Novel Biologically Active Bibenzyls from *Bauhinia saccocalyx* PIERRE

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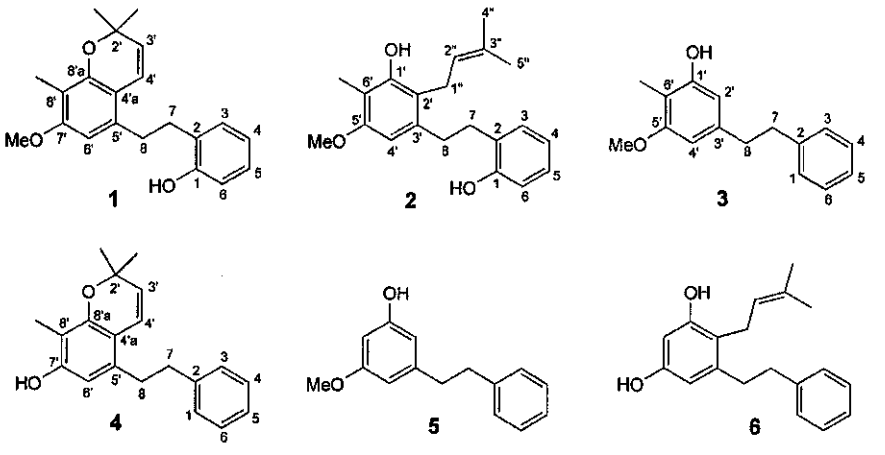
Four new bibenzyls, bauhinols A–D (**1**–**4**), together with the two known bibenzyls **5** and **6**, were isolated from the roots of *Bauhinia saccocalyx*, and their structures were elucidated by analyses of spectroscopic data. Bauhinol A (**1**) exhibits significant cytotoxicity towards NCI-H187 (small-cell lung cancer), BC (breast cancer), and KB (oral-cavity cancer) cell lines, with IC_{50} values of 2.7–4.5 μg/ml. Bauhinol B (**2**) is cytotoxic against NCI-H187 (IC_{50} = 1.1 μg/ml) and BC (IC_{50} = 9.7 μg/ml) cell lines, but inactive toward the KB cell line (at 20 μg/ml). Compound **2** also is mildly antifungal towards *Candia albicans* (IC_{50} = 28.9 μg/ml). Bibenzyl **6** is active against NCI-H187 (IC_{50} = 14.1 μg/ml) and BC (IC_{50} = 4.0 μg/ml) cells, but inactive (at 20 μg/ml) toward the KB cell line. Compounds **1**, **2**, and **6** show mild antimycobacterial activities, with *MIC* values of 25–50 μg/ml, but are inactive at 20 μg/ml against the K1 malarial parasite strain (*Plasmodium falciparum*). While bauhinol A (**1**) is inactive against cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2), compounds **2** and **6** inhibit both COX-1 and COX-2, with IC_{50} values comparable to those of the standard drug, aspirin (*Table 3*).

Introduction. – As part of our ongoing research program at BIOTEC [1–4] on biologically active substances from Thai bioresources, we have intensively screened biological activities of extracts from plants and microorganisms. Recently, we found that a crude CH_2Cl_2 root extract of *Bauhinia saccocalyx* PIERRE (Leguminosae-Caesalpinioideae) exhibited antimalarial (IC_{50} = 5.0 μg/ml) and antimycobacterial *MIC* = 25 μg/ml) activities[1]). Previous chemical investigations of active principles from the root extract of *B. saccocalyx* resulted in the isolation of two new antimycobacterial dibenzo[*b*,*f*]oxepins, bauhinoxepins A and B [5]. Further investigation on the minor metabolites in the roots of *B. saccocalyx* now led to the isolation of four new bibenzyls named bauhinols A–D (**1**–**4**), together with the two known bibenzyls **5** and **6**. We report herein the isolation and characterization of these biologically active metabolites.

Results and Discussion. – 1. *Structure Elucidation.* The CH_2Cl_2 root extract of *B. saccocalyx* was purified by *Sephadex LH-20* column chromatography and HPLC to yield bauhinols A–D (**1**–**4**), together with the two known bibenzyls **5** and **6**. The spectral data of **5** and **6** were identical in all respects to those published earlier [6–8].

[1]) IC_{50} and *MIC* designate 'inhibitory concentration fifty' and 'minimum inhibitory concentration', resp.



