Phytochemistry, Vol 27, No 11, pp 3449–3452, 1988
Printed in Great Britain

0031 9422/88 $3 00+0 00

ANTIMICROBIAL PTEROCARPANS OF NIGERIAN *ERYTHRINA MILDBRAEDII*

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(*Received in revised form* 18 *March* 1988)

Key Word Index—*Erythrina mildbraedii*, Leguminosae, antimicrobial agents, pterocarpans, erythrabyssin-II; isoneorautenol; erybraedin A, erybraedin B, erybraedin C.

Abstract—Bioassay-directed fractionation of ethanolic extracts of the roots of *Erythrina mildbraedii*, a plant used in the indigenous system of medicine in Nigeria, resulted in the isolation of the previously known pterocarpans erythrabyssin-II and isoneorautenol and the new pterocarpans erybraedins A, B, and C The structures of the new compounds were determined by chemical transformations and/or spectroscopy and their *in vitro* antimicrobial spectra and potencies are reported

INTRODUCTION

There has been much chemical study of various *Erythrina* species because of the reputed cardiovascular effects of their alkaloids [1]. Of late, survey of the neutral and phenolic components has gathered momentum [2]. Most particularly, a number of *Erythrina* species have been show to have various folkloric uses in indigenous medicinal systems [3]. In this context, our continuing interest in antimicrobial agents of potential medicinal use [4] led us to a study of *Erythrina mildbraedii*, a plant widely used in indigenous medical practice in Nigeria [5]. We report herein the properties of several pterocarpans which we find to be responsible for the bulk of the antimicrobial activity of extracts of this plant.

RESULTS AND DISCUSSION

Testing *in vitro* of extracts of the roots of *Erythrina mildbraedii* collected in Zaria, Nigeria, showed potent activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* so bioassay-directed fractionation was undertaken following our usual protocol (Table 1) [4]. The activity distributed into the polar lipids fraction which commonly, in our experience, contains phenolics [6]. These fractions were resolved by chromatography over silica gel. Five main bioactive fractions were readily obtained, one of which was quickly shown by comparison with an authentic specimen to be identical to erythrabyssin-II (**10**), first isolated as an antimicrobial constituent of the related medicinal plant *E abyssinica* [7]

Table 1. *In vitro* (agar-streak dilution) antibacterial activity of *Erythrina mildbraedii* components

Sample	Microorganism* 1	2	3	4	5	6	7
E mildbraedii root extract	100*	—	—	—	100	—	—
Alkaloids fractions	—	—	—	—	—	—	—
Water solubles fractions	—	—	—	—	—	—	—
Acids fractions	—	—	—	—	—	—	—
Non-polar lipids fractions	—	—	—	—	—	—	—
Polar lipids fractions	12.5	—	—	—	12.5	—	—
Erythrabyssin-II	3.12	—	—	—	0 78	—	—
Erybraedin A	12 5	—	—	—	6.25	—	—
Erybraedin B	12 5	—	—	—	12 5	—	—
Erybraedin C	12 5	—	—	—	12 5	—	—
Isoneorautenol	25.0	—	—	—	25 0	—	—
Streptomycin SO_4	5	5	50	2 5	1 25	—	—

*The units are micrograms/ml; —: no detectable activity The microorganisms employed are· 1 = *Staphylococcus aureus* ATCC 13709; 2, *Escherichia coli* ATCC 9637, 3, *Salmonella gallinarum* ATCC 9184, 4, *Klebsiella pneumoniae* ATCC 10031; 5, *Mycobacterium smegmatis* ATCC 607, 6, *Candida albicans* ATCC 10231, 7, *Pseudomonas aeruginosa* ATCC 27853

and, subsequently, in this laboratory, in *E. crista-galli* [6] and *E. variegata* [4] and a second was identified by literature comparison as isoneorautenol (**9**) previously reported as a phytoalexin of *Calopogonium mucunoides* [9]. The other constituents proved to be the novel and closely related pterocarpans erybraedin A (**1**), B (**5**), and C (**7**).

Erybraedin A, $C_{25}H_{28}O_4$, possessed an ultraviolet spectrum consistent with a pterocarpan ring system [10] along with the classical four proton pattern [11] and ^{13}C NMR peaks associated with the aliphatic rings of this system in addition to the necessary proton and carbon resonances associated with two prenyl groups. The presence of two phenolic hydroxyl moieties was readily confirmed by the formation of a diacetate (**2**) and a dimethyl ether (**3**). The lack of identity of erybraedin A with the previously known erycristagallin [8] and erythrabyssin-II (**10**) and the presence of four AB *ortho*-disposed aromatic hydrogens suggested the isomeric formulation **1**. In further support, the aromatic hydrogen signals lying farthest upfield underwent a significant downfield shift in the ^1H NMR spectrum of the acetate confirming the proximity of each to one of the phenolic hydroxyls [12].

