

ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. THE ISOLATION AND STRUCTURAL CHARACTERIZATION OF TWO ADDITIONAL PTEROCARPAN ANTIMICROBIAL AGENTS FROM NIGERIAN ERYTHRINA MILDBRAEDII.

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Abstract - Examination of bioactive mother-liquors from previous large scale experiments on the antimicrobial agents from the Nigerian medicinal plant, Erythrina mildbraedii, led to the isolation and structural characterization of two new pterocarpan, erybraedin D and E. Erybraedin D was initially isolated as its apparently artifactual hemiethyl phthalate ester. Both new pterocarpan possess marginal antimicrobial potency in vitro.

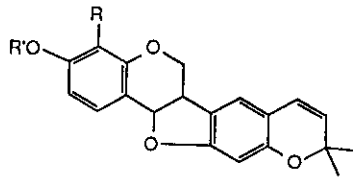
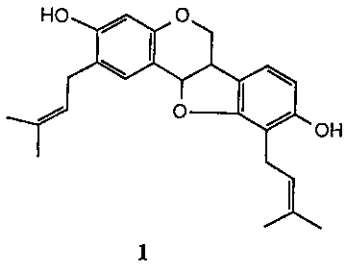
The genus Erythrina contains numerous attractive species distributed in tropical and subtropical habitats.^{1,2} Extracts of the leaves, bark and roots have a significant history of use in indigenous medical practice for the treatment of various diseases including applications likely to include microbial infections.³ In previous studies from this laboratory, we have shown that the roots of many species contain flavanoids, particular pterocarpan, which possess sufficient potency in vitro against human pathogens to rationalize the local popularity of extracts as medicaments.³ Other laboratories have made analogous findings.⁴ In particular, we have recently shown that Erythrina mildbraedii, collected in northern Nigeria, possessed several antimicrobial agents, three new to the literature. These are erythrabysin-II (1), isoneorautenol (2), and erybraedins A (3), B (4), and C (5).⁵

While examining the mother-liquors from the above study, we detected two quite minor antimicrobial agents which were not identical with the above and which proved to be new to the literature. This paper describes their properties and their structural characterization.

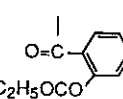
Erybraedin D hemiethyl phthalate (6) was isolated as a gum: ir $\text{CHCl}_3/\text{max cm}^{-1}$: 2960, 2800, 1710, 1601, 1470, 1435, 1360, 1280, 1260, 1150, 1110, 1065; $^1\text{H nmr}$ (300 MHz, CDCl_3) δ 1.38 (3H, t, J=7.1 Hz, CH_2CH_3), 1.42 (6H, s, H-3",4"), 1.73 (3H, s, H-4'), 1.81 (3H, s, H-5'), 3.42 (2H, d, J = 7.2 Hz, H-1'), 3.49 (1H, m, H-6a), 3.61 (1H, dd, J=10.8, 6.9 Hz, H-6ax), 4.28 (1H, dd, J=4.8, 10.8 Hz, H-6eq), 4.38 (2H, q, J=7.1 Hz, H- CH_2Me), 5.22 (1H, t, J=7.2 Hz, H-2'), 5.47 (1H, d, J=10 Hz, H-2"), 5.48 (1H, d, J=6.9 Hz, H-11a), 6.27 (1H, d, J= 10 Hz, H-1"), 6.34 (1H, s, H-10), 6.56 (1H, d, J=8.4 Hz, H-2), 6.85 (1H, s, H-7) 7.27 (1H, d, J=8.4 Hz, H-1), 7.54 (2H, m, Ar-H), 7.73 (2H, m, Ar-H); EIMS m/z (rel. int., %): 390 (59.2), 375 (100), 319 (33.9), 185

