

TWO NEW FLAVONOIDS FROM *ERYTHRINA ERIOTRIOCHA*<sup>1</sup>AUGUSTIN E. NKENGFAK,<sup>2</sup> DALE R. SANSON, MICHAEL S. TEMPESTA,\*

Department of Chemistry, University of Missouri, Columbia, Missouri 65211

and Z. TANEE FOMUM\*

Department of Organic Chemistry, University of Yaounde, B.P. 812, Yaounde, Cameroon

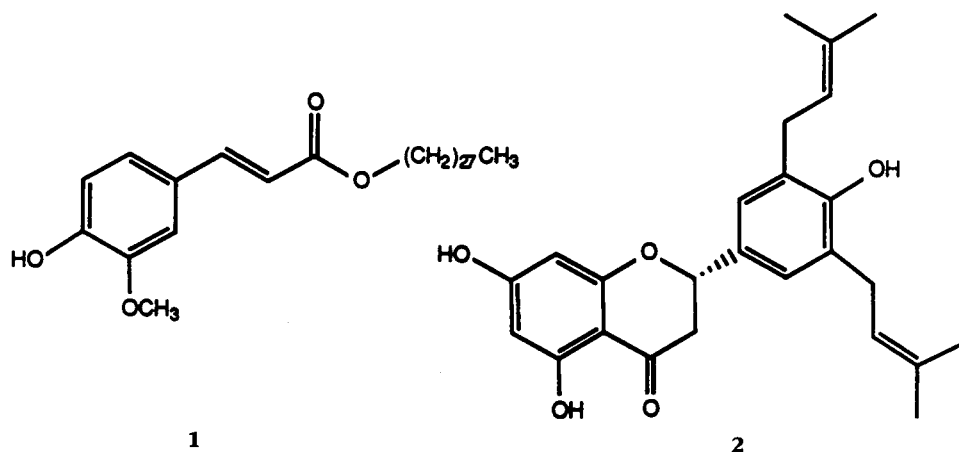
ABSTRACT.—In addition to the known compounds erythrinasinatate [1] and abyssinone V [2], two new flavonoids were isolated from the CHCl<sub>3</sub> extract of the stem bark of *Erythrina eriotriocha* and characterized by spectroscopic techniques as 3'-prenylnaringenin [3] and 2'-hydroxy-5'-methoxybiochanin A [4].

The genus *Erythrina* (Leguminosae) is well known for its alkaloids whose main physiological property is curare-like action (1). In recent years, however, there has been an increase in research efforts on the neutral biologically active compounds of this genus (2–10). In continuation of our phytochemical studies on the Cameroonian species of the genus (3–9), we have examined the constituents of *Erythrina eriotriocha*, a species which has not been investigated previously. In this paper we report the isolation and structural elucidation of two new compounds: a prenylated flavanone, 3'-prenylnaringenin [3] and a methoxyisoflavone, 2'-hydroxy-5'-methoxybiochanin A [4]. We also report <sup>13</sup>C-nmr spectral data of the co-occurring erythrinasinatate [1].

