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EXTRACTIVES FROM *ERYTHRINA ERIOTRIOCHA*[1]

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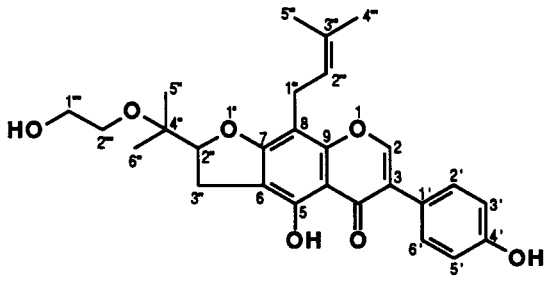
ABSTRACT.—From the stem bark of *Erythrina eriotriocha*, a novel isoflavone, eriotriochin [**1**], has been isolated and characterized, in addition to known compounds auriculatin, dihydroauriculatin, and 3′-*O*-methylorobol. The structure of compound **1** was determined by COSY, selective INEPT, 2D HMBC, NOESY, DEPT, and other spectroscopic techniques.

Studies in our laboratories of the neutral components of the genus *Erythrina* have resulted in the isolation of a number of new flavonoid compounds (1,2,4). The Cameroonian medicinal plant *Erythrina eriotriocha* Harms (Leguminosae) has now been studied. In this paper, we report the isolation and structural elucidation of a novel prenylated isoflavone, eriotriochin [**1**], bearing an unusual glycol unit. The known isoflavones auriculatin (3), dihydroauriculatin (4) and 3′-*O*-methylorobol (5) were also isolated.

RESULTS AND DISCUSSION

Preparative hplc of polar fractions of $CHCl_3$ extract of *E. eriotriocha*, followed by reversed-phase tlc, afforded four compounds including **1**.

Eriotriochin [**1**], oil, $[\alpha]^{22}D$ −7.5°, was analyzed as $C_{27}H_{30}O_7$. Its ir spectrum exhibited absorption bands at 3415 (free OH), 3280 (chelated OH), 1640 (conjugated carbonyl), and 1235 cm^{-1} (ether function). The downfield signals in the ^{1}H-nmr spectrum in $CDCl_3$ at δ 13.00 and δ 9.50 ppm confirmed the presence of an intramolecular hydrogen-bonded group at the C-5 position (δ 13.00) as well as the presence of one free phenolic group due to 4′-OH. The signal at δ 7.91 ppm was assigned to the C-2 proton of an isoflavone. This skeleton was supported by its uv spectrum [278, 310 nm (sh)] and by the following color tests: positive to $FeCl_3$ (greenish-brown) and negative to Mg-HCl. Major mass spectral fragments at *m/z* 423 $[M-43]^+$ and *m/z* 411 $[M-55]^+$ and ^{1}H-nmr signals at δ 1.79 (3H, s), 1.86 (3H, s), 3.40 (2H, d, $J=7.1$ Hz, H-1‴), and 5.22 ppm (1H, t, 7.1 Hz, H-2‴) were consistent with



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the presence of a 3,3-dimethylallyl group. Mass fragments at *m/z* 407 $[M-59]^+$ and *m/z* 59 were indicative of an

[1]Part 16 in the series "*Erythrina* studies." For Part 15 see Z.T. Fomum, A.E. Nkengfack, and J. Wandji, *Ann. Fac. Sci. Chem.*, **2**, 79 (1988).

hydroxyisopropyl-dihydrofuran substituent (6,7). The presence of this substituent was confirmed by the ^{1}H-nmr spectrum, which showed two methyl signals at δ 1.28 and 1.35 ppm, one methine proton next to an oxygen atom at δ 4.80 (1H, t, J = 8.5 Hz, H-2″), and two diastereotopic protons at δ 3.15 (1H, dd, J = 9.0 and 15.0 Hz, H-3″) and 3.21 ppm (1H, J = 9.0 and 15.0 Hz, H-3″). The isopropyl-dihydrofuran skeleton was also supported by the ^{13}C-nmr spectrum (see Experimental) which showed peaks for C-2″ and C-3″ at δ 91.3 and 29.7 ppm, respectively. Major peaks at *m/z* 422 $[M-44]^+$ and *m/z* 44 shown on the mass spectrum of compound **1** due to the loss of an C_2H_4O fragment and ^{1}H-nmr signals at δ 3.64 (2H, t, J = 8.5 Hz) and δ 3.77 (2H, t, J = 8.5 Hz), after D_2O exchange, were consistent with the presence of methylene protons coupled to a hydroxy proton (glycol unit). Before D_2O exchange the ^{1}H-nmr signal at δ 3.77 (2H) was a multiplet.

