Lipoxygenase Inhibitory Constituents from *Periploca aphylla*

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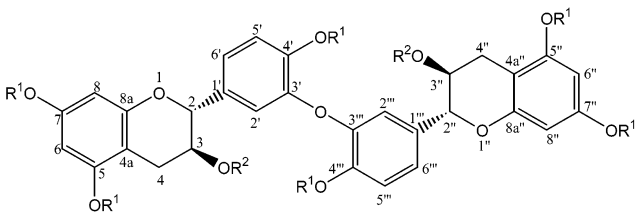
Bisflavan-3-ols **1** and **2** and norterpenoid **3** have been isolated from the methanolic extract of the whole plant of *Periploca aphylla*. Their structures have been assigned on the basis of spectroscopic analysis including 1D and 2D NMR techniques. In addition, *o*-phthalic acid bis(2-ethylnonyl) ester (**4**), 1,3,6-trihydroxy-2,5-dimethoxyxanthone (**5**), and (+)-lyoniresinol (**6**) have been reported for the first time from this species. Compounds **1**−**3** displayed evident inhibitory potential against the enzyme lipoxygenase in a concentration-dependent fashion with IC_{50} values 19.7, 13.5, and 150.1 μM, respectively.

The genus *Periploca*, belonging to the family Asclepiadaceae, comprises 12 species. All of these are erect leafless shrubs, distributed in Europe, Asia, and tropical Africa. Phytochemical studies on various species of the genus *Periploca* including *P. sepium*, *P. leavigatis*, and *P. gracea* have resulted in the isolation of various triterpenes, lignans, and flavonoids.[1−3] *P. aphylla* Decne is widely distributed in the northern part of Pakistan and finds various medicinal uses in the indigenous system of medicine.[4] The bark contains 8% tannin, and a decoction of it is given as a purgative. The leaves and stems contain 2.2% of resin alcohol, a bitter substance, and small quantities of a glucosidal principle, which produces first a decrease and then an increase in blood pressure.[4] It is a strange phenomenon, but no further clarification is provided in the literature. The milky juice is used as an external application against tumors and swellings and is said to be useful in cerebral fever and also as a stomachic.[5] Previously triterpenes have been reported from this species.[6] In the present investigation, a methanolic extract of the stem of *P. aphylla* showed positive activity in the brine shrimp lethality test.[7] Further biological screening of the methanolic and ethyl acetate fractions revealed significant inhibitory activity against the enzyme lipoxygenase. Herein we report the isolation and structure elucidation of bioactive compounds **1**, **2**, and **3** from the ethyl acetate fraction. In addition, *o*-phthalic acid bis(2-ethylnonyl) ester (**4**),[8] 1,3,6-trihydroxy-2,5-dimethoxyxanthone (**5**),[9] and (+)-lyoniresinol (**6**)[10] have been reported for the first time from this species.

Results and Discussion

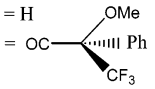
The ethyl acetate-soluble fraction of the methanolic extract of the whole plant of *P. aphylla* was subjected to column chromatography over flash silica eluting with different mobile phases. Compounds **1**−**6** were obtained, and their structures were established by UV, IR, mass, and NMR spectroscopy.

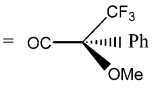
Compound **1** was isolated as a colorless gummy solid. The HRFABMS (positive ion mode) gave $[M + H]^+$ at *m/z* 563.1553, corresponding to the molecular formula $C_{30}H_{26}O_{11}$, which indicated 18 degrees of unsaturation. The UV absorptions of **1** were exhibited at 283, 276, and 216 nm, which indicated the presence of a conjugated aromatic system. The IR spectrum showed strong absorptions at 3356 (O−H), 2922 (C−H), 1623, 1517 (aromatic C=C), and 1230 (C−O) cm^{-1}. The dimeric nature of **1** was revealed

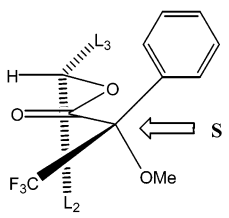


(**1**) $R^1 = R^2 = H$

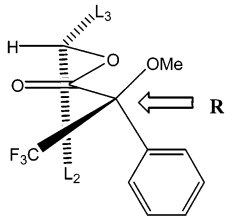
(**1a**) $R^1 = Me$, $R^2 = H$

(**1b**) $R^1 = Me$, $R^2 =$ 

(**1c**) $R^1 = Me$, $R^2 =$ 



1d



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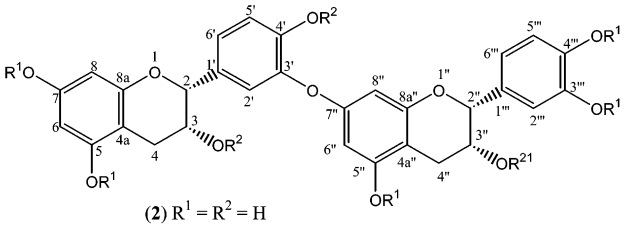


