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Short Communication

Two novel lupane triterpenoids from *Paullinia pinnata* L. with fibroblast stimulatory activity

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Abstract

Objectives Novel lupane triterpenoids from *Paullinia pinnata* L., a Ghanaian plant traditionally used for wound healing, were examined for in-vitro fibroblast stimulatory activity using the 142BR cell line.

Methods Bioactivity-guided isolation of the crude extract of *P. pinnata* L. was carried out in order to determine the nature of the compounds responsible for the stimulation of fibroblast proliferation.

Key findings Two novel compounds were isolated and characterised, namely, 6*β*-(3′-methoxy-4′-hydroxybenzoyl)-lup-20(29)-ene-one (**1**) and 6*β*-(3′-methoxy-4′-hydroxybenzoyl)-lup-20(29)-ene-ol (**2**), together with three known compounds, friedelin (**3**), *β*-sitosterol (**4**) and *β*-sitosterol-3-D-glucoside (**5**). The methanol extract of the roots of *P. pinnata* caused a significant in-vitro increase (94%) in 142BR cell line proliferation at 20 μg/ml compared with the control.

Conclusions Compounds **1** and **2**, which were isolated from the active chloroform fraction, have not previously been reported and showed a dose-dependent increase in proliferation of 142BR cells up to 3 μM; compounds **3**, **4** and **5** had no effect on the 142BR cell line at the concentrations tested.

Keywords fibroblast growth stimulation; 6*β*-(3′-methoxy-4′-hydroxybenzoyl)-lup-20(29)-ene-one; 6*β*-(3′-methoxy-4′-hydroxybenzoyl)-lup-20(29)-ene-ol; *Paullinia pinnata*; wound healing

Introduction

Wound healing is a complex process characterised by inflammation, proliferation and migration of different cell types. Fibroblast cells play a very important role in all these processes.[1] In many cultures around the world, plant extracts, juices and exudates are used in the treatment of both chronic and acute wounds, and scientific interest in plants traditionally used to treat wounds has increased in recent years, particularly with the introduction of some relevant in-vitro bioassays.[2] Attention in our laboratories has focussed on plants used in the Ashanti region of Ghana for healing wounds.[3–7] In a recent study, the methanol extract of *Paullinia pinnata* root demonstrated a strong stimulatory effect on the proliferation of fibroblasts, skin cells that play a vital role in the closure of wounds.[3] *P. pinnata* L. (Sapindaceae) is a tropical plant used traditionally in the treatment of infectious diseases, fractures and as an aphrodisiac.[8] It is also used in wound healing.[9] For this last purpose, the roots are pounded and mixed with water to form a paste that is applied as a poultice over the wound. Previous phytochemical investigations of *P. pinnata* have shown the presence of triterpene saponins and cardiotonic catechol tannins.[10] Other work reported the isolation of two diosmin-related flavone glycosides from the leaves[11] and the isolation of two ceramides, paullinoside A and paullinomide A, with *β*-sitosterol and *β*-amyrin, also from the leaves.[12] However, little is known about the chemical composition of the roots. In view of the activity displayed by the root extract, bioactivity-guided isolation was carried out in order to determine the nature of the compounds responsible for the stimulation of fibroblast proliferation.

Materials and Methods

Plant material

Plant material was collected in August 2005 near Akwapim Mampong, Ghana, and authenticated by Mr Ofori Lartey at the Centre for Scientific Research into Plant Medicine (CSRPM), Akwapim-Mampong, Ghana, where a voucher specimen (8/05/17) has been deposited.

General experimental conditions

Thin layer chromatography (TLC) was carried out with silica gel 60 GF254 (Merck Darmstadt, Germany)/petroleum ether (boiling range 40–60°C) (PE)–ethyl acetate (EtOAc) 8 : 2 using acidic anisaldehyde spray for visualisation. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ on a Bruker DPX 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR) using tetramethylsilane as internal standard. Electron ionisation mass spectrometry (EI-MS) was carried out on a Jeol AX505W mass spectrometer. Electrospray ionisation mass spectrometry (ESI-MS) and high-resolution electrospray ionisation mass spectrometry (HR-ESI-MS) were carried out on a Bruker Apex III FT ion cyclotron resonance mass spectrometer.

