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PRENYLATED ISOFLAVANONE FROM *ERYTHRINA ERIOTRICHA**

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Key Word Index—*Erythrina eriotricha*; Leguminosae; root bark; eriotrichin B; isoneorautenol; erybraedins A, C, D, E; isoflavanone and pterocarpan metabolites; antimicrobial agents.

Abstract—Bioassay-directed fractionation of a methylene chloride extract of the root bark of *Erythrina eriotricha* resulted in the isolation of a novel isoflavanone, named eriotrichin **B**, and the five known pterocarpans, isoneorautenol, erybraedin A, erybraedin C, erybraedin D and erybraedin E. The structure of the new compound has been investigated by extensive spectroscopic studies, including 2D NMR and chemical evidence. The *in vitro* antimicrobial spectrum and potencies of the isolated compounds are also reported.

INTRODUCTION

The genus *Erythrina* is well known for elaborating alkaloids with cardiovascular effects [1]. Recent studies on the neutral and phenolic components of this genus have revealed the occurrence of a wide range of flavanone [2, 3], isoflavanone, isoflavone [4–6] and antimicrobial pterocarpans [7–9].

Erythrina eriotricha, a species endemic to Cameroon, is a small tree growing in the savanna region of the northern part of the country where its various parts (leaves, bark and roots) are widely used in indigeneous medicinal practice [10]. Previous chemical studies in our laboratory of the stem bark of *E. eriotricha* led to the isolation and characterization of several prenylated and 5,7,4′-trioxygenated isoflavones and flavanones [4, 11]. The roots of this species, however, have not been studied previously. In continuation of our search for phenolic metabolites in this medicinal plant, we have examined the chemical constituents of the root bark. Herein, we report the isolation and structure elucidation of a new isoflavanone, eriotrichin **B** (**1**), along with the five known co-occurring pterocarpans, isoneorautenol (**2**), erybraedins A (**3**), C(**4**), D (**5**) and E (**6**) which we find to be responsible for the bulk of the antimicrobial activity of extracts of this species.

RESULTS AND DISCUSSION

The dried and powdered root bark of *E. eriotricha* was extracted with MeOH in a Soxhlet apparatus and the extract concentrated to dryness. The residue showed antimicrobial activity against *Staphylococcus aureus*, a Gram-positive bacterium, when tested using an agar streak-dilution technique [12, 13]. This residue was then suspended in a mixture of MeOH–H_2O (4:1) and successively partitioned into *n*-hexane-, CH_2Cl_2- and *n*-BuOH-soluble extracts. The activity was found to be distributed into the CH_2Cl_2 extracts which contains phenolic compounds. This extract, on chromatographic separations on silica gel, afforded six compounds including eriotrichin B (**1**) and five pterocarpans, isoneorautenol (**2**), erybraedin A (**3**), C (**4**), D (**5**) and E (**6**) previously isolated by Mitscher *et al.* [7, 9] from the root bark of *E. milbraedi*, collected in Nigeria.

Compounds (**2**)–(**6**) were identified by comparison of their physical and spectral data (IR, UV, ^{1}H NMR) with the corresponding literature values [7, 8, 9, 14]. The assignments of their ^{13}C NMR spectral data, reported here for the first time, were made on the basis of known related compounds, as well as the multiplicities in J Mod and DEPT spectra.

Eriotrichin B (**1**) was obtained as a brown sticky oil and gave a positive ferric chloride test. The high-resolution EI-mass spectrum displayed $[M]^+$ at *m/z* 408.1936 in agreement with the empirical formula $C_{25}H_{28}O_5$ (408.1937), indicating 12 degrees of unsaturation in the molecule. The broad-band decoupled ^{13}C NMR spectrum (75 Mz, $CDCl_3$, Table 1) showed 25 carbon signals. The analysis of his spectrum by the aid of J Mod and DEPT techniques unequivocally indicated the presence in the molecule of eleven sp^2 quaternary carbons amongst which were one carbonyl carbon (δ194.8), seven tertiary methine carbons with six sp^2 and one sp^3 (δ46.5), three secondary sp^3 methylene carbons (δ70.3, 25.7, 22.1) and four primary sp^3 methyl carbons. The IR spectrum exhibited bands at ν 3400 (OH), 1652 cm^{-1} (conjugated

*Part 31 in the series 'Erythrina Studies'.

