ANTIMICROBIAL AGENTS FROM AN EAST AFRICAN MEDICINAL PLANT ERYTHRINA ABYSSINICA

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Abstract: Two pterocarpans, five flavanones and a chalcone, all new natural products have been isolated from an East African folk medicinal plant. They exhibit antimicrobial activity or inhibition of platelet aggregation.

The roots of the East African medicinal plant *Erythrina abyssinica* (Leguminosae) are widely used in various folk remedies, such as malaria, syphilis.[1] Since the 60% aqueous methanol extract of the roots was found to possess strong activity against fungi and gram positive bacteria,[2] the extract was studied in greater detail. Separation of the root extract into hexane, ether and water-soluble fractions indicated the ether extract to be the active fraction. The ether-soluble portion was therefore separated into five fractions by column chromatography using a gradient of chloroform/methanol. Each fraction was further separated by flash chromatography[3] with hexane/ethyl acetate gradient, and respective eluates were further purified by reverse-phase HPLC, -Bondapak, MeOH : H_2O (65 : 35). This gave two new pterocarpans, erythrabyssin-I (1)and erythrabyssin-II (2)in addition to the well known phytoalexins phaseollin (3)[4] and phaseollidin (4)[5], five new flavanones, abyssinones I-V (5-9) and a chalcone, abyssinone-VI (10). Except for 7, all compounds exhibited either antimicrobial activity or inhibition of platelet aggregation induced by ADP, etc.

In the following text figures, we summarize the data which has led to the structure determination of these plant phenolics. The NMR spectra were measured with Brucker WP-80, WH-250 and the CD spectra with Jasco J-40 instruments.

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Structure of erythrabyssin-I (1)

a) Pterocarpan skeleton : From UV, ^{1}H-NMR[6] and ^{13}C-NMR.[7]

b) Aromatic OMe and isopentenyl groups : From ^{1}H-NMR.

c) Aliphatic OH: Treatment of 1 with CH_2N_2 gave monomethyl ether which still had OH (IR). Thus 1 contains phenolic OH, an aromatic OMe and an aliphatic OH. The two doublets at 3.98 and 4.23 ppm (J=11.5 Hz) and singlet at 5.25 showed that the aliphatic OH is at C-6a.

d) Aromatic substitution pattern : ^{1}H-NMR, $J_{1,2}$=8.3; $J_{2,4}$=2.4 and $J_{7,8}$=8.3 Hz.

e) OMe is at C-9 and OH's are at C-6a and C-3 : From the magnitude of pyridine induced shifts Δpy[8] (see 1). In monomethyl-1, Δpy values are still large for 6-H and 11a-H but are small for other protons.

f) Ring juncture is cis : From the large Δpy value for 11a-H.

g) Absolute configuration : The sign of the Cotton effect at 286 nm is the same as those of phaseollidin (4)(i.e., 6a-desoxy-9-desmethyl-1), ΔE_{286} +3.45, and phaseollin (3), ΔE_{286} +6.43, ΔE_{316} +2.87, which have known configurations.[5] An isomeric compound glyciollin-IV, with the same absolute configuration also exhibits a positive CD maximum at 291 nm.[9]

Structure of erythrabyssin-II (2)

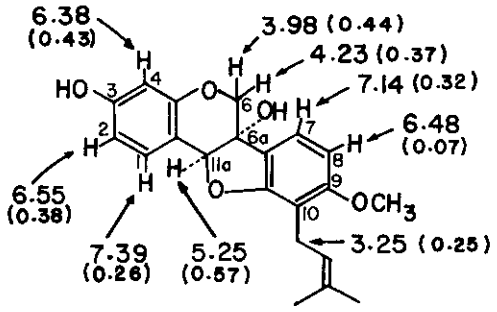
a) Pterocarpan skeleton and two isopentenyl groups : From UV, ^{1}H-NMR, and ^{13}C-NMR. The ^{1}H-NMR signals of 6/6a/11a-H's [4.22(dd,J=4.0,10.3), 3.61(dd,J=10.3,11.4)/ 3.50(ddd,J=4.0, 6.6,11.4)/5.45(d,6.6 Hz)] were almost identical with those of phaseollidin(4).[5]

b) Two phenolic OH's : Formation of diacetate.

