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Prostaglandin synthetase inhibitors in feverfew

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Abstract—The IC50 values for the in-vitro inhibition of the prostaglandin synthetase (bovine seminal vesicle mitochondrial fraction) mediated PGE_2 production from arachidonic acid by parthenolide, michefuscalide and chrysanthenyl acetate were $11{\cdot}0 \pm 0{\cdot}44$, $12{\cdot}1 \pm 0{\cdot}51$ and $14{\cdot}2 \pm 0{\cdot}58$ μM (mean ± 95% confidence limits), respectively.

Tanacetum parthenium (L.) Sch. Bip., commonly known as "feverfew", is used as a folk remedy in migraine and arthritis. Its botanical features have been described (Berry 1984) and trials have shown efficacy against migraine with minor side effects (Johnson et al 1985). Although the phytochemistry has now been extensively studied, notably by Bohlmann & Zdero (1982), who listed 39 terpene derivatives, reports of biochemical testing of isolated compounds are scarce. We report the activity of three compounds against the in-vitro conversion of arachidonic acid (AA) to prostaglandins (PGs) by the prostaglandin synthetase enzyme system.

Materials and methods

Materials. [^{3}H] arachidonic acid (AA) (Amersham), AA (99%, Sigma), scintillation cocktail T (BDH), other materials reagent grade.

Extraction. Following the suggestion of Blakeman & Atkinson (1979) that the sesquiterpenes are mainly produced by superficial leaf glands, a procedure was used which did not extract chlorophyll and other (presumably) inactive materials. Fresh leaves were dipped successively into three beakers of chloroform, and the combined extracts dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure in a rotary evaporator <40°C. Thin layer chromatography (TLC) on silica using three solvent systems: chloroform–ethyl acetate (8 : 1), chloroform–ethanol (20 : 1 and 9 : 1) followed by spraying with 50% sulphuric acid and heating at 110°C for 10 min separated the extract into six spots with R_F values from 0·08 to 0·91. It was fractionated by flash chromatography (Clark Still et al 1978) over silica (40–63 μm), column 25 cm × 3·5 mm, using successive 300 mL portions of chloroform-ethanol (20 : 1), ethyl acetate and methanol. 20 mL portions were collected, and TLC showed distinct fractions, I, II and III, corresponding to the eluting solvents. A preliminary PG inhibition test showed that by far the greatest activity was in the most polar fraction, III. Fraction III was streaked on to TLC plates (silica, 0·75 mm), developed firstly by chloroform–ethanol (2 : 1), dried at room temperature and re-developed by cyclohexane–chloroform–ethyl acetate (5 : 1 : 1). Examination under UV light, and spraying screened parts of the plates with 50% sulphuric acid, showed three major bands, which were scraped off and extracted into ether. TLC in a range of solvent systems showed each band to be a single component. The ether was removed by nitrogen and comparison of infra red, proton magnetic resonance and mass spectographic data with the literature values in square brackets (Bohlmann & Zdero 1982; Govindachari et al 1964; Iida & Ito 1982; Matsuo et al 1973; Ogura et al 1977; Uchio 1978) identified the compounds as parthenolide, michefuscalide and chrysanthenyl acetate (Fig. 1).

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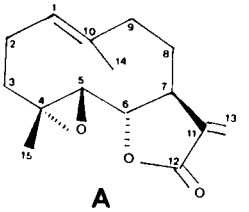

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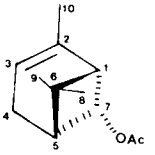

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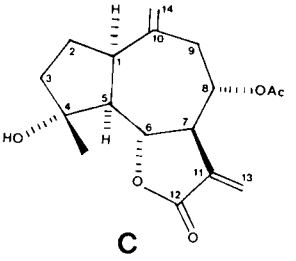