Preparation of extract

Dried powdered root (100 g) was packed into a cellulose thimble (28 × 100 mm) and was soxhlet-extracted with 500 ml methanol for 48 h until the material was exhausted, and the extract dried *in vacuo* to give a yield of 4.92% w/w. The fibroblast proliferative activity of this extract has previously been reported.[3]

The methanol extract (2.0 g) was suspended in methanol/water 1 : 1 (100 ml) and extracted with 3 × 100 ml PE, then with 3 × 100 ml chloroform. The three PE extracts were combined and evaporated to dryness (0.41 g) and the same procedure was applied to the chloroform extracts (0.58 g). The aqueous methanol layer was evaporated under reduced pressure to remove MeOH and then lyophilised to dryness (1.01 g).

For testing in the fibroblast proliferation assay, all extracts, fractions and compounds were initially dissolved in dimethylsulfoxide (DMSO), which was then diluted with the medium to give a final concentration of 1% DMSO. This concentration had been shown not to affect proliferation of cells in preliminary experiments (data not shown) and was used as a negative control in each experiment carried out.

Isolation of compounds

Compounds **1** (24 mg) and **2** (33 mg) were isolated from the active chloroform fraction (500 mg) by column chromatography (CC) (silica gel, 70–230 mesh, 200 g) using $CHCl_3$–MeOH (95 : 5) as the mobile phase and 5-ml aliquots were collected. Aliquots were analysed by TLC (silica gel 60 GF254 (Merck)/PE–EtOAc 4 : 1) and bulked into four sub-fractions, A–D, based on TLC zone profiles, and each subfraction was taken to dryness under reduced pressure. Subfraction A (120 mg) was subjected to CC (70–230 mesh, 60 g) using $CHCl_3$–MeOH (90 : 10), and each 5-ml eluant was monitored by TLC as above to yield an intermediate fraction (78 mg), which was further subjected to preparative TLC (silica gel, $CHCl_3$–MeOH, 90 : 10) to yield compounds **1** (24 mg) and **2** (33 mg). Subfraction B (132 mg) was also subjected to CC (silica gel $CHCl_3$–MeOH, 95 : 5) to obtain compounds **3** (16 mg) and **4** (29 mg), which were crystallised in acetone. Compound **5** (13 mg) was obtained after CC of subfraction C (42 mg) ($CHCl_3$–MeOH, 80 : 20), followed by preparative TLC (silica gel, $CHCl_3$–MeOH, 85 : 25).

In-vitro test for fibroblast growth stimulation

Confluent fibroblasts (142BR; Sigma, Dorset, UK) were trypsinised, centrifuged and resuspended in minimum essential medium (MEM)/15% fetal bovin serum (FBS)/1% L-glutamine. The cells were counted using a haemocytometer and the suspension standardised at a concentration of 1×10^4 cells/ml in MEM/15% FBS/1% L-glutamine.

Using a multichannel pipette, the cells were seeded at a density of 1×10^3 cells per well in a 96-well plate, excluding the first row. The plates were maintained at 37°C in a humidified incubator with a 5% CO_2 : 95% air atmosphere. The medium was replaced after 24 h with MEM containing 0.5% FBS and a range of concentrations of the chloroform fraction of the original methanol extract (1–50 μg/ml), six replicates of one concentration for each column on the microtitre well plate, except for two of the columns that were maintained at MEM/0.5% FBS and MEM/15% FBS to serve as negative and positive controls, respectively. The 0.5% FBS concentration is a maintenance dose needed for the production of healthy cells but does not significantly stimulate proliferation of cells, while 15% FBS stimulates proliferation of fibroblasts. The cells were incubated and assayed after 5 days using the neutral red assay method[13] to assess the effect of the extract on the growth of the cells. The procedure was repeated using the isolated compounds **1**–**5** over a concentration range of 0.1–5.0 μg/ml. The percentage proliferation for each well for each concentration tested was calculated according to the following formula:

% proliferation = (absorbance due to sample/absorbance due to 0.5% FBS alone) × 100

The mean value of percentage proliferation ± SEM was calculated for each concentration and these values were plotted against concentration for compounds **1**–**5**. The same calculation was done for the cells treated with 15% FBS, the positive control.

