Novel and Known Constituents from *Buddleja* Species and Their Activity against Leukocyte Eicosanoid Generation

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We have undertaken a systematic survey of the genus *Buddleja* used in traditional Chinese medicine for antiinflammatory and other indications by testing extracts and isolated natural products for their activity against the enzymes of the arachidonate cascade. This was done by using elicited rat peritoneal leukocytes, a physiologically relevant established whole cell system that expresses both cyclo-oxygenase (COX) and 5-lipoxygenase (5-LOX) activity. Lipophilic extracts of *B. globosa* roots and *B. myriantha* stem exhibited inhibitory activities in the 5-LOX and COX enzyme assays, whereas those of *B. officinalis* flowers, *B. yunanesis* stems, and *B. asiatica* stems showed inhibitory activities only against COX. The phytochemical investigation of these extracts, and consequent structure elucidation of isolated compounds using spectroscopic data, led to the isolation from *B. globosa* of three new terpenoid compounds named dihydrobuddledin A, buddledone A, and buddledone B and four known compounds—buddledins A, B, and C and zerumbone; 12 known compounds from *B. officinalis*—calceolarioside, campneoside, verbascoside, echinacoside, forsythoside B, angoroside A, crocetin monogentibiosyl ester, acacetin, acacetin-7-*O*-α-L-rhamnopyranosyl (1−6)-*β*-D-glucopyranoside, acacetin-7-*O*-α-L-rhamnopyranosyl (1−6)[α-L-rhamnopyranosyl (1−2)]-*β*-D-glucopyranoside, songarosaponin A, *δ*-amyrone; and eight known compounds from *B. yunanesis*—11,14-dihydroxy-8,11,13-abietatrien-7-one, *β*-sitosterol, verbascoside, echinacoside, forsythoside B, angoroside A, methylcatapol, and sucrose. Tests on the isolated compounds for inhibition of eicosanoid synthesis showed that buddledin A, crocetin monogentibiosyl ester, and acacetin exhibited an inhibitory effect on COX with IC_{50} values of 13.7 μM, 28.2 μM, and 77.5 μM, respectively, whereas buddledin A exhibited inhibitory effect on 5-LOX with an IC_{50} value of 50.4 μM.

The flowers, leaves, and roots of various species of *Buddleja* (family Buddlejaceae) are used in traditional medicine in several parts of the world.[1] "Mi-meng-hua", prepared from the flowers of *B. officinalis* Maxim., is a traditional Chinese medicine used for the treatment of conjunctival congestion and clustered nebulae,[2] whereas the roots of *B. asiatica* Lour. ("Qi-li-xiang") are used there as an antiinflammatory.[3] Similar usage for *B. globosa* Hope is reported from Chile, where the leaves and flowers are used for washing wounds and treating ulcers.[1]

Previous studies on *B. officinalis* and *B. globosa* have led to the isolation of various natural products, including triterpenoid glycosides,[4] phenylethanoids,[5,6] flavonoids,[7] phenolic fatty-acid esters,[8] and a diterpene.[9] Although *Buddleja* species are known to contain some compounds with known antiinflammatory activity, for example, kaempferol,[10] which inhibits both cyclo-oxygenase (COX) and 5-lipoxygenase (5-LOX),[11] the antiinflammatory activity of the extracts and constituents of this genus has not been adequately investigated.

We now report the isolation of three new compounds—dihydrobuddledin A (**4**) and buddledones A (**6**) and B (**7**)—and four known compounds—buddledins A (**1**), B (**2**), C (**3**), and zerumbone (**5**)—from *B. globosa*; 14 known compounds—calceolarioside, campneoside, verbascoside, echinacoside, forsythoside B, angoroside A, crocetin monogentibiosyl ester, acacetin, acacetin-7-*O*-α-L-rhamnopyranosyl (1−6)-*β*-D-glucopyranoside, acacetin-7-*O*-(α-L-rhamnopyranosyl (1−6), α-L-rhamnopyranosyl (1−2))-*β*-D-glucopyranoside, songarosaponin A, and *δ*-amyrone—from *B. officinalis*; and eight known compounds—11,14-dihydroxy-8,11,13-abietatrien-7-one, *β*-sitosterol, verbascoside, echinacoside, forsythoside B, angoroside A, methylcatalpol, and sucrose—from *B. yunanesis* . In addition, we have tested the inhibitory effect of these compounds, as well as the parent extracts from which they were derived, against the COX and 5-LOX pathways of arachidonate metabolism in elicited rat peritoneal leukocytes. These cells represent a physiologically relevant intact cell system that both expresses important pro-inflammatory pathways of arachidonic acid metabolism and can be stimulated by addition of calcium ionophore. COX and 5-LOX are the two key enzymes for the metabolism of arachidonic acid, generating prostaglandins and thromboxanes (COX) or leukotrienes (5-LOX), which have well-recognized and important actions as mediators of inflammation, as well as many other physiological and pathological properties.[12]