(**2**) $R^1 = R^2 = H$

(**2a**) $R^1 = Me$, $R^2 = H$

(**2b**) $R^1 = Me$, $R^2 =$ 

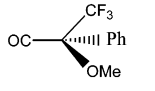
(**2c**) $R^1 = Me$, $R^2 =$ 

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Table 1. ^{1}H and ^{13}C NMR and HMBC Data for Compound **1** (CD_3OD)

position	$^{13}C^a$ δ	$^1H^b$ δ (mult., J in Hz)	HMBC^b (H−C)
2	81.7	4.43 (d, 7.2)	1′, 2′, 3, 4, 8a
3	68.0	4.00 (m)	1′, 2, 4
4	29.2	2.75 (dd, 16.2, 4.8)	2, 3, 4a, 5
		2.54 (dd, 16.2, 8.6)	2, 3, 4a, 5
4a	102.4		
5	157.0		
6	95.4	5.91 (d, 2.4)	4a, 5, 7, 8
7	156.7		
8	96.2	6.02 (d, 2.4)	6, 7, 4a, 8a
8a	157.3		
1′	131.2		
2′	120.3	7.02 (d, 2.1)	1′, 3′, 4′, 6′
3′	141.2		
4′	149.2		
5′	117.8	6.92 (d, 8.4)	1′, 3′, 4′, 6′
6′	124.0	7.31 (dd, 8.4, 2.1)	1′, 2′, 4′, 5′

[a] Recorded at 100 MHz. [b] Recoded at 400 MHz.

by a strong peak in the HREIMS at m/z 290.0790 ($C_{15}H_{14}O_6$) and 274.0841 ($C_{15}H_{14}O_5$) due to cleavage of an ether linkage. The ^{1}H NMR spectrum of **1** (Table 1) displayed a 1,3,4-trisubstituted phenyl group showing a doublet of doublets at δ 7.31 (2H, J = 8.4, 2.1 Hz), a doublet at δ 6.92 (2H, J = 8.4 Hz), and a doublet at δ 7.02 (2H, J = 2.1 Hz). Two doublets appeared at δ 5.91 (J = 2.4 Hz) and 6.02 (J = 2.4 Hz), integrating for two protons each, and could be assigned to meta-substituted protons. The two oxymethine protons resonated at δ 4.43 (2H, d, J = 7.2 Hz) and 4.00 (2H, m). The methylene protons that appeared at δ 2.75 (2H, dd, J = 16.2, 4.8 Hz) and 2.54 (2H, dd, J = 16.2, 8.6 Hz) were characteristic signals of a flavan-3-ol.[11] The ^{13}C NMR spectrum (Table 1) (both BB and DEPT) of **1** disclosed 15 highly resolved carbon signals comprising one methylene, seven methine, and seven quaternary carbon atoms. The downfield signals at δ 157.3, 157.0, 156.7, 149.2, and 141.2 indicated the presence of oxygenated aromatic quaternary carbon atoms. The ether linkage was deduced at 3′→O→3‴ due to the upfield shift of C3′ and C3‴ (δ 141.2) and downfield shift of C4′ and C4‴ (δ 149.2) and C2′ and C2‴ (120.3), compared to related flavan-3-ols.[12−15] The COSY-45° spectrum of **1** exhibited a cross-peak between H-5′ (δ 6.92) and H-6′ (δ 7.31). The H-3 oxymethine proton that resonated at δ 4.00 as a multiplet showed a cross-peak with H-2 (δ 4.43) and H_2-4 (δ 2.75, 2.54). The substitutions and the linkages at various positions of the dimer were confirmed by long-range HMBC experiments, the important HMBC correlations being illustrated in Table 1. Upon rationalizing the above data, it was evident that compound **1** is a catechin-(3′→0→3‴)-afzelechin. The relative 2,3-*trans* configuration was established by NOESY correlations and on the basis of similar spectroscopic data with related compounds.[15,16] The absolute configuration of **1** was determined by Mosher's method.[17−19] Reaction of **1** with diazomethane for protecting phenolic hydroxyl groups gave the hexamethyl ether **1a**, which on esterification with *R*-(+)-MTPACl and *S*-(−)-MTPACl afforded the esters **1b** and **1c**, respectively. In the configuration correlation models for correlating ^{1}H NMR shifts and absolute configurations of *S*-(−)-and *R*-(+)-MPTA esters **1d** and **1e**, the α-trifluoromethyl group, carbonyl, and carbinyl hydrogens are approximately eclipsed. The protons of the substituent that eclipses the phenyl ring [L^3 for the *S*-(−)-MTPA ester **1d** and L^2 for the *R*-(+)-MTPA ester **1e**] are, therefore, more highly shielded as a result of the diamagnetic shielding by the phenyl moiety.[20,21] The positive or negative difference in chemical shift of any set of like protons in the diastereomeric *S*- and *R*-MTPA esters ($\Delta\delta$) will thus provide evidence for absolute configuration. The comparison of the ^{1}H NMR data of **1b** and **1c** (Table 4) reveals a conspicuous shielding of the B-ring protons in the *R*-(+)-MTPA ester **1b** relative to the chemical shift of these protons in the *S*-(−)-MTPA ester **1c** [δ −0.12, H-2 (B); −0.90, H-5 (B); − 0.11, H-6 (B)], allowing us to assign the 3*S*-configuration to **1**. On the basis of this evidence the structure of **1** could be assigned as catechin-(3′→0→3‴)-afzelechin.