## RESULTS AND DISCUSSION

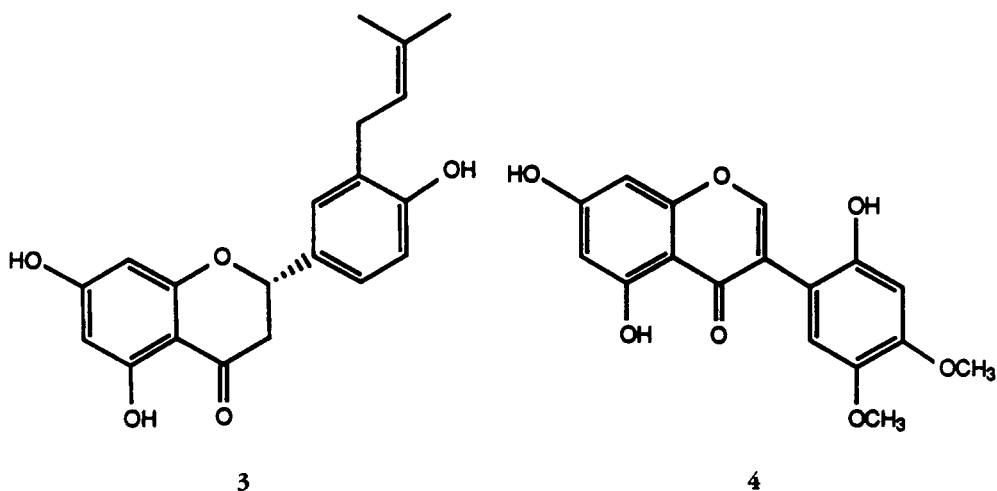
Si gel chromatography of the CHCl<sub>3</sub> extract of *E. eriotriocha* followed by preparative tlc afforded two new flavonoids 3 and 4 along with the previously known erythrinasinatate [1] (5) and abyssinone V [2] (2,9). <sup>13</sup>C-nmr spectral data [CDCl<sub>3</sub>, δ 167.4 (C-3'), 147.9 (C-3), 146.7 (C-4), 144.6 (C-1'), 127.0 (C-1), 123.1 (C-6), 115.7 (C-2), 114.7 (C-5), 109.3 (C-2'), 64.6 (C-1''), 55.9 (OMe), 31.9 (C-26''), 29.7 (C-5''→C-24''), 29.4 (C-4''), 29.3 (C-25''), 28.8 (C-2''), 26.0 (C-3''), 22.7 (C-27''), and 14.1 (C-28'')] of erythrinasinatate [1] is reported here for the first time.

3'-Prenylnaringenin [3], C<sub>20</sub>H<sub>20</sub>O<sub>5</sub> ([M]<sup>+</sup> 340.1315, calcd 340.1311), [α]<sup>23</sup><sub>D</sub>



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<sup>2</sup>On leave from the University of Yaounde.



– 1.1 ( $\epsilon = 2.2$ , MeOH), isolated as an oil, gave positive tests both with  $\text{FeCl}_3$  (greenish-brown) and  $\text{Mg-HCl}$  (purple). Its ir spectrum exhibited absorptions at 3410 (free OH), 3240 (chelated OH), and  $1640\text{ cm}^{-1}$  (conjugated carbonyl). The uv spectrum in MeOH (227 sh, 290, 312 nm) suggested the presence of a flavanone skeleton. This skeleton was also supported by its  $^{13}\text{C}$ -nmr spectrum (Table 1) which showed peaks for C-2 and C-3 carbons at  $\delta$  79.2 and 42.0, respectively. In the  $^1\text{H}$ -nmr spectrum in  $\text{DMSO}-d_6$  (Table 1), the downfield signals at  $\delta$  12.21, 9.50, and 8.35 confirmed the presence of a chelated hydroxy group at C-5 ( $\delta$  12.21) as well as the presence of two free phenolic groups. Two double doublets at  $\delta$  2.67 and 3.25 ( $J = 3.5, 16.4$  Hz and 13.8, 16.4 Hz) and a double doublet at  $\delta$  5.37 ( $J = 3.5, 13.8$  Hz) were assignable to  $\text{H}_2-3$  and H-2, respectively. The presence of one isoprenyl group was also shown in the  $^1\text{H}$ -nmr by two methyl signals ( $\delta$  1.69, 1.78), one 2H doublet ( $\delta$  3.42), and a 1H triplet ( $\delta$  5.25). In addition, a typical ABX system at  $\delta$  6.83 (d,  $J = 8.7$  Hz), 7.10 (dd,  $J = 2.8, 8.7$  Hz), and 7.13 (d,  $J = 2.8$  Hz) established the presence of three aromatic protons in ring B with ortho, ortho/meta, and meta coupling, respectively. Also, two 1H doublets with meta coupling ( $\delta$  2.1 Hz) at  $\delta$  5.84 and 5.86 were consistent with aromatic protons H-8 and H-6 in ring A, respectively. On biogenetic grounds (11), it was assumed that one of the two free phenolic groups is located at C-7 in ring A while the second one is in the B ring at C-4'. The chemical shifts of the B-ring protons were consistent with assignment of the ABX spin systems as H-2', H-5', and H-6', with OH at C-4' and prenyl at C-3'. The isoprenyl group was also shown to be located in the B ring by mass spectral studies. Confirmation of this structure was obtained by the mass spectrum of **3**, in which the molecular ion was detected at  $m/z$  340. Fragment ion peaks at  $m/z$  153 and 187 were caused by usual RDA fragmentation followed by hydrogen transfer. The former ion ( $m/z$  153) resulted from the ring-A moiety and showed that this ring possessed two hydroxyls and no isoprenyl. On the other hand, the latter ion ( $m/z$  187) arose from the B-ring moiety and showed that the B ring had an isoprenyl as well as an hydroxyl. On the basis of the above spectroscopic studies, structure **3** was assigned to be 5,7,4'-trihydroxy-3'-( $\gamma,\gamma$ -dimethylallyl) flavanone. The absolute stereochemistry at C-2 is assumed to be (*S*) in accord with known (–)-flavanones (12).