Results

Identification of compounds

Compound **1** was isolated as a white amorphous powder, $[\alpha]_D^{25}$ +152.2 ($CHCl_3$). Low resolution (LR) EI-MS m/z (rel. int., %) 590 (30) (accurate mass m/z 590.3970), 420 (55), 203 (40), 168 (75), 150 (100), 107 (20), 55 (15). The accurate mass of peak at m/z 590.3970 agrees with molecular formula $C_{38}H_{54}O_5$ (calculated mass m/z 590.3971). ^{1}H-NMR and ^{13}C-NMR: Table 1.

Compound **2** was isolated as a pale yellow amorphous powder, $[\alpha]_D^{25}$ + 62.7 ($CHCl_3$). LR-EI-MS m/z (rel. int., %) 592 (30) (accurate mass m/z 592.4130), 424 (46), 203 (15), 186 (100), 133 (23), 81 (20). The accurate mass of the peak at m/z 592.4130 agrees with molecular formula $C_{38}H_{56}O_5$ (calculated mass m/z 592.4128). ^{1}H-NMR and ^{13}C-NMR: Table 1.

Table 1 ^{1}H and ^{13}C NMR spectroscopic data (δ = pm in CDCl$_3$) for **1** and 2

Atom number	Type	δC	δH	δC	δH
		1	**2**		
1	CH_2	32.9	2.75 (H-α) *ddd* (*J* = 2.9, 6.4, 14.2 Hz), 2.30 (H-β) *ddd* (*J* = 2.9, 6.4, 14.2 Hz)	43.0	1.31 (H-α) *ddd* (*J* = 2.9, 6.4, 14.2 Hz, 1.34 (H-β) *ddd* (*J* = 2.9, 6.4, 14.2 Hz)
2	CH_2	39.9	1.90 (H-α) *ddd* (*J* = 2.9, 6.4, 15.0 Hz), 1.71(H-β), *ddd* (*J* = 2.9, 6.4, 15.0 Hz)	27.1	1.71(H-α) *m*[a], 1.60 (H-β) *m*[a]
3	C	218.4	–	78.4	3.23, *dd* (*J* = 3. 8, 11.7 Hz)
4	C	46.7	–	38.8	–
5	CH	56.1	2.13 *d* (*J* = 7. 4 Hz)	58.5	1.30 *d* (J = 7.2 Hz)
6	CH	72.4	5.35 *ddd* (*J* = 7.4, 7.0, 3.6 Hz)	71.6	5.59 *ddd* (*J* = 7.2, 7.0, 3.6 Hz)
7	CH_2	41.5	1.90 (H-α) *dd* (*J* = 12.0, 3.6 Hz), 1.54(H-β) *dd* (*J* = 12.0, 7.0 Hz)	42.9	1.90 (H-α) *dd* (*J* = 12.0, 3.6 Hz), 1.54(H-β) *dd* (*J* = 12.0, 7.0 Hz)
8	C	39.9	–	39.9	–
9	CH	49.0	1.53 *dt* (*J* = 12.0, 1.5 Hz)	49.0	1.39 *dt* (*J* = 12.0, 1.5 Hz)
10	C	35.3	–	35.3	–
11	CH_2	21.8	1.52, 1.27 *dd*[a]	21.8	1.52, 1.27 *dd*[a]
12	CH_2	25.1	1.51, 1.28 *dd*[a]	25.1	1.51, 1.28 *dd*[a]
13	CH	37.7	1.40 *m*[a]	37.7	1.40 *m*[a]
14	C	42.9	–	42.9	–
15	CH_2	29.8	1.26 *m*[a]	29.8	1.26 *m*[a]
16	CH_2	35.3	1.49 *m*[a], 1.34 *m*[a]	35.3	1.49 *m*[a], 1.34 *m*[a]
17	C	41.5	–	41.5	–
18	CH	47.9	1.43 *m*[a]	47.9	1.43 *m*[a]
19	CH	46.7	2.39 *ddd* (*J* = 2.59, 5.4, 11.1 Hz)	46.7	2.39 *ddd* (*J* = 2.39, 5.7, 10.9 Hz)
20	C	150.7	–	150.7	–
21	CH_2	29.8	1.63 (H-α) *m*[a], 1.38 (H-β) *m*[a]	29.8	1.63 (H-α) *m*[a], 1.38 (H-β) *m*[a]
22	CH_2	39.7	1.51(H-α) *d*[a], 1.40 (H-β) *d*[a]	39.7	1.51(H-α) *d*[a], 1.40 (H-β) *d*[a]
23	CH_3	31.5	1.33 *s*	30.8	1.23 *s*
24	CH_3	19.6	1.09 *s*	15.7	0.84 *s*
25	CH_3	17.6	0.85 *s*	18.0	0.78 *s*
26	CH_3	16.1	1.23 *s*	17.2	1.23 *s*
27	CH_3	14.4	0.98 *s*	14.5	0.98 *s*
28	CH_3	17.9	0.79 *s*	17.9	0.79 *s*
29	CH_2	109.4	4.54 *d* (*J* = 2.5 Hz) , 4.67 *d* (*J* = 2.5 Hz)	109.4	4.58 *d* (*J* = 2.5 Hz) , 4.69 *d* (*J* = 2.5 Hz)
30	CH_3	19.3	1.68 *s*	19.3	1.68 *s*
1′	C	124.0	–	124.0	–
2′	CH	111.8	7.58 *d* (*J* = 1.8 Hz)	111.8	7.58*d* (*J* = 1.8 Hz)
3′	C	150.7	–	150.7	–
4′	C	146.3	–	146.3	–
5′	CH	114.1	6.95 *d* (*J* = 8.3 Hz)	114.1	6.96 *dd* (*J* = 8.3 Hz)
6′	CH	122.7	7.63 *dd* (*J* = 8.3, 1.8 Hz)	122.7	7.61 *dd* (*J* = 8.3, 1.8 Hz)
	OCH_3	55.9	3.94 *s*	55.9	3.94 *s*
	OH	–	6.02 *br s*	–	6.02 *br s*
	C=O	165.4	–	165.4	–