Having, in a previous study, synthesized the monomethyl ether corresponding to structure **1** as a major byproduct in the prenylation of sandwicensin to erycristin [6], it was a simple matter to methylate this product further and to demonstrate the identity of this product with the dimethylether of erybraedin A completing an unambiguous structure proof.

Erybraedin C (**7**), also $C_{25}H_{28}O_4$, is clearly isomeric with erybraedin A in its molecular composition and in its major spectroscopic properties. As it was obviously neither erythrabyssin-II nor erybraedin A, the point of isomerism had to be in the other aromatic ring. This was clear from the proton magnetic resonance spectrum wherein the expected AB *ortho* aromatic hydrogen signals of H-1 and H-2 ($J = 8.1$ Hz) were joined by the signals of a *para* disposed pair of aromatic hydrogens (H-7 and H-10). Further, the proton signal farthest upfield in each pair underwent a substantial downfield shift upon acetylation to **8**. This leaves **7** as the only reasonable structural expression for erybraedin C.

The third new substance, erybraedin B (**5**), $C_{25}H_{26}O_4$, differed from A and C by possessing an additional degree of unsaturation. Further, it has an UV maximum at considerably longer wavelength than either A or C, suggesting strongly that an additional double bond is conjugated with one or more of the rings. The ring system was still that of a pterocarpan based upon the presence of the classical three signals in the carbon NMR (δ 40.24, 66.90 and 78.71) and the usual ABMX proton signals for the hydrogens attached to these carbons [11]. One prenyl group was present. Only one free phenolic hydroxyl group was present as shown by the formation of a monoacetate (**6**). The NMR signal for the upfield proton of one of the two aromatic *ortho* proton pairs was significantly moved downfield in the spectrum of the acetate whereas the others were relatively unaffected. Tellingly, there were two more unsaturated proton signals present in the olefinic region and their coupling made them *cis*. Their chemical shifts are characteristic of the *gem*-dimethylchromene system frequently encountered in this class [13]. The weak shoulder in the UV spectrum beyond 300 nm [10] and the *para* disposition of the hydrogens in this ring lead to structure **5** for erybraedin B.

The remaining pterocarpan of *E. mildbraedii* root extract, isoneorautenol (**9**), $C_{20}H_{18}O_4$, has the classical ABMX four proton signals for the aliphatic portion of that ring system. Instead, however, of the AB pattern associated with H-1 and H-2 of the other erybraedins, an ABM pattern was present and there were no prenyl signals. This suggested that, as with sandwicensin, this molecular feature was absent. The classical signals were present for a *gem*-dimethylchromene system along with singlets for hydrogens H-7 and H-10. In agreement with a linear annulation, the UV spectrum showed the usual succession of sharp maxima beyond 300 nm [10]. These features lead to expression **9** and these have been previously associated with isoneorautenol [9]. The alternate structure which satisfies these properties (in which the dimethylchromene ring is annulated to carbons 2 and 3 instead of carbons 8 and 9) has been assigned to neorautenol from *Neorautanenia edulis* [14]. The physicochemical properties and spectra of neorautenol differ significantly from those of isoneorautenol. All of the erybraedins described herein are levorotatory and their circular dichroism spectra are also in agreement with their possession of 6a (*R*), 11a (*R*) absolute stereochemistry [7, 15].

The *in vitro* antimicrobial potency of these substances is given in the table. It can be seen that erythrabyssin-II is much the more potent. Erybraedins A, B, and C are nearly equipotent and isoneorautenol is about half as active. All five compounds have the same antimicrobial spectrum as the crude root extracts and not only account for the majority of its potency but serve to validify, as far as these experiments go, the folkloric use of this material for certain infections in Nigeria [5]. Some minor, less bioactive, components are present and will be reported subsequently.

Thus, the antimicrobial potency of another *Erythrina* species has been shown to be due to the presence of apparently performed pterocarpans. A number of pterocarpans are phytoalexins [10] but the functional utility of the distinction between phytoalexin and constitutive agent in searching for new antimicrobial agents is weakened by these findings. Certainly antimicrobial activity is a common feature of pterocarpans whether they be present in the healthy plant or formed postinfectionally.