2'-Hydroxy-5'-methoxybiochanin A [**4**] was obtained as white needles from MeOH/ $\text{CH}_2\text{Cl}_2$ , mp  $250^\circ$ . Its molecular formula,  $\text{C}_{17}\text{H}_{14}\text{O}_7$ , was assigned from the hrms, which showed a molecular ion at  $m/z$  330.0736. Its ir spectrum (Nujol) displayed absorptions at 3464 (free hydroxyl), 3246 (chelated hydroxyl), and  $1647\text{ cm}^{-1}$  (conju-

TABLE 1.  $^1\text{H}$ -nmr Data and  $^{13}\text{C}$ -nmr Assignments for 3'-Prenylaringenin [3] and 2'-Hydroxy-5'-Methoxybiochanin A [4].

Atom	Compound					
	3 <sup>a</sup>			4 <sup>b</sup>		
	$^1\text{H}$	$J$ (Hz)	$^{13}\text{C}$	$^1\text{H}$	$J$ (Hz)	$^{13}\text{C}$
1 . . . . .	—	—	—	—	—	—
2 . . . . .	5.37,dd	3.5,13.8	79.2	8.02	—	155.5
3 . . . . .	3.25,2.67,dd	3.5,16.4;13.8,16.4	42.0	—	—	110.4
4 . . . . .	—	—	196.1	—	—	181.0
5 . . . . .	—	—	162.9	—	—	158.5
6 . . . . .	5.86,d	2.1	95.9	6.43,d	2.7	99.3
7 . . . . .	—	—	163.5	—	—	164.5
8 . . . . .	5.84,d	2.1	95.1	6.27,d	2.7	94.0
9 . . . . .	—	—	166.6	—	—	163.3
10 . . . . .	—	—	101.7	—	—	105.6
1' . . . . .	—	—	128.8	—	—	121.0
2' . . . . .	7.13,d	2.8	125.5	—	—	153
3' . . . . .	—	—	127.4	6.64	0	100.8
4' . . . . .	—	—	155.3	—	—	148.3
5' . . . . .	6.83,d	8.7	114.6	—	—	141.5
6' . . . . .	7.10,dd	2.8,8.7	122.6	6.94	0	116.4
1'' . . . . .	3.42,d	7.1	28.1	—	—	—
2'' . . . . .	5.25,t	7.1	128.2	—	—	—
3'' . . . . .	—	—	131.5	—	—	—
4'' . . . . .	1.69,s	—	17.7	—	—	—
5'' . . . . .	1.78,s	—	25.6	—	—	—
5-OH . . . . .	12.21	—	—	13.02	—	—
7-OH . . . . .	9.50	—	—	9.98	—	—
2'-OH . . . . .	—	—	—	7.95	—	—
4'-OH . . . . .	8.35	—	—	—	—	—
4'-OMe . . . . .	—	—	—	3.80	—	56.1
5'-OMe . . . . .	—	—	—	3.68	—	56.7