(14.1), 160 (35.4), 115 (15.2), 91 (10.9, 69 (21.6), 55 (18.2), 41 (39.4). Several of the pmr signals have not been seen for any pterocarpan known to us. The indicated OEt group (δ 1.38 and 4.38) was accompanied by an ir band at 1710 cm^{-1} suggesting an ethyl ester. Such esters are very rare in natural products. In addition, four aromatic hydrogens segregated into two groups of multiplets were seen at lower field (δ 7.54 and 7.78) than is seen with pterocarpan. It was surmised that these signals stemmed from a hemiethyl phthaloyl ester moiety arising most likely artifactually from reaction of erybraedin D with a plasticizer present in plastic containers used to transport plant material in the field and extracts from the laboratory to this country. There is no direct evidence supporting this hypothesis but this explanation is plausible and agrees with the known history of the extract. It is much less likely that the hemiethyl phthalate is of entirely natural occurrence. Why erybraedin D (7) occurs apparently only in the form of ester 6 is hard to understand. Thus, the sample was subjected to solvolysis with sodium ethoxide in MeOH at room temperature for 3 h. The resulting erybraedin D (7) now gave spectra completely characteristic for a pterocarpan (uv λ MeOH/max nm (log ϵ): 313 (3.69), 285 (3.72), 231 (4.31), 214 (4.50); λ MeOH-HCl/max: 314(3.64), 284 (3.68), 233 (4.26), 214 (4.49); λ MeOH-NaOH/max: 321 (3.67), 287 (3.76), 256 (3.84); λ MeOH-AlCl₃/max: 314 (3.66), 283 (3.68), 238 (4.15), 214 (4.49); λ MeOH-AlCl₃-HCl/max: 314 (3.69), 283 (3.72), 236 (4.20), 213 (4.47); ir CHCl₃/max cm^{-1} : 3400, 2960, 2920, 1601, 1470, 1430, 1370, 1260, 1250, 1150, 1110, 1070, 950; ¹H nmr (CDCl₃) δ 1.41 (6H, s, H-3",4"), 1.73 (3H, s, H-4'), 1.80 (3H, s, H-5'), 3.40 (2H, d, J=7.0 Hz, H-1'), 3.48 (1H, m, H-6a), 3.58 (1H, dd, J=10.8, 7.5 Hz, H-6ax), 4.27 (1H, dd, J=4.5, 10.8 Hz, H-6eq), 5.23 (1H, t, J=7.0 Hz, H-2'), 5.46 (1H, d, J=7.5 Hz, H-2"), 5.48 (1H, d, J=7.5 Hz, H-11a), 6.27 (1H, d, J=9.9 Hz, H-1"), 6.34 (1H, s, H-10), 6.56 (1H, d, J=8.1 Hz, H-2), 6.85 (1H, s, H-7), 7.27 (1H, d, J=8.1 Hz, H-1); HRMS m/z (rel. int., %): 390.1827 (M⁺, 44.7, calc. for C₂₅H₂₆O₄=390.1829): EIMS m/z (rel. int., %) 390 (45), 375 (61.5), 319 (24.0), 160 (28.8), 149 (33.5), 69 (32.2), 55 (47.1), 44 (100.0); optical rotation [α]_D²⁵-67° (c= 0.23, MeOH) and the properties led to assignment of novel structure 7. In essence, this compound is 4-prenylisoneorautenol. Isoneorautenol itself had been isolated by us previously from this plant.

The pterocarpan nucleus of erybraedin D was clear from the classical ABCM pattern (δ 3.48, 3.58, 4.27 and 5.48 with the appropriate couplings). The molecular formula (C₂₅H₂₆O₄) allowed for a prenyl group and a dimethylchromene moiety. These were confirmed in the pmr (prenyl = δ 1.73, 1.80, 3.40 and 5.23; dimethylchromene = δ 1.41, 5.46 and 6.27). These were assigned to be at carbons 3,4 and 8,9 based upon the classical H-1,H-2 ortho coupled pair (δ 7.27 and 6.56) and H-7,H-10 singlets (δ 6.85 and 6.34). These suggested locations were confirmed by the pmr of the

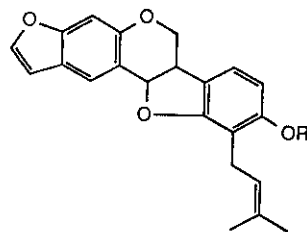
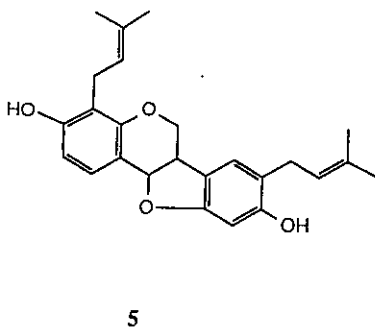
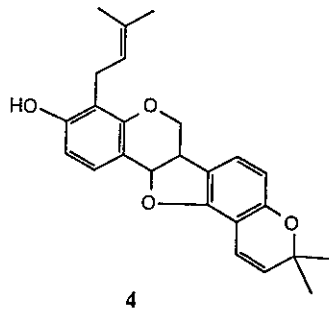
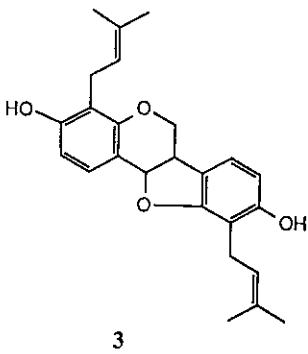