[a]Deduced from ^{1}H-^{1}H 2D COSY, HMQC and HMBC spectra but coupling constants cannot be calculated due to overlapping signals.

Compound **3** was isolated as fine needles, m.p 258–259°C. Melting point, MS, ^{1}H-NMR and ^{13}C-NMR data agreed with those for friedelin.[14] Compound **4** consisted of white needle crystals, m.p 138–139°C. Melting point, IR, MS, ^{1}H-NMR and ^{13}C-NMR data agreed with those for β-sitosterol.[15] Compound **5** was a white amorphous powder. The EI-MS (M^+ 456) and ^{13}C-NMR data agreed with literature values for β-sitosterol acetate.[15]

Fibroblast proliferation

When tested for fibroblast proliferation, the PE and aqueous methanol extracts were cytotoxic (data not shown), but the chloroform fraction showed a dose-dependent proliferative effect with an EC50 (the dose giving 50% increase in cell number determined by the neutral red assay) calculated as 7.6 μg/ml (data not shown).

Compounds **1**–**5** were isolated from the chloroform fraction and evaluated for their human fibroblast proliferation actions. Compounds **1** and **2** exhibited a dose-dependent proliferative action on the 142BR cell line over a relatively low concentration range, 0.17–3.4 μM (Figure 1), but compounds **3**, **4** and **5** showed no proliferative action on the 142BR cell line. Compounds **3**, **4** and **5** exhibited a cytotoxic effect at concentrations of 5 μg/ml and above (data not shown).

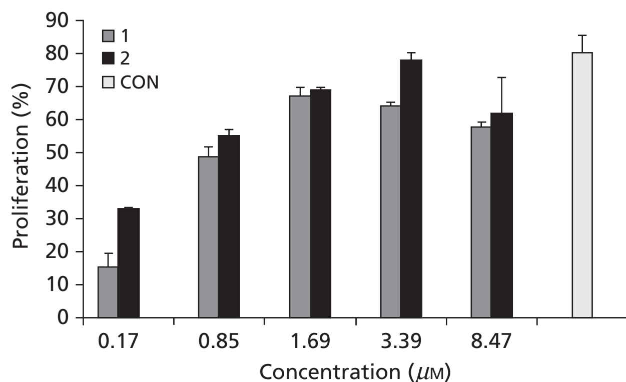

Figure 1 Effect of compounds **1** and **2** on 142BR cell proliferation. CON, 15% fetal bovine serum (positive control). Values represent the percentage increase in cell number compared with the negative control (0.5% fetal bovine serum) ± SEM, n = 6

