Glutathione *S*-Transferase, Acetylcholinesterase Inhibitory and Antibacterial Activities of Chemical Constituents of *Barleria prionitis*

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Z. Naturforsch. **2007**, *62b,* 580 – 586; received August 10, 2006

Phytochemical studies on the ethanolic extract of *Barleria prionitis* of Sri Lankan origin have resulted in the isolation of a new compound, balarenone (**1**), along with three known compounds, pipataline (**2**), lupeol (**3**) and 13,14-*seco*-stigmasta-5,14-diene-3-α-ol (**4**). The structures of **1** – **4** were elucidated with the aid of extensive NMR spectroscopic studies. Compounds **1** – **4** showed moderate inhibitory activity against glutathione *S*-transferase (GST) and acetylcholinesterase (AChE). Compounds **1**, **2** and **4** also exhibited antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* (25 μg/disk). Three different derivatives of compound (**2**), 7,8-epoxypipataline (**5**), 8-amino-7-hydroxypipataline (**6**) and 7,8-dibromopipataline (**7**) were synthesized to evaluate them for GST and AChE inhibitory activities. Household microwave radiations were used to synthesize compound (**6**). Among all tested compounds, 8-amino-7-hydroxypipataline (**6**) exhibited a significant AChE inhibitory activity with an IC_{50} value of 36.8 μM.

Key words: *Barleria prionitis*, Balarenone, Pipataline, Lupeol, 13,14-*seco*-Stigmasta-5,14-diene-3-α-ol, Glutathione *S*-Transferases, Acetylcholinesterase

Introduction

The naturally-occurring enzyme inhibitors play an important role in a drug discovery program. Glutathione *S*-transferases (GSTs) are a family of enzymes that catalyze the conjugation of the tripeptide, glutathione, to endogenous and xenobiotic substrates having electrophilic functional groups. They play an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds. The glutathione adducts produced have increased solubility in water and are subsequently enzymatically degraded and excreted. In general, GST binds a wide range of molecules of diverse structures hence its prospect as a target for chemotherapy [1]. Acetylcholine is the neurotransmitter at all preganglionic autonomic, parasympathetic and some sympathetic postganglionic nerve endings, at the neuromuscular junction and at some CNS synapses. Acetylcholinesterase (AChE) is an enzyme that degrades acetylcholine and is used as a marker for cholinergic neural function. Acetylcholinesterase inhibitors work by blocking the action of acetylcholine, and this mechanism is the most promising symptomatic therapeutic approach for the treatment of Alzheimer's disease. Based on this hypothesis, AChE inhibitors have extensively been studied over the last two decades and this has resulted in the discovery and development of several natural and synthetic AChE inhibitors [2].

Barleria prionitis is an annual shrub, 1 – 3 feet high, which is distributed throughout Africa, India, Sri Lanka and Tropical Asia [3]. *B. prionitis* Linn (Acanthaceae) is locally known as "Vajradanti" in India and "Katukaradu" in Sri Lanka, and is widely used in folk medicines. For instance, the dried plant is used to treat whooping cough and asthma in infants and children. The leaves are chewed to relieve toothache and the paste of the root is applied to disperse boils and glandular swellings. In India and Thailand, the decoction of leaves and flowers of *B. prionitis* is used in the treatment of viral fever [4]. The plant shows biological activity against respiratory syncytial virus [5] and also has been reported as anti-arthritic, anti-inflammatory [4] and anti-fertility [6] agent. The aqueous bioactive fractions are reported to possess hepatoprotective, anti-stress and immunorestorative properties [7]. Pre-

0932–0776 / 07 / 0400–0580 $ 06.00

viously, iridoid, iridoid glycosides and unsaponified materials were reported from various parts of *B. prionitis* [8 – 11].

We are involved in the discovery of new GST and AChE inhibitors from different natural sources including plants, microorganisms and marine organisms. During our screening program, the crude extract of *B. prionitis* exhibited GST inhibitory activity with an IC_{50} value 160 $\mu g\,mL^{-1}$. Additionally, the crude extract of this plant also showed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in our initial antibacterial screening. Our recent phytochemical studies on the ethanolic extract of *B. prionitis* yielded one new compound, balarenone (**1**) along with three known natural products, pipataline (**2**), lupeol (**3**), and 13,14-*seco*-stigmasta-5,14-diene-3-β-ol (**4**). The structures of compounds **1** – **4** were established with the aid of extensive NMR spectroscopic studies. Compounds **1** – **4** exhibited moderate GST and AChE inhibitory activities. Compounds **1**, **2** and **4** also showed antibacterial activity against *Bacillus cereus* and *P. aeruginosa*. Compound **2** was isolated in a quantity sufficient to prepare three derivatives and evaluate them for GST and AChE inhibitory activities. It was decided to synthesize 7,8-epoxypipataline (**5**), 8-amino-7-hydroxypipataline (**6**) and 7,8-dibromopipataline (**7**) by using compound **2** as a main precursor. Compound **6** was prepared by using household microwave radiations. During GST and AChE inhibition assays, it was discovered that compound **6** exhibited significant AChE inhibitory activity with an IC_{50} value of 36.8 μM. In this paper, we report the isolation, structure elucidation of compounds **1** – **4** and structural modifications of compound **2**, as well as their bioactivity data.