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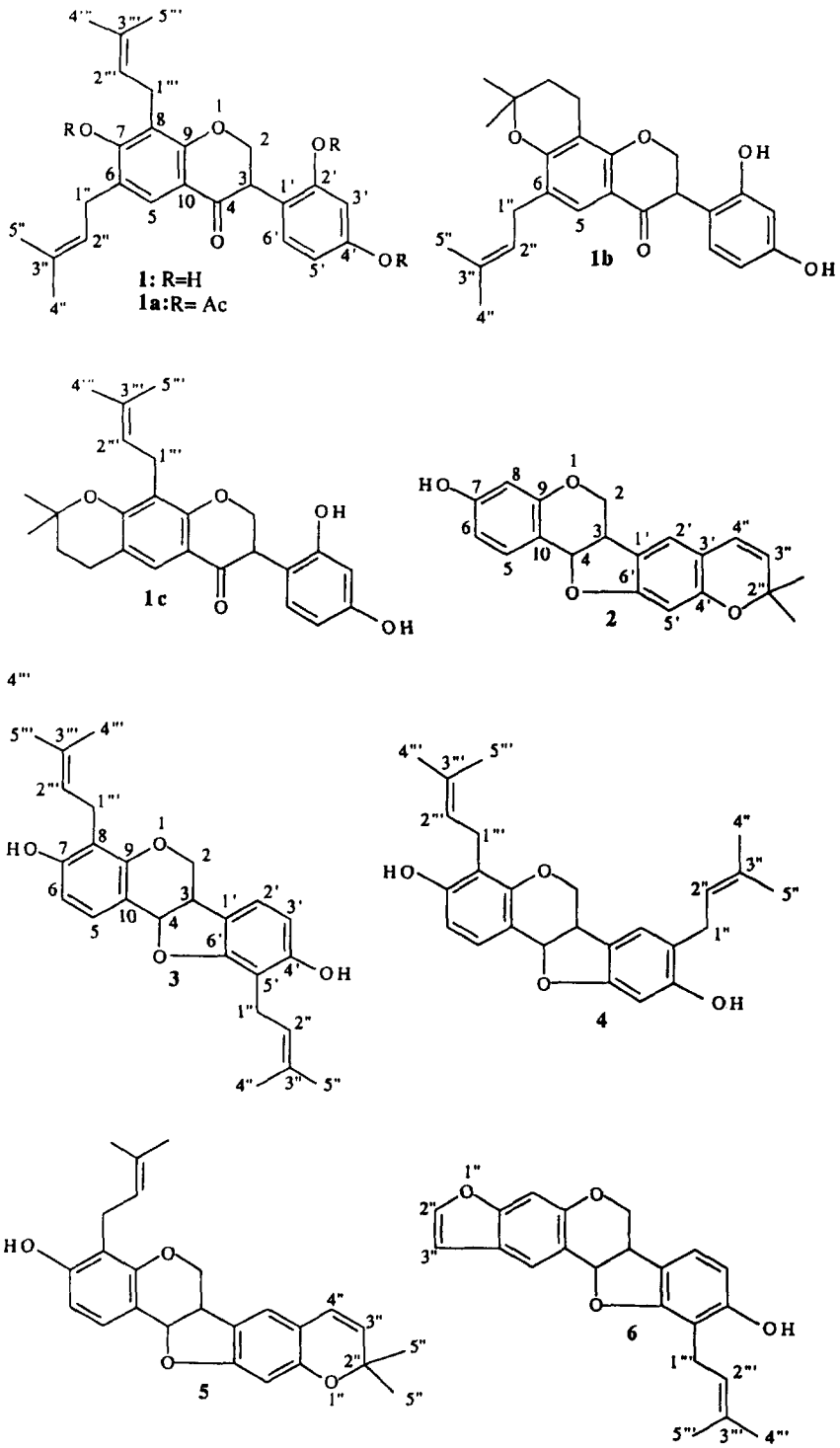

Scheme 1.

C=O) and several other bands at 1602, 1554, 1508 cm^{-1} assignable to an aromatic ring.

In the ^1H NMR spectrum analysed by ^1H–^1H COSY (300 Mz, $CDCl_3$, Table 1), a typical AMX spin system of three one-proton double doublets at δ3.99 (J = 11.6, 5.4 Hz), 4.60 (J = 11.4, 5.4 Hz) and 4.78 (J = 11.6 and 11.4 Hz) was characteristic of an isoflavanone skeleton and assignable to H-3, H-2 eq and H-2 ax, respectively [15]. This was supported both by the UV spectrum which showed an absorption band at λ_{max} (MeOH) 281 nm characteristic for the isoflavanone chromophore [16] and by HMQC NMR experiments which correlated the above NMR protons with the signals at δ46.5 and 70.3 due to C-3 and C-2, respectively. The small bathoch-

Table 1. ^{1}H NMR (300 MHz, $CDCl_3$) data for eriotrichin B (**1**) and ^{13}C NMR (75 Mz, $CDCl_3$) assignments for compounds (**1**)–(**6**)