c) Substituents on ring D : The ^{1}H-NMR peaks and Δpy values of 7 and 8-H, ($J_{7,8}$=8.1 Hz) were again very similar to those of phaseollidin (4).

d) Substituents on ring A : The 7.26 ppm singlet was assigned to 1-H because of its low chemical shift (compare with 1-H in 1); furthermore, it must be para to the 6.40 ppm singlet (4-H, compare with 4-H in 1) because of absence of coupling. The magnitudes of Δpy show that the ring A phenolic OH (as well as ring D phenolic OH) is flanked by isopentenyl and aromatic H. In the diacetate, the 1-H and 4-H signals are shifted downfield by 0.10 and 0.25 ppm, respectively. Hence it is the 4-H, and not 1-H, which is adjacent to the OH.

e) Absolute configuration : From the positive 289 nm CD extremum, ΔE+6.38.

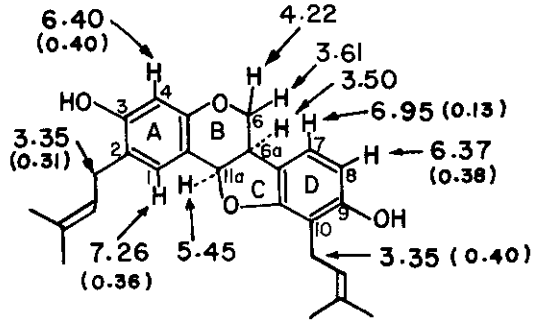


1 erythrabyssin-I

$C_{21}H_{22}O_5$ (M^+ 354)

UV (MeOH): 215 (28700), 280 (7500), 286 sh

CD (MeOH): $\Delta\epsilon_{286}$ +6.53

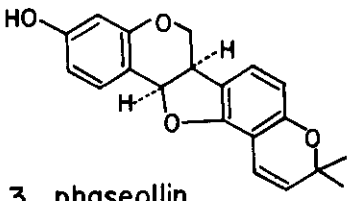


2 erythrabyssin-II

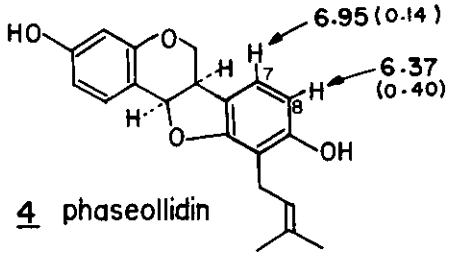
$C_{25}H_{28}O_4$ (M^+ 392)

UV (MeOH): 220 (18600), 287 (8130)

CD (MeOH): $\Delta\epsilon_{289}$ +6.38



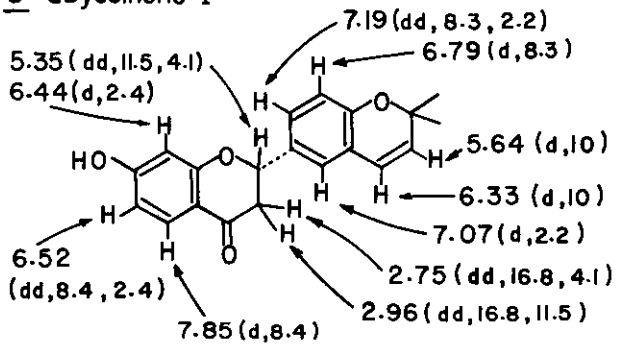
3 phaseollin



4 phaseollidin

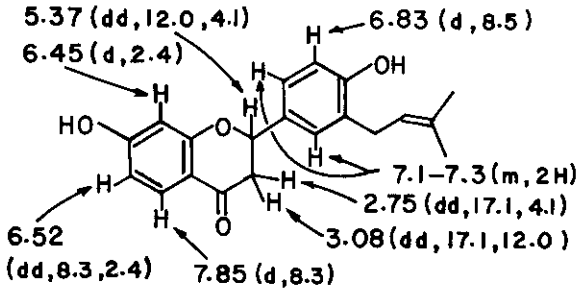
(numerals in paranthesis denote Δpy values[8])