Furthermore, a typical AA′BB′ system at δ 7.41 (2H, d, J = 8.5 Hz) and δ 6.89 (2H, d, J = 8.5 Hz) showed the presence of four aromatic protons in the B ring, while the lack of any signal at 5.90–6.10 ppm established that there were no aromatic protons in ring A. Thus, the hydroxyisopropyl-dihydrofuran unit was fused to ring A, on which a 3,3-dimethylallyl group is located.

It remained for us to establish unambiguously the position of the free prenyl group and to see if the fusion of the hydroxyisopropyl-dihydrofuran unit is linear or angular. An nmr technique based on ^{13}C-^{1}H long range spin-spin coupling, selective INEPT (8,9) (also called INAPT), and the 2D HMBC short and long range heteronuclear experiments (10) were used to verify the prenyl attachment. When H-2 is irradiated, we observed polarization transfer to the C-2 (δ 152.5), C-3 (δ 123.0), C-4 carbonyl (δ 181.3), and C-9 (δ 155.3). When H-1‴ was irradiated, polarization transfer was seen at C-1‴ (δ 21.9), C-2‴ (δ 122.5), C-3‴ (δ 132.3), C-7 (δ 164.1), and C-9 (δ 155.3). Irradiation of H-2″ saw enhancement through polarization transfer to C-2″ (δ 91.3), C-3″ (δ 29.7), and C-7 (δ 164.1), while polarization transfer to the C-3″ (δ 29.7), C-5 (δ 155.1), and C-4″ (δ 77.5) was observed when H-3″ was irradiated. These results showed clearly that the free prenyl group was attached at the C-8 position on ring A, and the fusion of hydroxyisopropyl-dihydrofuran ring was linear. Confirmation of the prenyl attachment on ring A was given by eims of compound **1**, which showed a weak molecular ion peak at *m/z* 466 and significant ion peaks at *m/z* 422, 407, 389, 363, 349, 295, 117, 59, 55, 44, and 43. Ion peaks at *m/z* 349 and *m/z* 117 arose from RDA cleavage followed by hydrogen transfer. The former ion (*m/z* 349) resulted from the ring A moiety and showed that this ring possessed the isoprenyl group, dihydrofuran ring, and glycol unit. On the other hand, the latter ion (*m/z* 117) arose from the B ring moiety and showed that this ring, in conjunction with the AA′BB′ ^{1}H-nmr spin pattern, had a hydroxyl group at position C-4′ and no isoprenyl group. From the above mass spectral evidence, we concluded that glycol unit was not attached at the C-4′ oxygen on B ring but attached at the cyclized prenyl end on ring A.

This was confirmed by the INEPT (8,9) technique as well as by spectral data. Irradiation of H-1⁗ saw enhancement through polarization transfer to C-2⁗ (δ 72.2), while a prominent peak at *m/z* 363 $[M-103]^+$ was due to the loss of the $C_5H_{11}O_2$ unit from the molecular ion. From the above spectroscopic studies, structure **1** was assigned to eriotriochin. Further confirmation of this structure came from the DEPT (11) experiment which established the presence of four methylene groups at δ 21.9 (C-1‴), δ 29.7 (C-3‴), δ 67.5 (C-1⁗), and δ 72.2 (C-2⁗) as well as the NOESY

(12) experiment which showed intense nOe's between H-2″ and H-3″, H-1‴ and H-2‴, H-1‴ and H-5‴, and H-2‴ and H-4‴. COSY (13) spectra and ^{13}C-nmr data (see Experimental) were also in accord with structure **1**. Assignments of the carbon chemical shifts in the ^{13}C-nmr spectra were made by using an INEPT long range experiment (8,9) and the 2D HMBC short and long range heteronuclear experiments (10). Spectral data identified auriculatin, dihydroauriculatin, and 3′-*O*-methylorobol, reported here for the first time as constituents of *E. eriotriocha*, and they correspond to the published data of these compounds.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were determined on a Kofler hot-stage apparatus and are uncorrected. Mass spectra are obtained with a Kratos MS-25 with a DS-55 data system. Ir spectra were run on a Nicolet 20 DBX, and uv spectra were run on a Beckman 25 spectrophotometer. Si gel GF_{254} (Merck) and Si gel 60 (70–230 mesh ASTM) (Merck) were used for tlc and cc, respectively. All nmr experiments were performed on a Nicolet NT-300 WB spectrometer equipped with a 5 mm ^{1}H and ^{13}C probe operating at 300.06 and 75.46 MHz, respectively. Samples were run in DMSO-d_6 or $CDCl_3$, and chemicals were referenced to internal TMS (0.0 ppm) for ^{1}H nmr and to deuterated solvents for ^{13}C nmr. The selective INEPT experiment used the pulse sequence by Bax and co-workers (8,9). A decoupler, field strength $\gamma H_2 = 25$ Hz, was used to generate a selective 90° proton pulse of 10 msec.