	1*		2+	3+	4+	5+	6+
Carbon	^{1}H	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C
2 ax	4.78 (*dd*, 11.6, 11.4)	70.3 *t*	66.5 *t*	66.8 *t*	66.8 *t*	66.9 *t*	66.9 *t*
2 eq	4.60 (*dd*, 11.4, 5.4)	—	—	—	—	—	—
3	3.99 (*dd*, 11.6, 5.4)	46.5 *d*	39.4 *d*	39.8 *d*	39.6 *d*	39.7 *d*	39.7 *d*
4	—	194.8 *s*	76.5 *d*	78.8 *d*	79.0 *d*	78.7 *d*	78.8 *d*
5	7.57 (*s*)	126.5 *d*	132.2 *d*	129.3 *d*	129.2 *d*	129.5 *d*	127.4 *d*
6	—	114.7 *s*	109.8 *d*	109.7 *d*	109.7 *d*	109.8 *d*	109.7 *s*
7	—	160.6 *s*	160.2[a] *s*	158.4[a] *s*	158.9[a] *s*	159.2[a] *s*	160.1[a] *s*
8	—	114.1 *s*	103.7 *d*	110.3 *s*	110.5[b] *s*	110.5 *s*	102.8 *d*
9	—	160.0 *s*	157.2[a] *s*	155.7[a] *s*	155.5[a] *s*	156.2[a] *s*	157.1[a] *s*
10	—	122.0 *s*	112.4[b] *s*	112.6[b] *s*	112.5[b] *s*	112.5[b] *s*	112.6[b] *s*
1′	—	113.5 *s*	119.4[b] *s*	118.8[b] *s*	119.0[b] *s*	118.0[b] *s*	118.7[b] *s*
2′	—	155.8 *s*	122.0 *d*	122.3 *d*	125.3 *d*	123.9 *d*	122.5 *d*
3′	6.37 (*d*, 2.3)	104.4 *d*	114.9[b] *s*	108.0 *d*	114.9 *s*	114.6[b] *s*	108.7 *d*
4′	—	156.6 *s*	156.6[a] *s*	153.9[a] *s*	153.9[a] *s*	153.7[a] *s*	154.2[a] *s*
5′	6.27 (*dd*, 8.4, 2.3)	108.0 *d*	99.4 *d*	114.9 *s*	98.5 *d*	98.9 *d*	114.7 *s*
6′	7.02 (*d*, 8.4)	129.0 *d*	154.4[a] *s*	155.5[a] *s*	155.1[a] *s*	154.8[a] *s*	155.1[a] *s*
1″	3.22 (*d*, 7.1)	29.0 *t*	—	22.0[b] *t*	29.4[b] *t*	—	—
2″	5.20 (*t*, 7.1)	121.3 *d*	78.4 *s*	121.7[c] *d*	121.7[c] *d*	79.2 *s*	147.7 *d*
3″	—	134.9 s	127.6 *d*	134.3[d] *s*	134.5[d] *s*	128.1 *d*	106.3 *s*
4″	1.77 (*s*)	25.7 *q*	122.1 *d*	25.0[e] *q*	25.8[e] *q*	122.3 *d*	—
5″	1.69 (*s*)	17.7 *q*	26.9 *q*	17.8 *q*	17.8[f] *q*	27.0 *q*	—
1‴	3.30 (*d*, 6.9)	22.1 *t*	—	23.1[b] *t*	22.1[b] *t*	22.5 *t*	22.1 *t*
2‴	5.23 (*t*, 6.9)	121.2 *d*	—	121.4[c] *d*	122.4[c] *d*	122.4 *d*	121.9 *d*
3‴	—	134.7 *s*	—	134.9[d] *s*	134.5[d] *s*	134.5 *s*	134.1 *s*
4‴	1.79 (*s*)	25.7 *q*	—	25.3[e] *q*	25.9[e] *q*	25.7 *d*	25.8 *q*
5‴	1.72 (*s*)	17.8 *q*	—	17.8 *q*	17.9[f] *q*	17.9 *q*	17.5 *q*

*δ7-OH, 2′-OH and 4′-OH: 5.50 (3H, *s*, exchangeable in D_2O).

†For these compounds, the isoflavone numbering system was preferred, since pterocarpans represent a subcategory of isoflavonoids compounds.

‡Chemical shifts (δ) are in ppm relative to TMS (multiplicity, *J*, in Hz).

[a–f]Assignments may be reversed within the same column.

romic shift ($\Delta\lambda$ = 3 nm) observed in the UV spectrum upon addition of NaOAc reagent, suggested that (**1**) had a free hydroxyl group at the C-7 position [16].