EXPERIMENTAL

Plant material. Erythrina mildbraedii roots were collected from the savana forests of Zaria, Nigeria, in June 1986. A voucher specimen is deposited in the Herbarium, Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria.

Extraction. The root powder (931 g) was subjected to cold (stationary) extraction using 95% EtOH. Concentration of the extract gave a residue (100.9 g) which was partitioned between 5% HCl and CH_2Cl_2. The organic layer was washed with H_2O and extracted with 5% Na_2CO_3. The organic layer was again washed with H_2O and evapd to dryness to give a dark solid (31.8 g). This last was partitioned between 90% MeOH and *n*-hexane to give 29.1 g of MeOH soluble solid material and 2.3 g of a *n*-hexane soluble viscous oil. The Na_2CO_3 extract was acidified with dilute HCl and extracted with CH_2Cl_2. The organic layer was washed with H_2O and evapd to dryness to give a dark brown solid (3.3 g). The initial aq. acid extract was basified with aq. ammonia to pH 9 and extracted with CH_2Cl_2. Evaporation

of the organic layer, after washing, gave a solid (1 8 g). The material insoluble in the initial partitioning of the total root extract weighed 23 9 g As shown in Table 1, the total extract and the polar lipids (containing the phenolic materials) contained all of the antimicrobial activity of the plant

Column chromatographic resolution of phenolic constituents A portion of the phenolic mixture (10 g) was dissolved in C_6H_6 (30 ml) and chromatographed over 300 g of silica gel The column was eluted with C_6H_6 (1 25 l) followed by mixtures of C_6H_6–EtOAc in 10 ml fractions Fractions 1–40 (19 1) gave group 1, 334 mg, fractions 41–50 gave group 2, 800 mg, fractions 51–61 gave group 3, 444 mg, and fractions 62–78 gave group 4, 1 46 g Fractions 79–106 (9 1) gave group 5, 1,03 g, and fractions 107–122 gave group 6, 1 06 g Fractions 123–132 (4 1) gave group 7, 660 mg and fractions 133–172 gave group 8, 2 86 g The column was finally washed with EtOAc (700 ml) to produce group 9, 556 mg. The various groups were combined on the basis of their TLC patterns (silica gel, C_6H_6–EtOAc 9 .1)

Isolation of erybraedin A (**1**) The residue identified as group 2 above was dissolved in CH_2Cl_2 and chromatographed on silica gel (25 g). The column was eluted with CH_2Cl_2 taking 3-ml fractions. Fractions 10–20 contained a single component upon TLC examination and were therefore combined and evapd to produce a solid residue which crystallized from *n*-hexane–CH_2Cl_2 to give pure erybraedin A (484 mg), mp 69–71, UV λ_{max}^{MeOH} nm (log ε) 285 (3 58), 280 sh (3 53), $\lambda_{max}^{MeOH-HCl}$ 285 (3 57), 280 (3 52), $\lambda_{max}^{MeOH-NaOH}$ 288 (3.69), 279 (3 59), $\lambda_{max}^{MeOH-AlCl_3}$ 285 (3.72), 279 (3 68), $\lambda_{max}^{MeOH-AlCl_3-HCl}$ 284 (3 68), 280 (3.65); IR ν_{max}^{neat} cm^{-1} 3585, 3440, 1605, 1480, 1465, 1445, 1420, 1380, 1354, 1265 (*br*), 1175, 1095, 1072, 1050, 1030, 895, ^1H NMR (300 MHz, $CDCl_3$) δ1 73 (6H, *s*, H-4′), 1 80 (6H, *s*, H-5′), 3.48 (4H, *m*, H-1′), 3 49 (1H, *m*, H-6ax), 3 56 (1H, *dd*, J = 10 8 Hz, H-6a), 4 29 (1H, *dd*, J = 4 8, 10 8 Hz, H-6eq), 5 18 (1 H, *t*, H-2′), 5 28 (1H, *m*, H-2′), 5 41 (1H, *s*, OH), 5.47 (1H, *d*, J = 7 2 Hz, H-11a), 5.50 (1H, *s*, OH), 6 37 (1H, *d*, J = 7 8 Hz, H-8), 6 55 (1H, *d*, J = 8 4 Hz, H-2), 6 95 (1H, *d*, J = 7.8 Hz, H-7), 7 29 (1H, *d*, J = 8 4 Hz, H-1); ^{13}C NMR (75 4 MHz, $CDCl_3$) 17 93, 18 03, 22 45, 23 25, 25.86, , 25.96, 40.10, 66.94, 78 93, 108 18, 109.79, 110 41, 112 76, 115 07, 118.91, 121.56, 121 85, 122 43, 129 46, 134 52, 135 10, 154 03, 155 16, 155 82, 158 53 ppm, HRMS *m/z* (rel): 392 1984 (M^+, 100%) (calc for $C_{25}H_{28}O_4$, 392 1986), EIMS *m/z* (rel. int)· 392 (100), 336 (32 8), 321 (14 5), 293 (19 1), 280 (82 2), 189 (21 9), 161 (28 3), 147 (74 2), 135 (37 7), 115 (21.4), 91 (28.5), 77 (31 7), 69 (22.5), 55 (30 4), 43 (69 0), and 41 (88 3), optical rotation: $[\alpha]_D^{25}$ −40 67 (MeOH, *c* 0.504), CD $[\theta]_{285}$ + 13 600, $[\theta]_{255}$ + 2650 and $[\theta]_{210}$ − 151 380 (MeOH)