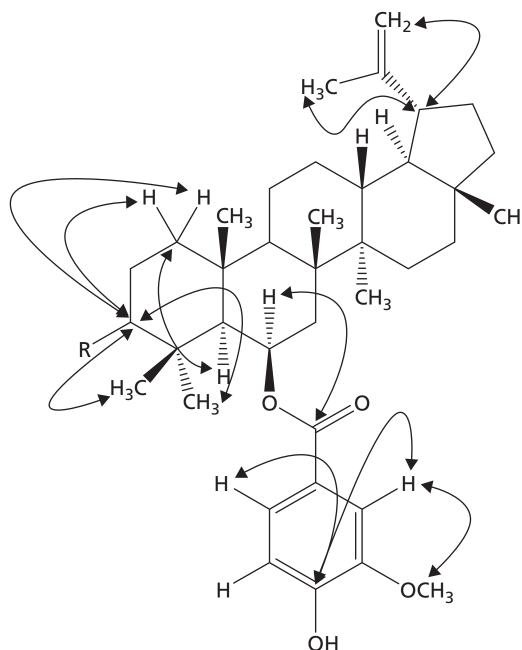

Figure 2 Important correlations from the HMBC spectrum of compound **1**

Discussion

Structure of compounds 1 and 2

Compound **1** was deduced to have molecular formula $C_{38}H_{54}O_5$ by comparison of the measured mass of the molecular ion at m/z 590.3970 with the calculated m/z 590.3971. The IR spectrum gave peaks at 3526 and 1701 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups, respectively. Total assignments were performed on the basis of heteronuclear multiple bond correlation (HMBC), heteronuclear multiple quantum correlation (HMQC) and ^1H–^1H correlation spectroscopy (COSY) spectra. The ^1H-NMR spectrum of compound **1** displayed a large cluster in the 'methylene envelope' (δ 2.6–0.7) region, suggesting the possibility of a steroidal or triterpenoid structure. The ^1H-NMR spectrum showed signals attributable to the presence of the lup-20(29)-ene system[16] at δ 4.69 (1H, *d*) and 4.58 (1H, *d*), typical of the 29-exomethylene in lupenes, and at 1.68 (3H, s) attributable to the 30-methyl group in this type of compound. A carbinol methine proton at δ 5.59, (1H, *m*) is similar to the chemical shift of the carbinol methine proton at H-6 in 6-hydroxy lupane derivatives,[17] which is at about δ 4.69. The more downfield signal in compound **1** indicates an ester attachment rather than OH at C-6. Characteristic aromatic proton signals at δ 7.63 (1H, *dd*, J = 8.3 Hz), 7.58 (1H, *d*, J = 1.83 Hz) and 6.95 (1H, *dd*, J = 8.3, 1.8 Hz) were observed, in addition to the signals usually shown by triterpenoids, indicating that the esterified group may comprise a substituted aromatic ring. The ^{13}C-NMR DEPT data indicated the presence of eight methyl carbons, including a methoxy at δ 56.0, 10 methylene carbons, nine methine carbons and 11 quaternary carbons, including one carbonyl carbon at δ 218.4 and another at δ 165.4, this latter signal further confirming an esterified substituent on the triterpenoid. Apart from six aromatic signals and the methoxy at δ 56.0, the signals observed were very similar to those given by a lupane ring system, for example the presence of a lowfield methylene carbon at δ 109.2 corresponding to the ^1H NMR signals seen at δ 4.69 and 4.58. The HMQC spectra enabled correspondence of assignment of the ^1H and ^{13}C NMR signals for each methyl group. It was noted that the signal for the 23-Me was more downfield than reported for the previously isolated 6β-hydroxy lupane derivative,[18] and this was ascribed to the proximity of the esterified aromatic ring. The position of the ketone (δ 218.4) at C-3 in the triterpenoid skeleton was confirmed by HMBC experiments, which showed a ^3J correlation of this quaternary carbon with methyl protons for H-23 (δ 1.33), H-24 (δ 1.09) and with a methylene group (δ 2.27 and 2.17) considered to be at position 1 (Figure 2). The ^{13}C NMR signal for C-1 (δ 42.9) is more downfield than that in the model compound reported by Machocho *et al.*,[17] but that paper does not make clear how signals were assigned and it is possible that a mistake was made. HMBC experiments show unequivocally that the signals can only be ascribed to the 1-CH_2, since the ^{13}C at δ 42.9 showed ^3J correlation with the ^1H signal at δ 2.13 (5-CH), which showed strong ^1H–^1H coupling with the signal at δ 5.59 (6-CH), which showed HMBC ^3J correlation with the ester carbonyl signal at δ 165.4. The 1-CH_2 proton signals at δ 2.75 and 2.30 also gave HMBC ^3J correlation with the C-3 carbonyl signal δ 218.4. The HMBC spectrum also confirmed the presence of the exomethylene and methyl side chain at C-19, characteristic of lupanoids. Based on the above deductions and comparison with literature values for a non-esterified 6β-hydroxy lupane,[18] a partial structure of compound **1** was suggested to be lup-20(29)-ene-3-one, with an esterified aromatic group.