5 abyssinone-I



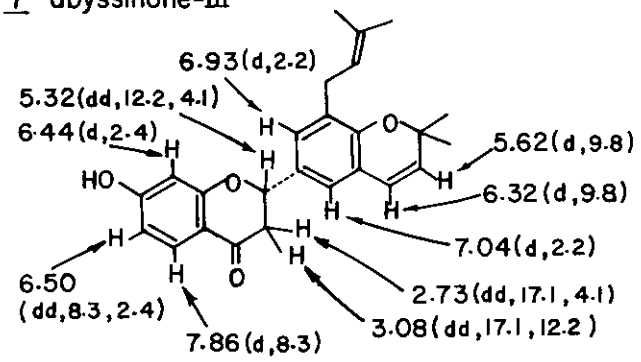
$C_{20}H_{18}O_4$ (M^+322)
IR($CHCl_3$) : 3350, 1680
UV(MeOH) : 275(12600), 310(7600)
(MeOH + NaOH) : 335(21000)
CD(MeOH) : 332($\Delta\epsilon$ +2.54), 303($\Delta\epsilon$ -6.1)

6 abyssinone-II



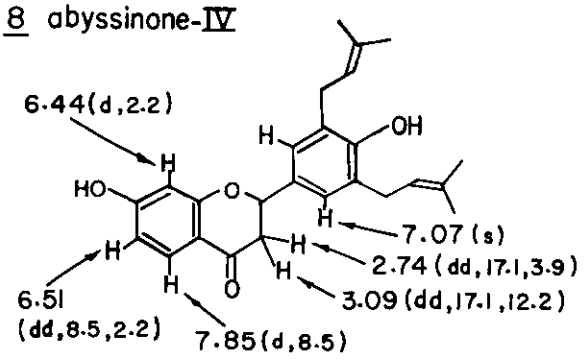
$C_{20}H_{20}O_4$ (M^+324)
IR($CHCl_3$) : 3300, 1680
UV(MeOH) : 276(12750), 310(6400)
(MeOH + NaOH) : 335(22000)

7 abyssinone-III



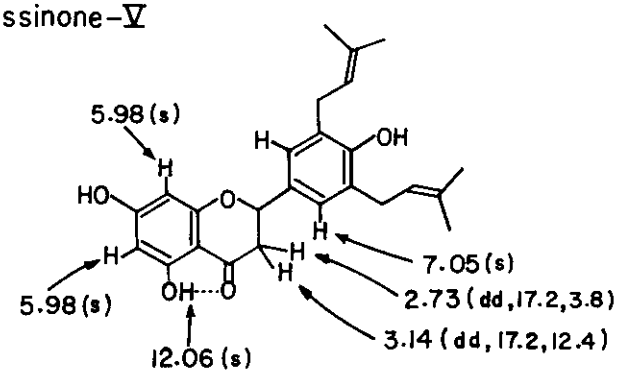
$C_{25}H_{26}O_4$ (M^+390)
IR(KBr) :3400,1680
UV(MeOH) :276(12500),312(6800)
(MeOH + NaOH) : 333(16500)
CD(MeOH) : 330($\Delta\epsilon$ + 0.88),303($\Delta\epsilon$ -2.64)

8 abyssinone-IV



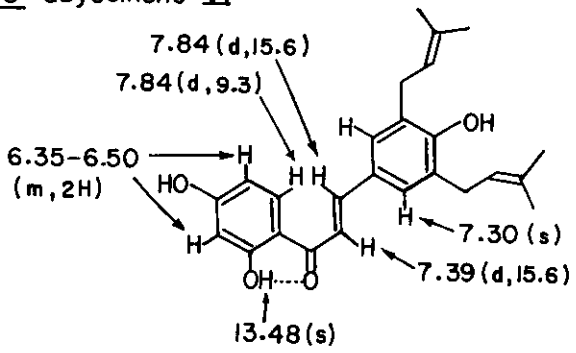
$C_{25}H_{28}O_4$ (M^+392)
IR($CHCl_3$) : 3400,1680
UV(MeOH) : 275(10000),312(60000)

9 abyssinone-Ⅴ



$C_{25}H_{28}O_5$ (M^+408)
IR(KBr) :3400(br.),1640,1600
UV(MeOH) : 288(16700),325 sh(5560)
(MeOH + NaOH) : 326 (34200)

10 abyssinone-Ⅵ



$C_{25}H_{28}O_4$ (M^+392)
IR(KBr) : 3400,1635,1605
UV(MeOH) :375(18700)
(MeOH + NaOH) : 462(22000)