<sup>a</sup>In  $\text{DMSO}-d_6$ .<sup>b</sup>In  $\text{Me}_2\text{CO}-d_6$ .

gated carbonyl). In the  $^1\text{H}$  nmr in  $\text{Me}_2\text{CO}-d_6$ , a 1H singlet at  $\delta$  8.02 was characteristic of an isoflavone and assignable to H-2. This skeleton was also supported by its uv spectrum (295, 266, 259 nm) and color tests (Mg-HCl and  $\text{FeCl}_3$ ). The downfield signals at  $\delta$  13.02, 9.98, and 7.95 confirmed the presence of a chelated hydroxyl at C-5 ( $\delta$  13.02) as well as the presence of two free phenolics. The presence of two methoxy groups in the  $^1\text{H}$  nmr of **4** was also shown by two sharp 3H signals at  $\delta$  3.68 and 3.80 ppm. Furthermore, two doublets with meta coupling ( $J = 2.8$  Hz) at  $\delta$  6.27 and 6.43 ppm were assigned as H-8 and H-6, respectively. Two sharp 1H singlets at  $\delta$  6.64 and 6.94 ppm established the presence of two para protons in the B ring. The mass spectrum of **3** exhibited a molecular ion at  $m/z$  330 and significant fragment peaks resulting from retro-Diels-Alder cleavage of the C ring at  $m/z$  152 and  $m/z$  178, indicating that the two methoxy groups were both located in the B ring. From the above spectroscopic studies and biogenetic considerations (11), three possible structures can be proposed.

To choose the correct structure a 2D nOe experiment (NOESY) was performed (13). We observed an intense nOe between each para proton and one methoxy group ( $\delta$  6.64 and 3.80;  $\delta$  6.94 and 3.68) as well as an nOe between the two methoxy groups. These results permitted us to assign structure **4** as 2'-hydroxy-5'-methoxybiochanin A.

## EXPERIMENTAL

**INSTRUMENTAL.**—Mass spectra were obtained with a Kratos MS-25 with a DS-55 data system. Ir spectra were run on a Nicolet 20 DBX and uv spectra on a Beckman 25 spectrophotometer. All nmr experiments were performed on a Nicolet NT 300 WB or JEOL-FX 90Q spectrometer equipped with 5-mm  $^1\text{H}$  and  $^{13}\text{C}$  probes operating at 300.06 and 75.45, or 90 and 22.5 MHz, respectively. Samples were run in  $\text{Me}_2\text{CO}-d_6$ ,  $\text{CDCl}_3$ , or  $\text{DMSO}-d_6$ , and chemical shifts were referenced to internal TMS 0.00 ppm for  $^1\text{H}$  nmr and to deuterated solvents for  $^{13}\text{C}$ -nmr spectra.

**PLANT MATERIALS.**—*E. eriotricha* stem bark was collected at Meiganga in Adamaoua province of Cameroon in June 1987. Voucher material documenting the collection was identified by the Director of the National Herbarium, Yaounde, Cameroon, and is on deposit there.

**EXTRACTION AND ISOLATION.**—Dried, ground stem bark (10 kg) was successively extracted in a MeOH Soxhlet extractor with *n*-hexane,  $\text{CHCl}_3$ , and MeOH. Concentration of extracts under reduced pressure gave, respectively, 60 g (0.6%) of hexane extract and 200 g of  $\text{CHCl}_3$  extract. The MeOH extract consisted mainly of tannins. Only the  $\text{CHCl}_3$  extract was examined in this investigation. Part of this extract (100 g) was subjected to cc over Si gel (900 g) packed in hexane. Gradient elution was effected with hexane/EtOAc, EtOAc, and MeOH/EtOAc mixtures. A total of 200 fractions (ca. 150 ml per fraction) were collected and mixed on the basis of tlc. The pure compounds were obtained from combined fractions after further purification by cc followed by tlc.

