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A NEW PRENYLATED ISOFLAVONE AND TRITERPENOIDS FROM *ERYTHRINA ERIOTRIOCHA*[1]

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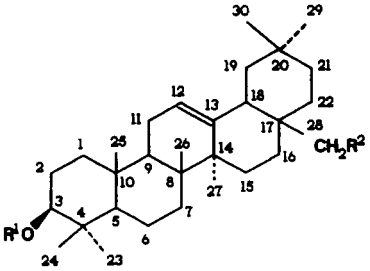
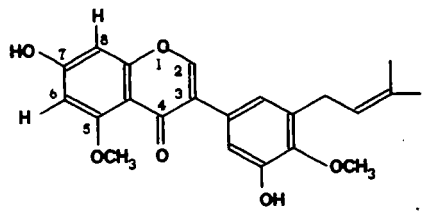
ABSTRACT.—In addition to known triterpenoids maniladiol [**6**] and serrat-14-ene-3β,21α-diol [**7**], two new compounds, one oleanane derivative designated as 28-acetoxyerythrodiol [**1**] and one isoflavone designated as 5,4′-dimethoxy-3′-prenylbiochanin A [**5**], have been isolated and characterized from the $CHCl_3$ extract of the stem bark of *Erythrina eriotriocha*. Their structures have been established on the basis of the spectrocopic techniques (ir, nmr, uv, ms) and chemical evidence.

As part of our chemical investigations on the neutral components of the Cameroonian medicinal plants of the genus *Erythrina* (Leguminosae) (1–8), we have recently reported (9,10) the isolation and structural elucidation of two new isoflavones (8-prenylluteone and 2′-hydroxy-5′-methoxybiochanin A) and one new flavanone (3′-prenylnaringenin) together with several known prenylated isoflavones from the $CHCl_3$ extract of the stem bark of *Erythrina eriotriocha* Harms. Further studies on this extract have led to the isolation of two more new compounds: 28-acetoxyerythrodiol [**1**] and 5,4′-dimethoxy-3′-prenylbiochanin A [**5**], along with the known but rare, maniladiol [**6**] (11), and serratenediol



1 R^1=H, R^2=OAc
2 R^1=H, R^2=OH
3 R^1=Ac, R^2=OH
4 R^1=Ac, R^2=OAc



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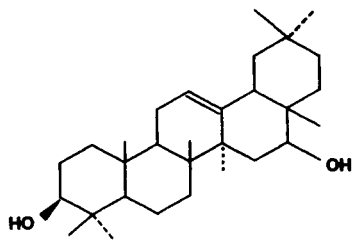

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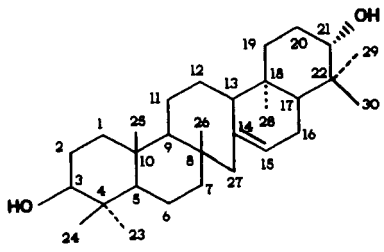

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[1]Part 17 in the series "Erythrina Studies." For Part 16, see A.E. Nkengfack, Z.T. Fomum, R. Ubillas, D.R. Sanson, and M.S. Tempesta, *J. Nat. Prod.*, **53**, 509 (1990).

[7] (12), which had not been reported previously as constituents of *E. eriotriocha*.

In this note, we described the isolation and structural elucidation of the new compounds **1** and **5** and as well as ^{13}C-nmr spectral data of the co-occurring known compounds **6** and **7** reported here for the first time.

RESULTS AND DISCUSSION

The $CHCl_3$ extract of the stem bark of *E. eriotriocha*, on repeated cc over Si gel followed by preparative tlc, afforded compounds **1**–**7**.