Results and Discussion

The first compound, balarenone (**1**), was obtained as a white amorphous solid from the crude ethanolic extract of *B. prionitis*. The molecular ion peak of this compound was determined with the aid of the chemical ionization mass spectrum (CIMS) which featured the $[M+H]^+$ ion at *m/z* = 463. The high resolution electron-impact mass spectrum (HREIMS) of **1** also showed the M^+ ion at *m/z* = 462.2403 corresponding to the molecular formula $C_{29}H_{34}O_5$ (calcd. 462.2406). This indicated the presence of thirteen double bond equivalents in **1**. The IR spectrum of **1** displayed intense absorption bands at 1739 (C=O) and 1652 (C=C) cm^{-1}.

The ^1H NMR spectrum ($CDCl_3$, 300 MHz) of **1** displayed a two-proton signal at δ = 5.91 due to the protons of the methylenedioxy moiety. A one-proton resonance at δ = 6.90 (d, J = 1.6 Hz) was assigned to the C-3 methine proton. Two signals at δ = 6.75 (J = 8.0, 1.6 Hz) and 6.74 (J = 8.0, 0.7 Hz) were due to the aromatic C-5 and C-6 methine protons, respectively. H-5 and H-6 showed vicinal couplings with each other in the COSY-45° spectrum. The C-5 methine proton (δ = 6.75) also exhibited a cross peak with the C-3 methine proton (δ = 6.90), representing a *meta* coupling between these protons. The doublet at δ = 6.32 (J = 16.0 Hz) was due to the C-7 olefinic proton and it exhibited vicinal coupling with the C-8 methine proton (δ = 6.08). The ^1H-^1H coupling constant data of H-7/H-8 (J = 16.0 Hz) indicated the *trans* orientation of a C-7/C-8 double bond. The C-8 methine proton further showed vicinal couplings with the allylic C-9 methylene protons (δ = 2.17). The rest of protons in **1** resonated at δ = 1.34 (s, H_2-10, H_2-11 and H_2-12). The C-13 methylene protons (δ = 2.20), adjacent to the carbonyl group, showed cross peaks with a signal appearing at δ = 1.34 due to the C-10, C-11 and C-12 methylene protons. The signal at δ = 1.34 also exhibited COSY-45° interactions with the C-9 methylene protons (δ = 2.17).

The ^{13}C NMR spectrum of compound **1** showed the resonance of fifteen carbon atoms. An attached proton test (APT) experiment was used to establish the multiplicity of each carbon signal. These ^{13}C NMR data indicated the presence of six CH_2, five CH and four quaternary carbon atoms in **1**. Two olefinic signals at δ = 129.6 and δ = 129.2 were due to the C-7 and C-8 carbons, respectively. The three sp^2 hybridized carbons that resonated at δ = 148.5, 148.0 and 128.5 were ascribed to C-1, C-2 and C-4 of the aromatic ring, respectively. The downfield chemical shift values of C-1 and C-2 indicated the substitution of methylenedioxy functionality on these carbons. The carbonyl carbon appeared at δ = 210.2 while a signal at δ = 100.5 was assigned to the methylenedioxy carbon atom. The HSQC spectrum of **1** was also recorded to establish $^1H/^{13}C$ one-bond shift correlations of all protonated carbon atoms. The mass spectrum of **1** provided the molecular formula $C_{29}H_{34}O_5$. The ^1H and ^{13}C NMR data indicated the presence of fifteen carbon atoms and seventeen protons in **1**. The EIMS showed two prominent ions at *m/z* = 217 and 245, which resulted from the cleavage of C-13/C-14 and C-14/C-13′ bonds (bonds on either side of the C-14 carbonyl group). A com-