Bauhinol A (**1**) was obtained as a brown viscous liquid, with the molecular formula $C_{21}H_{24}O_3$ as deduced from ESI-TOF-MS. As indicated in the ^{1}H-NMR spectrum ($CDCl_3$), **1** possessed a dimethylchromene (2*H*-1-benzopyran) unit (δ(H) 6.62 (*d*, J = 9.9), 5.56 (*d*, J = 9.9), 1.44 (*s*, 2 Me)), two groups of downfield-shifted methylenes (δ(H) 2.88 (*m*) and 2.93 (*m*)); a 1,2-disubstituted benzene ring (δ(H) 7.13 (*dd*, J = 7.3, 1.6), 6.90 (*td*, J = 7.3, 0.9), 7.12 (*td*, J = 7.7, 1.7), and 6.76 (br. *d*, J = 7.8)), and two Me groups (δ(H) 2.10 (*s*) and 3.79 (*s*)). Further, an aromatic *singlet* at δ(H) 6.25 suggested that the benzene ring of the chromene unit was triply substituted.

A total of 21 signals were observed in the ^{13}C-NMR spectrum of **1**, which were classified by DEPT as seven CH, four Me, two CH_2 groups, and eight quaternary C-atoms. The ^{1}H,^{1}H-COSY spectrum showed the connectivity from H–C(3) to H–C(6), and also demonstrated couplings between CH_2(7) and CH_2(8), and between H–C(3′) and H–C(4′). The HMBC spectral data were very informative concerning the assembly of the gross structure of **1**. The following ^{1}H,^{13}C long range correlations were observed: H–C(3) to C(1); both H–C(4) and H–C(6) to C(2); H–C(5) to C(1); CH_2(7) to C(1), C(2), and C(3); CH_2(8) to C(5′) and C(6′); the 2′-Me H-atoms to C(2′) and C(3′); H–C(3′) to C(4′a); H–C(4′) to C(2′), C(5′), and C(8′a); H–C(6′) to C(8) and C(7′); the 7′-OMe H-atoms to C(7′); and the 8′-Me H-atoms to C(7′), C(8′), and C(8′a). On the basis of these spectral data, bauhinol A (**1**) was identified as 2-[2-(7-methoxy-2,2,8-trimethyl-2*H*-1-benzopyran-5-yl)ethyl]phenol. The H- and C-atoms of **1** were completely assigned, as shown in *Table 1*.

Bauhinol B (**2**), a brown viscous liquid, exhibited, by analysis of the ESI-TOF mass spectrum, a molecular formula of $C_{21}H_{26}O_3$. Information from the ESI-TOF and ^{1}H-NMR spectral data readily indicated that **2** was a derivative of **1**. Careful analyses of the ^{1}H-NMR spectrum revealed the replacement of the dimethyl-2*H*-1-pyran ring in **1** with a 3-methyl-but-2-enyl moiety in **2** (ring opening). Again, the HMBC spectral data were very useful for the structure elucidation of **2**. The following correlations were observed: H–C(3) to C(1); both H–C(4) and H–C(6) to C(2); H–C(5) to C(1);

Table 1. *^{1}H- and ^{13}C-NMR Spectral Data for Bauhinols A* (**1**) *and B* (**2**). At 500 and 125 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	1			**2**	
	^{1}H	^{13}C		^{1}H	^{13}C
C(1)	–	153.7	C(1)	–	153.7
C(2)	–	128.0	C(2)	–	127.9
H–C(3)	7.13 (*dd*, *J* = 7.3, 1.6)	130.5	H–C(3)	7.13 (*dd*, *J* = 7.3, 1.5)	130.4
H–C(4)	6.90 (*td*, *J* = 7.3, 0.9)	120.9	H–C(4)	6.90 (*td*, *J* = 7.5, 0.9)	121.0
H–C(5)	7.12 (*td*, *J* = 7.7, 1.7)	127.4	H–C(5)	7.12 (*td*, *J* = 7.7, 1.7)	127.4
H–C(6)	6.76 (br. *d*, *J* = 7.8)	115.5	H–C(6)	6.77 (br. *d*, *J* = 8.0)	115.5
CH_2(7)	2.88 (*m*)	32.6	CH_2(7)	2.86 (*m*)	32.6
CH_2(8)	2.93 (*m*)	33.1	CH_2(8)	2.92 (*m*)	34.4
C(2′)	–	75.3	C(1′)	–	153.6
H–C(3′)	5.56 (*d*, *J* = 9.9)	128.3	C(2′)	–	117.5
H–C(4′)	6.62 (*d*, *J* = 9.9)	119.3	C(3′)	–	137.8
C(4′a)	–	113.1	H–C(4′)	6.31 (*s*)	104.4
C(5′)	–	135.3	C(5′)	–	156.6
H–C(6′)	6.25 (*s*)	104.1	C(6′)	–	110.8
C(7′)	–	157.9	CH_2(1″)	3.38 (*d*, *J* = 6.9)	25.4
C(8′)	–	112.3	H–C(2″)	5.12 (*td*, *J* = 5.5, 1.2)	122.9
C(8′a)	–	151.9	C(3″)	–	134.4
Me(2′)	1.44 (*s*)	27.8	Me(4″)	1.75 (*d*, *J* = 1.1)	25.8
Me(2′)	1.44 (*s*)	27.8	Me(5″)	1.84 (br. *s*)	17.9
Me(8′)	2.10 (*s*)	8.0	Me(6′)	2.12 (*s*)	8.2
OMe(7′)	3.79 (*s*)	55.6	OMe(5′)	3.78 (*s*)	55.6

