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Psilostachyin, acetylated pseudoguaianolides and their analogues: Preparation and evaluation of their anti-inflammatory potential

Renu Chib [a], Bhahwal Ali Shah *[,a], Naveen Anand [a], Anjali Pandey [a], Kamini Kapoor [b], Sarang Bani [a], Vivek K. Gupta [b], Rajnikant [b], Vijay K. Sethi [a], Subhash Chandra Taneja *[,a]

[a] Natural Product Microbes Division, Indian Institute of Integrative Medicine, Canal Road, Jammu 180 001, J&K, India
[b] X-ray Crystallography Laboratory, Post-Graduate Department of Physics, University of Jammu, Jammu, Tawi 180 006, India

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ABSTRACT

Naturally occurring acetylated pseudoguaianolides and psilostachyin including their analogues were synthesized. The structure of semi-synthetic psilostachyin was also confirmed by X-ray crystallography. The anti-inflammatory potential of all the derivatives has been evaluated through in vitro expression of TNF-α, IL-1β and IL-6 in murine neutrophils.

The occurrence of sesquiterpene lactones (SLs) is wide spread in nature, yet their physiological functions in plants and other sources have not been fully understood, though many are known to contribute towards defence mechanisms, may act as poisons for livestock and also act as insect feeding deterrents.[1] The natural SLs, which are having a diverse range of structures and bioactivities like anti-inflammatory, antibacterial, ameobicidal, anticancer etc., constitute interesting targets for further investigations and exploitations in the area of drug development.[2] The SLs, psilostachyin (**1**) and acetylated pseudoguaianolides (**2**, **3**) have been isolated from *Ambrosia psilostachya*[3] and *Parthenium hysterophorus*,[4] respectively, (Fig. 1). Psilostachyin was also isolated from *A. artimisifolia*,[5] *A. tenuifolia*[6] and other species of *Ambrosia*.[7] It is reported to possess anticancer,[8,9] anti-inflammatory,[10] leishmanicidal,[11] and molluscicidal[12] activities. Acetylated pseudoguaianolides have also been shown to possess significant anticancer activity.[4]

The activity of SLs especially cytotoxicity appears to be associated with their ability to act as alkylating agents by virtue of Michael addition of biological nucleophiles like cysteine to α-methylene part of γ-lactone moiety.[13] Their development as potential therapeutic agents was hampered because of the irreversible addition of biological nucleophiles to α-methylene moiety and any changes in its reactivity resulted in complete loss of the activity. Interestingly, recent studies indicate that irreversible binding may be an important factor against drug resistance especially in cancer therapy.[14] One important SL, that is, parthenin (**4**) isolated from *Parthenium hysterophorus* is known to possess anti-tumor,[15] anti-inflammatory,[16,17] ameobicidal activity[18] and trypanocidal activity.[19] It is also believed that this lactone is responsible for the allergic contact dermatitis in humans.[20] Since, *P. hysterophorus* is an obnoxious weed with an undemanding and extensive mode of adaptation behavior, its exploitation in the development of molecules of therapeutic potential will have enormous social and environmental impacts. Thus, in continuation of our work on the isolation and structural modifications of natural products to develop bioactive lead molecules particularly in the area of inflammation and cancer,[21] we envisaged semi-synthesis of naturally occurring acetylated pseudoguaianolides (**2** and **3**) and psilostachyin (**1**) including their analogues and evaluation of their anti-inflammatory potential through in vitro expression of TNF-α, IL-1β and IL-6 in murine neutrophils.

Our efforts initiated with the isolation of parthenin (**4**) and coronopilin (**12**) from *P. hysterophorus* by an earlier reported method.[21c] For semi-synthetic modifications, we firstly acetylated the hydroxyl group at C-1 in **4**. Since the hydroxyl at C-1 is tertiary, therefore, Lewis acid catalyzed acetylation at C-1 was visualized, which proceeded through acylium ion formation, a reaction similar to Friedel–Craft acylation. Thus, parthenin in presence of $InCl_3$ as catalyst was treated with acetic anhydride to facilitate the acetylation, giving the product (**5**) in almost quantitative yields (Scheme 1). Subsequently propionate and butyrate derivative, that is, **6** and **7** were also synthesized by using propionic and butyric anhydride, respectively.