Table 2. ^{1}H and ^{13}C NMR and HMBC Data for Compound **2** (CD_3OD)

position	$^{13}C^a$ δ	$^1H^b$ δ (mult., J in Hz)	HMBC^b (H−C)
2	79.8	4.92 (br s)	1′, 2′, 3, 4, 8a
3	67.6	4.21 (m)	1′, 2, 4, 4a
4	29.2	2.85 (dd, 9.4, 5.0)	2, 3, 4a, 5
		2.09 (dd, 9.4, 3.8)	2, 3, 4a, 5
4a	101.3		
5	157.4		
6	96.4	6.01 (s)	4a, 5, 7, 8
7	156.8		
8	96.4	6.01 (s)	6, 7, 4a, 8a
8a	157.6		
1′	131.9		
2′	120.9	6.99 (d, 2.1)	1′, 3′, 4′, 6′
3′	142.1		
4′	149.4		
5′	118.2	6.93 (d, 8.2)	1′, 3′, 4′, 6′
6′	124.7	7.21 (dd, 8.2, 2.1)	1′, 2′, 4′, 5′
2″	79.6	4.86 (br s)	1‴, 2‴, 3″, 4″, 8a″
3″	67.4	4.10 (m)	1‴, 2″, 4″, 4a″
4″	29.0	2.82 (dd, 9.4, 5.8)	2″, 3″, 4a″, 5″
		2.65 (dd, 9.2, 3.8)	2″, 3″, 4a″, 5″
4a″	100.5		
5″	157.2		
6″	96.6	5.98 (d, 2.2)	4a″, 5″, 7″, 8″
7″	156.8		
8″	95.9	6.12 (d, 2.2)	6″, 7″, 4a″, 8a″
8a″	157.9		
1‴	131.4		
2‴	116.0	6.87 (d, 2.1)	1‴, 3‴, 4‴, 6‴
3‴	145.4		
4‴	145.9		
5‴	116.8	6.81 (d, 8.4)	1‴, 3‴, 4‴, 6‴
6‴	119.7	7.02 (dd, 8.4, 2.1)	1‴, 2‴, 4‴, 5‴

[a] Recorded at 100 MHz. [b] Recoded at 400 MHz.

Table 3. ^{1}H and ^{13}C NMR and HMBC Data for Compound **3** (CD_3OD)

position	$^{13}C^a$ δ	$^1H^b$ δ (mult., J in Hz)	HMBC^b (H−C)
1	46.5	2.35 (dd, 12.8, 5.0)	2, 3, 6
		2.07 (dd, 12.8, 8.4)	2, 3, 6
2	66.9	4.62 (dd, 8.4, 5.0)	1, 3, 4, 6
3	202.9		
4	123.0	5.98 (s)	2, 3, 5, 6
5	161.1		
6	74.1		
1′	124.6	6.40 (d, 16.0)	5, 2′, 3′
2′	148.0	6.63 (d, 16.0)	5, 1′, 3′
3′	71.5		
Me-3′,3′	29.6	1.32 (s)	3′
Me-6	24.0	1.28 (s)	6

[a] Recorded at 100 MHz. [b] Recorded at 400 MHz.

Compound **2** was also isolated as a colorless gummy solid. The molecular formula $C_{30}H_{26}O_{11}$ was established by the data of the positive ion FABMS, showing a $[M + H]^+$ ion at 563.1557. The UV, IR, and EIMS spectra of **2** were identical to those of **1**, but some differences were observed in the ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectrum (Table 2), the signals of the 1,3,4-trisubstituted phenyl ring protons appeared at δ 6.87 (d, J = 2.1 Hz, H-2‴), 7.02 (dd, J = 8.4, 2.1 Hz, H-6‴), and 6.81 (d, J = 8.4 Hz, H-5‴) and δ 6.99 (d, J = 2.1 Hz, H-2′), 7.21 (dd, J = 8.2, 2.1 Hz, H-6′), and 6.93 (d, J = 8.2 Hz, H-5′). The other deshielded signals