Structures of abyssinones I-VI (5-10)

These structures were arrived by the analysis of their UV, IR, and ^{1}H-NMR spectra. The absolute configurations of 5 and 7 are based on CD curves.[10] Compounds 6, 8 and 9 were found to be optically inactive. The red-shifted λ_{max} of 9 as compared to other flavanones (5-8) and its 12.06 ppm ^{1}H-NMR signal are due to the chelated 5-OH. The chalcone abyssinone-VI (10) is a new natural product; however, its synthesis and its antipeptic activity are reported in a patent.[11]

Biological activity of compounds 1-10

The antimicrobial activity of erythrabyssin-I and other related compounds are listed in Table 1. The antiyeast and antifungal activities of erythrabyssin-I and phaseollin are noteworthy.

Inhibition of rabbit platelet aggregation induced by ADP, collagen and sodium arachidonate was also tested for the various phenolics isolated from *E. abyssinica*. The 50% inhibition concentrations (IC_{50}) which were obtained *in vitro* according to the method of Born and Cross[12] are listed in Table 2. In this test the chalcone 10 exhibited strong inhibitory activity against rabbit platelet aggregation.

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REFERENCES

1. J.O. Kokwaro, Medicinal Plants of East Africa, East African Literature Bureau, Nairobi, 1976.
2. M. Taniguchi, A. Chapya, I. Kubo and K. Nakanishi, Chem. Pharm. Bull. 1978, 26, 2910.
3. W.C. Still, M. Khan, and A. Mitra, J.Org.Chem., 1978, 43, 2923.
4. D.R. Perrin, Tetrhedron Lett., 1964, 29.
5. D.R. Perrin, C.P. Whittle and T.J. Batterham, Tetrahedron Lett., 1972, 1673.
6. K.G.R. Pachler and W.G.E. Underwood, Tetrahedron, 1967, 23, 1817.
7. A.A. Chalmers, G.J.H. Rall and M.E. Oberholzer, Tetrahedron, 1977, 33, 1735.
8. The Δpy value denotes the downfield shift observed in pyridine-d_5 as compared to $CDCl_3$; Ref. P.V. Demarco, E. Farkas, D. Doddrell, B.L. Mylari and E. Wenkert, J.Am.Chem.Soc., 1968, 90, 5480.
9. R.L. Lyne and L.J. Mulherin, Tetrahedron Lett., 1978, 3127.

Table 1 Antimicrobial activity (MIC. μg/ml) of compounds 1 - 10

Microorganisms	1	2	3	4	5	6	8	9
Bacteria								
Staphylococcus aureus	12.5	3.13	12.5	50	25	50	25	50
Bacillus subtilis	6.25	3.13	6.25	25	25	50	12.5	25
Micrococcus lysodeikticus	-	3.13	-	-	-	25	-	12.5
Yeast								
Saccharomyces cerevisiae	50	>100	25	>100	100	100	>100	>100
Candida utilis	50	>100	50	>100	100	100	>100	>100
Fungus								
Sclerotinia libertiana	6.25	>100	12.5	>50	12.5	12.5	>100	>100
Mucor mucedo	25	>100	12.5	>100	50	50	>100	>100
Rhizopus chinensis	>100	>100	12.5	>100	>100	100	>100	>100

a) - indicates not tested. b) 7 and 10 are not active at 100μg/ml.

Table-2 IC_{50} (μg/ml) of compounds 1 - 10 against rabbit platelet aggregation *(in vitro)*.

Aggregation induced by	1	2	3	4	6	10
ADP (10μM)	-	23	30	30	No inhibition	8.6
Collagen (38μg/ml)	74 (slight inhibition)	9	36	23	3.5	5.4
Arachidonic acid (0.57 mM)	74	34	80	34	74 (40% inhibition)	37

a) Compounds 5, 7, 8 and 9 have not been tested.

REFERENCES (Con't)

10. W. Gaffield, Tetrahedron, 1970, 26, 4093.

11. K. Kyogoku, K. Hatayama, S. Yokomori, T. Seki, Japan Kokai, 74126654; Chem. Abstr., 83, P 78864t

12. G.V.R. Born and M.J. Cross, J. Physiol., 1963, 168, 178.

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