyclohepta-[*b*]furan-6-carboxylate 22

Cyclohept-4-enecarboxylic acid, mp 66–68 °C (lit.,[34] mp 65–67 °C), was prepared from 1-(cyclopentenyl)pyrrolidine and acrolein according to the method of Stork and Landesman.[34] The acid (4.0 g) was converted into the corresponding ethyl ester **26** by reaction with ethanol (60 ml) in chloroform (40 ml) containing toluene-4-sulfonic acid (0.25 g) at reflux for 18 h. The ester **26** was isolated after standard work-up as a colourless oil, bp 41–43 °C at 0.4 mmHg (lit.,[35] bp 60 °C at 0.01 mmHg).

The *cis*-methylenelactone **22** was then prepared from this exactly as described above for the preparation of the related cyclodecane derivative **21**, by sequential cycloaddition to chloro(methyl)ketene to give the cyclobutanone **27**, Baeyer–Villiger oxidation to the lactone **28** and dehydrochlorination.[33] Similar yields to the foregoing preparations were obtained for each step. The final product was purified by CC ($CHCl_3$) to give the lactone **22** as a colourless oil, R_F 0.20 ($CHCl_3$); ν_{max}/cm^{-1} 1755, 1725, and 1655; δ_H(80 MHz) 1.25 (3 H, t, *J* 7, OCH_2CH_3), 3.25–3.52 (1 H, m, 3a-H), 4.13 (2 H, q, *J* 7, OCH_2CH_3), 4.57–4.85 (1 H, m, 8a-H), 5.59 (1 H, d, *J* 3, 9′-H) and 6.27 (1 H, d, *J* 3, 9-H); δ_C(100 MHz) 14.2 (Me), 26.7, 29.3, 29.7, 30.2, (4-, 5-, 7- and 8-CH_2), 42.9, 47.0 (3a- and 6-CH), 60.5 (11-CH_2), 81.5 (8a-CH), 122.5 (9-CH_2), 139.9 (3-C), 170.0 and 175.1 (2- and 10-CO); *m/z* 238 (M^+, 12%), 220 (30), 210 (43), 192 (91), 165 (100), 136 (71), 119 (94), 101 (53), 88 (55), 79 (76), 73 (60), 67 (58) and 41 (80) (Found: M^+, 238.1200. Calc. for $C_{13}H_{18}O_4$: 238.1205).

(3aβ,8aα)-Octahydro-3-methylene-2-oxo-2*H*-cyclohepta[*b*]-furan-6-carboxylic acid 23a

m-Chloroperoxybenzoic acid (12.94 g, 75 mmol) was added to a stirred solution of ethyl cyclohept-4-enecarboxylate **26** (8.41 g, 50 mmol) in dry dichloromethane (100 ml) and the resulting solution stirred at ambient temperature for 48 h. The resulting precipitate was filtered and the filtrate washed with 2 M aqueous sodium carbonate (2 × 50 ml), then dried and evaporated. The residue was distilled to give the epoxides **29** (7.80 g, 85%) as a colourless oil, bp 85 °C (oven temp.) at 0.3 mmHg (lit.,[35] bp 90 °C at 0.05 mmHg) (Found: C, 65.2; H, 8.8. Calc. for $C_{10}H_{16}O_3$: C, 64.8; H, 8.8%); ν_{max}/cm^{-1} 1730; δ_H(80 MHz) 1.23 and 1.24 (3 H total, 2 × t, both *J* 7, 2 × OCH_2CH_3), 3.05 (2 H, m, 2 × C*H*O), 4.09 and 4.11 (2 H total, 2 × q, both *J* 7, 2 × OCH_2CH_3); δ_C(23 MHz) 13.2 (Me), 25.3, 25.5, 25.6, 26.0, (2-, 3-, 5- and 6-CH_2), 45.1, 46.3, (1- and 7-CH), 53.9 and 54.3, (4-CH), 59.1 (10-CH_2), 173.6 and 174.3 (CO); *m*/*z* 184 (M^+, 2%), 156 (15), 139 (20), 128 (100), 111 (51), 93 (78), 67 (100) and 55 (77).