Erybraedin A diacetate (**2**) Erybraedin A (15 2 mg) was dissolved in pyridine (1 ml) and Ac_2O (2 ml) was added The reaction mixture was kept at 65° for 7 hr and then poured over crushed ice (20 g). The soln obtained was extracted with EtOAc and the organic layer washed with 5% HCl soln, and then brine. It was dried, and the solvent was removed *in vacuo* Purification of the residue by prep TLC over silica gel using *n*-hexane–EtOAc (5 1) gave the pure diacetate **2**, 7 0 mg, as a gummy solid IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3040, 2960, 2915, 2840, 1750, 1590, 1470, 1455, 1430, 1360, 1200, 1160, 1066, 1045, 1030, 885; ^1H NMR (300 MHz, $CDCl_3$): δ1 66 (6H, *s*), 1 73 (6H, *s*), 2.80 (3H, *s*), 2 32 (3H, *s*), 3.21 (2H, *d*, J = 6 9 Hz), 3 26 (2H, *d*, J = 6 9 Hz), 3 62 (2H, *br*), 4 35 (1H, *dd*, J = 3 9, 11 4 Hz), 5 15 (2H, *br*), 5.53 (1H, *d*, J = 6.3 Hz), 6.58 (1H, *d*, J = 7 8 Hz), 6 78 (1H, *d*, J = 8 4 Hz), 7 08 (1H, *d*, J = 7 8 Hz), 7 42 (1H, *d*, J = 8 4 Hz), HRMS *m/z* (rel int) 476 2203 [M^+, 25%], calcd for $C_{29}H_{32}O_6$ 476 2197, CIMS *m/z* (rel int)· 476 (25), 461 (18), 434 (45), 420 (13), 392 (33), 378 (23), 336 (50), 293 (15), 280 (31), 149 (45), 129 (14), 43 (100).

Erybraedin A dimethylether (**3**) Erybraedin A (10 mg), MeI (57 mg), Me_2CO (1 ml), K_2CO_3 (39 mg) were refluxed for 7 5 hr The mixture was filtered and the filtrate concn under red pres The residue obtained was dissolved in EtOAc, washed with brine, dried and the solvent was removed Purification by prep TLC over silica gel using *n*-hexane–EtOAc (5 1) yielded the ether (5 3 mg) as a gummy solid IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3050, 2960, 2930, 2860, 1725, 1610, 1480, 1460, 1440, 1370, 1350, 1200, 1170, 1100, 1080, 1040, 990, 895, 800; ^1H NMR (300 MHz, $CDCl_3$) δ1.66 (6H, *s*), 1.77 (6H, *s*), 3.28 (2H, *br*), 3.35 (4H, *d*, J = 6 0 Hz), 3.56 (2H, *br*), 3 81 (3H, *s*), 3 86 (3H, *s*), 4 29 (1H, *dd*, J = 2.1, 5.1 Hz), 5 22 (2H, *br*), 5 49 (1H, *d*, J = 5.4 Hz), 6 41 (1H, *d*, J = 8 7 Hz), 6.66 (1H, *d*, J = 7 9 Hz), 7.02 (1H, *d*, J = 7.9 Hz), 7 38 (1H, *d*, J = 8 7 Hz), HRMS *m/z* (rel int.)· 420 2285 (M^+, 100%, calcd for $C_{27}H_{32}O_4$ 420 2298), EIMS *m/z* (rel. int) 420 (25) (8 1), 364 (17 5), 349 (39 1), 321 (17.3), 161 (28.2), 149 (31), 115 (14 4), 91 (15 5), 69 (23.9), and 55 (18.3)