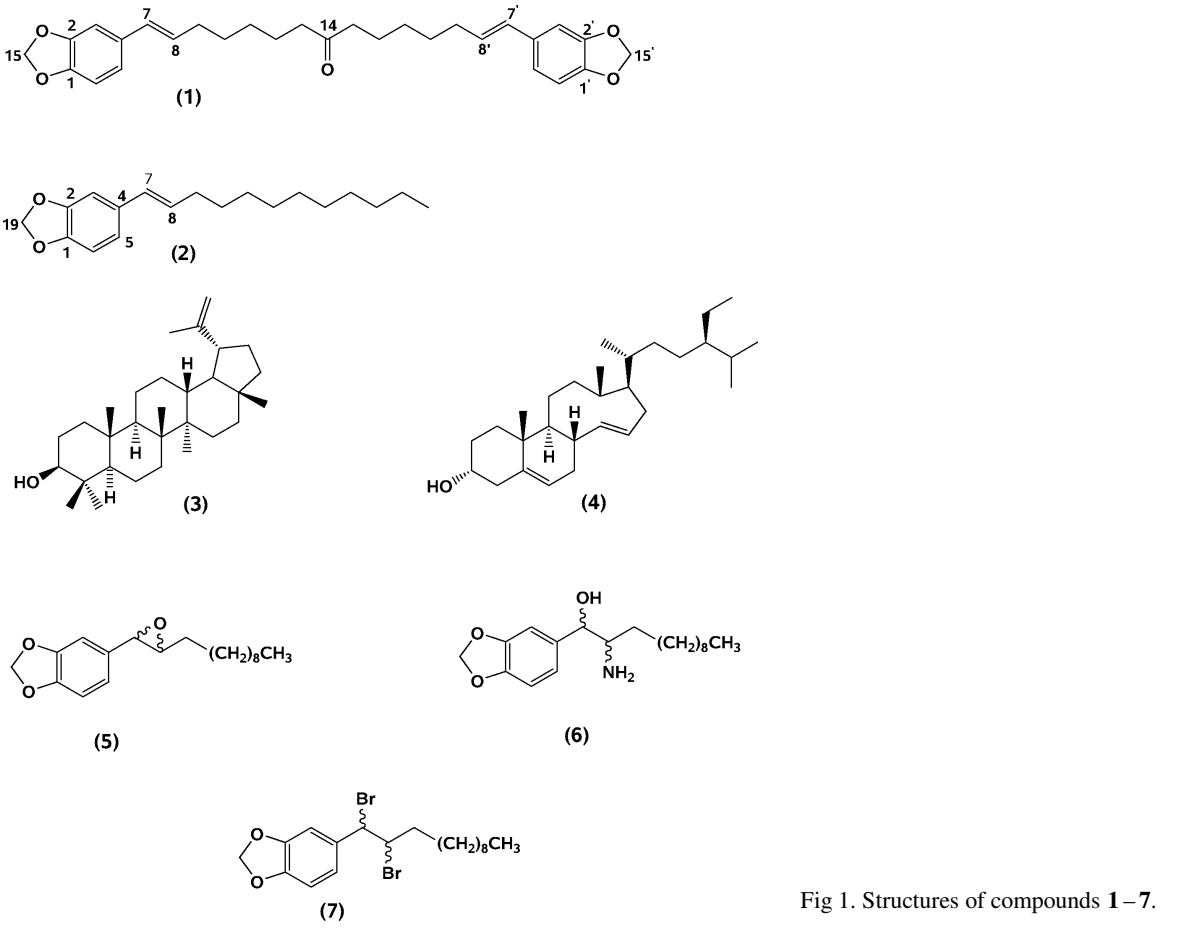

Fig 1. Structures of compounds **1** – **7**.

bination of mass, ^{1}H, ^{13}C NMR and HMBC spectral data indicated the presence of a plane of symmetry at the carbonyl carbon in **1**. Therefore, one half of the molecule was observed in the ^{1}H and ^{13}C NMR spectra. Based on these spectral data, structure **1** was proposed for this new natural product.

Additionally, we have also isolated three known natural products, pipataline (**2**), lupeol (**3**) and 13,14-*seco*-stigmasta-5,14-diene-3-α-ol (**4**). Their ^{1}H, ^{13}C NMR and mass spectral data were distinctly similar to those reported in the literature [12 – 14]. Previously, compounds **2** to **4** were isolated from *Piper peepuloides*, *Holarrhena floribunda* and *Phyllanthus amarus*, respectively [12 – 14]. To the best of our knowledge, we are reporting the isolation of compounds **2** – **4** for the first time from *B. prionitis*.