CH_2(7) to C(1), C(3), and C(3′); CH_2(8) to C(2), C(3′), and C(4′); the 6′-Me H-atoms to C(1′), C(5′), and C(6′); the 5′-OMe H-atoms to C(5′); CH_2(1″) to C(1′), C(2′), C(3′), and C(3″); H–C(2″) to C(2′); and both the Me(4″) and Me(5″) H-atoms to C(2″) and C(3″). The assignment of Me(4″) and Me(5″) was possible by means of NOESY experiments. Based on these spectral data, bauhinol B (**2**) was identified as a prenyl derivative of bauhinol A (**1**), corresponding to 3-[2-(2-hydroxyphenyl)ethyl]-5-methoxy-6-methyl-2-(3-methylbut-2-enyl)phenol. The complete assignments of the H- and C-atoms for **2** are shown in *Table 1*.

Bauhinol C (**3**), a brown viscous liquid, had the molecular formula $C_{16}H_{18}O_2$, as deduced from the ESI-TOF mass spectrum. ^{1}H- and ^{13}C-NMR resonances ascribed to two CH_2 groups at δ(H) 2.87 (δ(C) 37.8) and 2.94 (37.9) were characteristic of a bibenzyl moiety. Analyses of the ^{1}H- and ^{13}C-NMR spectral data revealed the replacement of the 1,2-substituted benzene rings in **1** and **2** with a mono-substituted benzene ring in **3**. Moreover, the NMR spectral data also indicated the replacement of the 2′-prenyl group of **2** with an aromatic H-atom (δ(H) 6.32 (br. *s*)). The ^{1}H,^{1}H-COSY spectrum of **3** indicated a correlation between H–C(2′) and H–C(4′), whose broad *singlets* implied *meta* coupling. The HMBC spectrum established the gross structure of **3**, showing the following correlations: H–C(1) to C(7); H–C(4) to C(2); CH_2(7) to C(3′); CH_2(8) to C(2); H–C(2′) to C(8); H–C(4′) to C(8) and C(6′); the 6′-Me H-atoms to C(1′), C(5′), and C(6′); and the 5′-OMe H-atoms to C(5′). The NOESY spectrum of **3** exhibited a cross-peak between the 5′-OMe and H–C(4′) H-atoms, suggesting close proximity of these groups. Based on these spectral data, the structure

Table 2. *^{1}H- and ^{13}C-NMR Spectral Data for Bauhinols C* (**3**) *and D* (**4**). At 500 and 125 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	3			**4**	
	^{1}H	^{13}C		^{1}H	^{13}C
H–C(1)	7.24 (*m*)	128.5	H–C(1)	7.17 (br. *d*, $J = 6.8$)	128.4
C(2)	–	141.8	C(2)	–	141.7
H–C(3)	7.24 (*m*)	128.5	H–C(3)	7.17 (br. *d*, $J = 6.8$)	128.4
H–C(4)	7.33 (*m*)	128.3	H–C(4)	7.28 (*m*)	128.3
H–C(5)	7.33 (*m*)	125.9	H–C(5)	7.18 (*m*)	125.9
H–C(6)	7.33 (*m*)	128.3	H–C(6)	7.28 (*m*)	128.3
CH_2(7)	2.94 (*m*)	37.9	CH_2(7)	2.82 (*m*)	37.5
CH_2(8)	2.87 (*m*)	37.8	CH_2(8)	2.82 (*m*)	34.2
C(1′)	–	154.0	C(2′)	–	75.4
H–C(2′)	6.32 (br. *s*)	103.6	H–C(3′)	5.50 (*d*, $J = 10.0$)	127.6
C(3′)	–	140.7	H–C(4′)	6.45 (*d*, $J = 10.0$)	119.2
H–C(4′)	6.36 (br. *s*)	108.1	C(4′a)	–	112.6
C(5′)	–	158.7	C(5′)	–	135.5
C(6′)	–	110.0	H–C(6′)	6.17 (*s*)	108.0
Me(6′)	2.13 (*s*)	7.8	C(7′)	–	153.9
OMe(5′)	3.82 (*s*)	55.7	C(8′)	–	109.7
			C(8′a)	–	152.2
			Me(2′)	1.40 (*s*)	27.7
			Me(2′)	1.40 (*s*)	27.7
			Me(8′)	2.07 (*s*)	7.7