Isoerycristin methyl ether (**3**) A synthetic sample of isoerycristin [6] (25 mg), MeI (142 5 mg), and K_2CO_3 (100 mg) in Me_2CO (2 ml) were refluxed at 60° for 7 hr After filtration and concn under red pres , the residue was dissolved in EtOAc, washed well with brine, dried, and the solvent removed Purification was accomplished by prep TLC using silica gel G and hexane–EtOAc (5 1) to produce isoerycristin methylether (15 7 mg) as a gummy solid· IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3020, 2970, 2930, 2866, 1610, 1485, 1460, 1440, 1380, 1350, 1260, 1170, 1085, 1030, 990 and 895, ^1H NMR (300 MHz, $CDCl_3$)· δ1.66 (6H, *s*), 1 76 (6H, *s*), 3 28 (2H, *br*), 3.80 (3H, *s*), 3 85 (3H, *s*), 4 29 (1H, *dd*, J = 1.8, 4 2 Hz), 5 22 (2H, *br*), 5 48 (1H, *d*, J = 5.4 Hz), 6.39 (1H, *d*, J = 8 4 Hz), 6.64 (1H, *d*, J = 7 9 Hz), 7 01 (1H, *d*, J = 7.9 Hz), 7 37 (1H, *d*, J = 8.4 Hz); EIMS *m/z* (rel int.). 420 ($M^+C_{27}H_{32}O_4$, 31.5%), 364 (5 8), 349 (13.5), 321 (6 4), 161 (18 8), 149 (16 4), 115 (14 8), 91 (21 7), 69 (31.1), 55 (36 2) and 41 (100). These spectra are identical to those obtained for erybraedin A dimethylether.

Isolation of erybraedin B (**5**). The residue obtained from group 1 fractions, on repeated CC over silica gel using *n*-hexane–CH_2Cl_2, produced erybraedin B (33 mg) as a gummy solid· UV λ_{max}^{MeOH} nm (log ε)· 313 sh (3.40), 288 (4.04), 283 (4 04), $\lambda_{max}^{MeOH-HCl}$ 320 (3 23), 308 (3.51), 285 (4 03), 283 (4 03), $\lambda_{max}^{MeOH-NaOH}$ 322 (3 31), 288 (4 03), 281 (4 00), 260 (3.94), 254 (3.93), $\lambda_{max}^{MeOH-AlCl_3}$ 319 (3 84), 310 (3.91), 283 (4 22), $\lambda_{max}^{MeOH-AlCl_3-HCl}$ 318 (3 74), 310 (3 82), 283 (4.15); IR ν_{max}^{neat} cm^{-1} 3440, 1601, 1580, 1475, 1465, 1442, 1372, 1275, 1155, 1110, 1080, 1050, 1030, 890, 870, 795, 775, 730, ^1H NMR (300 MHz, $CDCl_3$) δ1 34 (6H, *s*, H-4″, 5″), 1 66 (3H, *s*, H-4′), 1 71 (3H, *s*, H-5′), 3 28 (2H, *t*, J = 7 2 Hz, H-1′), 3.40 (1H, *m*, H-6ax), 3.54 (1H, *t*, J = 10.8 Hz, H-6a), 4.19 (1H, *dd*, J = 4.8, 10 8 Hz, H-6eq), 5.22 (1H, *t*, J = 7 Hz, H-2′), 5 29 (1H, *s*, OH), 5.35 (1H, *d*, J = 6.0 Hz, H-11a), 5.49 (1H, *d*, J = 10 8 Hz, H-2″), 6.29 (1H, *d*, J = 7.8 Hz, H-8), 6.45 (1H, *d*, J = 8.4 Hz, H-2), 6.55 (1H, *d*, J = 10 8 Hz, H-1″), 6.87 (1H, *d*, J = 7 8 Hz, H-7), 7.20 (1H, *d*, J = 8 4 Hz, H-1), ^{13}C NMR (75 4 MHz, $CDCl_3$): 18.11, 23.41, 26 05, 27 80, 28 00, 28.15, 40 24, 66.90, 78.71, 108.35, 108.45, 110 42, 110 60, 112 70, 116 80, 118.91, 121.63, 122.56, 129 34, 131.20, 135.31, 151 46, 154.23, 156.08, 158.71; HRMS *m/z* (rel. int): 390.18274 (M^+, 72%, calcd for $C_{25}H_{26}O_4$ 390.18296), EIMS *m/z* (rel. int)· 390 (70) 185 (42 2), 173 (53.3), 160 (46 6), 147 (26 6), 115 (28 8), 91 (28 8), 77 (34.4), 555 (41 1), 43 (98 8), 41 (100); optical rotation $[\alpha]_D^{25}$ −44 79 (MeOH, *c* 0.39), CD $[\theta]$ 290 (+10 420).