The identity of this aromatic group and its position of attachment to the lupanoid system were established mostly from HMBC and nuclear Overhauser effect spectrometry (NOESY) experiments (see Figures 2 and 3). The aromatic ring appeared to be substituted at 3′ and 4′, due to the ABX splitting pattern of the three protons. A peak in the ^1H-NMR spectrum indicated the presence of one aromatic methoxyl group in the molecule (singlet at δ 3.94) and a signal at δ 56.0 in the ^{13}C-NMR spectrum. The methoxyl group was

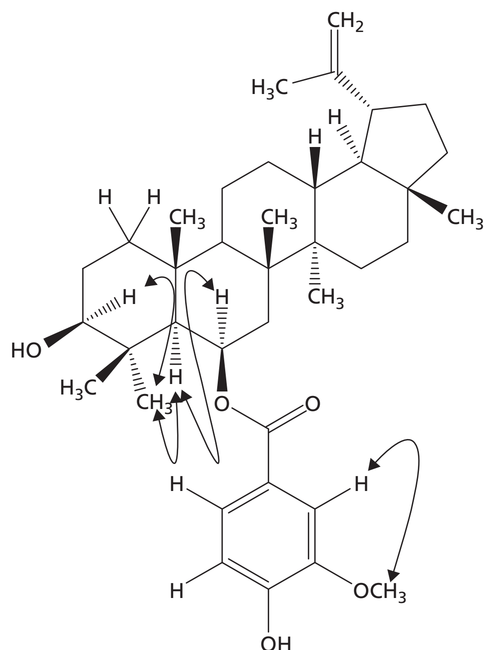

Figure 3 Important NOESY correlations for compound **2** (identical in compound **1** except for H-3)

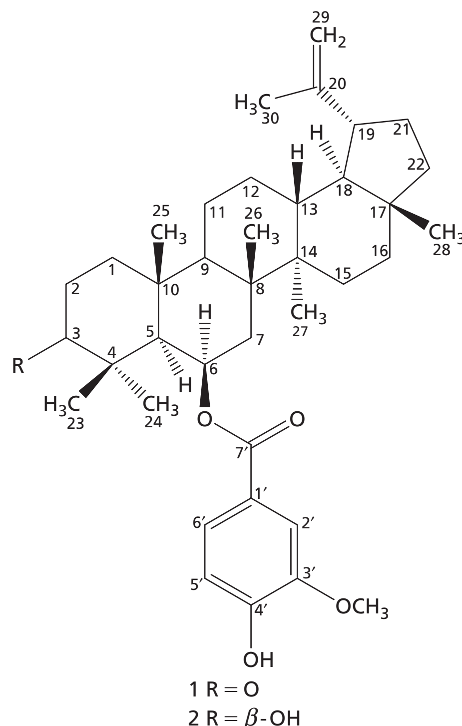

Figure 4 Structures of compounds **1** and **2**

considered to be attached to C-3′ due to its NOESY correlation with the 2′-H (δ 7.58). C-4′ had a HMBC correlation with the aromatic protons H-2′(δ 7.58) and H-6′ (δ 7.63). As well as the above considerations from the NMR spectra, 4-OH, 3-OMe substitution was favoured over 4-OMe, 3-OH because of the similarity in signals with ferulic acid rather than isoferulic acid. The HMBC spectrum showed that the ester carbon (δ 165.4) had a long range correlation with the methine proton at δ 5.35. The observed lowfield chemical shift of this proton is likely to be the result of its deshielding by the ester linkage to the 3-methoxy-4-hydroxybenzoyl substituent. HMBC data further indicated that C-6 was the most likely point of attachment, with the H in α configuration, since, in the NOESY spectrum the H-6 signal at δ 5.35 correlated with the C-H signal at δ 2.13 (5-CH), which is in α configuration.[17] The NOESY spectrum also showed in-space proximity between the 5-H and the 24-Me at δ 1.09, which has an axial (α) orientation.