Results and Discussion

The $CHCl_3$ extracts of *B. officinalis* flowers, *B. myriantha* stem, *B. asiatica* stem, *B. globosa* roots, and *B. yunanesis* stem and the MeOH extracts of *B. officinalis* flowers, *B. myriantha* stem, *B. asiatica* stem, and *B. yunanesis* stem were tested for their inhibitory activities against 5-LOX and COX (for results see Table 1). The inhibitory activities of the $CHCl_3$ extracts were superior to those of the MeOH extracts of the same plants. All the $CHCl_3$ extracts exhibited inhibitory activity against COX, whereas only two $CHCl_3$ extracts, those from *B. myriantha* and *B. globosa*, showed inhibition against 5-LOX. Furthermore, the $CHCl_3$

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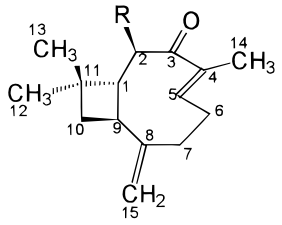
Table 1. 5-LOX and COX Inhibitory Activities of Different *Buddleja* Extracts

extract	extract rate % (w/w)	% Inhibition of COX at 50 μg/mL	% Inhibition of 5-LOX at 50 μg/mL
B. officinalis			
$CHCl_3$	3.4	67.8 ± 0.7	0
MeOH	11.5	30.7 ± 7.1	48.6 ± 12.7
B. myriantha			
$CHCL_3$	0.58	95.0 ± 1.7	81.7 ± 2.3
MeOH	6.75	37.8 ± 3.1	52.2 ± 5.4
B. asiatica			
$CHCL_3$	0.64	81.0 ± 0.3	15 ± 6.2
MeOH	4.60	35.6 ± 8.9	37.1 ± 19.7
B. yunanesis			
$CHCL_3$	0.25	74.9 ± 1.0	0
MeOH	3.31	29.3 ± 7.6	15.9 ± 10.4
B. globosa			
$CHCL_3$	1.55	86.7 ± 5.5	100

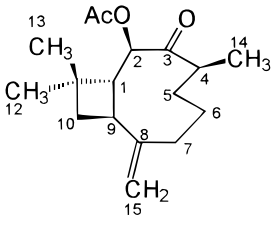
extracts, except that of *B. globosa*, exhibited stronger inhibition against COX than against 5-LOX at 50 μg/mL.

Buddledins A (**1**), B (**2**), and C (**3**) were identified as known sesquiterpenes with caryophyllane skeleton by comparing ^{1}H and ^{13}C NMR data and other physical constants with literature.[13] Compound **4** was obtained as



R

1 OAc
2 OH
3 H



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an oil, and its IR spectrum revealed the presence of a ketone (1716 cm^{-1}) and ester (1738 cm^{-1}) carbonyl groups. The formula of **4** was confirmed as $C_{17}H_{26}O_3$ by HREIMS. The DEPT spectrum of **4** exhibited four methyl carbons at δ 31.6, 23.0, 21.0, and 15.8; four methylene carbons at δ 39.4, 32.9, 30.7, and 26.4; four methine carbons at δ 79.6, 50.5, 44.4, and 38.2; one quaternary carbon at δ 34.5; two olefinic carbons at δ 153.2 and 111.4; and two carbonyl carbons at δ 213.4 and 171.0. This pattern of signals was very similar to that given by buddledin A (**1**),[13] apart from the absence of signals in **4** that corresponded to those of C-4 and C-5 in **1**. However, **4** showed an extra methine at δ 30.7 and an extra methylene at δ 26.4 compared with **1**. As a result of this, **4** was considered analogous to buddledin A but lacking the C-4−C-5 double bond. The ^{1}H−^{1}H COSY spectrum exhibited correlations between H-1 (δ 2.07) and H-2 (δ 5.12), H-1 (δ 2.07), and H-9 (δ 3.03) and between H-9 (δ 3.03) and H-10 (δ 1.73 and 1.92). Significantly, the signal for H-14 (δ 1.11) showed coupling with a multiplet at δ 2.96, and this showed coupling with a geminal signal at δ 1.40 and 1.53, which in turn coupled with one at δ 1.87 and 2.01. This strongly supported the proposed C-4−C-5 saturated bond and the coupling of signals observed would be due to the H-4, CH_2-5, and CH_2-6 concatenation. The HMBC spectrum (see Figure 1) confirmed the proposed structure of **4**. The relative stereochemistry of **4** was established by a NOESY experiment (see Figure 2).