Fractions 5–15, eluted with hexane-EtOAc (10:1) were concentrated to give an oil (100 mg). Preparative tlc on Si gel of this oil with toluene- $\text{Me}_2\text{CO}$  (10:1) afforded 20 mg of a compound whose mp (76°), ir, uv, and  $^1\text{H}$ -nmr spectral data matched with those published (5) for erythrinasinatone [1]. Its  $^{13}\text{C}$ -nmr spectra data is reported for the first time. Fractions 20–30, eluted with hexane-EtOAc (17:3), were concentrated to give a sticky yellow oil (200 mg). Repeated cc of this oil, followed by preparative tlc on Si gel eluted with toluene- $\text{Me}_2\text{CO}$  (10:2) yielded a compound whose physical and spectroscopic properties were identical with the previously known abyssinone V [2] (2). The combined fractions 15–48, eluted with hexane-EtOAc (10:4), were concentrated to give 100 mg of sticky brown oil. Further cc on Si gel eluted with hexane and increasing concentration of EtOAc yielded 2 fractions, A and B.

Fraction A was rechromatographed by cc on Si gel followed by preparative tlc on Si gel eluted with toluene- $\text{Me}_2\text{CO}$  (10:3) to yield 7 mg of 3. Fraction B, eluted with hexane-EtOAc (3:4) was subjected to reversed-phase cc eluted with MeOH- $\text{H}_2\text{O}$  (10:3), yielding compound 4 (10 mg).

**3'-PRENYLNARINGENIN [3].**—Oil;  $[\alpha]_D^{25} - 1.1$  ( $c = 2.2$ , MeOH); uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 312 (3.14), 290 (3.24);  $/\text{AlCl}_3$  310 (4.08), 232 (4.46);  $/\text{NaOMe}$  323 (4.09), 242 (4.06); ir  $\nu$  max ( $\text{CDCl}_3$ ) 3410, 3240, 1640, 1215, 1020, 756  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (300 MHz, DMSO) see Table 1;  $^{13}\text{C}$  nmr (75.45, DMSO) see Table 1; eims  $m/z$   $[\text{M}]^+$  340 (58), 323 (77), 297 (27), 285 (44), 284 (37), 267 (35), 213 (22), 188 (33), 187 (6), 175 (100), 153 (90), 152 (40), 133 (56); hrms calcd for  $\text{C}_{20}\text{H}_{20}\text{O}_5$ ,  $m/z$   $[\text{M}]^+$  340.1311, found  $m/z$  340.1315.

**2'-HYDROXY-5'-METHOXYBIOCHANIN A [4].**—White needles ( $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ); mp 250°; ir (Nujol) 3464, 3246, 1647, 1627, 1445  $\text{cm}^{-1}$ ; uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 295 (3.76), 266 (4.60), 259 (4.75), 253 (4.64);  $/\text{NaOMe}$  323 (3.74), 269 (4.72), 260 sh (4.78), 253 (4.64);  $/\text{AlCl}_3$  296 (3.81), 269 (4.69), 263 (4.77), 253 (4.62);  $/\text{NaOAc}$  296 (3.88), 268 (4.60), 260 (4.72), 252 (4.60);  $^1\text{H}$  nmr and  $^{13}\text{C}$  nmr see Table 1; eims  $m/z$   $[\text{M}]^+$  330 (100), 315 (36), 300 (48), 178 (8), 177 (12), 168 (8), 153 (44), 152 (14), 135 (22); hrms calcd for  $\text{C}_{17}\text{H}_{14}\text{O}_7$ ,  $m/z$   $[\text{M}]^+$  330.0734, found  $m/z$  330.0736.

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