* Corresponding authors.
E-mail addresses: bashah@iiim.ac.in (B.A. Shah), sctaneja@iiim.ac.in (S.C. Taneja).

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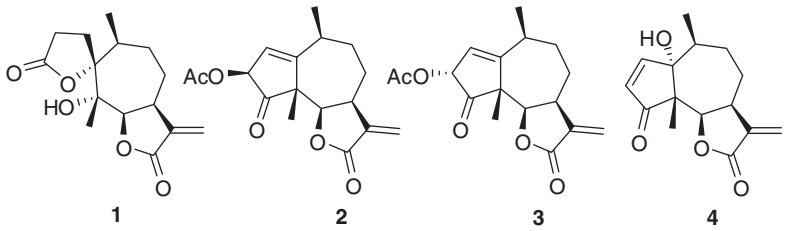
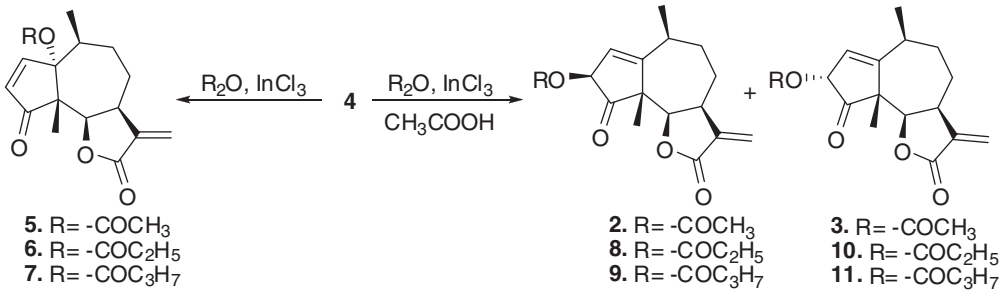


Figure 1. Naturally occurring SLs.



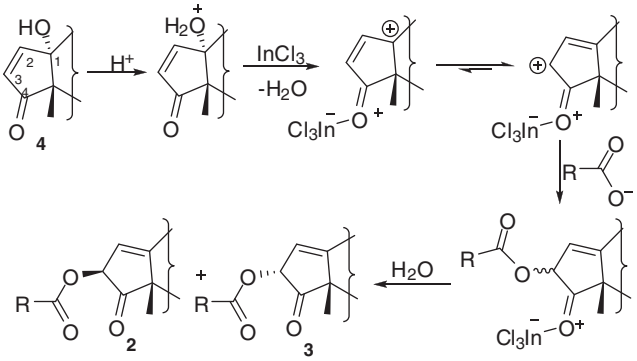
Scheme 1. Synthesis of various acylated pseudoguaianolides

The same reaction facilitated the migration of double bond and acylation at C-3, when performed in presence of acetic acid, acetic anhydride and $InCl_3$, thus leading to the direct synthesis of acetylated pseudoguaianolides (Schemes 1 and 2). The reaction probably proceeds via protonation of C-1 hydroxyl by acetic acid, which facilitates its elimination and generation of carbocation at C-3. At first glance the formation of **2** and **3** appears as a consequence of the reactivity umpolung. However, the formation of the carbocation at C-1 followed by 1, 3-shift of the double bond facilitates the generation of the carbocation at C-3 and finally leading to the synthesis of both the isomers of the acetylated pseudoguaianolides (**2** and **3**) in equal proportions. The presence of the Lewis acid ($InCl_3$) also facilitates the stabilization of the carbocation and the generation of acetate ion from anhydride, which concomitantly attacks at C-3, resulting in the formation of the desired isomers **2** and **3**. The plausible mechanism is depicted in Scheme 2. In order to clear the ambiguity that the acetate anion is derived from anhydride and not from the corresponding acid, the reaction was carried out in presence of trifluoroacetic acid instead of acetic acid. The reaction afforded the same products, that is, **2** and **3**, thereby supporting the observation that the acetate ion is derived from anhydride. Moreover, the formation of propionate and butyrate analogues (**8**, **9**, **10** and **11**) using propionic and butyric anhydride, respectively, in presence of acetic acid as shown in Scheme 1 also supports the plausible mechanism.