Pipataline (**2**) showed a higher GST inhibitory activity as compared to compounds **1**, **3** and **4**. It was decided to synthesize three derivatives, 7,8-epoxypipataline (**5**), 8-amino-7-hydroxypipataline (**6**) and 7,8-dibromopipataline (**7**) in order to investigate the role of a double bond adjacent to the benzene ring in its bioactivity. 7,8-epoxypipataline (**5**) was prepared by the reaction of compound **2** with *m*-chloroperbenzoic acid [15]. Compound **5** was identified with the aid of its mass spectrum, which showed the molecular ion peak at *m/z* = 304. The presence of an epoxide at C-7/C-8 was also confirmed from the ^{1}H NMR spectrum which showed up-field resonances of C-7 and C-8 methine protons at δ = 3.37 (1H, d, J = 2.0 Hz) and 2.88 (1H, ddd, J = 8.4, 2.4 and 2.0 Hz), respectively. The ^{13}C NMR spectrum also featured the resonances of C-7 and C-8 at δ = 63.0 and 58.6, respectively. These spectroscopic studies helped to identify compound **5** as 7,8-epoxypipataline.

8-Amino-7-hydroxypipataline (**6**) was synthesized by reacting compound **5** with a 28 % solution of NH_3 using household microwave radiations as a cat-

Table 1. GST and AChE inhibitory activities (IC_{50}= μmol) of compounds **1** – **7**.

Compound	Inhibition activity (μmol) GST	AChE
1	48.50 ± 0.381	200.09 ± 0.570
2	57.0 ± 0.370	135.00 ± 0.5
3	60.00 ± 0.270	89.97 ± 0.121
4	248.00 ± 0.130	105.16 ± 0.121
5	86.9 ± 0.215	164.00 ± 0.112
6	53.2 ± 0.225	36.75 ± 0.272
7	90.4 ± 0.470	208.0 ± 0.372

alyst [15]. The formation of **6** was confirmed by recording the mass, ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of **6** showed the resonance of H-7 and H-8 at δ = 3.70 (1H, d, J = 2.30 Hz) and δ = 2.87 (ddd, J = 9.7, 3.2 and 2.30 Hz), respectively. EIMS also showed the molecular ion peak at m/z = 321. These spectroscopic studies aided the characterization of compound **6** as 8-amino-7-hydroxypipataline. 7,8-dibromopipataline (**7**) was prepared by the method reported by Motsarev *et al.* [16]. The ^{1}H, ^{13}C NMR and mass spectral data (see Experimental Section) confirmed the formation of compound **7**.

Enzyme inhibition assays

The crude extract of *B. prionitis* showed weak GST inhibitory activity (IC_{50} = 160 μg/mL). Compounds **1** – **7** exhibited enzyme inhibitory activity to certain degrees (Table 1). During this bioassay, we discovered a promising GST inhibitory activity of compounds **2** and **6** with IC_{50} values of 57.0 and 53.2 μM, respectively. Compounds **1** – **7** also exhibited moderate AChE inhibitory activity (Table 1). Compound **6** showed a significant AChE inhibitory activity with an IC_{50} value of 36.8 μM.

Antibacterial activity

Compounds **1** – **4** were also screened against seven pathogenic bacteria (listed in the Experimental Section), and compounds **1**, **2** and **4** exhibited antibacterial activity against *B. cereus* and *P. aeruginosa* (Table 2).

In summary, we have isolated and characterized four compounds (**1** – **4**). Compound **1** was found to be new and **2** – **4** were known compounds, isolated for the first time from *B. prionitis*. Additionally, we have attempted the preparation of three derivatives of compound **2**, 7,8-epoxypipataline (**5**), 8-amino-7-hydroxypipataline (**6**) and 7,8-dibromopipataline (**7**). Unfortunately, we could not establish the stereochemistry at C-7 and C-8 in compounds **5** – **7**, primarily due to low quantities of these compounds, and because we were interested primarily in the study of the bioactivities of these derivatives. Compounds **1** – **7** were found to be active in GST and AChE inhibition assays. Compounds **1**, **2** and **6** showed higher enzyme inhibitory activity as compared to **3** – **5** and **7**. The significant increase in bioactivity of compound **2** and **6** may be attributed to the presence of a $\Delta^{7,8}$ double bond and an amino group, respectively. We also discovered higher AChE inhibitory activity of compound **6** and this may be due, probably, to the presence of the C-8 amino group.

Table 2. Antibacterial activities of crude extract, **1**, **2** and **4**.

Compound	Diameter of zone inhibition (mm) *S. aureus*	*B. cereus*	*P. aeruginosa*
Crude extract	9 ± 0.1	–	15 ± 0.26
1	–	19 ± 0.17	18 ± 0.03
2	–	15 ± 0.26	20 ± 0.10
4	–	25 ± 0.20	–
Standard	25	11 ± 0.26	24 ± 0.20

Standard = Ceftriaxone (30 μg) per disk.