Table 4. ^{1}H NMR Data of the *R*-(+)-, and *S*-(−)-MTPA Esters **1b**, **1c**, **2b**, and **2c**

no.	**1b**[a]	**1c**[a]	**2b**[a]	**2c**[a]
2	4.78 (d, 8.5)	4.84 (d, 9.0)	5.03 (br s)	5.01 (br s)
3	5.52 (ddd, 9.0, 8.7 6.0)	5.42 (ddd, 9.0, 8.8, 6.0)	5.50 (ddd, 4.0, 2.5, 1.5)	5.52 (ddd, 4.1, 2.7, 1.0)
4	3.11 (dd, 16.0, 6.0)	3.18 (dd, 16.2, 6.0)	3.02 (dd, 9.0, 2.5)	3.01 (dd, 9.1, 2.7)
	2.71 (dd, 16.0, 9.0)	2.56 (dd, 16.2, 9.0)	2.90 (dd, 9.0, 4.0)	2.92 (dd, 9.1, 4.1)
6	6.05 (d, 2.4)	6.04 (d, 2.4)	6.01 (d, 2.2)	6.05 (d, 2.2)
8	6.07 (d, 2.4)	6.06 (d, 2.4)	6.06 (d, 2.2)	6.10 (d, 2.2)
2′	6.84 (d, 2.1)	6.96 (d, 2.1)	6.95 (d, 2.1)	6.78 (d, 2.1)
5′	6.81 (d, 8.4)	6.90 (d, 8.4)	6.79 (d, 8.2)	6.67 (d, 8.2)
6′	7.17 (dd, 8.4, 2.1)	7.28 (dd, 8.4, 2.1)	7.19 (dd, 8.2, 2.1)	7.08 (dd, 8.2, 2.1)
2″			5.05 (br,d, 1.0)	5.03 (br,d, 1.0)
3″			5.53 (ddd, 3.9, 2.4, 1.6)	5.55 (ddd, 4.0, 2.5, 1.1)
4″			3.05 (dd, 9.2, 2.4)	3.03 (dd, 9.2, 2.5)
			2.93 (dd, 9.2, 3.9)	2.95 (dd, 9.2, 4.0)
6″			6.03 (d, 2.2)	6.07 (d, 2.2)
8″			6.08 (d, 2.2)	6.12 (d, 2.2)
2‴			6.85 (d, 2.1)	6.74 (d, 2.1)
5‴			6.78 (d, 8.2)	6.64 (d, 8.2)
6‴			7.01 (dd, 8.2, 2.1)	6.89 (dd, 8.2, 2.1)
OMe	3.75(C-5), 3.81(C-7), 3.84(C-4′), each s	3.75(C-5), 3.82(C-7), 3.86(C-4′), each s	3.72(C-5), 3.74(C-7), 3.86(C-4′), 3.73(C-5″), 3.76(C-7″), 3.87(C-4‴), each s	3.78(C-5), 3.75(C-7), 3.86(C-4′), 3.74(C-5″), 3.77(C-7″), 3.85(C-4‴), each s

[a] Recorded in CD_3OD at 400 MHz.

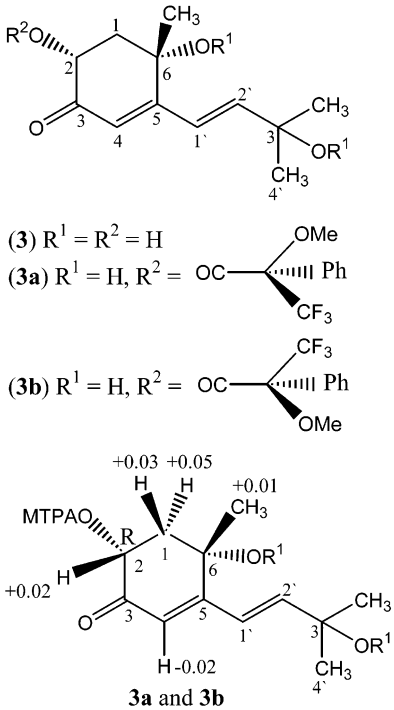
Table 5. ^{1}H NMR Data of the *R*-(+)- and *S*-(−)-MTPA Esters **3a** and **3b**

no.	**3a**[a]	**3b**[a]
1	2.05 (dd, 13.2, 4.7)	2.02 (dd, 13.1, 5.0)
	1.49 (dd, 13.2, 7.8)	1.44 (dd, 13.1, 8.1)
2	5.68 (dd, 7.8, 4.7)	5.66 (dd, 8.1, 5.0)
4	5.75 (s)	5.77 (s)
2′	6.39 (d, 16.2)	6.40 (d, 16.1)
3′	6.61 (d, 16.2)	6.63 (d, 16.1)
Me-3′,3′	1.31 (s)	1.30 (s)
Me-6	1.08 (s)	1.07 (s)

[a] Recorded in CD_3OD at 400 MHz.