of bauhinol C (**3**) was identified as 3-methoxy-2-methyl-5-(2-phenylethyl)phenol. The assignment of the H- and C-atoms for **3** is shown in *Table 2*.

Bauhinol D (**4**) was obtained as a yellow viscous liquid. Since it was not very stable in solution, all necessary spectral data had to be collected rapidly. The pseudo-molecular ion of **4** could not be observed in the ESI-TOF mass spectrum, possibly due to the unstable nature of the molecule. Analysis of ^{1}H- and ^{13}C-NMR, DEPT, and HMQC spectral data, as well as analogous correlation of the NMR data of **4** with those of compounds **1**–**3**, readily established the molecular formula of **4** as $C_{20}H_{22}O_2$. Its ^{1}H-NMR spectrum clearly revealed signals of a dimethylchromene unit (δ(H) 6.45 (*d*, $J = 10.0$), 5.50 (*d*, $J = 10.0$), 1.40 (*s*, 2 Me)) and two downfield methylene groups (δ(H) 2.82 (*m*, 4 H)), suggesting that **4** was a (phenylethyl)-substituted dimethylchromene. Analysis of the ^{1}H- and ^{13}C-NMR spectral data indicated that **4** was not only a desmethyl derivative of bauhinol A (**1**), but also that the 1,2-disubstituted benzene ring of **1** was replaced with a mono-substituted benzene ring in **4**. Similar to the structures of bauhinols A–C (**1**–**3**), the structure of bauhinol D (**4**) was substantiated by HMBC spectral data, in which the following correlations were identified: both H–C(1) and H–C(3) to C(7); both H–C(4) and H–C(6) to C(2); CH_2(7) to C(1) and C(3); CH_2(8) to (6′); the 2′-Me H-atoms to C(2′) and C(3′); H–C(3′) to C(2′) and C(4′a); H–C(4′) to C(2′), C(5′), and C(8′a); H–C(6′) to C(8), C(4′a), C(5′), and C(8′); and the 8′-Me H-atoms to C(7′), C(8′), and C(8′a). On the basis of these spectral data, bauhinol D (**4**) was identified as 2,2,8-trimethyl-5-(2-phenylethyl)-2*H*-1-benzopyran-7-ol. The H- and C-atoms of **4** were completely assigned (*Table 2*).

2. *Biological Activity.* Bauhinol A (**1**) exhibited significant cytotoxicity towards NCI-H187 (small-cell lung cancer), BC (breast cancer), and KB (oral-cavity cancer) cell lines, with IC_{50} values[1]) of 2.7–4.5 μg/ml (*Table 3*). Bauhinol B (**2**) was cytotoxic against NCI-H187 (IC_{50} = 1.1 μg/ml) and BC (IC_{50} = 9.7 μg/ml) cell lines, but was inactive toward the KB cell line (at 20 μg/ml). Compound **2** also demonstrated mild antifungal activity (IC_{50} = 28.9 μg/ml) against *Candida albicans*. Bibenzyl **6** was active against NCI-H187 (IC_{50} = 14.1 μg/ml) and BC (IC_{50} = 4.0 μg/ml) cell lines, and was inactive (at 20 μg/ml) toward the KB cell line (*Table 3*). Compounds **1**, **2**, and **6** showed mild antimycobacterial activities with *MIC* values of 25–50 μg/ml, and they were inactive (at 20 μg/ml) *in vitro* against the malarial parasite *Plasmodium falciparum* (*Table 3*). While bauhinol A (**1**) was inactive against cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2), compounds **2** and **6** inhibited both COX-1 and COX-2, with IC_{50} values (9.0 and 2.5 μg/ml for COX-1, and 1.3 and 1.8 μg/ml for COX-2, resp.) comparable to those of the standard drug *aspirin* (*Table 3*). The biological activities of bibenzyls **3** and **5** were not evaluated due to the limited amount of sample isolated, and the activity of **4** could not be determined due to its instability in the test systems. Previously, bibenzyls isolated from *B. malabarica* were reported to exhibit antimalarial activity [9], and leaves of *B. racemosa* have been used in the treatment of malaria [10].