The polarization transfer delays Δ_1 and Δ_2 were optimized for $J_{CH} = 6$ Hz. Between 600 and 2000 16 K acquisitions were signal-averaged in double precision acquisition mode and processed in floating point mode with standard Nicolet software.

PLANT MATERIAL.—*E. eriotriocha* stem bark was collected at Meiganga in Adamaoua Province of Cameroon, in June 1986. 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 a Soxhlet extractor 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 (2%) of $CHCl_3$ extract. The MeOH extract consisted mainly of tannins. Only the $CHCl_3$ extract was examined. Part of this extract was column chromatographed 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 about 150 ml per fraction were collected and united on the basis of tlc. The combined fraction 150–175 eluted with hexane-EtOAc (3:17) was concentrated to give a yellow sticky oil (1 g). This oil was subjected to repeated cc on Si gel eluted with *n*-hexane and increasing concentrations of EtOAc in hexane, yielding two fractions, A and B.

Fraction A (0.25 g), eluted with hexane-EtOAc (3.2), was subjected to hplc [normal SiO_2, toluene-Me_2CO (10:1)]. Fractions (15 ml each) were collected and combined on the basis of tlc. Fractions 10–14 were subjected to preparative tlc on Si gel with toluene-Me_2CO (10:3) and yielded dihydroauriculatin as yellow crystals (10 mg), mp 101° [lit. (4) mp 100–101°], $[\alpha]^{22}D$ 0.0° ($c = 0.02$, MeOH).

Preparative tlc over Si gel of fractions 16–20 eluted with toluene-Me_2CO (10:3) afforded auriculatin as yellow crystals, mp 135° [lit. (3) mp 135–136°].

Fraction B (0.25 g), eluted with hexane-EtOAc (3:7), was rechromatographed over Si gel. Elution of the column with $CHCl_3$-hexane (1:1) afforded a residue (100 mg) that contained two compounds, R_f 0.45 and 0.32 [toluene-Me_2CO (10:3), double development]. Hplc of this residue [normal SiO_2, toluene-Me_2CO (10:1)] followed by reversed-phase tlc over C-18 bonded phase, eluted with MeOH-H_2O (10:3), yielded eriotriochin [**1**] and 3′-*O*-methylorobol, mp 218° [lit. (4) mp 217–219°].

Eriotriochin [**1**], yield 8 mg; $[\alpha]^{23}D$ −7.5° ($c = 0.01$, MeOH); R_f 0.32 (toluene/Me_2CO); uv λ max (MeOH) (log ε) 278 (4.10), 310 nm (sh) (3.62); ir ($CHCl_3$) 3415, 3373, 1640, 1235 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.91 (1H, s, H-2), 7.41 (2H, d, $J = 8.4$, H-2′ and H-6′), 6.89 (2H, d, $J = 8.4$, H-3′ and H-5′), 4.80 (1H, t, $J = 8.5$, H-2″), 3.21 (1H, dd, $J = 9.0$ and 15.0, H-3″), 3.15 (1H, dd, $J = 9.0$ and 15.0, H-3″), 1.28 (3H, s, H-5″), 1.35 (3H, s, H-6″), 3.40 (2H, d, $J = 7.4$, H-1‴), 5.22 (1H, t, $J = 7.4$, H-2‴), 1.86 (3H, s, H-4‴), 1.79 (3H, s, H-5‴), 3.64 (2H, t, $J = 8.5$, H-1⁗), 3.77 (2H, m, H-2⁗), 13.00 (1H, s, exchangeable to D_2O, 5-OH), 9.65 (1H, d, exchangeable to D_2O, 4′-OH), 1.58 (1H, m, exchangeable to D_2O, 1⁗-OH); ^{13}C nmr 17.8 (C-5‴), 21.9 (C-1‴), 23.9 (C-5″), 25.6 (C-4‴), 25.7 (C-6″), 29.7 (C-3″), 64.5 (C-1⁗), 72.2 (C-2⁗), 77.5 (C-4″), 91.3 (C-2″), 102.0 (C-6), 106.0 (C-10), 108.0 (C-8), 115.7 (C-3′ and C-5′), 121.4 (C-1′), 122.5 (C-2‴), 123.0 (C-3), 130.2 (C-2′ and C-6′), 132.3 (C-3‴), 152.5 (C-2), 155.1 (C-5), 155.3 (C-9),

156.0 (C-4'), 164.0 (C-7), 181.3 (C-4); eims *m/z* (rel. int.) 466 (10), 423 (35), $[M-C_2H_4O]^+$ 422 (95), 411 (20), 407 (18.3), 389 (15), 363 (27), 349 (32), 335 (20), 321 (25), 307 (16), 295 (32), 118 (13), 117 (11), 59 (66), 55 (49), 44 (97), 43 (100).

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