The correlations between H-1 and H-13 (δ 1.12), between H-9 and H-12 (δ 1.14), and between H-2 (δ 5.12) and H-9 and the lack of correlation between H-9 and H-1, indicated

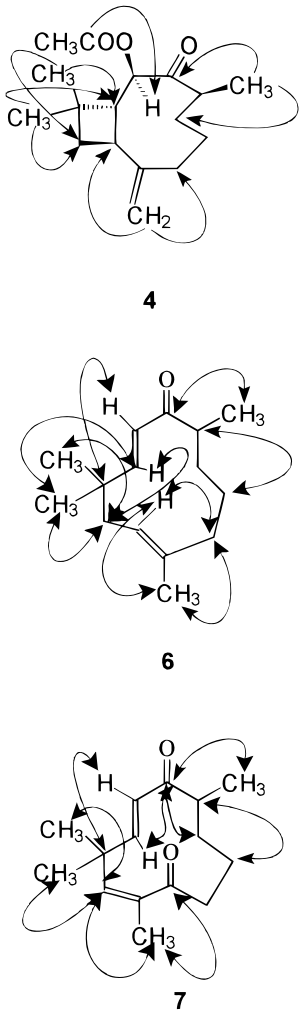


Figure 1. Important correlations in HMBC spectra of **4**, **6**, and **7**.

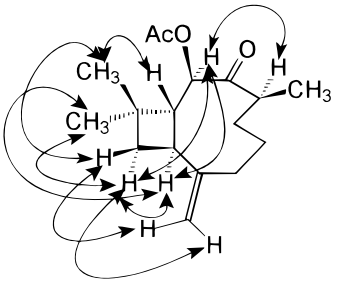
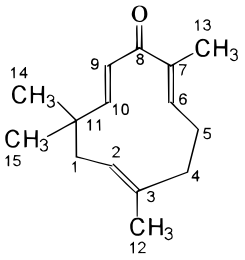


Figure 2. Key correlations in NOESY of **4**.

that H-9 was on a different side of the molecule from H-1 but on the same side as H-2, while the correlations between H-2 and H-4 showed that H-4 was on the same side as H-2.

Zerumbone (**5**) was identified through full agreement of its ^{1}H and ^{13}C NMR data with those reported in the literature.[14] Two compounds with similar spectral values, **6** and **7**, were obtained as oils. The IR spectrum of **6** showed a strong absorption band at 1680 cm^{-1}. The molecular formula of $C_{15}H_{24}O$ was established from HREIMS and the DEPT spectrum. The ^{13}C NMR and DEPT experiments revealed the presence of 15 carbons, of which four were methyl carbons, four methylene carbons, four vinyl carbons (corresponding to two double bonds), one a methine carbon, one a quatenary carbon, and one ketone carbonyl, a pattern very similar to the signals given by zerumbone (**5**), apart from the absence of two double-bond carbons and the presence of an extra methylene and extra methine signal.



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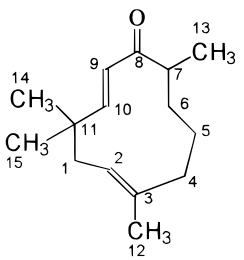

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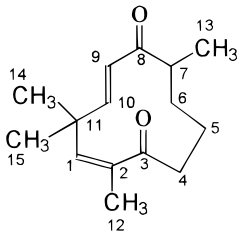

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The molecular formula of $C_{15}H_{24}O$ agreed with the evidence of a degree of unsaturation of four, rather than six as in zerumbone (**5**), and hence **6** was considered to consist of one ring with two double bonds and one ketone group. Thus, **6**, like zerumbone (**5**), could be an 11-membered ring sesquiterpene.