appeared at δ 5.98 (d, J = 2.2 Hz, H-6″), 6.12 (d, J = 2.2 Hz, H-8″), and 6.01 (2H, s, H-6, H-8). The spectroscopic studies suggested that compound **2** could be a bisflavan-3-ol with a (3′→O→7″) linkage. The ^{13}C NMR spectrum also supported the structure with an upfield shift of C-3′ (142.1) and downfield shifts of C-2′ (120.9) and C-4′ (149.4).[11] Upon rationalizing the above data, it was evident that compound **2** is epicatechin-(3′→0→7″)-epiafzelechin. The relative 2,3-*cis* configuration was established by NOESY and on the basis of similar spectroscopic data with related compounds.[11,12,22] The NOESY spectrum showed correlations between H-2, H-3, and H-4$_{ax}$. The absolute configuration assignment was verified by the Mosher ester procedure.[20,21] Methylation of **2** followed by esterification with *R*-(+)-MTPACl and *S*-(−)-MTPACl provided the esters **2b** and **2c**; their ^{1}H NMR data are reported in Table 4. The differences in chemical shifts of ring B and E protons in **2b** and **2c** are now opposite those of **1** [δ +0.17, H-2(B); +0.12, H-5(B); +0.11, H-6(B) and δ +0.11, H-2(E); +0.14, H-5(E); +0.12, H-6(E)], revealing a 3*R*-configuration at C-3. On the basis of the above evidence, compound **2** could be assigned the structure epicatechin-(3′→0→7″)-epiafzelechin.

Compound **3** was isolated as a colorless gummy solid. The high-resolution mass spectrum showed a molecular ion peak $[M]^+$ at *m*/*z* 226.1205 corresponding to the molecular formula $C_{12}H_{18}O_4$. The EIMS showed peaks at *m*/*z* 208 $[M - H_2O]^+$ due to the loss of a water molecule. The IR spectrum displayed the absorption for a hydroxyl group (3381 cm^{-1}), an α,β-unsaturated carbonyl (1730 cm^{-1}), and a double bond (1667, 1596 cm^{-1}). The UV spectrum showed absorption at λ_{max} (278, 203 nm) due to a cyclic α,β-unsaturated ketone. The ^{1}H NMR spectrum (Table 3) of **3** showed a singlet at δ 5.98 due to Δ^4 unsaturation, while



3a and **3b**

Figure 1. ^{1}H NMR chemical shift differences [δ(−MTPA) − δ-(+MTPA)] of the MTPA esters.

two doublets at δ 6.63 (1H, J = 16.0 Hz) and 6.40 (1H, J = 16.0 Hz) were indicative of a *trans* disubstituted double bond. The oxymethine proton appeared as a doublet of doublets at δ 4.62 (J = 8.4, 5.0 Hz), while the singlet at δ 1.32, which integrated for six protons, was due to two angular methyl groups. Another methyl group resonated at δ 1.28, while methylene protons appeared as a doublet of doublets at δ 2.35 (J = 12.8, 5.0 Hz) and 2.07 (J = 12.8, 8.4 Hz). The ^{13}C NMR spectrum (BB and DEPT) of **3** corroborated the presence of three methyl, one methylene, four methine, and four quaternary carbon atoms. The downfield signals at δ 202.9, 161.1, and 123.0 confirmed the presence of a Δ^4- 3-one system, while the signals at δ 148.0 and 124.6 were due to the side chain double bond between C-1′ and C-2′. The positions of various substituents were confirmed by HMBC correlations; important HMBC interactions are shown in Table 3. The remaining problem

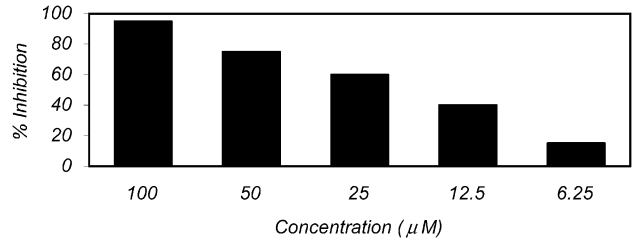


Figure 2. Inhibition (%) of lipoxygenase by compound **1** at various concentrations.

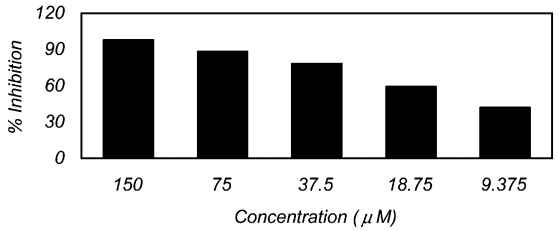


Figure 3. Inhibition (%) of lipoxygenase by compound **2** at various concentrations.

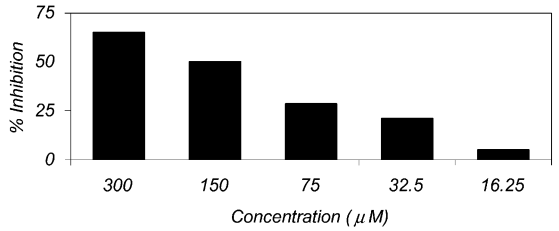


Figure 4. Inhibition (%) of lipoxygenase by compound **3** at various concentrations.