Erybraedin B acetate (**6**). Erybraedin B (11 mg) was dissolved in pyridine (1 ml) and Ac_2O (2 ml) was added. The reaction mixture was kept at 65° for 7 hr and worked-up as described above. The residue obtained was purified by prep TLC using *n*-hexane–EtOAc 5 .1 to give the acetate (6.2 mg) as a gummy solid: IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3040, 2980, 2920, 2850, 1760, 1710, 1630, 1605, 1580, 1465, 1440, 1420, 1370, 1210, 1160, 1115, 1085, 1095, 1050, 1035, 905, ^1H NMR (300 MHz, $CDCl_3$)· δ1.44 (6H, *s*), 1.67 (3H,

s), 1 73 (3H, s), 2 29 (3H, s), 3 21 (2H, br), 3 56 (1H, br), 3 71 (1H, dd, J = 11 Hz), 4 29 (1H, dd, J = 10 5 Hz), 5 13 (1H, d, J = 7 8 Hz), 6 64 (1H, d, J = 10 8 Hz), 7 09 (1H, d, J = 7 8 Hz), 7 29 (1H, d, J = 6 9 Hz), HRMS m/z (rel int) 432 1925 ([M^+], 13, calcd for $C_{27}H_{28}O_5$ 432 1935), EIMS m/z (rel int) 432 (25), 417 (16 6), 389 (6 5), 375 (6 7), 319 (7 7), 185 (1 1), 173 (11 5), 43 (100)

Isolation of erybraedin C (**7**) The residue from group 6 was chromatographed on silica gel (50 g) using *n*-hexane–EtOAc (17 3) and taking 2-ml fractions Fractions 48–68 were combined and evapd to produce a gum (309 mg) Further chromatography of this material on silica gel (30 g) using *n*-hexane- EtOAc (9 1), gave erybraedin C (179 mg) as a gum, UV λ_{max}^{MeOH} nm (log ε) 287 (3 95), 233 (4.25), $\lambda_{max}^{MeOH-HCl}$ 287 (3 92), 233 (4 24) $\lambda_{max}^{MeOH-NaOH}$ 296 (4 01), 253 (4 16), 248 (4 21), $\lambda_{max}^{MeOH-AlCl_3}$ 300 (3 89), 287 (4 07), 234 (4 31), $\lambda_{max}^{MeOH-AlCl_3-HCl}$ 300 (3 84), 287 (4 01), 234 (4 27), IR ν_{max}^{neat} cm^{-1} 3560, 3040, 2980, 1601, 1590, 1440, 1390, 1310, 1230, 1180, 1100, 100, 920, 820, 790, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1 68 (3H, s, H-4'), 1 73 (3H, s, H-5'), 1 78 (3H, s, H-4'), 1 80 (3H, s, H-5'), 3 28 (2H, d, J = 7 80 Hz, H-1'), 3 40 (2H, d, J = 7 8 Hz, H-1'), 3 43 (1H, m, H-6ax), 3 55 (1H, dd, J = 10 80 Hz, H-6a), 4 29 (1H, dd, J = 4 8, 10 8 Hz, H-6eq), 5 24 (1H, s, OH), 5 22 (1H, t, J = 7 Hz, H-2'), 5 29 (1H, t, J = 7 Hz, H-2'), 5 43 (1H, s, OH), 5 47 (1H, d, J = 6 0 Hz, H-11a), 6 36 (1H, s, H-8), 6 55 (1H, d, J = 8 1 Hz, H-2), 6 95 (1H, s, H-7), 7 27 (1H, d, J = 8 1 Hz, H-1) ^{13}C NMR (75 7 MHz, $CDCl_3$) 17 70, 22 21, 25 58, 25 66, 29 04, 39 48, 66 70, 79 00, 98 33, 109 61, 112 30, 115 06, 118 89, 119 08, 121 67, 122 16, 125 11, 129 06, 133 98, 134 05, 153 85, 154 81, 155 36, 158 59, HR MS m/z (rel int) 392 19848 (M^+, 88, calcd for $C_{25}H_{28}O_4$ 392 19860), EIMS m/z (rel int) 392 (85), 336 (46 5), 281 (52 6), 161 (30 0), 147 (42 1), 135 (21 2), 115 (20 5), 91 (23 9), 69 (74 5), 55 (38 1), 41 (100), optical rotation $[\alpha]_D^{25}$ −125 55 (MeOH, c 3 75), CD $[\theta]_{286}$ (+9685) and $[\theta]_{236}$(−33 500) (MeOH)