Experimental Section

General

The UV spectra were recorded on a Shimadzu UV-250 PC spectrophotometer. IR spectra were recorded in a Michelson-Bomen Hartmann and Braun (MB-series) spectrometer on KBr disks. EI/CI MS data were obtained on a Hewlett Packard 5989B spectrometer using the direct insertion probe (DIP) method. 1D and 2D NMR data were recorded on a Bruker Avance 300 MHz, and ^{13}C NMR spectra were recorded on the same instrument at 75 MHz. Enzyme inhibitory activity for both bioassays was measured spectrophotometrically using a HP 8452 Diode Array spectrophotometer. Column chromatography was carried out on silica gel (200 – 400 mesh). Thin-layer chromatography (TLC) was performed on TLC aluminum sheets coated with a 0.25 mm layer of silica gel G_{60} as purchased from Merck. Fractions were monitored by TLC and spots were visualized by heating the silica gel plate and spraying it with 10 % H_2SO_4. All solvents used were purchased from Fisher Scientific. Equine liver glutathione *S*-transferase (GST), acetylthiocholine iodide, acetylcholinesterase, sodium phosphate, and *m*-chloroperbenzoic acid (*m*CPBA) were purchased from Sigma Aldrich. Sodium phosphate and 5,5′-dithiobis[2-nitrobenzoic acid] were purchased from VWR, glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) from MP Biomedicals, ammonium hydroxide (28 %) and bromine solution from EMD chemicals.

Plant materials

The aerial part of *B. prionitis* was collected from Gampaha, Western province of Sri Lanka in September 2004. The plant was identified by Dr. Radhika Samarasakera, and a voucher specimen was deposited with the Natural Products Development Group at Industrial Technology Institute, Colombo, Sri Lanka.

Extraction and isolation

The aerial part of *B. prionitis* (2.2 kg) was dried in air and macerated into a powdered form, which was extracted with 95 % ethanol at r. t. Filtration and evaporation of the solvent *in vacuo* afforded the gum (72.5 g). The crude extract was fractionated by column chromatography over silica gel (200 – 400 mesh) using hexane-ethyl acetate (0 – 100 %) and ethyl acetate-methanol (0 – 100 %). This yielded several fractions, which were pooled on the basis of similarity in R_f values on analytical TLC. Fraction F-2 (230 mg), obtained on elution of the primary silica gel column with hexane-ethyl acetate (3 : 1), was re-chromatographed over another silica gel column using a stepwise gradient solvent system of hexane-ethyl acetate (0 – 100 %) to purify compound **1** (8.2 mg, R_f = 0.41 in 9 : 1 hexane-ethyl acetate) as a white amorphous powder. Fraction F-3 (102.5 mg), also obtained on elution of the primary silica gel column with hexane-ethyl acetate (1 : 1), was subjected to silica gel column chromatography using hexane-ethyl acetate (0 – 100 %) to afford compound **2** (9.1 mg, R_f = 0.7 in 3 : 2 hexane-ethyl acetate) as a white powder. Compounds **3** (white crystalline solid, 6.2 mg, R_f = 0.67 in 4 : 1 hexane-ethyl acetate) and **4** (white powder, 6.2 mg, R_f = 0.34 in 1 : 1 hexane-ethyl acetate) were purified using prep. TLC of a fraction obtained on elution of the primary silica gel column with hexane-ethyl acetate (4.5 : 5.5).

*Balarenone (**1**)*

White amorphous powder. – UV (MeOH): λ_{max} = 264 nm. – IR (KBr): ν_{max} = 1739 (C=O), 2921 (C–H), 1652 (C=C), 1248 (C–O) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.90 (s, 1H, H-3), 6.75 (dd, *J* = 8.0, 1.6 Hz, 1H, H-5), 6.74 (dd, *J* = 8.0, 0.7 Hz, 1H, H-6), 6.32 (d, *J* = 16.0 Hz, 1H, H-7), 6.08 (dt, *J* = 16.0, 6.87, 3.2 Hz, 1H, H-8), 5.91 (s, 2H, H_2-15), 2.20 (t, *J* = 5.7, 2.6 Hz, 2H, H-13), 2.17 (m, 2H, H-9), 1.34 (s, 6H, H_2-10, H_2-11, H_2-12). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 210.2 (–C–, C-14), 148.5 (–C–, C-1), 148.0 (–C–, C-2), 129.6 (CH, C-7), 128.5 (–C–, C-4), 129.2 (CH, C-8), 105 (CH, C-6), 103.2 (CH, C-5), 100.5 (CH_2, H_2-15), 42.6 (CH_2, C-13), 33.3 (CH_2, C-9), 29.7 (CH_2, C-10), 29.3 (CH_2, C-11), 23.7 (CH_2, C-12). – HREIMS *m/z* = 462.2403 (calcd. 462.2406 for $C_{29}H_{34}O_5$). – EIMS: *m/z* (%) = 462 (20), 245 (35), 217 (100), 161 (31), 131 (10).