Table 3. *Biological Activities of Compounds* **1**, **2**, *and* **6**. IC_{50} and *MIC* values[1]) are expressed in μg/ml.

Property	Parameter	Cell type	**1**	**2**	**6**
Cytotoxicity[a])	IC_{50}	NCI-H187	3.4	1.1	14.1
		BC	2.7	9.7	4.0
		KB	4.5	> 20	> 20
Antimycobacterial activity[b])	*MIC*		50	25	25
Antifungal activity[c])	IC_{50}		> 50	28.9	11.7
Antimalarial activity[d])	IC_{50}		> 20	> 20	> 20
COX Inhibition[e])	IC_{50}	COX-I	> 20	9.0	2.5
		COX-II	> 20	1.3	1.8

[a]) Cell types: NCI-H187, small-cell lung cancer; BC, breast cancer; KB, oral-cavity cancer. The reference compound ellipticine exhibited activity toward Vero, KB, and BC cell lines, with IC_{50} values of 0.2–0.3 μg/ml. [b]) Tested against *Mycobacterium tuberculosis* H37Ra; the standard drugs isoniazid and kanamycin sulfate showed *MIC* values of 0.040–0.090 and 2.0–5.0 μg/ml, resp. [c]) Tested against *Candida albicans*; the standard drug amphotericin B exhibited an IC_{50} value of 0.04 ± 0.01 μg/ml (n = 3). [d]) Tested against *Plasmodium falciparum* (K1, multidrug-resistant strain); an IC_{50} value of 1.2 ± 0.02 ng/ml (n = 3) was determined for the reference compound dihydroartemisinin. [e]) *Aspirin* was used as standard drug, exhibiting IC_{50} values of 2.06 and 3.57 μg/ml for COX-1 and COX-2, resp.

Interestingly, *Bauhinia* species are able to produce cyclic compounds with a seven-membered ring fusion between rings A and B (*Figure*), representatives being racemosol and oxepin derivatives (*e.g.*, bauhinoxepin). Racemosol is produced by *B. malabarica*, *B. racemosa*, and *B. rufescens* [9][11][12], while oxepin derivatives are found in *B. saccocalyx*, *B. racemosa*, and *B. variegata* [5][13][14]. The biosynthesis of racemosol from bibenzyl precursors was proposed earlier (*Figure*) [9][12]. As mentioned, the dibenzo[*b*,*f*]oxepins, bauhinoxepins A and B, were previously isolated from *B. saccocalyx* [5]. The co-existence of bauhinols A–D (**1**–**4**) and their structurally related dibenzo[*b*,*f*]oxepins within the same plant, *B. saccocalyx*, suggests

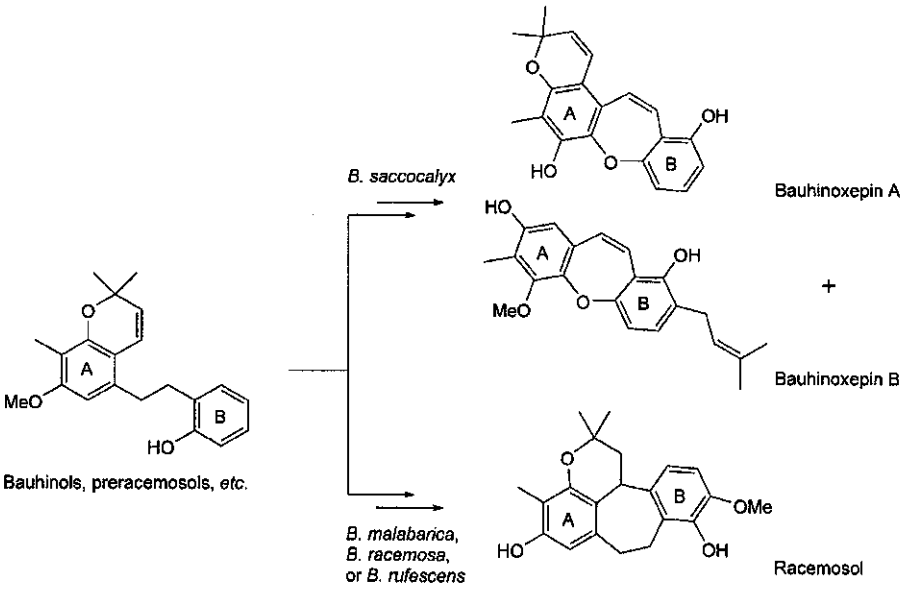


Figure. *Biosyntheses of bauhinoxepins and racemosol from bibenzyl precursors by* Bauhinia *species*