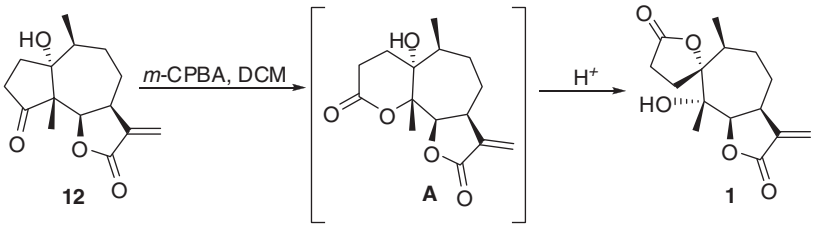
In the literature, the synthesis of psilostachyin (**1**) is reported from coronopilin (**12**) using peracetic acid.[3] In the present work, we synthesized **1** by subjecting **12** to Bayer–Villiger oxidation[21b] using *m*-CPBA. The intermediate δ-lactone (**A**) formed equilibrated into the more stable spirolactone **1** in presence *m*-chlorobenzoic acid formed in situ (Scheme 3). The higher stability of the spiro γ-lactone scaffold prompted us to study the structural features including the conformations of the three rings in **1**. Therefore, it was envisaged to carry out its X-ray crystallographic analysis. The energy minimization calculations using impact minimization tool of Schrodinger software with OPLS_2005 force field disclosed that the spiro derivative (**1**) is at a lower energy state (33.3 kcal) compared to δ-lactone (**A**, 40.5 kcal) which is apparently more strained.

An ORTEP view of **1** with atomic labeling is shown in Figure 2.[22] The XRD studies showed that in **1**, bond lengths and bond angles are in agreement with those reported for other structure determinations of sesquiterpene lactones.[23,24] The torsion angles of the cycloheptane ring have C_2 symmetrical values [$\Delta C_2 = 8.50$] and the ring is therefore in a twist-chair conformation. The pseudo-twofold axis bisects the C1–C10 bond and passes through C7. The spiro lactone ring has a half-chair conformation [$\Delta C_2 = 1.67$] with a pseudo-twofold axis running through the C4 carbonyl atom. The cis-fused lactone ring at C6–C7 has envelope conformation with C7 at the flap [$\Delta C_s = 4.78$]. Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-795880.

Parthenin was further subjected to a series of reactions (Scheme 4) to generate a library of analogues based on psilostachyin and related δ-valerolactones. Thus, Bayer–Villiger oxidation[21b] of parthenin in presence of *m*-CPBA, gave δ-valerolactone (**13**) (Scheme 4). In the next step it was treated with TFA under reflux to form a rearranged product, that is, spirolactone **14** in almost quantitative yield. Besides, **13** was acylated at C-1 using acyl anhydride in presence of $InCl_3$ as catalyst to afford corresponding acylates **16-18** (Scheme 4). Modifications were also envisaged in the α-methylene-γ-lactone part of the lactones via 1,3-dipolar cycloaddition