*Pipataline (**2**)*

White amorphous solid. – UV ($CHCl_3$): λ_{max} = 260 nm. – IR (KBr): ν_{max} = 1602 (C=C) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.91 (s, 1H, H-3), 6.72 (dd, *J*= 8.0, 1.7 Hz, 2H, H-5, H-6), 6.32 (d, *J* = 16.0 Hz, 1H, H-7), 6.08 (dt, *J*= 16.0, 6.8, 3.0 Hz, 1H, H-8), 5.91 (s, 2H, H_2-19), 2.17 (m, 2H, H_2-9), 1.34 (s, 14H, H_2-10 to H_2-16), 0.82 (t, *J* = 5.7 Hz, 3H, H-18). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 148.5 (–C–, C-1), 148.0 (–C–, C-2), 105.3 (CH_2, C-6), 129.6 CH, C-7), 129.2 (CH, C-8), 128.5 (–C–, C-4), 120.2 (CH, C-5), 100.5 (CH_2, C-19), 33.1 (CH_2, C-9), 32.9 (CH_2, C-10), 31.9 (CH_2, C-11), 29.1 (CH_2, C-12), 29.5 (CH_2, C-13), 29.5 (CH_2, C-14), 29.3 (CH_2, C-15), 29.2 (CH_2, C-16), 22.7 (CH_2, C-17), 14.2 (CH_3, C-18). – EIMS: *m/z* (%) = 288 (100), 131 (92), 161 (80), 135 (43).

*Lupeol (**3**)*

White crystalline solid. – UV ($CHCl_3$): λ_{max} = 228 nm. – IR (KBr): ν_{max} = 3354 (OH), 2940 (C–H) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.75 (s, 3H, H-23), 0.78 (s, 3H, H-24), 0.84 (s, 3H, H-25), 0.95 (s, 3H, H-26), 0.98 (s, 3H, H-28), 1.04 (s, 3H, H-29), 1.70 (s, 3H, H-30), 2.38 (m, 1H, H-19), 3.19 (dd, *J* =10.8, 4.7 Hz, 1H, H-3), 4.56 (s, 1H, H-29 b), 4.65 (s, 1H, H-29a). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 39.0 (CH_2, C-1), 27.8 (CH_2, C-2), 79.3 (CH, C-3), 39.2 (–C–, C-4), 55.6 (CH, C-5), 18.7 (CH_2, C-6), 34.6 (CH_2, C-7), 41.2 (–C–, C-8), 50.7 (CH, C-9), 37.5 (–C–, C-10), 21.3 (CH_2, C-11), 25.5 (CH_2, C-12), 38.4 (CH, C-13), 43.2 (–C–, C-14), 27.8 (CH_2, C-15), 35.9 (CH, C-16), 43.4 (–C–, C-17), 48.3 (CH, C-18), 48.6 (CH, C-19), 151.1 (CH, C-20), 30.2 (CH_2, C-21), 40.4 (CH_2, C-22), 28.4 (CH_3, C-23), 15.8 (CH_3, C-24), 16.5 (CH_3, C-25), 16.3 (CH_3, C-26), 14.9 (CH_3, C-27), 18.4 (CH_3, C-28), 109.6 (CH_2, C-29), 19.7 (CH_3, C-30). – EIMS: *m/z* (%) = 425 (18), 409 (23), 218 (68), 207 (60), 189 (100).

*13,14-Seco-stigmasta-5,14-diene-3α-ol (**4**)*

White amorphous powder. – UV ($CHCl_3$): λ_{max} = 210 nm. – IR (KBr): ν_{max} = 3419 (OH), 2965 (C–H), 1598 (C=C) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.68 (d *J* = 6.7 Hz, 3H, H_3-18), 0.80 (d *J* = 6.5 Hz, 3H, H_3-26), 0.81 (d, *J* = 6.5 Hz, 3H, H_3-27), 0.86 (d, *J* = 6.5 Hz, 3H, H_3-29), 0.92 (d, *J* = 6.5 Hz, 3H, H_3-21), 1.00 (s, 3H, H_3-19), 3.47 (m, 1H, H-3), 5.02 (dd *J* = 15.2, 5.2 Hz, 1H, H-15), 5.12 (dd, *J* = 15.2, 4.5 Hz, 1H, H-14), 5.35 (d, *J* = 5.8 Hz, 1H, H-6). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 36.7 (CH_2, C-1), 31.3 (CH_2, C-2), 71.2 (CH, C-3), 140.2 (–C–, C-5), 121.1 (CH, C-6), 31.3 (CH_2, C-7), 51.4 (CH, C-8), 52.6 (CH, C-9), 35.9 (C, C-10), 22.5 (CH_2, C-11), 33.4 (CH_2, C-12), 39.2 (CH, C-13), 128.7 (CH, C-14), 137.7 (CH, C-15), 35.6 (CH_2, C-16), 56.2 (CH, C-17), 19.2 (CH_3, C-18), 20.5 (CH_3, C-19), 35.9 (CH, C-20),

