Bauhinoxepins A and B: New Antimycobacterial Dibenzo[*b*,*f*]oxepins from *Bauhinia saccocalyx*

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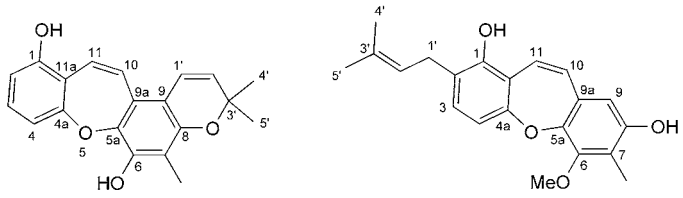
Two new antimycobacterial dibenzo[*b*,*f*]oxepins, bauhinoxepins A (= 3,3,5-trimethylbenzo[*b*]pyrano[*g*][1]-benzoxepin-6,11-diol; **1**) and B (= 6-methoxy-7-methyl-2-(3-methylbut-2-enyl)dibenzo[*b*,*f*]oxepine-1,8-diol; **2**), were isolated from the roots of *Bauhinia saccocalyx*, and their structures were elucidated by analysis of spectroscopic data. Bauhinoxepins A and B exhibited antimycobacterial activities with respective minimum-inhibitory concentrations (*MIC*) of 6.25 and 12.5 μg/ml. They were inactive (at 20 μg/ml) against the malarial parasite, and also inactive (at 20 μg/ml) towards the Vero, KB, and BC cell lines.

Introduction. – The occurrence of dibenzo[*b*,*f*]oxepins in nature is rare [1–6], and, therefore, biological activities of naturally occurring oxepins have hardly been evaluated. However, synthetic dibenzo[*b*,*f*]oxepins have been reported to possess interesting biological activities such as anti-inflammatory [7][8], antipsychotic [9][10], angiotensin-II-receptor-antagonist [11], and neuroprotective properties [12–14]. The pronounced neuroprotective activity of certain synthetic dibenzo[*b*,*f*]oxepins is of great interest. These compounds may have the potential of inhibiting the progression of the neurodegenerative process in patients with *Parkinson*'s disease [12][13].

We have intensively investigated biologically active substances from Thai plants and microorganisms [15–19]. Our routine biological-screening program revealed 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]). Investigation of active principles from the root extract of *B. saccocalyx* resulted in the isolation of antimycobacterial oxepins. However, for unknown reasons, the antimalarial agent(s) were lost during purification. We report herein the isolation and characterization of two new antimycobacterial dibenzo[*b*,*f*]oxepins, bauhinoxepins A (**1**) and B (**2**), from the roots of *B. saccocalyx*.

Results and Discussion. – Bauhinoxepin A (**1**) was obtained as a colorless solid. The molecular formula $C_{20}H_{18}O_4$ was deduced by mass spectrometry (ESI-TOF-MS). Bauhinoxepin A (**1**) exhibited distinctive UV adsorptions at λ_{max} 205.1, 231.7, and 316.6 nm. The ^{1}H-NMR spectrum ($CDCl_3$) revealed the presence of a dimethylchromene unit (δ_H 6.49 (*d*, $J = 10.0$), 5.58 (*d*, $J = 10.0$), 1.41 (*s*, 2 Me)); *cis*-olefinic H-atoms

[1]) IC_{50} stands for inhibitory concentration causing 50% reduction in parasite growth. *MIC* is the minimum-inhibitory concentration.



1 **2**

at δ_H 6.96 (*d*, $J = 11.7$) and 7.00 (*d*, $J = 11.7$); an *ABC* system of aromatic H-atoms (δ_H 6.61, 6.74, and 7.12); a *s* for a Me group at δ_H 2.14; and two exchangeable H-atoms at δ_H 5.50 and 6.15. The ^{13}C-NMR spectrum of **1** revealed 20 signals attributable to seven methine, three Me, and ten quaternary C-atoms (classified by DEPT spectra). Analysis of coupling constants and the ^{1}H,^{1}H COSY spectrum enabled us to assign the connectivities from H–C(2) to H–C(4), H–C(10), and H–C(11), and from H–C(1′) to H–C(2′). The NOESY spectrum of **1** revealed the proximity of H–C(1′) to H–C(10), showing intense cross-peaks for H–C(1′) to H–C(10). The HMBC spectral data of bauhinoxepin A (**1**) showed correlations from H–C(2) to C(11a); H–C(3) to both C(1) and C(4a); H–C(4) to C(4a); 6-OH to C(5a), C(6), and C(7), resp.; the 7-Me H-atoms to C(6), C(7), and C(8), resp.; H–C(10) to both C(5a) and C(11a); H–C(11) to both C(4a) and C(9a); H–C(1′) to both C(8) and C(3′); H–C(2′) to C(9), C(3′), C(4′), and C(5′), resp.; and both H–C(4′) and H–C(5′) to C(3′) and C(2′), resp. These ^{1}H,^{13}C long-range correlations, together with information from ^{1}H,^{1}H COSY and NOESY spectral data, led to the assignment of the gross structure of **1**. All H- and C-atoms in bauhinoxepin A (**1**) were completely assigned, as shown in *Tables 1* and *2*, respectively.