The broad three-proton signal in the ^{1}H NMR at δ5.50, exchangeable in D_2O, established the presence of three hydroxyl groups in the molecule. This was confirmed by acetylation of (**1**) and formation of compound (**1a**), which did not respond positively to the Fe^{3+} test and which had a molecular formula, $C_{31}H_{34}O_8$, assigned from the $[M]^+$ at *m/z* 534.2067 (calc. 534.2069) in the HR-mass spectrum, corresponding to a triacetate derivative. The ^{1}H NMR spectrum of eriotrichin B also showed a set signals [δ1.69 (3H, *s*, Me, *cis*), 1.72 (3H, *s*, Me, *cis*), 1.77 (3H, *s*, Me, *trans*), 1.79 (3H, *s*, Me, *trans*), 3.22 (2H, *d*, *J* = 7.1 Hz), 3.30 (2H, *d*, *J* = 6.9 Hz), 5.20 (1H, *t*, *J* = 7.1 Hz), 5.23 (1H, *t*, *J* = 6.9 Hz)] which, together with certain peaks in the EI-mass spectrum at *m/z* 229 (A_1 − 44), 217 (A_1 − 56), 175 [A_1 − (55 + 43)] and 161 [A_1 − (56 + 56)] (Scheme 1), indicate the presence of two separated γ,γ-dimethylallyl groups. Furthermore, the typical ABX spin system at δ7.02 (1H, *d*, *J* = 8.4 Hz), 6.27 (1H, *dd*, *J* = 8.4 and 2.3 Hz) and 6.37 (1H, *d*, *J* = 2.3 Hz) established the presence of three aromatic protons in ring B with *ortho*-, *ortho*-/*meta*- and *meta*-coupling, respectively. The evidence of a one-proton singlet at δ7.57 and a fragment ion at *m/z* 273 (A_1) due to retro-Diels–Alder cleavage of ring C in the EI-mass spectrum indicated that (**1**) had a 7-hydroxy-6.8-di-(γ,γ-dimethyl) moiety in the A-ring. Cleavage also resulted in a fragment ion at *m/z* 135 (B_1) showing that the B-ring possessed two hydroxyl groups. On the other hand, it was assumed on biogenetic grounds that, one of the two hydroxyl groups in ring B should occupy the C-4′ position [17], thus leading to two possible substitution patterns, an *ortho*- or *meta*-dihydroxyl system. The chemical shifts of the B-ring protons were consistent with the ABX spin system as H-5′ (δ6.27), H-3′ (δ6.37) and H-6′ (δ7.02) respectively, and the formulation that the two phenolic groups in ring B are not *ortho*. Support of the *meta*-position of these two phenolic groups was given by the ^{13}C NMR spectrum which showed diagnostic signals at δ155.8 and 156.6 characteristic of *meta*-aromatic carbons bearing on oxygen atom [15].

Treatment of (**1**) with formic acid afforded two compounds (**1b**) and (**1c**) formed by cyclization between the isoprenyl groups and a neighbouring hydroxyl group.

This result confirmed that in ring A, the two isoprenyl groups are located in adjacent positions (positions 6 and 8) to the hydroxyl group, which occupies position 7. Further confirmation came from the HMBC spectrum of eriotrichin B (**1**) which revealed long-range correlations (^2J and ^3J) between C-7 (δ160.6) and ^{1}H NMR signals at δ3.22 (H-1″), 3.30 (H-1″), 7.57 (H-5), the latter which also showed appreciable correlations to ^{13}C signals at δ194.8 (C-4), 160.0 (C-9) and 114.7 (C-6). In addition, ^{13}C signals at δ126.5 (C-5) and 114.1 (C-8) were correlated to ^{1}H signals at δ3.22 (H-1″) and 3.30 (H-1‴), respectively, which is also consistent with the substitution pattern in ring A. Similarly, long-range correlations observed between H-6′ (δ7.02) and ^{13}C NMR signals at δ155.8 (C-2′), 156.6 (C-4′) and 46.5 (C-3) and between C-1′ (δ113.5) and ^{1}H signals at δ6.27 (H-5′), 6.37 (H-3′), 4.60 (H-2eq) and 3.99 (H-3) also supported the substitution pattern in ring B.

On the basis of the above spectroscopic studies, the novel compound eriotrichin B (**1**), was assigned as 6, 8-di (3,3-dimethylally)-7,2′,4′-trihydroxyisoflavanone.

All the compounds isolated were tested *in vitro* for their antimicrobial activities and the bioassay results are given in Table 2. Eriotrihin B (**1**) is much more potent against *S. aureus*. Erybraedins A (**3**) and C (**4**) are nearly equipotent. Isoneorautenol (**2**) and erybraedin E (**6**) are about half as active, whereas erybraedin D (**5**) is six times less active. Although the activity of each compound is moderate, the total quantity is quite large and they may therefore account not only for the potency of the crude root extract but can also serve to validate, as well as experiments go, the folklore use of this material for certain infections in Cameroon [18].

Table 2. *In vitro* antibacterial activity (MIC, μg ml^{-1}) of *Erythrina eriotricha* components against *Staphylococcus aureus* 209 P*

	MIC
Crude root extract	220.4
CH_2Cl_2 root extract	155.4
Eriotrichin B	8.3
Isoneorautenol	28.4
Erybraedin A	13.6
Erybraedin C	12.8
Erybraedin D	78.3
Erybraedin E	22.1
Penicillin	6.0

*No activity was found when tested against *Escherischa coli* RL65.

EXPERIMENTAL

General. All mps are uncorr. Optical rotations were measured at room temp. in MeOH. ^{1}H and ^{13}C NMR were recorded with TMS as int. standard and chemical shifts are given in δ values. ^{1}H–^{1}H shift correlation (COSY), JMod, DEPT, HMQC and HMBC experiments were performed with the usual pulse sequence and data processing was obtained with standard software.