18.8 (CH_3, C-21), 31.1 (CH_2, C-22), 25.5 (CH_2, C-23), 49.5 (CH, C-24), 31.2 (CH, C-25), 18.4 (CH_3, C-26), 18.4 (CH_3, C-27), 31.87 (CH_3, C-28), 11.4 (CH_3, C-29). – EIMS: *m/z* (%) = 414 (41), 396 (21), 381 (16), 43 (100).

*Synthesis of 7,8-epoxepipataline (**5**)*

Compound **5** was synthesized by the procedure described in [15]. Pipataline (**2**) (3 mg) dissolved in 10 mL of CH_2Cl_2 was mixed with an equal amount of water containing 1 g of $NaHCO_3$; to this reaction mixture was cautiously added 3.58 mg of *m*-chloroperbenzoic acid. The reaction mixture was stirred at r.t. for 24 h. Afterwards, Na_2SO_3(10 mL) was added to the reaction mixture, which was then extracted with 2 × 10 mL of CH_2Cl_2. The combined organic phase was washed with 2 × 25 mL aqueous $NaHCO_3$ and water and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent under vacuum gave **5** as a white powder (2.59 mg, 86.4 % yield). – UV ($CHCl_3$): λ_{max} = 305 nm. – IR (KBr): ν_{max} = 2919 (CH) and 1275(C–O) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.91 (s, 1H, H-3), 6.72 (dd, *J* = 8.00,1.5Hz, 2H, H-5, H-6), 3.37 (d, *J* = 2.0 Hz, 1H, H-7), 2,8 (dd, *J* = 7.3, 2.4, 2.0 Hz, 1H, d H-8), 5.91 (s, 2H, H-15), 2.17 (m, 2H, H-9), 1.34 (s, 14H, H-10 to H-16), 0.82 (t, *J*= 6.0 Hz, 3H, H-18). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 147.8 (–C–, C-1), 147.9 (–C–, C-2), 105.3 (CH_2, C-6), 62.9 (CH, C-7), 58.6 (CH, C-8), 128.5 (–C–, C-4), 120.2 (CH, C-5), 100.5 (CH_2, C-19), 33.1 (CH_2, C-9), 32.9 (CH_2, C-10), 31.9 (CH_2, C-11), 29.1 (CH_2, C-12), 29.5 (CH_2, C-13), 29.5 (CH_2, C-14), 29.3 (CH_2, C-15), 29.2 (CH_2, C-16), 22.7 (CH_2, C-17), 14.2 (CH_3, C-18). – EIMS: *m/z* (%) = 304 (15), 150 (100), 135 (82), 63 (45), 177 (20).

*Synthesis of 8-amino-7-hydroxypipataline (**6**)*

Epoxide **5** (2.5 mg) and 28 % NH_3 (5 mL) were placed in a 20 mL closed vial [15]. The vial was put into a modified standard household microwave oven at the chosen power (1200 KW). The reaction was carried out for 2 min and 10 sec with 5 sec intervals. After cooling, the solution was extracted with ethyl acetate and the organic layer dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to afford **6** as a colorless solid (2.48 mg, 97.2 % yield). Colorless solid. – UV ($CHCl_3$): λ_{max} = 284 nm. – IR (KBr): ν_{max} = 3452 (OH), 3323 (NH), 2900 (CH) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.91 (s, 1H, H-3), 6.72 (dd, *J* = 7.8, 1.4 Hz, 2H, H-5, H-6), 3.70 (d, *J* = 2.3 Hz, 1H, H-7), 2.87 (dt, *J*= 9.7, 3.2, 2.3 Hz, 1H, H-8), 5.91 (s, 2H, H_2-15), 2.17 (m, 2H, H_2-9), 1.34 (s, 14H, H_2-10 to H_2-16), 0.82 (t, *J*= 6.0 Hz, 3H, H-18). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 148.5 (–C–, C-1), 148.0 (–C–, C-2), 105.3 (CH_2, C-6), 78.0 (CH, C-7), 60.4 (CH, C-8), 128.5 (–C–, C-4), 120.2 (CH, C-5), 100.5 (CH_2, C-19), 33.1 (CH_2, C-9), 32.9 (CH_2, C-10), 31.9 (CH_2, C-11), 29.1 (CH_2,C-12), 29.5 (CH_2, C-13), 29.5 (CH_2, C-14), 29.3 (CH_2, C-15), 29.2 (CH_2, C-16), 22.7 (CH_2, C-17), 14.2 (CH_3, C-18). – EIMS: *m/z* (%) = 321 (12), 305 (44), 288 (64), 179 (16), 161 (15), 151 (93), 150 (33).