was to assign the configurations at C-2 and C-6. The ^{1}H NMR spectrum of **3** showed a double doublet at δ 4.62 (1H, $J_{ax,ax} = 8.4$ Hz and $J_{ax,eq} = 5.0$ Hz) for H-2, allowing us to assign the α and equatorial position for the secondary hydroxyl group, the β-configuration being assigned to Me-6 on the basis of biogenetic grounds.[23] The stereochemical features were further confirmed by the NOESY correlations of the corresponding protons. The α-configuration at C-2 and β-configuration at C-6 were confirmed by the presence of significant NOESY correlations between H-2, H-4, and Me-6, confirming their *trans* relationship. The absolute configuration at C-2 was achieved by Mosher's method.[17−19] Formation of esters **3a** and **3b** was confirmed by a significant downfield shift of the signal for H-2 and appearance of the expected new signals in the ^{1}H NMR spectra (Table 5). Comparison of the ^{1}H NMR chemical shifts for **3a** and **3b** ($\Delta\delta$ values shown in Figure 1) led to the assignment of the *R*-configuration at C-2. On the basis of these data, compound **3** could be assigned the structure 2α,6α-dihydroxy-5-[(*E*)-3′-hydroxy-3′-methyl-1′-butenyl]-6-methyl-4-cyclohexen-3-one.

Lipoxygenases constitute a family of non-haem iron-containing dioxygenases that are widely distributed in animals and plants. These are involved in arachidonic acid metabolism, generating various biologically active lipids that play important roles in inflammation.[24] Thrombosis and tumor angiogenesis, the formation of new capillary vessels from preexisting ones, underpins a number of physiological processes and participates in the development of several pathological conditions such as arthritis and cancer.[25] Lipoxygenases are, therefore, potential targets for rational drug design and discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, inflammation, cancer, and autoimmune diseases.

Table 6. In Vitro Quantitative Inhibition of Lipoxygenase by Compounds **1**−**3**

compound	IC_{50} (μM) ± SEM[a]
1	19.7 ± 0.2
2	13.5 ± 0.5
3	150.1 ± 0.3
baicalein[b]	22.6 ± 0.5

[a] Standard mean error of five assays. [b] Standard inhibitor of lipoxygenase.

In the lipoxygenase inhibition assay baicalein (Aldrich Chem. Co.) was used as positive control. The percentage inhibitions of lipoxygenase at five different concentrations of compounds **1**−**3** are given in Figures 2−4. From the results (Table 6) it is clear that bis-flavan-3-ols **1** and **2** have higher inhibitory potential than the norterpenoid **3**. Furthermore, compound **2** is more potent than compound **1**, which shows that the stereochemistry of **2** is probably more favorable for the inhibition of lipoxygenase enzyme than **1**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EIMS and HRFABMS were recorded on JMS-HX-110 and JMS-DA 500 mass spectrometers. The ^{1}H and ^{13}C NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C NMR, respectively. The chemical shift values are reported in ppm (δ) units, and the coupling constants (*J*) are in Hz. Aluminum sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck) were used for TLC, and flash silica (230−400 mesh) was used for column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and also by spraying with ceric sulfate solution (with heating). For the enzyme inhibition assay, all chemicals used and lipoxygenase (1.13.11.12) type I-B were purchased from Sigma (St. Louis, MO).

Plant Material. The whole plant material was collected from Swat and identified as *Periploca aphylla* by Mr. Habib Ahmad. A voucher specimen is deposited in the herbarium (accession no. 1997-1294) of the Department of Botany, Government Degree College Matta, Swat, Pakistan.

Extraction and Isolation. The shade-dried whole plant material (25 kg) was extracted three times with MeOH at room temperature. The combined MeOH extract (1 kg) was partitioned between *n*-hexane and H_2O. The H_2O fraction was further extracted with chloroform and EtOAc. The EtOAc-soluble fraction (245 g) was subjected to column chromatography over flash silica using *n*-hexane−$CHCl_3$, $CHCl_3$, $CHCl_3$−MeOH, and MeOH as eluents in increasing order of polarity. The fraction obtained from *n*-hexane−$CHCl_3$ (4:6) was rechromatographed over flash silica using *n*-hexane−$CHCl_3$ (4.5:5.5) to afford compound **3** (18 mg). The fraction obtained from $CHCl_3$−MeOH (9.5:0.5) was a mixture of two components, which were separated by column chromatography over flash silica using $CHCl_3$−MeOH (9.8:0.2) to afford compounds **4** (20 mg) and **5** (27 mg). The fraction obtained from $CHCl_3$−MeOH (9:1) was rechromatographed using $CHCl_3$−MeOH (9.5:0.5) to obtain compound **6** (14 mg). The fractions obtained from $CHCl_3$−MeOH (8.5:1.5) were subjected to preparative TLC ($CHCl_3$−MeOH−H_2O; 80:19.5:0.5) to afford **1** (15 mg) and **2** (12 mg), respectively.

Compound 1: colorless gummy solid; $[\alpha]^{25}_D$ −24.2° (*c* 0.031, MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (3.8), 276 (4.08), 216 (3.62); IR (KBr) ν_{max} 3356, 2922, 1623, 1230 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 290 (9), 274 (13), 153 (37), 137 (100), 129 (19); HRFABMS *m*/*z* 563.1553 (calcd for $C_{30}H_{27}O_{11}$ 563.1550).