Plant material. Erythrina eriotricha Harm root bark was collected at Meiganga in northern Cameroon in February 1994. Voucher material documenting the collection was identified by the director of the National Herbarium, Yaounde, Cameroon, and is deposited there.

Extraction and isolation. Dried ground root bark (10 kg) of *E. eriotricha* was extracted in a Soxhlet with MeOH and the extract concd to dryness. The residue (800 g) was dissolved in MeOH–H_2O (4:1) and the aq. soln re-extracted successively with *n*-hexane, CH_2Cl_2 and *n*-BuOH. Evpn of these various extracts under red. pres. gave 65 g of hexane extract (0.6%) and 250 g (2.5%) of CH_2Cl_2 extract. The *n*-BuOH extract consisted mainly of tannins, while the CH_2Cl_2 extract, examined during this investigation, contained the microbial activity of the plant, when tested against *S. aureus*, using agar streak-dilution technique [12]. A portion of this extract (100 g) was fractionated by CC over silica gel (230–400 mesh) packed in cyclohexane and eluted with a cyclohexane–EtOAc mixt. A total of 160 frs of *ca* 250 ml each were collected and combined on the basis of TLC analysis leading to five main series A–E. Frs 1–80 eluted with benzene–EtOAc (17:3) gave series A (55.0 g), frs 81–96 (4:1) gave series B (10 g) and frs 97–112 (3:1) gave series C (8.5 g). Frs 113–128 eluted with cyclohexane–EtOAc (3:2) gave series D (10 g), while frs 129–160 eluted with EtOAc produced series E (10 g).

During this investigation, we focused on the residue identified as series A above. This residue was dissolved in CH_2Cl_2 and chromatographed on silica gel. The column was eluted with cyclohexane–EtOAc (10:1) taking 80 × 20 ml frs. Combined frs 1–14 were evapd and the residue (3.7 g) recrystallized from cyclohexane–EtOAc to give isoneorautenol (**2**) (1.2 g). Frs 15–25 were subjected to repeated CC over silica gel using cyclohexane–EtOAc (10:1) followed by prep. TLC over silica gel employing cyclohexane–Me_2CO (7:3) as eluent to yield erybraedin C (**4**) (900 mg) and erybraedin A (**3**) (955 mg). Frs 26–32 were purified by prep. TLC on silica gel with cyclohexane–EtOAc (7:3) to afford erybraedin D (**5**) (9 mg) and erybraedin E (**6**) (12 mg). Finally frs 33–40 were purified by prep. TLC over silica gel using cyclohexane–Me_2CO (7:3) to give eriotrichin B (**1**) (250 mg).

Eriotrichin B (**1**). Brown sticky oil $[\alpha]_D^{22} = 0°$ (MeOH; *c* 0.50). UV λ_{max}^{MeOH} nm (log ε): 281 (4.27), 210 (4.20); (+NaOAc): 284 (3.89), 215 (4.55); (+$AlCl_3$): 280 (3.76), 212 (4.11), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3400, 2924, 1652, 1602, 1554, 1508, 1498, 1450, 1334, 1222, 1166, 1110, 1080, 840. ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1. HRMS *m/z*: 408.1935 $[M]^+$ (calc. for $C_{25}H_{28}O_5$, 408.1937). EIMS *m/z* (rel. int.): 408 $[M]^+$ (100), 390 (32), 334 (16), 319 (20), 273 (70), 229 (21), 217 (46), 203 (22), 188 (40), 175 (44), 161 (75), 147 (28), 135 (54), 110 (39), 91 (29), 77 (39), 69 (32), 55 (36).

Eriotrichin B triacetate (**1a**). Eriotrichin B (**1**) (12 mg) was dissolved in pyridine (1 ml) and Ac_2O(2 ml) added. The reaction mixt. was kept at room temp. for 48 hr and