that bibenzyls may be precursors of bauhinoxepins A and B (*Figure*). Enzymatic cyclization for the formation of seven-membered rings in *Bauhinia* species is extremely interesting and deserves more attention, as well as other potential cyclization reactions to other dibenzo[*b*,*f*]oxepin derivatives.

Experimental Part

General. HPLC Separation was performed on a *Waters 600* pump, equipped with a *Waters 996* photodiode-array detector and a C_{18} reverse-phase column (*LiChroCART 250-10*; *Merck*). UV Spectra were recorded on a *Cary-1E* UV/VIS spectrophotometer; λ_{max} [nm], (log ε). IR Spectra were recorded on a *Perkin-Elmer 2000* spectrometer; in cm^{-1}. ^1H-, ^{13}C-, DEPT, $^1H,^1H$-COSY, NOESY, HMQC, and HMBC NMR experiments were carried out on a *Bruker DRX-400* spectrometer, operating at 400 MHz (^1H) and 100 MHz (^{13}C), resp.; chemical shifts δ in ppm rel. to Me_4Si, coupling constants J in Hz. Electrospray-ionization time-of-flight mass spectrometry (ESI-TOF-MS) was performed on a *Micromass-LCT* mass spectrometer, and the lock mass calibration was applied for the determination of accurate mass (in *m/z*).

Plant Material. Roots of *B. saccocalyx* were collected from Nakhon Sawan Province, Thailand, and identified by *P. Charoenchai* (BIOTEC). A voucher specimen (BRU521) was deposited at BIOTEC, Thailand.

Extraction and Isolation. Dried roots of *B. saccocalyx* (2.33 kg) were macerated in CH_2Cl_2 (8 l) for 2 d. The extract was filtered and evaporated to dryness, yielding 19.8 g of crude extract, which was purified on a *Sephadex LH-20* (*Amersham Biosciences*) column (MeOH as eluent), from which 20 fractions (A_1–A_{20}; 80 ml each) were collected. Fraction A_7 was sequentially purified on a *Sephadex LH-20* column (eluting with MeOH, 50 ml for each fraction) and HPLC (eluting with $MeCN/H_2O$ 55:45), to yield bauhinols A (**1**; 36.4 mg), C (**3**; 1.3 mg), and D (**4**; 9.2 mg). Fraction A_8 was re-chromatographed on *Sephadex LH-20* (MeOH, 30 ml for each fraction), followed by HPLC ($MeCN/H_2O$ 50:50), to afford bauhinol B (**2**; 13.2 mg) and bibenzyls **5** (1.4 mg) and **6** (42.0 mg).

Bauhinol A (=*2-[2-(7-Methoxy-2,2,8-trimethyl-*2H*-1-benzopyran-5-yl)ethyl]phenol*; **1**): Brown viscous liquid. UV (MeOH): 203 (4.47), 278 (3.94). IR (nujol): 3443, 2972, 1602, 1455, 1132, 753. ^1H- and ^{13}C-NMR: see *Table 1*. ESI-TOF-MS: 347.1667 ($[M+Na]^+$, $C_{21}H_{24}NaO_3^+$; calc. 347.1623).

Bauhinol B (= *3-[2-(2-Hydroxyphenyl)ethyl]-5-methoxy-6-methyl-2-(3-methylbut-2-enyl)phenol*; **2**): Brown viscous liquid. UV (MeOH): 206 (4.06), 274 (3.53). IR (nujol): 3417, 2934, 1614, 1581, 1458, 1127, 743. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-TOF-MS: 349.1795 ($[M+Na]^+$, $C_{21}H_{26}NaO_3^+$; calc. 349.1780).

Bauhinol C (= *3-Methoxy-2-methyl-5-(2-phenylethyl)phenol*; **3**): Brown viscous liquid. UV (MeOH): 205 (5.11), 278 (2.79). IR (nujol): 3448, 2934, 1618, 1507, 1456, 1112, 760. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-TOF-MS: 243.1388 ($[M+H]^+$, $C_{16}H_{19}O_2^+$; calc. 243.1385).