*Synthesis of dibromopipataline (**7**)*

Pipataline (3 mg) was dissolved in of ethyl acetate (5 mL) and the mixture was stirred with Br_2 (0.5 mL) under UV (λ = 366 nm) for 24 h [16]. After 24 h, the reaction mixture was stopped by adding water and extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to yield **7** as a brown solid (2.3 mg, 97.2 % yield). Brown solid. – UV ($CHCl_3$): λ_{max} = 293 nm. – IR (KBr): 2919 (C–H) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.91 (s, 1H, H-3), 6.72 (dd, *J*= 7.8, 1.4 Hz, 2H, H-5, H-6), 5.58 (d, *J*= 7.0 Hz, 1H, H-7), 4.39 (dt, *J*= 7.0, 5.4, 3.4 Hz, 1H, H-8), 5.91 (s, 2H, H-15), 2.17 (m, 2H, H_2-9), 1.34 (s, 14H, H_2-10 to H_2-16), 0.82 (t, *J* = 6.0 Hz, 3H, H_3-18). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 148.4 (–C–, C-1), 147.8 (–C–, C-2), 105.3 (CH_2, C-6), 57.5 (CH, C-7), 55.6 (CH, C-8), 128.5 (–C–, C-4), 120.2 (CH, C-5), 100.5 (CH_2, C-19), 33.1 (CH_2, C-9), 32.9 (CH_2, C-10), 31.9 (CH_2, C-11), 29.1 (CH_2, C-12), 29.5 (CH_2, C-13), 29.5 (CH_2, C-14), 29.3 (CH_2, C-15), 29.2 (CH_2, C-16), 22.7 (CH_2, C-17), 14.2 (CH_3, C-18). – EIMS: *m/z* (%) = 448 (15), 368 (35), 228 (20), 213 (6).

GST Inhibition assay

The inhibitory activity of GST was assayed according to a spectrophotometeric method [17]. Specific concentrations of the compounds were incubated with the enzyme at 22 °C for 30 min after which an assay was carried out. The final assay mixture contained the following concentrations in a 3000 μL solution: 5 mM GSH, 1 mM CDNB, 100 mM phosphate buffer (pH = 6.5) and GST (with initial effective assay activity of 0.12106 U mL^{-1}). The assay measured the activity of GST in conjugating CDNB to GSH, and the product of conjugation was measured at 340 nm using a HP 8452 Diode Array spectrophotometer equipped with a thermostated cell compartment (22 °C). The reaction was monitored for 20 sec and GST inhibition was calculated with reference to a control assay. Basal coupling between the substrates was also analyzed, and was observed to be insignificant under these assay conditions. Substrate limitation was also taken into consideration, and was observed not to occur during the initial 30 sec of the assay under the same assay conditions. All assays were carried out in triplicates.

AChE Inhibition assay

AChE inhibitory activity was measured according to a modified Ellman's assay [18]. All assays were carried out in triplicates.

Antimicrobial assay

Ethanolic extract of *B. prionitis* and the pure compounds **1** – **4** were screened against seven bacterial strains, *Escherischia coli* (ATCC 25922), *S. aureus* (ATCC25923), *Corynebacteriun xerosis* (ATCC 373), *Streptococcus agalactiae* (ATCC13813), *Enterococcus faecalis* (ATCC19433), *B. cereus*, (ATCC14579), *P. aeruginosa* (ATCC27853) using the paper disk diffusion method. Ceftriaxone (30 μg/disk) was used as positive control, and ethanol and ethyl acetate were used as negative controls. The antibacterial activity was determined by measuring the diameters of zones of inhibition around each paper disk [19]. Each experiment was performed in triplicate.

Acknowledgements

Funding provided for this research by Natural Sciences and Engineering Research Council of Canada (NSERC) and Research Corporation, Tucson, USA, is gratefully acknowledged. A. A. also acknowledges the Department of Chemistry, University of Manitoba for granting him adjunct professor status. Students (S. Z., C. C. U. and S. A.) are registered at the Department of Chemistry, University of Manitoba. We are also thankful to Dr. Kirk Marat, for his help in doing NMR experiments.

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