then poured over crushed ice. The soln obtained was extracted with CH_2Cl_2 and the organic layer washed with 5% HCl soln and then brine. It was dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification of the residue by prep. TLC over silica gel using cyclohexane–EtOAc (7:3) gave the triacetate derivative (**1a**) (12 mg) as a sticky oil. $[\alpha]_D^{22} = 0°$ (MeOH; *c* 0.7). R_f 0.60 (cyclohexane–EtOAc, (7:3)). $FeCl_3$ test: negative. IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2935, 1772, 1769, 1654, 1602, 1556, 1445, 1384, 1250, 1160. ^1H NMR (300 MHz, $CDCl_3$): δ7.70 (1H, *s*, H-5), 7.21 (1H, *d*, *J* = 8.9 Hz, H-6'), 7.01 (1H, *d*, *J* = 2.4 Hz, H-3'), 6.98 (1H, *dd*, *J* = 8.9 and 2.3 Hz, H-5'), 5.10 (1H, *t*, *J* = 7.3 Hz, H-2''), 5.19 (1H, *t*, *J* = 7.3 Hz, H-2'''), 4.65 (1H, *dd*, *J* = 11.6 and 11.4 Hz, H-2ax), 4.52 (1H, *dd*, *J* = 11.4 and 5.3 Hz, H-2eq), 4.10 (1H, *dd*, *J* = 11.6 and 5.3 Hz, H-3), 3.10 (2H, *d*, *J* = 7.3 Hz, H-1''), 3.18 (2H, *d*, *J* = 7.3 Hz, H-1'''), 2.10 (3H, *s*, Ac), 2.20 (3H, *s*, Ac), 2.65 (3H, *s*, Ac) 1.60 (3H, *s* = C – Me , *cis*), 1.65 (3H, *s*, =C–Me, *cis*), 1.72 (3H, *s*, =C–Me, *trans*), 1.77 (3H, *s*, =C–Me, *trans*). HRMS *m/z*: 534.2067 $[M]^+$ (calc. for $C_{31}H_{34}O_8$, 534.2069. EIMS *m/z* (rel. int.): 534 $[M]^+$ (100), 519 (28), 492 (70), 450 (55), 436 (37), 408 (52), 273 (71), 229 (43), 217 (37), 161 (64), 135 (23).

Acid-catalysed cyclization of compound (**1**). A soln of (**1**) (20 mg) and 98% HCO_2H (2 ml) was stirred at room temp. for 24 hr. The reaction mixt. was poured into H_2O, extracted with CH_2Cl_2, concd *in vacuo* and purified by CC over silica gel eluted with cyclohexane–EtOAc (9:1) followed by prep. TLC over silica gel (cyclohexane–EtOAc, 9:1) to afford compounds (**1b**) (6 mg) and (**1c**) (7 mg), both as brown sticky oils.

Compound **1b**. $[\alpha]_D^{22} = 0°$ (MeOH; *c* 0.1). R_f 0.56 (cyclohexane–EtOAc, 7:3). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3382, 2947, 1654, 1604, 1560, 1434, 1365, 1210, 1104, 1100. ^1H NMR (300 MHz, $CDCl_3$): δ7.48 (1H, *s*, H-5), 6.95 (1H, *d*, *J* = 8.6 Hz, H-6'), 6.51 (1H, *d*, *J* = 2.3 Hz, H-3'), 6.41 (1H, *dd*, *J* = 8.6 and 2.3 Hz, H-5'), 5.20 (1H, *t*, *J* = 6.8 Hz, H-2''), 4.70 (1H, *dd*, *J* = 11.6 and 11.3 Hz, H-2ax), 4.61 (1H, *dd*, *J* = 11.3 and 5.2 Hz, H-2eq), 4.01 (1H, *dd*, *J* = 11.6 and 5.2 Hz, H-3), 3.21 (2H, *d*, *J* = 6.8 Hz, H-1''), 2.67 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2$-), 1.89 (2H, *t*, *J* = 7.0 Hz, -CH_2CH_2Ar), 1.78 (3H, *s*, Me, *trans*) 1.69 (3H, *s*, Me, *cis*), 1.39 [6H, *s*, -$C(Me)_2$]. EIMS *m/z* (rel. int.): 408 $[M]^+$ (100).

Compound **1c**. $[\alpha]_D^{22} = 0°$ (MeOH; *c* 0.3), R_f 0.60 (cyclohexane–EtOAc, (7:3). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3382, 2947, 1654, 1604, 1560, 1434, 1365, 1210, 1104, 1100. ^1H NMR (300 MHz, $CDCl_3$): δ7.49 (1H, *s*, H-5), 6.88 (1H, *d*, *J* = 8.4 Hz, H-6'), 6.51 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.39 (1H, *dd*, *J* = 8.4 and 2.5 Hz, H-5'), 5.24 (1H, *t*, *J* = 6.8 Hz, H-2'''), 4.72 (1H, *dd*, *J* = 11.5 and 11.1 Hz, H-2ax), 4.61 (1H, *dd*, *J* = 11.1 and 5.4 Hz, H-2eq), 4.05 (1H, *dd*, *J* = 11.5 and 5.4 Hz, H-3), 3.19 (2H, *d*, *J* = 6.8 Hz, H-1'''), 2.64 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2$-), 1.89 (2H, *t*, *J* = 7.0 Hz, -CH_2CH_2Ar) 1.76 3H, *s*, Me, *trans*), 1.66 (3H, *s*, Me, *cis*), 1.39 (6H, *s*, -$C(Me)_2$. EIMS *m/z* (rel. int.): 408 $[M]^+$ (100).