The unequivocal determination of the structure of **6** was made using the HMBC, $^{13}C-^{1}H$ one-bond correlation and COSY experiments. The HMBC experiment (Figure 1) established that the ketone was located at C-8, two double bonds at C-2−C-3 and C-9−C-10 and the four methyl groups at C-3, C-7, and C-11, respectively. The COSY experiment supported the existence of three spin coupling systems—H-13/H-7/H-6/H-5/H-4, H-1/H-2, and H-9/H-10, supporting structure **6**. Compound **6** was named buddledone A.

The ^{1}H and ^{13}C NMR spectra of compound **7**, which had the formula of $C_{15}H_{22}O_2$ from MS data, were similar to those of **6**. However, there was evidence of two ketone groups (δ 203.7 and 207.9) and the isolated olefinic proton at δ 5.07, comparable to H-2 in **6**, coupled only with a methyl group at δ 1.61 which, from its downfield shift, is likely to be that attached to a double-bond carbon such as C-3 in **6**. From the HMBC experiment (Figure 1), the correlations between H-14 (δ 1.25) and C-1 (δ 100.8) and between H-15 (δ 1.20) and C-1 supported the presence of Δ^1, and the correlations between H-12 and C-3 (δ 203.7) indicated that there existed a ketone group at C-3. The other correlations were identical to those of **6**. Compound **7** was named buddledone B.

The known phenylethanoid compounds calceolarioside,[15] campneoside,[16] verbascoside,[17] echinacoside,[16] forsythoside,[18] angoroside A;[19] the isoprenoid glycoside crocetin monogentibiosyl ester;[20] the flavonoids acacetin,[7] acacetin -7-*O*-α-L-rhamnopyranosyl (1−6)-*β*-D-glucopyranoside,[8] acacetin -7-*O*-α-L-rhamnopyranosyl (1−6)[α-L-rhamnopyranosyl (1−2)]*β*-D-glucopyranoside;[21] the diterpene 11,14-dihydroxy-8,11,13-abietatrien-7-one;[22] the iridoid methylcatalpol;[23] the steroid *β*-sitosterol; and the triterpenoids songarosaponin A[24] and *δ*-amyrone[25] were identified by comparison of their respective spectral data (IR, MS, ^{1}H and ^{13}C NMR) with literature values. Verbascoside and echinacoside have previously been reported from the flowers of *B. officinalis*.[6]

Most of the isolated compounds were tested for possible inhibitory activity against 5-LOX and COX, and their inhibitions, given by a concentration of 50 μg/mL, together with IC_{50} values of active compounds, are listed in Table 2.

Buddledin A (**1**), crocetin monogentiobiosyl ester, and acacetin exhibited inhibitory effects on COX, with IC_{50} values of 13.7 μM, 28.2 μM, and 77.5 μM, respectively, and buddledin A (**1**) also exhibited an inhibitory effect on 5-LOX, with an IC_{50} value of 50.4 μM. Crocetin monogentiobiosyl ester showed a selective inhibition of COX. This is the first report of the inhibitory effect of these compounds on COX and 5-LOX.

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 400 MHz (^{1}H) and 100 (^{13}C) on AMX 400 spectrometer using $CDCl_3$, DMSO-d_6, or CD_3OD as solvents, with TMS as internal standard. A DEPT experiment was carried out with the polarization pulse $\theta = 45°$, 90°, and 135°. FABMS and EIMS data were recorded on Kratos MS890MS and JEOL JMS-AX505W high-resolution mass spectrometers. UV spectra were obtained on a Perkin−Elmer Lambda-2 spectrometer. IR spectra were measured with with a Perkin−Elmer 1420 spectrometer.

Plant Material. Roots of *B. globosa* Hope were obtained from a specimen growing in a London garden; stems of *B. yunanesis* Gagne, *B. asiatica* Hemsley, and *B. myriantha* Diels were collected from the mountains of Yunan province, China, in October, 1997; and flowers of *B. officinalis* Maxim. were bought from a drugstore in Beijing. All five plants were authenticated by Dr. Peter Houghton, and their voucher specimens PH97-115−PH97-119, respectively, are deposited in the Herbarium of the Department of Pharmacy, King's College London. Voucher specimens for the four species collected in China are also held in the herbarium of the Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. Column chromatography was carried out on Si gel (220−400 mesh, Fluka), on polyamide (ICN Biochemicals), and on Sephadex LH-20 (Pharmacia). Preparative TLC was carried out using plates of Si gel PF_{254} (Merck) made in the laboratory.