Erybraedin C diacetate (**8**) Erybraedin C (20 mg) was dissolved in pyridine (1 ml) and Ac_2O (2 ml) was added The reaction mixture was kept at 65 for 7 hr and worked-up as above The residue was chromatographed on a short column of silica gel using *n*-hexane–EtOAc (5 1) to produce the pure acetate ester (12 mg) as a gummy solid IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3060, 2970, 2850, 1760, 1620, 1595, 1480, 1430, 1365, 1330, 1200, 1140, 1160, 1070, 1015, 970, 910, 890, 810, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1 65 (3H, s) 1 70 (3H, s), 1 73 (3H, s), 1 75 (3H, s), 2 29 (3H, s), 2 30 (3H, s), 3 19 (2H, d, J = 7 4 Hz), 3 28 (2H, d, 7 4 Hz), 3 61 (2H, br), 4 37 (1H, dd, J = 11 Hz), 5 11 (1H, t, J = 7 4 Hz), 5 22 (1H, t, J = 7 4 Hz), 5 52 (1H, d, J = 6 3 Hz), 6 53 (1H, s), 6 76 (1H, d, J = 7 8 Hz), 7 08 (1H, s), 7 39 (1H, d, J = 8 4 Hz), EIMS m/z (rel int) 476 (M^+, 6 2), 434 (7 8), 392 (9 0), 336 (9 5), 281 (8 5), 147 (8 0), 135 (4 3), 115 (3 3), 91 (4 5), 69 (19 4), 43 (100)

Isolation of isoneorautenol (**9**) Further chromatography of the group 4 residue on silica gel (30 g), eluting with C_6H_6, and taking 3-ml fractions, led to fractions 29–94 which were combined and evapd to give an impure gummy material (900 mg) Repeated CC of this last on silica gel with *n*-hexane–EtOAc (9 1) produced isoneorautenol (656 mg) recrystallizable from $CHCl_3$–cyclohexane as white flakes, mp 154–155°, UV λ_{max}^{MeOH} nm (log ε) 326 (3 65), 321 (3 72), 313 (3 78), 294 (3 87), 286 (4 00), 281 (3 99), $\lambda_{max}^{MeOH-HCl}$ 325 (370), 312 (3 81), 286 (4 02), 280 (4 01), 258 (3 00), $\lambda_{max}^{MeOH-NaOH}$ 325 (3 72), 312 (384), 291 (4 02), 253 (4 06), $\lambda_{max}^{MeOH-AlCl_3}$ 326 (3 94), 312 (4 03), 286 (4 17), 281 (4 17), $\lambda_{max}^{MeOH-AlCl_3-HCl}$ 325 (3 90), 313 (3 97), 287 (4 13), 281 (4 13), IR ν_{max}^{neat} cm^{-1} 3402, 1620, 1549, 1481, 1377, 1271, 1211, 1155, 1113, 1080, 1034, 958, 843, 736, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1 40 (3H, s, H-5'), 1 42 (3H, s, H-4'), 3 49 (1H, m, H-6ax), 3 41 (1H, t, J = 10 8 Hz, H-6a), 4 21 (1H, dd, J = 4 8, 10 8 Hz, H-6eq), 5 30 (1H, overlapped d, H-11a), 5 45 (1H, d, J = 10 Hz, H-2'), 6.24 (1H, d, J = 10 Hz, H-1'), 6 33 (1H, s, H-10), 6 40 (1H, d, J = 1 5 Hz, H-4), 6 53 (1H, dd, J = 1 5, 8 4 Hz, H-2), 6 84 (1H, s, H-7), 7 37 (1H, d, J = 8 4 Hz, H-1), HRMS m/z (rel int) 322 1212 (M^+, 35 9%, calcd for $C_{20}H_{18}O_4$ 322 1204), EIMS m/z (rel int) 322 (35), 307 (100), 279 (5 2), 185 (13 5), 173 (10 4), 153 (22 8), 145 (6 3), 115 (14 9), 91 (15 3), 77 (16 5), 69 (17 7), 52 (21 6), optical rotation $[\alpha]_D^{25}$ −163 47 (MeOH, c 0 49), CD $[\theta]_{310}$ (+5440), $[\theta]_{285}$ (+3870) (MeOH)

