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TWO BIOACTIVE PTEROCARPANS FROM *ERYTHRINA BURANA*

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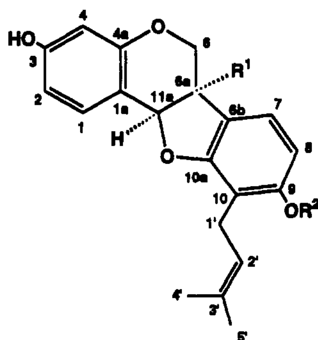
ABSTRACT.—Bioactivity-directed fractionation of the $CHCl_3$ extract of the bark of *Erythrina burana* afforded phaseollidin [**1**] and cristacarpin [**2**]. Both **1** and **2** exhibited moderate but selective activity towards DNA repair-deficient yeast mutants, whereas only **1** was found to be cytotoxic. ^{13}C-nmr spectra of both compounds were assigned.

In a continuing search for potential anticancer agents from natural sources, we are employing a mechanism-based screen with DNA repair-deficient and repair-proficient yeast mutants (1). An important feature of many tumor cells is that they have defects in their ability to repair damage to DNA as compared with normal cells, so agents with selective toxicity towards repair-deficient cells might be potential antitumor agents. Strongly supporting this rationale is the fact that repair-deficient yeast mutants have been demonstrated to be hypersensitive to most known DNA-damaging agents (2).

The genus *Erythrina* (Fabaceae) is well known for elaborating alkaloids with cardiovascular effects (3). Recent studies on the neutral and phenolic components of this genus have revealed the occurrence of flavanones and isoflavones (4–6) and antimicrobial pterocarpans (7). *Erythrina burana* Chiov. is known to occur only in Ethiopia (8), and the seeds of this plant have been shown to contain the *Erythrina* alkaloids erysosalvine, erythraline, erythratidine, and erythrinine (9). A $CHCl_3$ extract of the bark of *E. burana* showed selective activity against the repair-deficient rad 52 yeast strain. Successful bioactivity-directed fractionation of this extract was achieved by flash chromatography over Si gel and Sephadex LH-20 gel permeation chromatography, followed by Si gel preparative tlc, to afford two bioactive compounds **1** and **2** (Table 1).

^1H-nmr spectra of both compounds (Table 2) suggested a pterocarpan skeleton (10). In its ^1H-nmr spectrum, the heterocyclic ring protons of **1** exhibited an ABMX pattern whereas in **2** this system was replaced by a singlet at δ 5.26 and two doublets at δ 4.00 and 4.23. This suggested that **2** is a 6a-hydroxypterocarpan; the presence of a significant peak in the ms of **2** due to the loss of a



1 $R^1=R^2=H$
2 $R^1=OH, R^2=Me$

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TABLE 1. Bioactivity Data of *Erythrina burana* $CHCl_3$ Extract and Pterocarpans **1** and **2**.[a]

Compound	Organism or Cell Line[b]						
	RS 322 YK rad 52	RS 167N rad 6	RS 321 rad 321	RS 188N RAD$^+$	P-388	CHOC	CHOC-PGO
$CHCl_3$ extract	800	NT[c]	NT[c]	7000	NT[c]	NT[c]	NT[c]
1	500	>1000	400	5500	>10.0	4.0	7.6
2	80	540	108	330	>10.0	>20.0	4.0

[a]Results are expressed as IC_{12} (RAD 52Y, RAD 6, RAD 321, RAD$^+$) (μg/ml) or IC_{50} (P-388, CHOC, CHOC-PGO) (μM) values.
[b]P-388, wild-type P-388 murine leukemia cells; CHOC, wild-type Chinese hamster ovary cells; CHOC-PGO, P glycoprotein overproducing Chinese hamster ovary cells.
[c]Not tested.

molecule of H_2O further supported this substructure (11). The presence of prenyl-substituted aromatic rings was evident in the ^{1}H-nmr spectra of both compounds. The OMe group in **2** was located at C-9 as the irradiation of the signal at δ 3.80 due to the OMe group resulted in a significant enhancement of the aromatic signal at δ 6.38 due to H-8. Both **1** and **2** exhibited large negative [α]D values which established their absolute configurations as 6a*R*:11a*R* and 6a*S*:11a*S*, respectively (12). This evidence and comparison of spectral data with those re-

TABLE 2. ^{1}H- and ^{13}C-nmr Data for **1** and **2** in $CDCl_3$.[a]