C

Parthenolide (A). Colourless crystals. m.p. 115°C. ^{1}H NMR ($CDCl_3$): δ (ppm) 1·30 [1·29–1·30] (s, 3H, C_4-Me), 1·67 [1·67] (m, 2H, C_8-H_a, C_8-H_b), 1·72 [1·72–1·73] (br s, 3H, C_{10}-Me), 2·12 [2·13] (m, 2H, C_9-H_a, C_9-H_b), 2·41 [2·42] (m, 2H, C_2-H_a, C_2-H_b), 2·73 [2·73] (m, 1H, C_7-H), 2·78 [2·78–2·79] (d, 1H, C_5-H), 3·90 [3·88–3·91] (t, 1H, C_6-H), 5·26 [5·25–5·27] (br, 1H, C_1-H), 5·62 [5·62–5·65,] (d, 1H, C_{13}-H_a), 6·32 [6·31–6·33] (d, 1H, C_{13}-H_b). IR (KBr disc): 1765, 1660 (α, β-unsaturated γ-lactone). MS m/z 248 (M^+, $C_{15}H_{20}O_3$).

Michefuscalide (B). Pale green oil. ^{1}H NMR ($CDCl_3$): δ (ppm) 1·30 [1·36] (s, 3H, C_4-Me), 2·10 [2·16] (s, 3H, $OCOCH_3$), 2·10 (m, 2H, C_2-H_a, C_2-H_b), 2·39 [2·39] (C_5-H), 2·75 (m, 2H, C_3-H), 2·85 (m, 1H, C_7-H), 4·10 [4·04–4·12] (dd, 1H, C_6-H), 4·95 [4·86–5·09] (m, 1H, C_8-H), 5·08 [4·62–5·11] (s, 1H, C_{14}-H_a), 5·20 [4·77–5·17] (br s, 1H, C_{14}-H_b), 6·05 [5·75–6·15] (d, 1H, C_{13}-H_a), 6·25 (d, 1H, C_{13}-H_b). IR ($CHCl_3$): 3450 (O-H), 1770, 1660 (α, β-unsaturated γ-lactone). MS: m/z 306 (M^+, $C_{17}H_{22}O_5$).

cis-Chrysanthenyl acetate (C). Pale yellow-greeen fragrant oil. ^{1}H NMR ($CDCl_3$): δ (ppm) 0·90 [0·88–0·91] (s, 3H, C_9-Me), 1·60 [1·57–1·70] (q, 3H, C_8-Me), 2·01 [2·01] (s, 3H, OCH_3), 2·13 (m, 2H, C_1-H, C_5-H), 2·33 [2·27–2·29] (ddq, 1H, C_4-H_a), 2·43 [2·39–2·43] (ddq, 1H, C_4-H_b), 4·45 [4·45] (s, 1H, C_7-H), 5·20 [5·23] (m, 1H, C_3-H). IR ($CHCl_3$): 3050, 1730, 1380, 1370 [3030, 1740, 1375, 1360]. MS: m/z 194 (M^+, $C_{12}H_{18}O_2$).

Prostaglandin biosynthesis. The method was based on that of Tomlinson et al (1972). Prostaglandin synthetase, a complex containing cyclo-oxygenase, peroxidase and reductase activity, present in bovine seminal vesicular mitochondrial fraction (BSVM), was prepared from frozen material by the method of Hassid & Dunn (1980). Mixed toluene solutions of AA (33 μmol) and [^{3}H]AA (5 μCi) were evaporated under nitrogen in an incubation tube. The residue was mixed with reduced glutathione (7 μmol), adrenaline tartarate (7 μmol) and BSVM (100 mg) in Tris-HCl buffer (0·1 M, pH 8·2, 5 mL) and incubated aerobically with gentle shaking (37°C, 60 min). Controls contained boiled BSVM. The reaction was stopped by citric acid (0·2 M, 5 mL) and the PGs extracted with three portions of ethyl acetate (5 mL), dried over anhydrous sodium sulphate, filtered, and the solvent removed by a stream of nitrogen. The residue was dissolved in acetone (0·2 mL) and resolved by TLC on silica by chloroform-isopropanol-ethanol-formic acid (45:5:0·5:0·3) alongside authentic samples of PGE_2, $PGF_{2\alpha}$ and AA. Spots were visualized by exposure to iodine vapour and by spraying with phosphomolybdic acid (10% in ethanol) followed by heating (120°C, 15 min). Spots developed at R_F 0·22 ($PGF_{2\alpha}$), 0·35 (PGE_2), 0·79 (AA), 0·88 (impurity in AA) and 0·51 (literature values (Flower et al 1973; Srivastara & Awasthi 1983; Harris & Baenziger 1983) suggest PGA_2 or PGD_2). They were scraped into vials of scintillation cocktail T and radioactivity measured in an LKB Wallace 1217 Rackbeta liquid scintillation counter. Typically 8–12% of the AA was converted into PGs in the approximate ratio $PGE_2 : PGF_{2\alpha} : PG_{unidentified}$ of 7:2:1 in terms of counts min^{-1}. This proportion was not significantly changed by the inhibitors, implying blockade at an early step in the AA cascade.