Scheme 2. Plausible mechanism of synthesis of acylated pseudoguaianolides



Scheme 3. Synthesis of psilostachyin

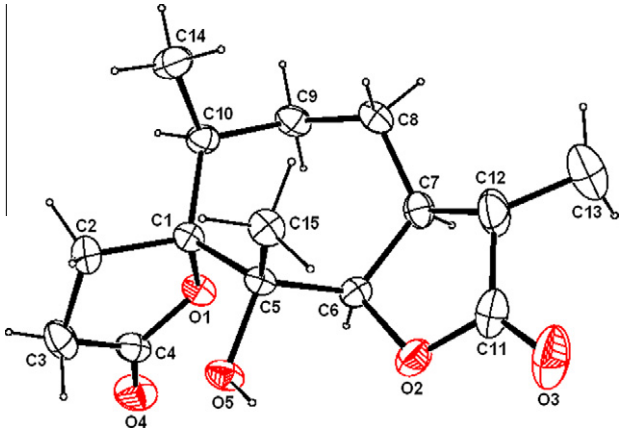


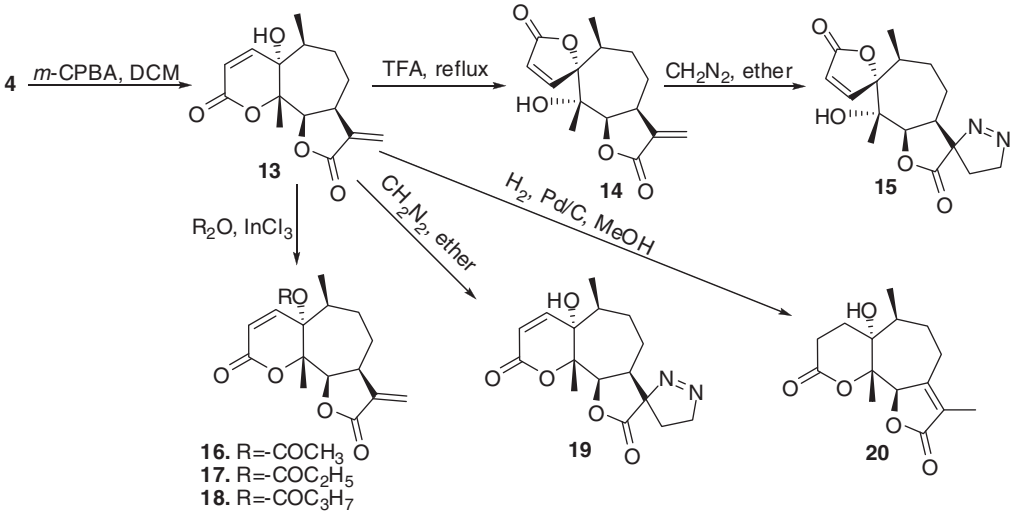
Figure 2. *ORTEP* view of the molecule, showing the atom-labeling scheme. Note: Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

of diazomethane on exocyclic double bond. Thus, lactones **13** and **14** on treatment with diazomethane afforded corresponding azaspiro lactones **15** and **19**, respectively, (Scheme 4). Furthermore, through ene migration the exocyclic double bond in the α-methylene-γ-lactone moiety **13** underwent a facile isomerization to butenolide **20** during catalytic hydrogenation with Pd/C and hydrogen gas at atmospheric pressure (Scheme 4).

The flow cytometry (FCM) studies were carried out on all the analogues to determine TNF-α cytokine expression in LPS activated human neutrophils. The neutrophils are involved in the phagocytosis, release of proteolytic enzymes, generation of active free radicals, synthesis of cytokines, chemokines and lipid mediators causing inflammation. Studies have demonstrated that the recruitment of leukocytes into an inflamed area is mediated by various peptides and other factors which in turn amplify the inflammatory response by their effects on macrophages and lymphocytes.[25] Initially, TNF-α was chosen as the target for anti-inflammatory activity screening. The analogues displayed a range of TNF-α expression inhibition from low to high. It was quite interesting to observe that some of the compounds displayed better TNF-α inhibition than parthenin and were found to be efficacious in suppressing intracellular TNF-α level at the dose of 1 μg/ml when compared to the LPS control. Parthenin itself displayed significant inhibition of the expression of TNF-α in the range of 39.45%. From the results given in Table 1, it is clear that at 1 μg/ml concentrations, the analogues **15**, **19** and **20** displayed maximum inhibitory effects, that is, 49.21%, 59.76% and 53.12%, respectively, on TNF-α cytokine secretion in murine isolated neutrophils in response to LPS stimulant.

Since, analogues **15**, **19** and **20** displayed maximum inhibitory effect on TNF-α cytokine secretion; these samples were further evaluated for their effect on the expression of extracellular IL-β and IL-6 levels. The results displayed that the three compounds **15**, **19** and **20** significantly suppressed extracellular IL-1β expression level in LPS activated neutrophils at dose level of 1 μg/ml. Rolipram a standard inhibitor showed 64.27% inhibition when compared with lipopolysaccharide (LPS) stimulated untreated neutrophils (Table 2). The molecules **15**, **19** and **20** also suppressed extracellular IL-6 expression level at the dose of 1 μg/ml but the inhibition of expression was not significant (Table 3).