Table 1. *^{1}H-NMR Spectral Data* (400 MHz, $CDCl_3$) *for Bauhinoxepins A* (**1**) *and B* (**2**)

1		**2**	
H–C(2)	6.61 (*d*, $J = 7.9$)	H–C(3)	6.99 (*d*, $J = 8.3$)
H–C(3)	7.12 (*t*, $J = 8.1$)	H–C(4)	6.50 (*d*, $J = 8.3$)
H–C(4)	6.74 (*d*, $J = 8.0$)	H–C(9)	6.30 (*s*)
H–C(10)	6.96 (*d*, $J = 11.7$)	H–C(10)	6.54 (*d*, $J = 11.5$)
H–C(11)	7.00 (*d*, $J = 11.7$)	H–C(11)	6.90 (*d*, $J = 11.5$)
7-Me	2.14 (*s*)	7-Me	2.19 (*s*)
H–C(1′)	6.49 (*d*, $J = 10.0$)	H–C(1′)	3.73 (*d*, $J = 7.3$)
H–C(2′)	5.58 (*d*, $J = 10.0$)	H–C(2′)	5.41 (br. *t*, $J = 7.3$)
Me(4′)	1.41 (*s*)	Me(4′)	1.75 (*s*)
Me(5′)	1.41 (*s*)	Me(5′)	1.78 (*s*)
1-OH	5.50 (br. *s*)	6-(MeO)	3.92 (*s*)
6-OH	6.15 (br. *s*)	1-OH	5.25 (br. *s*)
		8-OH	5.05 (br. *s*)

Bauhinoxepin B (**2**) was obtained as a colorless solid. The ESI-TOF mass spectrum of **2** established the molecular formula $C_{21}H_{22}O_4$. Analyses of the ^{1}H-NMR spectrum revealed that **2** was a derivative of bauhinoxepin A (**1**). However, the signals of the

dimethylchromene unit in **1** were replaced by prenyl signals. Moreover, the *ABC* system (H–C(2) to H–C(4)) in **1** changed to an *AB* system (H–C(3) and H–C(4)), and there was an additional *s* at δ_H 6.30 (H–C(9)) in **2**. The ^{1}H-NMR spectrum of **2** showed typical prenyl signals at δ_H 3.73 (*d*, $J = 7.3$, H–C(1′)), 5.41 (br. *t*, $J = 7.3$, H–C(2′)), 1.75 (*s*, H–C(4′)), and 1.78 (*s*, H–C(5′)). The ^{1}H- and ^{13}C-NMR spectral data also indicated an additional MeO group in **2**. Analyses of the HMBC spectrum revealed that oxygenated C-atoms (C(1), C(4a), C(5a), C(6), and C(8)) in **2** remained unchanged (similar to those of **1**), demonstrating the correlations from H–C(11) to both C(1) and C(4a), H–C(10) to C(5a), and the 7-Me protons to both C(6) and C(8). Interestingly, the ^{1}H- and ^{1}H,^{1}H COSY spectra indicated that H–C(2) in compound **2** was substituted, and analyses of the HMBC spectrum unambiguously placed the prenyl unit at C(2) (correlations observed from H–C(3) to C(1′); and from H–C(1′) to C(1), C(2), and C(3), resp.). H–C(9) in **2** was assigned by HMBC and NOESY experiments; and HMBC experiments showed correlations from H–C(10) to both C(5a) and C(9); and H–C(9) to C(5a), C(7), C(8), and C(9a), resp., while the NOESY spectrum demonstrated an intense cross-peak between H–C(9) and H–C(10). The MeO group in **2** was also assigned by HMBC (correlation from MeO H-atoms to C(6)) and NOESY (cross-peak between MeO and 7-Me H-atoms) spectral data. The NOESY spectrum showed a cross-peak between H–C(5′) and H–C(2′), leading to the assignment of Me(4′) and Me(5′). Based upon these spectral data, the structure of bauhinoxepin B (**2**) was secured. The complete assignment of H- and C-atoms for **2** is shown in *Tables 1* and *2*.

Table 2. *^{13}C-NMR Data* (100 MHz, $CDCl_3$) *for Bauhinoxepins A* (**1**) *and B* (**2**)

1		**2**	
C-Atom	δ_C [ppm]	C-Atom	δ_C [ppm]
C(1)	153.7	C(1)	158.1
C(2)	112.2	C(2)	126.6
C(3)	129.8	C(3)	130.2
C(4)	113.0	C(4)	111.6
C(4a)	159.4	C(4a)	151.2[a])
C(5a)	139.1	C(5a)	145.3
C(6)	146.0	C(6)	150.6[a])
C(7)	113.8	C(7)	119.3
C(8)	148.6	C(8)	150.7[a])
C(9)	110.8	C(9)	109.9
C(9a)	122.6	C(9a)	130.1
C(10)	126.4	C(10)	129.1
C(11)	123.8	C(11)	124.5
C(11a)	118.5	C(11a)	118.6
C(1′)	118.7	C(1′)	27.4
C(2′)	129.0	C(2′)	123.3
C(3′)	75.3	C(3′)	132.2
C(4′)	27.5	C(4′)	17.8
C(5′)	27.5	C(5′)	25.7
7-Me	8.4	7-Me	9.0
		MeO	61.2

[a]) May be exchangeable.