Incubation time. Since sampling at 5 min intervals up to 40 min showed no further increase in PGE_2 production after 15 min, an incubation time of 30 min was used for inhibition studies.

BSVM concentration. PGE_2 production was directly proportional to BSVM concentration up to 1 mg mL^{-1}, above which it remained constant. A BSVM concentration of 5 μg mL^{-1} was used for inhibition studies.

Inhibition studies. [H^3]AA (1 μCi) mixed with AA (20 μmol) in toluene was dried under nitrogen and mixed with inhibitor, which had been pre-incubated for 10 min in 2 mL final volume of reduced glutathione (7 μmol), adrenaline tartarate (7 μmol) and BSVM (10 μg) in Tris-HCl buffer (0·1 M, pH 8·2). The mixture was incubated aerobically with gentle shaking (37°C, 30 min) and the reaction stopped by citric acid (0·2 M, 0·5 mL). Controls contained boiled BSVM. Extraction and TLC were as described above. A channel 1 cm wide was marked along the TLC plate and a 1 cm square marked around the PGE_2 spot. This small area and the remainder of the channel were scraped into counting vials 1 and 2, respectively. The yield of PGE_2 was calculated by:

$$\text{yield } PGE_2 = \text{cpm}(1)/[\text{cpm}(1)+\text{cpm}(2)]$$

where cpm = count min^{-1}.

Aspirin and feverfew fractions I, II and III were used at 200 μg mL^{-1}, and a range of concentrations of III, parthenolide, michefuscalide and chrysanthenyl acetate used to interpolate the concentrations needed to reduce PGE_2 production by 50% (IC50s). The results are in Table 1.

Table 1. Inhibition of PGE_2 production. Confidence limits (p = 0·05) and number of replicates in parentheses.

	% Inhibition	
Inhibitor	IC_{50} (μM)	IC_{50} (μg mL^{-1})
Aspirin 200 μg mL^{-1} 28 (2·0, 4)		
Fraction I 200 μg 0		
Fraction II 200 μg < 1		
Fraction III 200 μg 34 (1·8, 4)		
Parthenolide	11·0 (0·44, 8)	2·73 (0·11, 8)
Michefuscalide	12·1 (0·51, 8)	3·70 (0·16, 8)
Chrysanthenyl acetate	14·2 (0·58, 8)	2·76 (0·11, 8)

Discussion

Collier et al (1980) showed that aqueous extracts blocked PG production from AA in-vitro without blocking cyclo-oxygenase. This activity was lost after boiling. Platelet aggregation studies (Makheja & Bailey 1981) implied a thermostable phospholipase blocker, which inhibited the production of AA from platelet lipids, and the associated release of 5-hydroxytryptamine (5-HT), although later workers could not reproduce their results (Jessup 1982; Heptinstall et al 1985). Capasso (1986) showed that aqueous extracts inhibited the cyclo-oxygenase- and lipoxygenase-controlled pathways, and Heptinstall et al (1985) showed inhibition of 5-HT secretion by platelets and polymorphonucleocytes, which are present in synovial fluid in rheumatoid arthritis. They supported Makheja's view that the thromboxane pathway was unaffected. Jessup (1982) reported activity of three novel methylenic sesquiterpenes which blocked the effect of acetylcholine, 5-HT and histamine on guinea-pig ileum. It is generally accepted that the various biological activities of the sesquiterpene lactones are attributable to Michael addition between a conjugated methylene group and sulphydryl and amino groups on enzymes, and Heptinstall et al (1987) propose this mechanism to explain inhibition of platelet aggregation by the major sesquiterpene present, parthenolide. Inhibition of the prostaglandin synthetase-mediated pathway by parthenolide and michefuscalide could be explained in this way, although it is also possible that they inactivate the cofactor (reduced glutathione) used in the assay by reaction with its -SH groups. We consider this to be unlikely as the inhibitor is present at a much higher concentration than the co-factor (≈ 10 μM and 0·014 μM, respectively) and at this apparently swamping difference level we would not expect to see a dose-related response. It seems possible that chrysanthenyl acetate might act by an acetylation reaction similar to that attributed to aspirin (Roth et al 1975; Roth & Siok 1978).