The flowers of *B. officinalis* (1.3 kg); the stems of *B. myriantha* (1.2 kg), *B. asiatica* (1.4 kg), and *B. yunanesis* (2.0 kg); and the roots *of B. globosa* (1.1 kg) were extracted with $CHCl_3$ under reflux (3 × 5 L) to produce 44-g, 7-g, 9-g, 5-g, and 17-g extracts, respectively, after evaporation under reduced pressure. The marc from the $CHCl_3$ extraction of the first four species was then extracted with MeOH under reflux (3 × 5 L) to give 149-g, 81-g, 65-g, and 66-g extracts, respectively.

The $CHCl_3$ extracts of *B. globosa* were chromatographed on a Si gel column (120 g) eluted with light petroleum ether−$CHCl_3$ mixtures. From the fractions eluted with 43% $CHCl_3$, 4.5 g of residue A was obtained, while the fractions eluted with 33% $CHCl_3$ afforded 2.6 g of residue B. Residue A was rechromatographed over a Si gel column, eluting with light petroleum ether−EtOAc (96:4), then purified on a Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1) to give **4** (60 mg) and buddledin A (**1**) (800 mg). The residue B was fractionated into six parts (BGBF1−BGBF6) over a Si gel column eluting with light petroleum ether−EtOAc (97:3). The BGBF2 fraction was purified on Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1), followed by preparative TLC (hexane−$CHCl_3$, 55:45 × 4) to give compounds **6** (6.4 mg) and **7** (7.3 mg). Purifications of BGBF3, BGBF4, and BGBF5 by preparative TLC (hexane−$CHCl_3$, 1:1 × 3) yielded buddledin C (**3**) (25 mg), buddledin B (**2**) (22 mg), and zerumbone (**5**) (17 mg), respectively.

The $CHCl_3$ extracts of *B. officinalis* (28 g) were fractionated into eight parts (BOCF1−BOCF8) by Si gel column chromatography using hexane−$CHCl_3$ mixtures as eluent. Purification of BOCF2 collected from the 33% $CHCl_3$ fractions yielded *δ*-amyrone (1000 mg). The BOCF8 fraction was purified by

Table 2. Inhibition (% ± S.E.M.) and IC_{50} Values of the Isolated Compounds from *Buddleja* Genus and Positive Controls ($n = 3$)

compound	% COX at 50 μg/mL	% 5-LOX at 50 μg/mL	COX IC_{50} [μM]	5-LOX IC_{50} [μM]
sesquiterpenes				
buddledin A (**1**)	89.3 ± 4.4	98.0 ± 0.2	13.7	50.4
buddledin B (**2**)	47.9 ± 7.6	49.3 ± 7.8	>200	>200
buddledin C (**3**)	9.5 ± 7.1	16.4 ± 2.1	>200	>200
dihydrobuddledin A (**4**)	0	0		
zerumbone (**5**)	0	0		
diterpene				
11,14-dihydroxy-8,11,13-abietatrien-7-one	50.0 ± 15.1	27.1 ± 13.8		
flavonoids				
acacetin	69.5 ± 9.5	77.6 ± 6.9	77.5	123.2
acacetin-7-O-α-L-rhamnopyranosyl (1−6)-β-D-glucopyranoside	0	0		
acacetin-7-O-α-L-rhamnopyranosyl (1−6)[α-L-rhamnopyranosyl (1−2)]-β-D-glucopyranoside	0	0		
phenylethanoids				
calceolarioside	0	0		
campneoside	0	0		
verbascoside	0	0		
echinacoside	0	0		
forsythoside B	0	0		
angoroside A	0	0		
miscellaneous				
δ-amyrone	16.8 ± 6.7	0		
crocetin monogentibiosyl ester	85.1 ± 1.9	0	28.2	n. a.[a]
methylcatalpol	0	0		
positive controls				
dazoxiben	89.6 ± 0.9	0		
indomethacin	100	0		
ZM211965[26]	0	100		
ZM230487[26]	47.8 ± 4.9	100		

[a] n.a. = not assessed.