Compound 2: colorless gummy solid; $[\alpha]^{25}_D$ −41.3° (*c* 0.040 MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (3.6), 274 (4.02), 218 (3.60);

IR (KBr) ν_{max} 3354, 2923, 1625, 1231 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m/z* 290 (7), 274 (16) 153 (39), 137 (100), 129 (17); HRFABMS *m/z* 563.1557 (calcd for $C_{30}H_{27}O_{11}$ 563.1552).

Compound 3: colorless gummy solid; $[\alpha]^{25}_D$ +9.3° (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 278 (3.42), 203 (4.8); IR (KBr) ν_{max} 3381, 1730, 1667, 1596 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS *m/z* 208 (4), 190 (25), 175 (100), 137 (81), 107 (18), 59 (58); HREIMS *m/z* 226. 1205 (calcd for $C_{12}H_{18}O_4$ 226.1201).

Methylation of Biflavan-3-ols 1 and 2. An excess of diazomethane in ether (10 mL) was added to a solution of **1** and **2** (10 mg each) in MeOH (3 mL) separately, and the solution was allowed to stand at 5 °C for 10 h. The reaction mixture was concentrated in vacuo, and the residue was chromatographed on Sephadex LH-20 to furnish the corresponding methyl ethers **1a** and **2a**, respectively.

Hexamethyl ether 1a: colorless gummy solid; $[\alpha]^{25}_D$ −26.2° (*c* 0.023, MeOH); IR (KBr) ν_{max} 3026, 2916, 2880, 1598, 1450 cm^{-1}; HRFABMS *m/z* 647.2497 (calcd for $C_{36}H_{39}O_{11}$ (M + H$^+$) 647.2491).

Hexamethyl ether 2a: colorless gummy solid; $[\alpha]^{25}_D$ −40.7° (*c* 0.031 MeOH); IR (KBr) ν_{max} 3029, 2916, 2881, 11596, 1448 cm^{-1}; HRFABMS *m/z* 647.2495 (calcd for $C_{36}H_{39}O_{11}$ (M + H$^+$) 647.2491).

Preparation and Purification of Mosher Ester. (*R*)-MTPACl or (*S*)-MTPACl was added to methyl ethers of **1** and **2** and norterpenoid **3** in DCM (0.1 mL/0.01 mmol of substrate) and triethylamine (6 equiv) separately. The mixture was left at room temperature for 1 h and the reaction progress monitored by TLC. HCl (0.1 M, 50 mL) was added, and the products were extracted with EtOAc. The organic phase was washed with $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated under reduced pressure. Subsequent purification by preparative TLC ($CHCl_3$−MeOH, 8:1) afforded the (*R*)-MTPA esters (**1b**, **2b**, **3a**) or the (*S*)-MTPA esters (**1c**, **2c**, **3b**), respectively.

(*R*)-MTPA ester 1b: $[\alpha]^{25}_D$ +31.2° (*c* 0.021, MeOH); IR (KBr) ν_{max} 2916, 2882, 1600, 1450, 1250 cm^{-1}; ^{1}H NMR (CD_3-OD, 400 MHz), see Table 5; HRFABMS *m/z* 1079.3291 (calcd for $C_{56}H_{53}F_6O_{15}$ (M + H$^+$) 1079.3286).

(*S*)-MTPA ester 1c: $[\alpha]^{25}_D$ −28.4° (*c* 0.023, MeOH); IR (KBr) ν_{max} 2918, 2881, 1598, 1451, 1249 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table 5; HRFABMS *m/z* 1079.3295 (calcd for $C_{56}H_{53}F_6O_{15}$ (M + H$^+$) 1079.3286).

(*R*)-MTPA ester 2b: $[\alpha]^{25}_D$ +48.1° (*c* 0.038, MeOH); IR (KBr) ν_{max} 2914, 2879, 1596, 1446, 1250 cm^{-1}; ^{1}H NMR (CD_3-OD, 400 MHz), see Table 5; HRFABMS *m/z* 1079.329089 (calcd for $C_{56}H_{53}F_6O_{15}$ (M + H$^+$) 1079.3285).

(*S*)-MTPA ester 2c: $[\alpha]^{25}_D$ −43.6° (*c* 0.036, MeOH); IR (KBr) ν_{max} 2916, 2879, 1697, 1446, 1248 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table 5; HRFABMS *m/z* 1079.3288 (calcd for $C_{56}H_{53}F_6O_{15}$ (M + H$^+$) 1079.3285).

(*R*)-MTPA ester 3a: $[\alpha]_D^{25}$ +13.6° (*c* 0.021, MeOH); IR (KBr) ν_{max} 3380, 1730, 1666, 1598 1250 cm^{-1}; ^{1}H NMR (CD_3-OD, 400 MHz), see Table 6; HREIMS *m/z* 442.1609 (calcd for $C_{22}H_{25}F_3O_6$ 442.1602).