Bauhinol D (=*2,2,8-Trimethyl-5-(2-phenylethyl)-2*H*-1-benzopyran-7-ol*; **4**): Yellow viscous liquid, poorly stable in soln. UV (MeOH): 210 (4.40), 234 (4.09), 284 (3.67). IR (nujol): 3444, 2926, 1602, 1496, 1455, 1418, 1103, 750. ^{1}H- and ^{13}C-NMR: see *Table 2*.

Bioassays. Antifungal activities were assessed against a clinical isolate of *Candida albicans* by means of a method modified from the soluble formazan assay [15]. Briefly, 100 µl of 2×10^6 CFU/ml *C. albicans* in *RPMI-1640* medium, containing 34.53 g/ml of 3-[*N*-morpholino]propanesulfonic acid (MOP; *Sigma*, USA), was added to each well of 96-well microculture plate containing 100 µl of test compound diluted in 10% DMSO; *Sigma*, USA. Plates were incubated at 37° for 4 h, before 50 µl of a soln. containing 1 mg/ml of 2,3-bis-[2-methoxy-4-nitro-5-sulfonylphenyl]-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT tetrazolium; *Sigma*, USA) and 0.025 mM of *N*-methylphenazolium methosulfate (PMS; *Sigma*, USA) were added. After an additional 4-h incubation at 37°, the number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. Amphotericin B (*Sigma*, USA) and 10% DMSO were used as positive and negative controls, resp. In our system, the IC_{50} value of the standard drug, amphotericin B, was 0.04 ± 0.01 µg/ml ($n = 3$). The antimycobacterial activity was assayed against *Mycobacterium tuberculosis* H37Ra, using the Microplate Alamar-Blue Assay (MABA) [16]. The twofold dilution technique, starting at a concentration of 200 µg/ml, was used, and the *MIC* value[1]) was recorded at the minimum concentration of the tested compound inhibiting bacterial growth. The standard drugs, isoniazid (*Sigma*, USA) and *kanamycin sulfate* (*Sigma*, USA), used as reference compounds for the antimycobacterial assay, showed *MIC* values of 0.040–0.090 and 2.0–5.0 µg/ml, resp. Cytotoxicity was determined by employing the colorimetric method described by *Skehan et al.* [17]. The reference compound, *Ellipticine* (*Sigma*, USA), exhibited activity toward Vero, KB, and BC cell lines, with IC_{50} values of 0.2–0.3 µg/ml.

Antimalarial activities were evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), which was cultured continuously according to the method of *Trager* and *Jensen* [18]. Quant. assessment of antimalarial activity *in vitro* was determined by the microculture radioisotope technique based upon the method described by *Desjardins et al.* [19]. Briefly, a mixture of 200 µl of 1.5% of erythrocytes with 1% parasitemia at the early ring stage was pre-exposed to 25 µl of the medium containing a test sample dissolved in DMSO (0.1% final concentration) for 24 h, employing the incubation conditions described above. Subsequently, 25 µl of [^{3}H]hypoxanthine (*Amersham*, USA) in culture medium (10 µCi) was added to each well, and the plates were incubated for an additional 24 h. Levels of incorporated radioactively labeled hypoxanthine, indicating parasite growth, were determined by means of a *TopCount* microplate scintillation counter (Packard, USA). Inhibition concentrations (IC_{50}) represent the concentrations required for 50% reduction in parasite growth. The standard sample was dihydroartemisinin (*Sigma*, USA).