Isolation of erythrabyssin-II (**10**) The combined EtOAc washings from the second column chromatography of the group 4 residue was evapd to give a solid (149 mg) Chromatography of this material on 20 g silica gel using *n*-hexane–EtOAc (17 3), gave erythrabyssin-II (58 mg) It crystallized from $CHCl_3$–cyclohexane as a white powder, mp 157 5–158 5° (lit 152–153°, from cyclohexane) Co-TLC with an authentic sample gave a single spot, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1 75 (3H, s, H-4'), 1 80 (9H, s, H-4', 5'), 3 35 (4H, m, H-1'), 3 48 (1H, m, H-6ax), 3 59 (1H, dd, J = 10 8 Hz, H-6a), 4 21 (1H, dd, J = 4 8, 10 8 Hz H-6eq), 5 34 (4H, m, H-2'), 5 40 (1H, d, J = 6 0 Hz, H-11a), 6 38 (1H, d, J = 8 4 Hz, H-8), 6 41 (1H s, H-2), 6 95 (1H, d, J = 8 4 Hz, H-7), 7 26 (1H, s, H-1), ^{13}C NMR (75 4 MHz, $CDCl_3$) 18 01, 18 07, 23 41, 25 94, 26 02, 29 42, 40 29, 66 75, 78 42, 104 09, 108 34, 110 43, 112 60, 118 95, 121 20, 121 61, 122 12, 122 54, 132 20, 134 99, 135 35, 155 22, 155 86, 156 01, 158 61, HRMS m/z (rel int) 392 19840 (M^+, 59 1, calc for $C_{25}H_{28}O_4$ 392 19860), EIMS m/z (rel int) 392 (60), 336 (23 2), 281 (40 1), 161 (29 2), 147 (42 1), 115 (18 7), 91 (27 6) 69 (53 5), and 41 (100)

Acknowledgements—The authors thank the National Institutes of Health (U S A.) for partial support of this work under grant AI-13155 and one of us (S K O) thanks the Fogarty International Center, NIH, for a fellowship (TW 03859) and the Nigerian Defence Academy, Kaduna, Nigeria, for a leave of absence

REFERENCES

1 Cordell, G A (1981) *The Alkaloids, A Biogenetic Approach*, p 450 Wiley, New York
2 Fomum, Z T, Ayafor, J F and Wandji, J (1987) *J Nat Prod* **50**, 921
3 Morton, J F (1981) *Atlas of Medicinal Plants of Middle America Bahamas to Yucatan*, p 316 Charles C Thoms, Springfield, Il
4 Mitscher, L A, Drake, S, Gollapudi, S R and Okwute S K (1987) *J Nat Prod* **50**, 1025
5 Ayensu, E S. (1978) *Medicinal Plants of West Africa*, p 153 Reference Publications, Algonac, Michigan
6 Mitscher, L A, Gollapudi, S R, Gerlach, D C, Drake, S D, Veliz, E A and Ward, J A (1988) *Phytochemistry* **27**, 381
7 Kamat, V S, Chuo, F V, Kubo, I and Nakanishi, K (1981) *Heterocycles* **15**, 1163
8 Mitscher, L A, Ward, J A, Drake, S and Rao, G S (1984) *Heterocycles* **15**, 1673
9 Ingham, J L and Tahara, S (1985) *Z Naturforsch (C)* **40C**, 482
10 Ingham, J L (1982) in *Phytoalexins* (Bailey, J A, and Mansfield, J W, eds), p 21 Wiley, New York
11 Afzal, M and Al-Oriquat, G (1986) *Heterocycles* **24**, 2911
12. Brown, K S, Cameron, D W and Weiss, U (1969) *Tetrahedron Letters* 471
13 Perrin, D. R, Whittle, C P and Batterham, T J (1972) *Tetrahedron Letters* 1673
14 Brink, A J, Rall, G J H and Engelbrecht, J P (1974) *Phytochemistry* **13**, 1581
15 Pelter, A and Amenechi, P I (1969) *J Chem Soc (C)*, 887