Compound **1**, $C_{32}H_{52}O_3$ ($[M]^+$ at *m/z* 484), from its positive response in the Lieberman-Burchard test, was found to be a triterpenoid and did not show any absorption above 220 nm in the uv spectrum. A broad band at 3480–3400 cm^{-1} in its ir spectrum was ascribed to an alcoholic function (OH), which was proved to be equatorial and present at the C-3 position of an A/B trans-triterpene [13,14], by the C-OH stretching vibrations at 1012 and 1018 cm^{-1}, as well as from a biogenetic point of view. On acetylation with Ac_2O and pyridine, a monoacetate (mp 187°) was formed. This indicates that compound **1** contains only one hydroxyl group. The ir spectrum of **1** also showed absorption bands at 1741 cm^{-1} due to the carbonyl function of an ester and at 1390, 1382, and 1368 cm^{-1} due to a *gem*-dimethyl and/or an isopropyl group in the molecule. Furthermore, the C-3 axial proton appeared in the ^{1}H-nmr spectrum as a doublet of doublets at δ 3.18 with coupling constants of 10.3 Hz (J_{aa}) and 6.1 Hz (J_{ae}) supporting the equatorial orientation of the C-3 hydroxyl group. The ^{1}H nmr also showed the presence of seven tertiary methyl singlets at δ 0.71–1.18 (that excludes the presence of an isopropyl group), an acetoxyl group at 2.06 (3H, s), and a vinylic proton centered at δ 5.14 ppm. The presence of this trisubstituted double bond was confirmed by ^{13}C nmr which showed peaks at δ 122.8 (C-12) and 143.6 (C-13) due to sp^2 carbon atoms. In addition, the presence of diastereotopic protons of the acetoxy methylene group in compound **1** was inferred from the AB quartet (J = 11 Hz) at δ 3.64 and 3.91 ppm (15).

The high resolution mass spectrum of **1** indicated that by typical retro-Diels-Alder fragmentation of ring C, compound **1** produced a fragment at *m/z* 276.0207 ($C_{18}H_{28}O_2$) and a fragment at *m/z* 208. The presence of only seven tertiary methyl singlets together with the ^{13}C-nmr data (Table 1) and mass spectra fragmentation (16,17) confirmed that compound **1** was

TABLE 1. ^{13}C-nmr Assignment for 28-Acetoxyerythrodiol [**1**], Maniladiol [**6**], and Serratenediol **7**.

Carbon	Compound		
	1 ($CDCl_3$)	**6** ($CDCl_3$)	**7** (C_6D_6)
C-1 . . .	38.8 t	38.6 t	38.9 t
C-2 . . .	27.2 t	27.2 t	27.2 t
C-3 . . .	78.9 d	78.9 d	78.5 d
C-4 . . .	38.8 s	38.8 s	38.9 s
C-5 . . .	55.1 d	55.1 d	55.9 d
C-6 . . .	18.3 t	18.3 t	18.6 t
C-7 . . .	32.5 t	32.6 t	33.1 t
C-8 . . .	39.7 s	39.7 s	40.1 s
C-9 . . .	47.6 d	47.6 d	48.0 d
C-10 . . .	36.9 s	36.9 s	37.1 s
C-11 . . .	23.5 t	23.5 t	23.9 t
C-12 . . .	122.9 d	122.3 d	38.9 t
C-13 . . .	143.6 s	144.2 s	33.4 d
C-14 . . .	41.6 s	41.2 s	144.4 s
C-15 . . .	27.4 t	31.1 t	122.8 d
C-16 . . .	22.2 t	70.0 d	28.4 t
C-17 . . .	46.2 s	46.5 s	44.9 d
C-18 . . .	42.2 d	42.3 d	37.6 q
C-19 . . .	45.1 t	48.0 t	41.9 t
C-20 . . .	30.9 s	30.9 s	46.7 t
C-21 . . .	33.9 t	34.1 t	76.4 d
C-22 . . .	31.4 t	31.0 t	42.3 s
C-23 . . .	28.1 q	28.1 q	28.2 q
C-24 . . .	15.6 q	15.5 q	15.7 q
C-25 . . .	15.4 q	15.5 q	15.9 q
C-26 . . .	16.7 q	16.7 q	17.1 q
C-27 . . .	25.9 q	25.9 q	30.1 t
C-28 . . .	70.7 t	29.7 q	25.9 q
C-29 . . .	33.1 q	33.2 q	28.9 q
C-30 . . .	25.6 q	26.1 q	20.6 q
-OCOMe .	174.1 s	—	—
$OCOCH_3$.	21.0 q	—	—

an olean-12-ene derivative carrying one hydroxyl group in ring A/B with one of its tertiary methyls in ring D/E transformed into an acetoxymethylene function. It remained for us to establish unambigiously the position of this acetoxymethylene group.