Sephadex LH-20 ($CHCl_3$−MeOH) to give acacetin (13 mg). The MeOH extracts of *B. officinalis* (50 g) were fractionated into eight parts (BOMF1−BOMF8) by polyamide column chromatography using H_2O−MeOH gradient elution. The BOMF2−BOMF7 fractions were further chromatographed on Si gel ($CHCl_3$−MeOH−H_2O), then purified by repetitive column chromatography on Sephadex LH-20 (MeOH) to give calceolarioside (35 mg), campneoside (55 mg), verbascoside (3000 mg), echinacoside (150 mg), forsythoside B (100 mg), angoroside A (80 mg), crocetin monogentibiosyl ester (110 mg), acacetin-7-*O*-α-L-rhamnopyranosyl (1−6)-β-D-glucopyranoside (100 mg), acacetin-7-*O*-α-L-rhamnopyranosyl (1−6)[α-L-rhamnopyranosyl (1−2)]-β-D-glucopyranoside (35 mg), and songarosaponin A (120 mg).

The $CHCl_3$ extracts of *B. yunanesis* (5 g) were chromatographed on Si gel (hexane−EtOAc), then purified by repetitive column chromatography on Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) to give 11,14-dihydroxy-8,11,13-abietatrien-7-one (17 mg) and β-sitosterol (1 g).

The MeOH extracts of *B. yunanesis* (50 g) were chromatographed on Si gel ($CHCl_3$−MeOH−H_2O), then purified by repetitive column chromatography on Sephadex LH-20 (MeOH) to give verbascoside (200 mg), echinacoside (400 mg), forsythoside B (300 mg), angoroside A (150 mg), methylcatalpol (1.5 g), and sucrose (2 g).

Dihydrobuddledin A (4): obtained as a colorless oil, $[\alpha]^{20}_D$ −64.7° (*c* 0.51, $CHCl_3$); UV (EtOH) no absorption; IR ν_{max} (KBr) 2955, 2935, 1738, 1716, 1455, 1371, 1244, 1020, 890 cm^{-1}; ^1H NMR ($CDCl_3$, 400 mHz) δ 5.12 (1H, d, $J = 11.4$ Hz, H-2), 4.75 (1H, s, H-15), 4.62 (1H, t, $J = 1.8$ Hz, H-15), 3.03 (1H, m, H-9), 2.96 (1H, m, H-4), 2.08 (3H, s, OAc), 2.07 (1H, dd, $J = 11.4$, 10.0 Hz, H-1), 2.01−1.53 (8H, m, CH_2-5, CH_2-6, CH_2-7, CH_2-10), 1.14 (3H, s, H-12), 1.12 (3H, s, H-13), 1.11 (3H, d, $J = 6.8$ Hz, H-14); ^{13}C NMR ($CDCl_3$, 400 mHz) δ 213.4 (C-3), 171.0 (2-OO***C***CH_3), 153.2 (C-8), 111.4 (C-15), 79.6 (C-2), 50.5 (C-1), 44.4 (C-4), 39.4 (C-10), 38.2 (C-9), 34.5 (C-11), 32.9 (C-7), 31.6 (C-12), 30.7 (C-5), 26.4 (C-6), 23.0 (C-13), 21.0 (2-OO***C***CH_3), 15.8 (C-14); FABMS *m*/*z* 279 $[M + 1]^+$; HRFABMS *m*/*z* 279.1945 (calcd for $C_{17}H_{27}O_3$ 279.1960).

Buddledone A (6): obtained as a colorless oil, UV ($CHCl_3$) λ_{max} (log ε) 240 nm (3.12); IR (KBr) ν_{max} 2955, 2935, 1680, 1455, 1371, 1244, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 400 mHz) δ 6.24 (1H, d, $J = 16.1$ Hz, H-10), 6.05 (1H, d, $J = 16.1$ Hz, H-9), 5.07 (1H, m), 2.56 (1H, m, H-2), 2.06 (2H, d, $J = 8.2$ Hz, H-6), 1.90 (2H, m, H-4), 1.69 (2H, m, H-6), 1.45 (3H, d, $J = 1.0$ Hz, H-12), 1.24−1.35 (2H, m, CH_2-5), 1.17 (3H, s, H-14), 1.15 (3H, s, H-15), 1.03 (3H, d, $J = 6.7$ Hz, H-13); ^{13}C NMR ($CDCl_3$, 100 mHz) δ 205.8 (C-8), 152.4 (C-10), 137.4 (C-3), 127.1 (C-9), 122.7 (C-2), 47.7 (C-7), 42.1 (C-1), 40.9 (C-4), 40.0 (C-11), 32.8 (C-6), 22.3 (C-5), 26.8 (C-14), 26.3 (C-15), 17.0 (C-12), 14.5 (C-13); FABMS *m*/*z* 221 $[M + 1]^+$; HRFABMS *m*/*z* 221.1900 (calcd for $C_{15}H_{25}O$ 221.1905).