Isoneorautenol (**2**). White needles (cyclohexane–EtOAc), mp 156° (lit. [7] 154–155°). HRMS, *m/z*: 322.1212 (calc. for $C_{20}H_{18}O_4$: 322.1204). EIMS, *m/z* (rel. int.): 322 $[M]^+$ (35), 307 (100), 279 (6), 185 (10), 173 (10), 165 (11), 153 (22), 145 (6), 115 (14), 88 (10), 86 (65). IR, UV and ^1H NMR matched well with lit. values [6, 14]; for ^{13}C NMR, see Table 1.

Erybraedin A (**3**). Crystals, mp 72° (lit. [7] 69–71°). IR, UV and ^1H NMR identical to published data [7]; for ^{13}C NMR see Table 1.

Erybraedin A diacetate (**3a**). Erybraedin A (**3**) (10 mg) was dissolved in pyridine (1 ml) and Ac_2O (2 ml) added. The reaction mixt. was kept at room temp. for 48 hr and worked-up in the usual manner. The residue was purified by CC over siliva gel eluted with cyclohexane–EtOAc followed by prep. TLC (cyclohexane–EtOAc, 7:3) to yield pure erybraedin A diacetate (**3a**) as a sticky oil (13 mg). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2943, 1769, 1605, 1590, 1478, 1360, 1208, 1160, 1066. ^1H NMR (300 MHz, $CDCl_3$): δ7.42 (1H, *d*, *J* = 8.3 Hz, H-5), 7.03 (1H, *d*, *J* = 8.0 Hz, H-2'), 6.75 (1H, *d*, *J* = 8.3 Hz, H-6), 6.58 (1H, *d*, *J* = 8.0 Hz, H-3'), 5.51 (1H, *d*, *J* = 6.5 Hz, H-4), 5.08 (1H, *t*, *J* = 8.2 Hz, H-1''), 5.06 (1H, *t*, *J* = 7.1 Hz, H-1'''), 4.32 (1H, *dd*, *J* = 9.4 and 4.4 Hz, H-2eq), 3.65 (2H, *m*, H-2ax and H-3), 3.25 (2H, *d*, *J* = 7.1 Hz, H-2'''), 3.20 (2H, *d*, *J* = 8.2 Hz, H-2''), 2.29 (3H, *s*, Ac-7), 2.26 (3H, *s*, Ac-4'), 1.71 (3H, *s*, Me, *trans*), 1.65 (3H, *s*, Me, *cis*). HRMS *m/z* 476.2204 $[M]^+$ (calc. for $C_{29}H_{32}O_6$, 476.2206). EIMS *m/z* (rel. int.): 476 $[M]^+$ (55), 461 (22), 434 (100), 417 (24), 392 (89), 378 (35), 336 (92), 319 (26), 293 (23), 280 (60) 202 (15), 189 (40), 173 (20), 159 (18), 147 (45) 135 (37).

Erybraedin C diacetate (**4a**). Compound **4** (15 mg) was dissolved in pyridine (1 ml) and Ac_2O (2 ml) added. The reaction mixt. was kept at room temp. for 48 hr and worked-up in the usual manner. The residue was purified by CC on silica gel followed by prep. TLC to afford the diacetate derivative (**4a**) as a sticky oil (11 mg). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2924, 1768, 1602, 1554, 1492, 1476, 1372, 1208, 1156, 1066, 1016, 898. ^1H NMR (300 MHz, $CDCl_3$): δ7.32 (1H, *d*, *J* = 8.4 Hz, H-5), 7.03 (1H, *s*, H-2'), 6.70 (1H, *d*, *J* = 8.4 Hz, H-6), 6.49 (1H, *s*, H-5'), 5.50 (1H, *d*, *J* = 6.5 Hz, H-4), 5.20 (1H, *t*, *J* = 6.9 Hz, H-2''), 5.09 (1H, *t*, *J* = 7.1 Hz, H-2'''), 4.30 (1H, *dd*, *J* = 11.1 and 5.8 Hz, H-2 eq), 3.60 (1H, *t*, *J* = 11.1 Hz, H-2ax), 3.55 (1H, *m*, H-3), 3.20 (2H, *d*, *J* = 6.9 Hz, H-1''), 3.15 (2H, *d*, *J* = 7.1 Hz, H-1'''), 2.24 (3H, *s*, Ac-7), 2.22 (3H, *s*, Ac-4'), 1.78 (3H, *s*, Me, *trans*), 1.70 (3H, *s*, Me, *trans*), 1.67 (3H, *s*, Me, *cis*), 1.65 (3H, *s*, Me, *cis*). EIMS *m/z* (rel. int.): 476 $[M]^+$ (100), 434 (98), 420 (19), 392 (70), 378 (6), 336 (23), 281 (5), 189 (14), 149 (30), 147 (4), 135 (4), 115 (18), 84 (9), 69 (21).

Erybraedin D (**5**). Sticky oil. IR, UV and ^1H NMR identical to published data [9]. For ^{13}C NMR data, see Table 1.