Beside peaks at *m/z* 276 and 208, the eims of compound **1** also showed other diagnostically prominent peaks at *m/z* 424 $[M-HOAc]^+$, 411 $[M-CH_2OAc]^+$, 216, 203, 189, and 133. The base peak at *m/z* 203, which originated from ion *m/z* 276 by the loss of acetoxymethylene group (CH_2OAc), led us to conclude that the acetoxymethylene group is located unambigiously at the C-17 position (16,17). From the spectral data described above, compound **1** was deduced to be 28-acetoxyerythrodiol. This structure was supported by the ^{13}C-nmr data (Table 1) as well as the monoacetate derivative of **1**, whose physical properties (mp 187°) matched with the one of the diacetate of erythrodiol (mp 186°) previously published (18). The ^{13}C-nmr assignments were made on the basis of the known related compounds (19–21) as well as the observed multiplicities in the off-resonance spectrum of compound **1**. Thus, compound **1** is a new naturally occurring monoacetate of erythrodiol [**2**], a pentacyclic triterpene isolated for the first time from the beans of *Erythroxylum novogranatense* (22), of which another monoacetate, 3-acetoxyerythrodiol [**3**] and the diacetate, 3,28-diacetoxyerythrodiol [**4**], are described in the literature (17).

Maniladiol [**6**] and serrat-14-ene-3β,21α-diol [**7**], which also respond positively in the Liebermann-Burchard test, were found to be unsaturated pentacyclic triterpenes. Their structures were identified on the basis of their physical and spectral properties. For ^1H-nmr data of maniladiol [**6**] and serratene-diol [**7**] see Experimental. For their ^{13}C-nmr data, reported here for the first time, see Table 1. Up to now and with isolation of compounds **1**, **6**, and **7**, this is the first time that the *Erythrina* genus is reported as a source of pentacyclic triterpenes.

5,4′-Dimethoxy-3′-prenylbiochanin A [**5**] was obtained as white needles from $MeOH/CH_2Cl_2$, mp 278°. Its molecular formula, $C_{22}H_{22}O_6$, was assigned from the hrms, which showed a molecular ion at *m/z* 382.1070 (calcd 382.0141). Its ir spectrum (Nujol) displayed absorptions at 3460 (free hydroxyl) and 1647 cm^{-1} (conjugated carbonyl). In the ^1H nmr in the Me_2CO-d_6 spectrum, a 1H singlet at δ 8.03 was characteristic of an isoflavone and assignable to H-2. This skeleton was supported by its uv spectrum [335 (sh), 286, 256 nm] and color tests (Mg-HCl and $FeCl_3$). The lack of a downfield signal at δ 12–13 ppm establishes the absence of a chelated hydroxyl group at C-5 position, while signals at δ 9.30 and δ 10.01 ppm, exchangeable with D_2O, indicate the presence of two free phenolic OH. The presence of two methoxy groups in the ^1H nmr of **5** was also shown by two sharp 3H signals at δ 3.81 and 3.96 ppm. Furthermore, two doublets with meta coupling ($J=2.8$ Hz) at 6.27 and 6.49 ppm were assigned as H-8 and H-6, respectively. One 2H signal at δ 7.38 ppm was assigned to H-2′ and H-6′ in the B ring. The presence of one prenyl group was also shown by two methyl signals (δ 1.69, 1.78), one 2H doublet (δ 3.60), and a 1H triplet (δ 5.45). On biogenetic grounds (23), 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. The absence of a chelated OH at C-5 position led us to locate one methoxy group at C-5 position. The mass spectrum of **5**, which exhibited a molecular ion at *m/z* 382 and significant fragment peaks resulting from retro-Diels-Alder cleavage of the ring C at *m/z* 166 and *m/z* 216, indicated that one methoxy group, one hydroxyl group, and a prenyl group were located in the B ring. It remained for us to locate on ring B the position of the prenyl group, one methoxy group, and one free OH group. Again, from a