Sodium (1.21 g, 53 mmol) was added to dry methanol (100 ml). When the metal had all dissolved, freshly distilled dimethyl malonate (11.4 ml, 100 mmol) was added and the solution stirred for 10 min at ambient temperature before the addition of the foregoing epoxides (6.45 g, 35 mmol) in methanol (20 ml) in one portion. The resulting solution was boiled under reflux for 10 days then cooled and poured into a mixture of 2 M hydrochloric acid (200 ml) and chloroform (200 ml). The organic layer was separated and the aqueous phase extracted with fresh chloroform (3 × 100 ml). The combined organic solutions were dried and evaporated and the residue treated with excess ice-cold diazomethane in ether. After 0.5 h at 0 °C, the solvent was removed using a stream of nitrogen and the residue filtered through a pad of silica gel with the aid of ether as eluent. Evaporation of the filtrate gave the lactone diesters **30** (4.90 g) in a semi-pure state. Without further purification, a sample of this product (4.05 g) was dissolved in water (20 ml) and methanol (20 ml) and the solution treated with potassium hydroxide (2.19 g, 30 mmol) then stirred at ambient temperature for 3 h. Aqueous formaldehyde (75 mmol) and diethylamine (7.76 ml, 75 mmol) were then added and the resulting mixture refluxed for 0.5 h, at which point glacial acetic acid (13 ml) and sodium acetate (0.88 g) were added and heating continued for a further 0.25 h. The cooled mixture was neutralized using saturated aqueous sodium hydrogen carbonate (100 ml), washed with ether (2 × 50 ml) then acidified using 3 M hydrochloric acid and extracted with dichloromethane (4 × 50 ml). The combined extracts were dried and evaporated and the residue purified by CC (5% AcOH in $CHCl_3$) to give the required lactone carboxylic acid **23a** (2.08 g, 66%) as a pale solid, crystallization of which gave pure product, mp 113–114 °C (ether–petrol) (lit.,[35] mp 111–114 °C) (Found: C, 62.4; H, 6.9. Calc. for $C_{11}H_{14}O_4$: C, 62.8; H, 6.7%); R_F 0.17 ($CHCl_3$); ν_{max}/cm^{-1} 3700–2400, 1755 and 1685; δ_H(400 MHz) 4.08–4.22 (1 H, m, 8a-H), 5.49 and 5.52 (1 H total, 2 × d, both *J* 3, 9′-H), 6.21 and 6.23 (1 H total, 2 × d, both *J* 3.5, 9-H), *m*/*z* 210 (M^+, 4%), 192 (100), 164 (93), 156 (47), 136 (34), 119 (47), 98 (43), 95 (61), 93 (44), 79 (69), 73 (76) and 67 (77) (Found: M^+, 210.0896. Calc. for $C_{11}H_{14}O_4$: 210.0892).

The corresponding sodium and potassium salts (**23b,c**) were obtained by careful addition of an aqueous solution of one equivalent of either sodium or potassium hydroxide to a suspension of the acid **23a** in water. The resulting solutions were washed with chloroform then evaporated to dryness (below 35 °C) to leave solid, colourless residues of the salts.

Assay for anti-secretory activity

The test compound (2 mg) was suspended in phosphate-buffered saline (500 μl). After centrifugation, a sample (100 μl) of the supernatant was added to platelet-rich plasma (PRP) (460 μl) and the resulting mixture stirred at 37 °C. The PRP was prepared from citrated human blood in which the platelets had been labelled with [^{14}C]-5-hydroxytryptamine (serotonin).[36] After 2 min, a solution of stimulant (adrenaline, arachidonic acid or adenosine diphosphate) (40 μl, to give a final concentration of 100 μM) was added and stirring continued for a further 6 min. Acetylsalicylic acid (50 μl of a 14 mM aqueous solution) was then added and the sample cooled in ice, centrifuged and the amount of [^{14}C]-5-hydroxytryptamine present in the supernatant determined by standard means. A sample of PRP that contained phosphate-buffered saline in place of a test compound was taken through this procedure to quantify the level of secretion in the absence of inhibitory material.

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