In conclusion, subsequent to the successful semi-synthesis of three natural SLs viz., acetylated pseudoguaianolides and psilostachyin, a series of analogues were prepared for the evaluation of their anti-inflammatory potential. Interestingly, the compounds



Scheme 4. Synthesis of analogues of parthenin

Table 1
In vitro tumor necrosis factor-α (TNF-α) expression

Entry	Compound	Concn (μg/ml)	Mean ± SE	%TNF-α expression
	Naïve control	–	0.65 ± 0.07	–
	LPS control	–	2.56 ± 0.04	–
1	**1**	1	1.55 ± 0.04	39.45↓
2	**2**	1	2.42 ± 0.04	5.46↓
3	**3**	1	2.50 ± 0.03	2.34↓
4	**4**	1	2.57 ± 0.02	0.39↑
5	**5**	1	2.59 ± 0.03	1.17↑
6	**6**	1	3.00 ± 0.07	17.18↑
7	**7**	1	2.20 ± 0.07	14.06↓
8	**8**	1	2.29 ± 0.01	10.54↓
9	**9**	1	2.73 ± 0.07	6.64↑
10	**10**	1	2.80 ± 0.05	9.37↑
11	**11**	1	1.74 ± 0.05	32.03↓
12	**12**	1	2.74 ± 0.04	7.03↑
13	**13**	1	1.50 ± 0.07	41.41↓
14	**14**	1	1.76 ± 0.06	31.25↓
15	**15**	**1**	**1.30 ± 0.07**	**49.21**↓
16	**16**	1	2.13 ± 0.04	16.79↓
17	**17**	1	1.87 ± 0.04	26.95↓
18	**18**	1	2.24 ± 0.05	12.50↓
19	**19**	**1**	**1.03 ± 0.07**	**59.76**↓
20	**20**	**1**	**1.20 ± 0.05**	**53.12**↓
21	Rolipram (standard)	100	0.73 ± 0.08	71.48

No. of observations-3; LPS: lipopolysaccharide; ↓ – inhibition of TNF-α expression.

Table 2
Effect of compounds **15**, **19** and **20** on expression of extracellular Interleukin-1β (IL-1β)

Compd	Concn (μg/ml)	Concn of IL-1β (pg/ml) Mean ± SE	% IL-1β inhibition against LPS control
LPS control	–	115.01 ± 3.06	–
15	1	73.55 ± 2.87	36.04↓
19	1	67.43 ± 4.01	41.37↓
20	1	68.01 ± 2.55	40.86↓
Rolipram		(standard)	100
		41.09 ± 3.09	64.27↓

No. of observations-3; LPS: lipopolysaccharide; ↓ – decrease in extracellular IL-1β expression.

Table 3
Effect of compounds **15**, **19** and **20** on expression of Interleukin-6 (IL-6)

Compd	Concn (μg/ml)	Concn of IL-6 (pg/ml) Mean ± SE	% IL-6 inhibition against LPS control
LPS Control	–	317.39 ± 6.81	–
15	1	281.44 ± 4.51	11.32↓
19	1	272.91 ± 3.11	14.01↓
20	1	263.23 ± 7.04	17.06↓
Rolipram (standard)	100	202.81 ± 4.71	36.10↓

No. of observations-3; LPS: lipopolysaccharide; ↓ – decrease in extracellular IL-6 expression.

without α-methylene moiety viz., **15**, **19** and **20** displayed better anti-inflammatory potential compared to the compounds having α-methylene moiety when evaluated through in vitro expression of TNF-α, IL-1β and IL-6 in murine neutrophils. Moreover, there was a significant improvement in anti-inflammatory activity compared to the parent molecules, thus, underlining the need for further development. As mentioned earlier the development of SLs with α-methylene-γ-lactone moiety was hampered because of the irreversible addition of biological nucleophiles to α-methylene group, but in our case it was interesting to observe that the dipolar cycloaddition on exocyclic double bond (compounds **15** and **19**) and its migration during catalytic hydrogenation (**20**) increased the activity of the resulting molecules. Thus, is significant to note that the presence of α-methylene-γ-lactone moiety is not essential for TNF-α activity in the present series of molecules, besides, retaining of bioactivity even after the modifications of exocyclic double bond is also noteworthy. The presence of spiro-diazo group also did not affect the expression TNF-α in compounds **15** and **19**. Thus, SLs with interesting structural profiles and ubiquitous presence in natural kingdom present an important platform for further development into the products of therapeutic significance.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.037.

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