Buddledone B (7): obtained as an oil, UV ($CHCl_3$) λ_{max} 240 nm; IR ν_{max} (KBr) cm^{-1} 2955, 2935, 1680, 1675, 1455, 1371, 1244, 1020; EIMS *m*/*z* (rel int) ^1H NMR ($CDCl_3$, 400 mHz) δ 6.47 (1H, d, $J = 16.5$ Hz, H-10), 6.01 (1H, d, $J = 16.5$ Hz, H-9), 5.07 (1H, m, H-1), 2.76 (1H, m, H-7), 1.93−2.29 (2H, m, H-4), 1.61 (3H, d, $J = 2.7$ Hz, H-12), 1.43−1.60 (2H, m, H-5), 1.29−1.68 (2H, m, H-6), 1.25 (3H, s, H-14), 1.20 (3H, s, H-15), 1.10 (3H, d, $J = 6.6$ Hz, H-13); ^{13}C NMR ($CDCl_3$, 100 mHz) δ 207.9 (C-8), 203.7 (C-3), 153.9 (C-10), 124.9 (C-9), 101.7 (C-2), 100.8 (C-1), 43.2 (C-7), 39.0 (C-11), 33.2 (C-6), 32.2 (C-4), 28.3 (C-15), 26.3 (C-14), 24.6 (C-5), 19.0 (C-12), 15.6 (C-13); FABMS *m*/*z* 235 $[M + 1]^+$; HRFABMS *m*/*z* 235.1706 (calcd for $C_{15}H_{23}O_2$ 235.1699).

Preparation of Suspensions of Rat Peritoneal Leukocytes. A suspension of leukocytes containing approximately 85% polymorphonuclear leukocytes (PMNs) and 15% mononuclear cells was elicited from male Wistar rats (200−300 g) by an ip injection of 10 mL of a solution of 6% oyster glycogen in saline,[11] followed 16−20 h later by 60 mL ice-cold modified Hank's balanced salt solution (HBSS) free of Ca^{2+} and Mg^{2+}. After about 90 s of vigorous massage, the peritoneal washing was removed, centrifuged at 400 g for 10 min at 4 °C, and the contaminating erythrocytes in the pellet lysed after resuspension in a small volume of 0.2% saline for 30 s. The isotonicity of the pellet suspension was then reestablished by adding excess HBSS. After further centrifugation and washing, the mixed peritoneal leukocytes were resuspended in complete HBSS at 2.5×10^6 cells/mL, containing 1.26 mM Ca^{2+} and 0.9 mM Mg^{2+}. Cell viability based on trypan blue exclusion was greater than 95%.

Stimulation of the Release of Eicosanoids and Their Radioimmunoassay. Triplicate aliquots of 0.5 mL of leukocytes were preincubated at 37 °C for 10 min with the test compounds or extracts. They were added prior to the cells in 5 mL of dimethylsulfoxide (DMSO) or $CHCl_3$ to the 3-mL polypropylene incubation tubes. In the case of solvents added in $CHCl_3$, the solvent was allowed to evaporate before adding the cells. After this drug preincubation, 1 mL of calcium ionophore A23187 was added in DMSO to give a final concentration of 1 mM for further 10 min of incubation. The cells were pelleted by centrifugation at 2500 *g* for 10 min at 4 °C, and the supernatants were decanted and frozen. Aliquots (5−15 μL) of the thawed samples were subjected to radioimmunoassay for TXB_2 and LTB_4 by making up to 100 μL with 50 mM phosphate buffer (pH 7.5) containing 0.1% human γ-globulin and 0.9% saline, adding 200 μL polyclonal rabbit anti-eicosanoid serum (diluted 1:1500), 100 μL tracer containing 10 nCi $^3H_8$-TXB_2 or 4 nCi $^3H_8$-LTB_4 (NEN or Amersham), respectively, mixing and incubating at 4 °C for 18 h. After this, bound label was separated from free using 200 μL dextran-coated charcoal and the bound dpm counted in a Packard model 1900TR liquid scintillation analyzer. Dazoxiben and indomethacin were used as positive controls for COX inhibition and methoxyalkylthiazole ZM-211965 and methoxytetrahydropyran ZM 230487 for 5-LOX inhibition.[26]