biogenetic point of view (19), position C-4′ has to be occupied either by an OH or a methoxy group. There remained on ring B only positions 3′ and 5′, which are equivalent, to be occupied by a prenyl group. From the above spectroscopic studies and biogenetic considerations (19), two possible structures can be proposed. To choose the correct structure, a 2D nOe experiment (NOESY) was performed (24). We observed an intense nOe between H-2′ (or H-6′) and methylene protons of the prenyl group as well as an nOe between methylene protons of the prenyl group and the methoxy group. These results permitted us to assign structure **5** as 5,4′-dimethoxy-3′-prenyl-biochanin A.

EXPERIMENTAL

INSTRUMENTAL.—All mp's were determined on a Kofler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Kratos MS-25 with a DS-55 data system. Ir spectra were run on Nicolet 20 DXB and uv spectra on a Beckman 25 spectrophometer. All nmr experiments were performed on a Nicolet NT 300 WB or JEOL-FX 90 Q spectrometer equipped with 5 mm ^{1}H and ^{13}C probes operating at 300.06 and 75.45 MHz or and 90 and 2.25 MHz, respectively. Samples were run in Me_2CO-d_6, C_6D_6, or $CDCl_3$, and chemical shifts were referenced to internal TMS 0.00 ppm for ^{1}H-nmr and deteurated solvents for ^{13}C-nmr spectra.

PLANT MATERIALS.—*E. eriotriocha* stem bark was collected at Meiganga, in Adamaoua Province of Cameroon, in June 1985. Voucher material documenting the collection was identified by the Director of National Herbarium, Yaounde, Cameroon, and is on deposit there.

EXTRACTION AND ISOLATION.—Dried, ground stem bark (10 kg) was successively extracted in an MeOH Soxhlet with *n*-hexane, $CHCl_3$, and MeOH. Concentration of various extracts under reduced pressure gave 60 g (0.6%) of hexane extract and 200 g of $CHCl_3$ extract. The MeOH extract consists mainly of tannins. Only the $CHCl_3$ 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 of ca. 150 ml each were collected and mixed on the basis of tlc. The pure compounds were obtained from combined fractions after further purifications by cc followed by tlc.

The combined fractions 40–80, eluted with a mixture of hexane-EtOAc (20:3), were concentrated to give a yellow sticky oil (1.5 g). This oil was subjected to repeated cc on Si gel and eluted with *n*-hexane, yielding two fractions, A and B.

Fraction A (0.8 g), eluted with a mixture of hexane-EtOAc (15:1), was rechromatographed over Si gel. Elution of the column with *n*-hexane with increasing concentrations of $CHCl_3$ in hexane yielded compounds **1**, **6**, and **7**.

28-ACETOXYERYTHRODIOL [**1**].—Compound **1** (10 mg): oil; ir ν max (CH_2Cl_2) 3480–3400 (OH), 1741 (−C=O, acetate), 1650 (C=C), 1390, 1382, 1368 (*gem*-dimethyl), 1245, 1042, 1018 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 0.72 (3H, s), 0.81 (3H, s), 0.87 (3H, s), 0.89 (3H, s), 0.92 (3H, s), 1.11 (3H, s), 1.18 (3H, s), 2.08 (3H, s, OAc), 3.18 (1H, t, J_{aa} = 10.3 Hz, J_{ae} = 6.1 Hz, H-3), 3.64 (1H, d, J = 11 Hz, H-28), 3.91 (1H, d, J = 11 Hz, H-28), 5.14 (1H, t, H-12); ^{13}C nmr (75.45 MHz, $CDCl_3$) see Table 1; eims *m/z* (rel. int.) [M]$^+$ 484 (4), 424 (50), 411 (20), 307 (21), 276 (18), 255 (9), 216 (70), 208 (30), 203 (100), 189 (30), 133 (20), 119 (31), 69 (30); hrms calcd for $C_{32}H_{52}O_3$ [M]$^+$ *m/z* 484.0391, found *m/z* 484.0389.