The anti-inflammatory activity assay (COX-1 and COX-2) was performed by means of the radioimmunoassay method previously described by *Kirtikara et al.* [20]. Immortalized COX-1$^{-/-}$ and COX-2$^{-/-}$ mouse-lung fibroblast cells (prepared as described in [20]) were used to produce prostaglandin E_2 (PGE_2), representing COX-2 and COX-1 activity, resp. Briefly, immortalized COX-1$^{-/-}$ and COX-2$^{-/-}$ mouse-lung fibroblast cells were plated at 1×10^5 cells/ml in complete *Dulbelcco's Modified Eagle Medium* (DMEM) containing 0.1 mM nonessential amino acids, 292 mg/ml L-glutamine, 50 mg/ml ascorbic acid, and 10% fetal bovine serum (*PAA*, Austria), in 96-well flat-bottomed tissue-culture plates at 83 µl/well. The cells were incubated at 37° for 72 h in a humidified incubator with 5% CO_2. Subsequently, the cells were washed with phosphate buffer saline soln. and incubated for 30 min in 83 µl serum-free DMEM containing test compounds. DMEM Media containing drug vehicle, DMSO (0.1%), and aspirin were used as a control for 100% COX activities and a positive control, resp. The medium was then replaced with serum-free DMEM containing the same amount of drugs or DMSO and 20 µM of arachidonic acid (*Sigma*, USA), and the cells were incubated for 30 min. Culture supernatants were collected at the end of incubation time and assayed for PGE_2 concentrations by the radioimmunoassay method [20]. The inhibition of COX activity was determined from the percent reduction of PGE_2 produced by drug-treated cells relative to PGE_2 produced by cells treated with DMSO alone. IC_{50} Values of COX-1 and -2 were determined with the SOFTmax software (*Molecular Devices*, Sunnyvale, CA). *Aspirin* (*Sigma*, USA) was used as a positive control and was almost equally effective against COX-1 and COX-2. Typical IC_{50} values of aspirin for COX-1 and COX-2 were 2.06 and 3.57 µg/ml, resp.

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REFERENCES

[1] C. Thawai, P. Kittakoop, S. Tanasupawat, K. Suwanborirux, K. Sriklung, Y. Thebtaranonth, *Chem. Biodiv.* **2004**, *1*, 640.
[2] M. Chinworrungsee, P. Kittakoop, M. Isaka, P. Maithip, S. Supothina, Y. Thebtaranonth, *J. Nat. Prod.* **2004**, *67*, 689.
[3] A. Puntumchai, P. Kittakoop, S. Rajviroongit, S. Vimuttipong, K Likhitwitayawuid, Y. Thebtaranonth, *J. Nat. Prod.* **2004**, *67*, 485.
[4] C. Limmatvapirat, S. Sirisopanaporn, P. Kittakoop, *Planta Med.* **2004**, *70*, 276.
[5] P. Kittakoop, S. Nopichai, N. Thongon, P. Charoenchai, Y. Thebtaranonth, *Helv. Chim. Acta* **2004**, *87*, 175.
[6] Y. Asakawa, K. Kondo, M. Tori, T. Hashimoto, S. Ogawa, *Phytochemistry* **1991**, *30*, 219.
[7] Y. Asakawa, T. Hashimoto, K. Takikawa, M. Tori, S. Ogawa, *Phytochemistry* **1991**, *30*, 235.
[8] M. Takasugi, S. Kawashima, K. Monde, N. Katsui, T. Masamune, A. Shirata, *Phytochemistry* **1987**, *26*, 371.
[9] P. Kittakoop, K. Kirtikara, M. Tanticharoen, Y. Thebtaranonth, *Phytochemistry* **2000**, *55*, 349.
[10] S. K. Jain, R. A. DeFilipps, 'Medicinal Plants of India', Reference Publications, Inc., Algonac, Michican, USA, 1991, p. 207.
[11] A. S. R. Anjaneyulu, A. V. Rahgava Reddy, D. S. K. Reddy, T. S. Cameron, S. P. Roe, *Tetrahedron* **1986**, *42*, 2417.
[12] M. P. Maillard, M.-C. Recio-Iglesias, M. Saadou, H. Stoeckli-Evans, K. Hostettmann, *Helv. Chim. Acta* **1991**, *74*, 791.
[13] M. V. Reddy, M. K. Reddy, D. Gunasekar, C. Caux, B. Bodo, *Phytochemistry* **2003**, *64*, 879.
[14] A. S. R. Anjaneyulu, A. V. R. Reddy, D. S. K. Reddy, R. S. Ward, D. Adhikesavalu, S. T. Cameron, *Tetrahedron* **1984**, *40*, 4245.
[15] S. P. Hawser, H. Norris, C. J. Jessup, M. A. Ghannoum, *J. Clin. Microbiol.* **1998**, *36*, 1450.
[16] L. Collins, S. G. Franzblau, *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
[17] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
[18] W. Trager, J. B. Jensen, *Science* **1976**, *193*, 673.
[19] R. E. Desjardins, C. J. Canfield, J. D. Haynes, J. D. Chulay, *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
[20] K. Kirtikara, S. G. Morham, R. Raghow, S. J. Laulederkind, T. Kanekura, S. Goorha, L. R. Ballou, *J. Exp. Med.* **1998**, *187*, 517.

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