2 R = R' = H

6 R = Prenyl, R' = 

7 R = Prenyl, R' = H

8 R = Prenyl, R' = Ac



9 R = H

10 R = Ac

acetate ester (8): ir $\text{CHCl}_3/\text{max cm}^{-1}$: 2940, 2920, 2840, 1760, 1620, 1595, 1480, 1434, 1365, 1260, 1205, 1155, 1115, 1075, 1050, 1010, 885, 760; $^1\text{H nmr (CDCl}_3)$: δ 1.41 (6H, s, H-3",4"), 1.65 (3H, s, H-4'), 1.73 (3H, s, H-5'), 2.31 (3H, s, H-COCH₃), 3.28 (2H, d, J=6.0 Hz, H-1'), 3.49 (1H, m, H-6a), 3.61 (1H, dd, J=10.8, 6.9 Hz, H-6ax), 4.30 (1H, dd, J=4.8; 10.8 Hz, H-6eq), 5.10 (1H, t, J=7.4 Hz, H-2'), 5.46 (1H, d, J=10 Hz, H-2''), 5.47 (1H, d, J=6.9 Hz, H-11a), 6.26 (1H, d, J= 10 Hz, H-1''), 6.33 (1H, s, H-10), 6.75 (1H, d, J= 7.8 Hz, H-2) 6.85 (1 H, s, H-7), 7.40 (1H, d, J=7.8 Hz, H-1); HRMS m/z (rel. int., %): 432.1934 (M^+ 4.1, Calc. for $\text{C}_{27}\text{H}_{28}\text{O}_5 = 432.1935$); EIMS m/z (rel. int., %): 432 (M^+ , 28.9), 417 (32.1), 375 (23.8), and 319 (9.4) in which the H-1,H-2 AB pair had undergone a substantial downfield shift (H-1 $\Delta\delta$ =0.53; H-2 $\Delta\delta$ =0.19). Thus the prenyl group must be at C-4 as C-3 has a free phenolic moiety in erybraedin D. The dimethylchromene ring, by difference, is at C-8,9.

Erybraedin E (9) has 3 carbons fewer than erybraedin D (uv $\lambda_{\text{MeOH}/\text{max nm (log } \epsilon)}$): 301 (4.07), 295 (4.09), 286 (4.00), 279 (3.86), 252 (4.31), 244 (4.37); $\lambda_{\text{MeOH-HCl}/\text{max}$: 300 (4.06), 295 (4.08), 285 (4.00), 279 (3.86), 253 (4.30), 244 (4.36), $\lambda_{\text{MeOH-NaOH}/\text{max}$: 295 (4.19), 253 (4.46), 246 (4.41); $\lambda_{\text{MeOH-AlCl}_3}/\text{max}$: 296 (4.17), 286 (4.12), 279 (4.01), 252 (4.37), 244 (4.42); $\lambda_{\text{MeOH-AlCl}_3\text{-HCl}/\text{max}$: 296 (4.14), 286 (4.10), 279 (3.99), 252 (4.35), 244 (4.40); ir $\text{CHCl}_3/\text{max cm}^{-1}$: 3420, 2960, 2901, 1601, 1530, 1440, 1375, 1360, 1280, 1255, 1230, 1150, 1065, 1020; $^1\text{H nmr (CDCl}_3)$: δ 1.73 (3H, s, H-4'), 1.81 (3H, s, H-5'), 3.42 (2H, d, J= 6.9 Hz, H-1'), 3.66 (2H, m, H-6eq,H-6a), 4.34 (1H, m, H-6eq), 5.23 (1H, t, J= 6.9 Hz, H-2'), 5.44 (1H, s, OH), 5.57 (1H, d, J= 5.7 Hz, H-11a), 6.58 (1H, d, J= 8.4 Hz, H-8), 6.69 (1H, d, J = 2.4 Hz., H-3''), 6.99 (1H, s, H-4), 7.31 (1H, d, J= 8.4 Hz, H-7), 7.41 (1H, s, H-1), 7.53 (1H, d, J = 2.4 Hz., H-2''); HRMS m/z (rel. int. %): 348.13595 (M^+ , 49.7, Calc. for $\text{C}_{22}\text{H}_{20}\text{O}_4=348.13604$); EIMS m/z (rel. int., %) 348 (50%), 292 (38.7), 291 (22.9), 158 (32.6), 71 (45.7), 57 (79.5), 44 (97.2), 43 (100.0); Optical rotation $[\alpha]_D^{25} - 104^\circ$ (c = 0.29, MeOH). In addition to the ABCM signals in the pmr which identify the compound as a pterocarpan, the signals at δ 6.69 and 7.53 which are mutually coupled by 2.4Hz clearly indicate a fused benzofuran moiety. The signals at δ 1.73, 1.81, 3.42 and 5.23 are attributable to a prenyl moiety. The signal at δ 7.41 resonates where H-1 of pterocarpan is found and, being an apparent singlet, indicates that C-2 is substituted. The ortho coupled AB pair at δ 6.58 and 7.31 are assigned to H-7 and H-8. This leaves open only the question of the location of the prenyl moiety and the furan ring. This was quickly settled by preparation of a monoacetyl ester (10) (ir $\text{CHCl}_3/\text{max cm}^{-1}$: 2985, 2920, 1760, 1620, 1595, 1535, 1450, 1430, 1360, 1285, 1200, 1150, 1065, 1020, 890, 880, 760, 730; $^1\text{H nmr (CDCl}_3)$: δ 1.65 (3H, s, H-4'), 1.73 (3H, s, H-5'), 2.31 (3H, s, OCOCH₃), 3.27 (2H, d, J= 6.9 Hz, H-1'), 3.60 (2H, m, H-6eq,H-6a), 4.38 (1H, dd, J=4.8, 10.8 Hz, H-6eq), 5.11 (1H, t, J=