Acetylation of compound **1**.—Compound **1** (5 mg) was treated with Ac_2O/pyridine and refluxed for 5 h. After the reaction was complete, the product was purified to give compound **4**: mp 187° [lit. (16) mp 186°]; *m/z* [M]$^+$ 526; ir ν max ($CHCl_3$) 1740, 1650, 1365, 1380, 1245 cm^{-1}.

Maniladiol [**6**].—Colorless crystals; mp 222° [lit. (11), mp 221°]; ν max ($CHCl_3$) 3480–3200 (OH); 1650, 1385, 1380, 1368 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 0.79 (3H, s), 0.87 (3H, s), 0.90 (3H, s), 0.94 (3H, s), 0.96 (3H, s), 1.01 (3H, s), 1.18 (3H, s), 1.25 (3H, s), 3.24 (1H, b, H-3), 3.53 (1H, b, H-16); ^{13}C nmr see Table 1; eims *m/z* (rel. int.) [M]$^+$ 422 (4), 234 (100), 203 (60), 149 (20).

Serrat-14-ene-3β,21α-diol [**7**].—[α]^{22}D + 19° (*c* = 0.8, $CHCl_3$); white needles (MeOH); mp 300° [lit. (12) mp 302–304°]; ir ν max ($CHCl_3$) 3480–3350 (OH), 1460, 1380, 1358, 1028 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 0.79 (3H, s), 0.87 (3H, s), 0.90 (3H, s), 0.94 (3H, s), 0.97 (3H, s), 1.03 (3H, s), 1.11 (3H, s), 2.07 (1H, b, H-27), 2.11 (1H, b, H-27), 3.22 (1H, t, J_{ae} = 5.75 Hz, J_{ea} = 5.34 Hz, H-21), 3.41 (1H, t, H-3), 5.24 (1H, t, H-15); ^{13}C nmr (75.45, C_6D_6-d_6) see Table 1; eims *m/z* (rel. int.) [M]$^+$ 442 (4), 234 (100), 220 (39), 207 (9), 176 (29); hrms calcd for $C_{30}H_{50}O_2$ [M]$^+$ *m/z* 442.0381, found *m/z* 442.0371.

Fraction B, eluted with hexane-EtOAc (1:1), was subjected to reversed-phase cc eluted with MeOH-H_2O (10:3), yielding compound **5**.

5,4′-Dimethoxy-3′-prenylbiochanin A [**5**].—

Compound **5** (6 mg): colorless crystals from MeOH-CH_2Cl_2; mp 278°; uv λ max (MeOH) (log ε) 335 (sh) (3.54), 286 (3.65), 256 (3.63); ir (Nujol) 3460, 1647, 1445, 1235 cm^{-1}; ^1H nmr (300 MHz, Me_2CO-d_6) δ 1.69 (3H, s), 1.78 (3H, s), 3.60 (2H, d, J = 7.1 Hz), 3.81 (3H, s, 4′-OMe), 3.96 (3H, s, 5-OMe), 5.45 (1H, t, J = 7.1 Hz), 6.27 (1H, d, J = 2.5 Hz, H-6), 6.49 (1H, d, J = 2.5 Hz, H-8), 7.38 (2H, s, H-2′ and H-6′), 8.03 (1H, s, H-2), 9.30 (1H, b, OH, exchangeable with D_2O), 10.01 (1H, s, OH exchangeable with D_2O); hrms calcd for $C_{22}H_{22}O_6$ $[M]^+$ *m/z* 382.0141, found *m/z* 382.1070; eims *m/z* (rel. int.) $[M]^+$ 382 (94), 351 (5), 364 (6), 339 (7), 326 (100), 283 (15), 216 (10), 166 (9), 153 (12), 69 (20), 43 (22).

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