(*S*)-MTPA ester 3b: $[\alpha]^{25}_D$ −11.2° (*c* 0.022, MeOH); IR (KBr) ν_{max} 3382, 1729, 1665, 1598 1249 cm^{-1}; ^{1}H NMR (CD_3-OD, 400 MHz), see Table 6; HREIMS *m/z* 442.1611 (calcd for $C_{22}H_{25}F_3O_6$ 442.1602).

In Vitro Lipoxygenase Inhibition Assay. Lipoxygenase-inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by A. L. Tappel.[26] Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from Sigma (St. Louis, MO). All other chemicals were of analytical grade. The reaction mixture contained 160 μL (100 nM) of sodium phosphate buffer (pH 8.0), 10 μL of test compound solution, and 20 μL of lipoxygenase solution and was incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 μL of linoleic acid (substrate) solution, with the formation of (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate. The change of absorbance at 234 nm was followed for 6 min. Test compounds and the control were dissolved in methanol. All the reactions were performed in triplicate in a 96-well microplate in a SpectraMax 340 (Molecular Devices). The IC_{50} values were then calculated using the EZ-Fit Enzyme kinetics program (Perrella Scientific Inc., Amherst). The percentage (%) inhibition was calculated as $(E - S)/E \times 100$, where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

References and Notes

(1) Zorina, L. G.; Matyukhino, L. G.; Pyabinin, A. A. *Khim. Prir Soedin.* **1996**, *2*, 291−295.
(2) Barrera, J. B.; Funes, J. B.; Fuente, G. D.; Genzales, G. A. *An. R. Soc. Espan. Fis. Quim., Ser. B* **1966**, *82*, 859−864.
(3) Askari, M.; Bui, A. M.; Mighri, Z. *J. Soc. Chem. Tunis.* **1982**, *8*, 23−28.
(4) Shastri, B. N. *Wealth of India; Sree*; Saraowaty: New Dehli, 1966; p 313.
(5) Baquar, S. R. *Medicinal and Poisonous Plants of Pakistan*; Printas Press: Karachi, 1989.
(6) Mustafa, G.; Anis, E.; Ahmed, S.; Anis, I.; Ahmed, H.; Malik, A.; Hassan, S. S.; Choudhary, M. I. *J. Nat. Prod.* **2000**, *63*, 881−883.
(7) Meyer, B. N.; Ferrigni, J. E.; Jacobsen, L. B.; Nicholas, P. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.
(8) Wahidulla, S.; Souza, L. D.; Govenker, M. *Phytochemistry* **1998**, *48*, 1203−1206.
(9) Pinto, D. C. G.; Fuzzati, N.; Pazmino, X. C.; Hostettmann, K. *Phytochemistry* **1994**, *37*, 875−878.
(10) Ohashi, K.; Watanabe, H.; Okumura, Y.; Uji, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 1924−1926.
(11) De Bruyne, T.; Cimanga, K.; Pieters, L.; Claeys, M.; Dommisse, R.; Vlietinck, A. *Nat. Prod. Lett.* **1997**, *11*, 47−52.
(12) Hori, K.; Satake, T.; Saiki, Y.; Murakami, T.; Chen, C. M. *Chem. Pharm. Bull.* **1988**, *36*, 4301−4306.
(13) Czochanska, Z.; Foo, L. Y.; Newman, R. H.; Porter, L. J. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2278−2286.
(14) Jayaprakasam, B.; Damu, A. G.; Rao, K. V.; Gunasekar, D.; Blond A.; Bodo B. *J. Nat. Prod.* **2000**, *63*, 507−508.
(15) Rao, K. V.; Sreeramula, K.; Rao, C. V.; Gunasekar, D. *J. Nat. Prod.* **1997**, *60*, 632−634.
(16) Malan, E.; Roux, D. G. *Phytochemistry* **1975**, *14*, 1835−1841.
(17) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.
(18) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(19) Rieser, M. J.; Hui, Y.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203−10213.
(20) Hundt, A. F.; Burger, J. F. W.; Steynberg, J. P.; Steenkamp, J. A.; Ferreira, D. *Tetrahedron Lett.* **1990**, *31*, 5073−5076.
(21) Rossouw, W.; Hundt, A. F.; Steenkamp. J. A.; Ferreira, D. *Tetrahedron* **1994**, *50*, 12477−12488.
(22) Steynberg, J. P.; Burger, J. F. W.; Cronje, A.; Bonnet, S. L.; Malan, J. C. S.; Young, D. A.; Ferreira, D. *Phytochemistry* **1990**, *29*, 2979−2989.
(23) Itokawa, H.; Xu, J.; Takeya, K. *Chem. Pharm. Bull.* **1987**, *35*, 4524−4529.
(24) Nie, D.; Honn, K. V. *Cell Mol. Life Sci.* **2002**, *59*, 799−807.
(25) Steinhilber, D. *Curr. Med. Chem.* **1999**, *6*, 71−85.
(26) Tappel, A. L. *Methods of Enzymology*; Academic Press: New York, 1962; Vol. 5, pp 539−541.

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