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The local modulation of vascular permeability by endothelial cell derived products

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Abstract—Endothelin has been shown to suppress increased vascular permeability in the rat at doses of 0·01 pmol. The agonists used were nitric oxide and nitroprusside, which have the same activity as endothelial-derived relaxing factor. Histamine, 5-hydroxytryptamine, platelet activating factor and carrageenan were the other agonists used. It is proposed that endothelin and EDRF act as local hormones produced by endothelial cells to control local vascular permeability.

Yanagisawa et al (1988) isolated a peptide from cultured porcine aortic endothelial cells, which displayed potent vasoconstrictor activity in numerous arterial strips. This peptide, which they named endothelin, has been cloned and sequenced. Furthermore the expression of the endothelin gene was shown to be regulated by several vasoactive agents. In view of its potent vasoconstrictor activity, we have investigated its ability to influence increased vascular permeability induced by an endothelial cell product endothelial-derived relaxing factor, EDRF (Furchgott & Zawadzki 1980), and several other mediators. It has recently been shown that nitric oxide (NO) accounts for the activity of EDRF (Palmer et al 1987). The relaxation effect of EDRF on vascular smooth muscle is mediated by activation of guanyl cyclase, leading to increased intracellular cyclic guanosine monophosphate (cGMP) (Griffith et al 1985).

Materials and methods

Vascular permeability. Male Hooded Lister rats (250 ± 10 g; n = 6) were anaesthetized with ether and their abdominal surfaces shaved. Evans blue (0·5 mL of 0·5% solution in sterile saline) was administered intravenously into the tail. Intradermal injections were given in a volume of 0·1 mL sterile saline according to a Latin square design. After 30 min the animals were killed, the injection sites excised, and the dye was extracted with formamide and assayed spectrophotometrically according to Lykke & Cummings (1969). The permeability enhancing factors were used in amounts to give a leakage of protein-bound dye, expressed in μg Evans blue, at between 5·0 and 7·0. In absolute doses these were histamine 10 μg; 5-hydroxytryptamine (5-HT) 120 ng; bradykinin 2 μg; platelet activating factor (PAF) 40 ng; NO 0·05 mL of a saturated solution in saline mixed with an equal volume of saline; nitroprusside 100 ng; carrageenan 1·0 μg; endothelin 0·01–1 pmol. To study the inhibitory effect of endothelin, it was used at 0·5 pmol injected together with each mediator.

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Results and discussion

Injections of endothelin caused an intense vasoconstriction at all doses tested. In contrast, the permeability inducing agents histamine, 5-HT, bradykinin and PAF all gave a maximal leakage of protein-bound Evans blue. Carrageenan, a commonly used inflammatory irritant, also caused a leakage of Evans blue. Nitric oxide and nitroprusside, both reflecting EDRF activity, also provoked an increase in vascular permeability (see Table 1).

Table 1. Permeability effects of various factors in the presence or absence of endothelin, as judged by leakage of protein bound dye.

	Leakage of protein-bound dye (μg Evans blue	
Substance	Control	With Endothelin
Histamine	6·2 ± 0·7	1·8 ± 0·2
5-HT	7·2 ± 0·6	1·7 ± 0·2
Bradykinin	6·3 ± 0·5	1·2 ± 0·1
PAF	5·9 ± 0·4	1·3 ± 0·1
NO	4·9 ± 0·3	1·8 ± 0·2
Nitroprusside	7·1 ± 0·9	1·6 ± 0·1
Carrageenan	7·4 ± 1·0	1·3 ± 0·1