Erybraedin E (**6**). Sticky oil. $[\alpha]_D^{22} = -108°$ (MeOH; *c* 0.32). UV λ_{max}^{MeOH} nm (log ε): 302 (4.07), 295 (4.09), 286 (4.00), 278 (3.87), 254 (4.32), 2.46 (4.48). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3425, 2967, 1603, 1534, 1446, 1378, 1360, 1285, 1255, 1230, 1150, 1067, 1027, ^1H NMR (300 MHz, $CDCl_3$): δ: 7.54 (1H, *d*, *J* = 2.4 Hz, H-2''), 7.42 (1H, *s*, H-5), 7.30 (1H, *d*, *J* = 8.5 Hz, H-2'), 6.98 (1H, *s*, H-8), 6.69 (1H, *d*, *J* = 2.3 Hz, H-3''), 5.59 (1H, *d*, *J* = 5.8 Hz, H-4), 5.20 (1H, *s*, exchangeable in D_2O, OH-4'), 5.21 (1H, *t*, *J* = 6.8 Hz,

H-2‴), 4.35 (1H, *m*, H-2 eq), 3.68 (2H, *m*, H-2-ax, H-3), 3.20 (2H, *d*, $J = 6.9$ Hz, H-1‴), 1.78 (3H, *s*, Me *trans*), 1.72 (3H, *s*, Me, *cis*). ^{13}C NMR: see Table 1. HRMS *m/z*: 348.1359 $[M]^+$ (calc. for $C_{22}H_{20}O_4$, 348.1360). EIMS *m/z* (rel. int.): 348 $[M]^+$ (50), 292 (39), 291 (23), 158 (33), 71 (47), 57 (80), 43 (100).

Antimicrobial activity screening. Extracts and purified active compounds were tested at 1 mg ml^{-1} against *Staphylococcus aureus* 209P and *Escherischa coli* RL65. The two strains of bacteria were cultured in Mueller Hinton agar medium at 37°. After 1 day, their growth was examined with the naked eye. The lowest concentration of the test compounds in which no growth occurred was defined as the MIC.

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REFERENCES

1. Cordell, G. A. (ed.) (1981) in *The alkaloids, a biogenetic approach*, pp. 450–462. John Wiley & Sons, New York.
2. Fomum, Z. T., Ayafor, J. F. and Mbafor, J. T. (1983) *Tetrahedron Letters* **24**, 4127.
3. Fomum, Z. T., Ayafor, J. F. and Mbi, C. N. (1986) *J. Chem. Soc. Perkin Trans I* 33.
4. Nkengfack, A. E., Sanson, D. R., Fomum, Z. T. and Tempesta, M. S. (1989) *J. Nat. Prod.* **52**, 320.
5. Nkengfack, A. E., Vouffo, W. T., Fomum, Z. T., Meyer, M., Bergendorff, O. and Sterner, O. (1994) *Phytochemistry* **36**, 1047
6. Wandji, J., Fomum, Z. T., Tillequin, F., Seguin, E. and Koch, M. (1993) *Phytochemistry* **35**, 245
7. Mitscher, L. A., Okwute, S. K., Gollapudi, S. R., Drake, S. and Avona, E. (1988) *Phytochemistry* **27**, 3449.
8. Mitscher, L. A., Gollapudi, S. R., Gerlach, D. C., Drake, S. D., Veliz, A. E. and Ward, J. A. (1988) *Phytochemistry* **27**, 381.
9. Mitscher, L. A., Okwute, S. K., Gollapudi, S. R. and Keshavarz-Shokri, A. (1988) *Heterocycles* **27**, 2517.
10. Ayensu, E. S. (ed.) (1978) in *Medicinal plants of west Africa*, p. 153, Reference Publication Inc, Algonac Michigan.
11. Nkengfack, A. E., Fomum, Z. T., Ubillas, R. and Tempesta, M. S. (1990) *J. Nat. Prod.* **53**, 1552.
12. Mitscher, L. A., Leu, R. P., Batchala, M. S., Wu, W. N., Beal, J. L. and White, R. (1972) *J. Natl. Prod.* **35**, 157.
13. Marchal, N., Bourdon, J. L. and Richard, Cl. (eds) (1982) in *Les milieus de culture pour l'Isolement et l'identification biochimique des bacteries*, p. 38. Doin editeurs, Paris.
14. Ingham, J. L. and Tahara, S. (1985) *Z. Natursforch* **40c**, 482.
15. Agrawal, P. K. (ed.) in *Carbon-13 NMR of flavonoids*, p. 219. Elsevier, Amsterdam.
16. Mabry, T. J., Markham, K. R. and Thomas, M. D. (eds) (1970 in *The systematic identification of flavonoids*. Springer, Berlin.
17. Ebel, E. and Halhbrock, K. (1982) in *The flavonoids advances in research* (Harborne, J. B. and Mabry, T. J., eds), p. 649. Chapman & Hall, London.
18. Dalziel, J. M. (ed.) (1937) in *The useful plants of West Tropical Africa*, p. 612. Crown Agents for Colonies, London.