The complete structure of compound **1** is therefore proposed to be 6β-(3′-methoxy-4′-hydroxybenzoyl)-lup-20 (29)-ene-one (Figure 4).

The IR, ^{1}H-NMR and ^{13}C-NMR signals for compound **2** were similar to those seen for compound **1**, with the notable absence of the carbonyl signal at 1701 cm^{-1} in the IR spectrum, and, in the ^{13}C-NMR spectrum, the signal for the C-3 ketone seen at δ 218.4 in compound **1**. However an additional methine signal at δ 78.4 was observed in the ^{13}C NMR spectrum. In the ^{1}H-NMR spectra of compound **2**, a sharp peak at δ 3.23, not seen in compound **1**, was observed, attributable to a methine proton with a geminal OH, and in the HMBC spectrum this was seen to correspond with the methine signal at δ 78.4 in the ^{13}C-NMR spectrum. Differences in the shifts of the ^{1}H and ^{13}C signals for positions 1 to 6 in the lupane skeleton between compounds **1** and **2** were observed (see Table 1), which consolidates the argument that the C-3 ketone has been replaced by OH, and the presence of two extra H atoms seen in the molecular formula derived from the accurate MS measurement of the molecular ion (calculated mass m/z 592.4128 conforms most closely with $C_{38}H_{56}O_5$) supports this.

The position at C-3 of the methine carbon (δ 78.4) in the triterpenoid skeleton was confirmed by HMBC experiments, which established that the methine carbon C-3 had a ^{3}J correlation with methyl protons H-23 (δ 30.8, 1.23), H-24 (δ 15.7, 0.84) and a ^{3}J correlation with the methylene protons H-1 (δ 1.31 and 1.34). H-3 was considered to be in axial α configuration because it showed NOESY correlation with the 23-CH_3 signal at δ 1.33. Compound **2** was therefore considered to be the C(3)-α-OH analogue of compound **1**, that is 6β-(3-methoxy-4-hydroxybenzoyl)-lup-20(29)-ene-3-ol (Figure 4).

This is the first report of lupane triterpenes with an aromatic ring esterified at C-6.

Proliferative effect on fibroblasts

Compounds **1** and **2** displayed considerable proliferative activity (Figure 1) giving EC50 values of 0.87 μM and 0.81 μM, respectively, with a maximum effect at 1.69 μM for compound **1** and 3.39 μM for compound **2**. Above these concentrations, there was an indication of a decrease in proliferative activity, which might be due to cytotoxicity. Insufficient amounts of the compounds prevented a greater number or range of concentrations being tested to determine whether higher concentrations decreased proliferation proportionately. The two compounds appear to have very similar activity. These effects on the 142BR cell line are remarkable and are comparable with the 15% FBS control used in the experiment, which gave an increase in proliferation of 82%.

Conclusions

Although a paste made with water is traditionally used to help wounds to heal, in this study, a methanolic extract was shown to induce proliferation, with the highest activity being shown by a fraction eluted from silica with chloroform. It was from this fraction that the two active compounds were isolated. The possibility of such relatively non-polar compounds being present in significant amounts in a water-based paste could be argued to be unlikely, but it must not be forgotten that solubilisation of relatively insoluble compounds by other ingredients in the plant material may occur, as happens for artemisinin in aqueous extracts of *Artemisia annua*. These findings suggest that fibroblast cell proliferation may be one of the ways by which *P. pinnata* exerts its wound healing effects, and compounds **1** and **2** play a part in this. The fact that cytotoxicity might occur at higher concentrations raises questions as to the safety of the traditional use of *P. pinnata* plant material since dosage cannot be controlled in these situations.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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