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References and Notes

(1) Houghton, P. J. *J. Ethnopharmacol.* **1985**, *11*, 293−308.
(2) Jiangshu Botanic Institute, Institute of Materia Medica CAMS, Kunming Botanic Institute. *Xinhua Bencao Gangyao II*; Shanghai Scientific and Technical Press: Shanghai, 1991; p 383.
(3) Li, G. *Yunnan Zhiwuzi*; Yunnan Technical and Scientific Press: Yunnan, China, 1990; p 491.
(4) Ding, N.; Yahara, S.; Nohara, T. *Chem. Pharm. Bull.* **1992**, *40*, 780−782.
(5) Pardo, F.; Perich, F.; Villarroel, L.; Torres, R. *J. Ethnopharmacol.* **1993**, *19*, 221−222.
(6) Li, J.; Zhao, Y.; Ma, L. *J. Chin. Pharm. Sci.* **1997**, *6*, 178−181.
(7) Matsuda, H.; Cai, H.; Kudo, M.; Tosa, H.; Iinuma, M. *Biol. Pharm. Bull.* **1995**, *18*, 463−466.
(8) Houghton, P. J. *Phytochemistry* **1989**, *28*, 2693−2695.
(9) Houghton, P. J.; Woldemariam, T. Z.; Candau, M.; Barnardo, A.; Khen-Alafun, O.; Shangxiao, L. *Phytochemistry* **1996**, *42*, 485−488.
(10) Harborne, J. B.; Williams, C. A. *Phytochemistry* **1971**, *10*, 367−368.
(11) Hoult, J. R. S.; Moroney, M. A.; Paya, M. *Methods Enzymol.* **1994**, *234*, 443−454.
(12) Moore, P. K. *Prostanoids: Pharmacological, Physiological and Clinical Relevance*; Cambridge University Press: Cambridge, 1985; p 225.
(13) Yoshida, T.; Nobuhara, J.; Uchida, M.; Okuda, T. *Chem. Pharm. Bull.* **1978**, *26*, 2535−2544.
(14) Matthes, H. W. D.; Luu, B.; Ourisson, G. *Phytochemistry* **1980**, *19*, 2643−2650.
(15) Shoyama, Y.; Matsumoto, M.; Nishioka, I. *Phytochemistry* **1986**, *25*, 1633−1636.
(16) Kitagawa, S.; Tsukamoto, H.; Hisada, S.; Nishibe, S. *Chem. Pharm. Bull.* **1984**, *32*, 1209−1213.
(17) Liu, Z.; Jia, Z. *Phytochemistry* **1991**, *30*, 1341−1344.
(18) Endo, K.; Takahashi, K.; Abe, T.; Hikino, H. *Heterocycles* **1982**, *19*, 261−264.
(19) Calis, I.; Hosny, M.; Khalifa, T.; Ruedi, P. *Phytochemistry* **1987**, *26*, 2057−2061.
(20) Pfander, H.; Wittwer, F. *Helv. Chim. Acta* **1975**, *58*, 2233−2236.
(21) Li, J., Zhao, Y.; Wang, B.; Li, X.; Ma, L. *Yaoxuexuebao* **1996**, *31*, 849−854.
(22) Kuo, Y.; Chen, C.; Huang, S. *J. Nat. Prod.* **1998**, *61*, 829−831.
(23) Duff, R. B.; Bacon, J. S. D.; Mudie, C. M.; Farmer, V. C.; Russell, J. D.; Forrester, A. R. *Biochem. J.* **1965**, *96*, 1−4.
(24) Seifert, K.; Preiss, A.; Johne, S.; Schmidt, J.; Lien, N. T.; Lavaud, C.; Massoit, G.; *Phytochemistry* **1991**, *30*, 3395−3400.
(25) Ames, T. R.; Halsall, T. G.; Jones, E. R. H. *J. Chem. Soc.* **1951**, 450−457.
(26) Bird, T. G. C.; Bruneau, P.; Crawley, G. C.; Edwards, M. P.; Foster, S. J.; Girodeau, J. M.; Kingston, J. F.; McMillan, R. M. *J. Med. Chem.* **1991**, 2176−86.

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