7.4 Hz, H-2'), 5.59 (1H, d, J= 6.3 Hz, H-11a), 6.69 (1H, d, J= 2.4 Hz, H-3"), 6.79 (1H, d, J= 7.8 Hz, H-8), 7.00 (1H, s, H-4), 7.42 (1H, s, H-1), 7.43 (1H, d, J= 7.8 Hz, H-7), 7.54 (1H, d, J = 2.4 Hz, H-2"); HRMS m/z (rel. int. %): 390.1469 (M⁺, 39.5; Calc. for C₂₄H₂₂O₅: 390.1466), EIMS m/z (rel. int. %) 390 (40), 348 (37.2), 292 (42.2), 158 (18.4), 147 (13.8) and 43 (100.0.) in the usual fashion and examination of its pmr spectrum. By structural necessity only the hydrogen ortho to the hydroxyl flanked by a prenyl group could undergo the usual downfield shift in the pmr spectrum. It will be noted that the doublet at δ 6.58, assigned to H-8, shifted to 6.79 ($\Delta\delta$ 0.21) and its companion doublet at δ 7.31 shifted to 7.43 ($\Delta\delta$ =0.11). At the same time the prenyl CH₂ signal shifted from δ 3.42 to 3.60 ($\Delta\delta$ =0.18) while the apparent singlets at δ 7.41 and δ 6.99 were virtually unaffected. This leaves structure 9 as the only structural expression consistent with these findings. In effect erybraedin is C-10-prenylneodunol. Neodunol has not previously been found in this plant.⁶

The in vitro antimicrobial spectra of erybraedin D and E are compared with the other E. mildbraedii substances and streptomycin SO₄ are given in the table.

Table. In vitro (agar-streak dilution) antibacterial activity of Erythrina mildbraedii products.

Substance	Microorganism*						
	1	2	3	4	5	6	7
Erythrabyscin-II(1)	3.12	i	i	i	0.78	i	i
Isonoeorautenol(2)	25.0	i	i	i	25.0	i	i
Erybraedin A(3)	12.5	i	i	i	6.25	i	i
Erybraedin B(4)	12.5	i	i	i	12.5	i	i
Erybraedin C(5)	12.5	i	i	i	12.5	i	i
Erybraedin D(7)	100	i	i	i	25.0	i	i
Erybraedin E(9)	25.0	i	i	i	i	i	i
Streptomycin SO ₄	5.0	5.0	50.0	2.5	1.25	i	i

*Microorganisms: (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.

Of this group of pterocarpans, erythrabyscin-II (1) is dramatically the more potent, being

under these conditions more potent even than streptomycin, although at the same time being considerably narrower in spectrum. Interestingly, erybraedins A (3) and C (5), which are isomeric with 1 with respect to the placement of their prenyl groups, are much less potent. The importance of having two free phenolic hydroxyl groups is also clear. Erybraedins B (4), D (7) and E (9) as well as isoneorautenol (2) have only one free hydroxyl and all of these are comparatively weakly active compounds. In our hands, then, erythrabysin-II and erycristagallin (10)⁷ have proven to be the most active antimicrobial pterocarpan from Erythrina sp. in vitro and their very close structural relationship is apparent. The possession of a pterocarpan vs pterocarpene ring system appears to be much less critical than the possession of two free phenolic hydroxyl groups each flanked on the proper side by a prenyl moiety.

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