Position	**1**		**2**	
	^{1}H nmr[b]	^{13}C nmr[c]	^{1}H nmr[d]	^{13}C nmr[c]
1	7.39 (1H, d, *J*=8.4)	132.4 (d)	7.39 (1H, d, *J*=8.4)	132.4 (d)
1a	—	112.6 (s)	—	113.2 (s)
2	6.57 (1H, dd, *J*=8.4, 2.4)	110.0 (d)	6.55 (1H, dd, *J*=8.4, 2.5)	110.4 (d)
3	—	158.5 (s)	—	158.8 (s)
4	6.44 (1H, d, *J*=2.4)	103.7 (d)	6.49 (1H, d, *J*=2.5)	103.8 (d)
4a	—	157.4 (s)	—	157.3 (s)
6α	4.24 (1H, dd, *J*=11.0, 4.9)	66.6 (t)	4.23 (1H, d, *J*=11.7)	69.8 (t)
6β	3.64 (1H, t, *J*=11.0)		4.00 (1H, d, *J*=11.7)	
6a	3.49 (1H, m)	40.1 (s)	—	77.3 (s)
6b	—	118.7 (s)	—	120.8 (s)[e]
7	6.93 (1H, d, *J*=7.8)	121.6 (d)	7.14 (1H, d, *J*=8.2)	120.8 (d)[e]
8	6.38 (1H, d, *J*=7.8)	108.2 (d)	6.38 (1H, d, *J*=8.2)	104.5 (d)
9	—	156.5 (s)	—	160.2 (s)
10	—	110.9 (s)	—	114.3 (s)
10a	—	155.5 (s)	—	155.9 (s)
11a	5.46 (1H, d, *J*=6.8)	78.2 (d)	5.26 (1H, s)	84.6 (d)
1′	3.37 (2H, d, *J*=6.4)	23.1 (t)	3.25 (2H, d, *J*=7.3)	22.7 (t)
2′	5.28 (1H, t, *J*=6.5)	122.3 (d)	5.19 (1H, m)	122.3 (d)
3′	—	134.4 (s)	—	131.5 (s)
4′	1.79 (3H, s)	17.9 (q)	1.73 (3H, s)	17.7 (q)
5′	1.72 (3H, s)	25.6 (q)	1.64 (3H, s)	25.6 (q)
OMe	—	—	3.80 (3H, s)	56.2 (q)

[a]Chemical shifts are in ppm from internal TMS, *J* values are in Hz.
[b]At 270 MHz.
[c]At 100.57 MHz; multiplicities determined by a DEPT sequence.
[d]At 400 MHz.
[e]Overlapping signals.

ported (10,12) established the identities of **1** and **2** as phaseollidin and cristacarpin, respectively. The structures proposed for **1** and **2** were further supported by their ^{13}C-nmr spectra, assignments of which were made based on multiplicities and on chemical shift data reported for related pterocarpans (13). These assignments along with ^{1}H-nmr data are given in Table 2.

The biological activity data for pterocarpans **1** and **2** in our mechanism-based yeast mutant bioassays are summarized in Table 1. Both **1** and **2** showed selective activities in rad 52 and rad 321 assays compared with the wild-type RAD$^+$ strain. Cytotoxic activities of **1** and **2** were determined in three cell lines (Table 1). Phaseollidin [**1**] was found to be moderately active in the wild-type CHOC cytotoxicity assay. Interestingly, cristacarpin [**2**] was inactive against wild-type CHOC but showed activity against a P-glycoprotein overproducing cell line; this reversal of activity is unusual.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Unless otherwise stated, instrumentation, general isolation, and bioassay procedures were the same as those described in our previous publication (1). Ir spectra were recorded as KBr disks. The ^{1}H-nmr spectra were recorded on a Bruker XL 270 spectrometer (at 270 MHz) and on a Varian Unity 400 spectrometer (at 400 MHz). The ^{13}C-nmr spectra were measured using the latter instrument at 100.57 MHz. Sephadex LH-20 (Sigma) was employed for gel permeation chromatography.

BIOACTIVE EXTRACT OF *E. BURANA*.—The bark of *E. burana* (ED-S425) was collected in March 1992 at Alemaya in eastern Ethiopia, and a voucher specimen has been deposited at the National Herbarium in Addis Ababa University. Powdered bark (170 g) was extracted for 8 h with $CHCl_3$ in a Soxhlet apparatus. Evaporation gave 12 g of the $CHCl_3$ extract as a brown semisolid.

BIOASSAYS.—The mechanism-based yeast bioassays were similar to those described by us previously (1) and were carried out at Virginia Polytechnic Institute and State University. The cytotoxicity assays were performed at SmithKline Beecham Pharmaceuticals, Philadelphia.

ISOLATION OF BIOACTIVE PTEROCARPANS.—The isolation process was guided throughout by the results of the rad 52 bioassay. The bioactive $CHCl_3$ extract (12.0 g) was subjected to flash chromatography on Si gel (120 g) and eluted with mixtures of petroleum ether and EtOAc of increasing polarities; only the fractions eluted with 20% EtOAc in petroleum ether were found to be bioactive. The bioactive fractions were combined and subjected to gel permeation chromatography on Sephadex LH-20 using a step gradient of $CHCl_3$, 50% MeOH in $CHCl_3$, and MeOH. The bioactivity was found to be concentrated only in the fractions eluted with 50% MeOH in $CHCl_3$. These combined fractions were then subjected to preparative tlc [hexane-toluene-EtOAc (4:3:3)] to give the bioactive pterocarpans **1** (280 mg) and **2** (100 mg).

Phaseollidin [**1**].—Colorless oil: $[\alpha]^{20}$D $-178°$ (c=0.1, $CHCl_3$) [lit. (12) -150 to $-250°$]; ir ν max 3350, 2950, 1620, 1460, 1380, 1230, 1160, 1120, 1020 cm^{-1}; eims m/z $[M]^+$ 324 (96%), 309 (4), 281 (12), 268 (100), 267 (61), 239 (6); ^{1}H and ^{13}C nmr see Table 2.

Cristacarpin [**2**].—Colorless oil: $[\alpha]^{20}$D $-250°$ (c=0.2, MeOH) [lit. (10) $-220°$]; eims m/z $[M]^+$ 354 (14%), 336 (100), 299 (4), 298 (5), 293 (6), 281 (5); ^{1}H and ^{13}C nmr see Table 2.

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