Bauhinoxepins A (**1**) and B (**2**) exhibited antimycobacterial activity, with respective *MIC* values of 6.25 and 12.5 μg/ml (three replicates of bioassay, two-fold dilution technique). Both **1** and **2** were inactive (at 20 μg/ml) against the malarial parasite, and also inactive (at 20 μg/ml) towards the Vero, KB, and BC cell lines. As mentioned earlier, biological activities of naturally occurring dibenzo[*b*,*f*]oxepins have rarely been investigated and, to our knowledge, this is the first report on antimycobacterial properties of naturally occurring dibenzo[*b*,*f*]oxepins. Bauhinoxepins A (**1**) and B (**2**) are derivatives of pacharin, a dibenzo[*b*,*f*]oxepin previously isolated from *B. racemosa* [2].

Experimental Part

General. 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}. ^{1}H-, ^{13}C-, DEPT, ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC NMR experiments were carried out on a *Bruker DRX-400* spectrometer, operating at 400 MHz (^{1}H) and 100 MHz (^{13}C), resp.; chemical shifts δ in ppm rel. to $SiMe_4$, coupling constants *J* in Hz. Electrospray-ionization time-of-flight mass spectrometry (ESI-TOF-MS) was performed on a *Micromass LCT* mass spectrometer, with lock mass calibration; values in *m*/*z*.

Plant Material. Roots of *B. saccocalyx* were collected from Nakhon Sawan Province, Thailand, and identified by *P.C.* A voucher specimen (BRU521) was deposited at the *National Center for Genetic Engineering and Biotechnology (BIOTEC)*, Thailand.

Extraction and Isolation. Dried roots of *B. saccocalyx* (1 kg) were macerated in CH_2Cl_2 (5 l) for 2 d. The extract was filtered and evaporated to dryness, yielding 36.4 g of a crude extract, which was purified on a *Sephadex LH-20* column (MeOH as eluent), from which twelve fractions (A_1 – A_{12}, 80 ml each) were collected. Fraction A_6 was chromatographed on a *Sephadex LH-20* column (50 ml MeOH for each fraction) to yield fractions B_1 – B_{10}. Fraction B_6 was again rechromatographed (same column) (30 ml MeOH for each fraction) to yield fractions C_1 – C_{15}. Fractions C_{10} – C_{12} were combined and purified by column chromatography (CC) (SiO_2; CH_2Cl_2/AcOEt 95:5), furnishing 13.1 mg of bauhinoxepin A (**1**). Fraction B_5 was subjected to CC (SiO_2; CH_2Cl_2/AcOEt 95:5) to yield 48.4 mg of bauhinoxepin B (**2**).

Bauhinoxepin A (*3,3,5-Trimethylbenzo[*b*]pyrano[*g*][1]benzoxepin-6,11-diol*; **1**). Colorless solid. M.p. 183.8–186.2. UV (MeOH): 205.1 (4.42), 231.7 (4.00), 316.6 (3.69). IR (KBr): 3385, 1602, 1452, 1279, 1213, 1098, 1022, 756. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-TOF-MS: 321.1120 ($[M-H]^-$, $C_{20}H_{17}O_4^-$; calc.: 321.1127).

Bauhinoxepin B (*6-Methoxy-7-methyl-2-(3-methylbut-2-enyl)dipenzo[*b*,*f*]oxepine-1,3-diol*; **2**). Colorless solid. M.p. 165.2–167.7. UV (MeOH): 206.1 (4.40), 233.1 (3.97), 315.2 (3.60). IR (KBr): 3423, 1607, 1459, 1274, 1209, 1134, 1008, 758. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-TOF-MS: 337.1441 ($[M-H]^-$, $C_{21}H_{21}O_4^-$; calc: 337.1440).

Bioassays. Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra by means of the Microplate Alamar Blue Assay (MABA) [20]. Two-fold dilution technique, starting at a conc. of 200 μg/ml, was employed, and the *MIC* value was read at the minimum concentration of the tested compound inhibiting the bacterial growth. The standard drugs, isoniazid and kanamycin sulfate, used as reference compounds, showed *MIC* values of 0.040–0.090 and 2.0–5.0 μg/ml, respectively. Cytotoxicity was determined by employing the colorimetric method described by *Skehan et al.* [21]. The reference compound, ellipticine, exhibited activity toward Vero, KB, and BC cell lines, with IC_{50} values of 0.2–0.3 μg/ml. The antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), which was cultured continuously according to the method of *Trager* and *Jensen* [22]. Quantitative assessment of antimalarial activity *in vitro* was made by the microculture radioisotope technique based on the method described by *Desjardins et al.* [23]. IC_{50} represents the concentration causing 50% reduction in parasite growth, as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*; an IC_{50} value of 1 ng/ml was observed for the standard compound, artemisinin, in the same test system.

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