ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. ERYCRISTAGALLIN, A NEW PTEROCARPENE FROM THE ROOTS OF THE BOLIVIAN CORAL TREE, *ERYTHRINA CRISTA-GALLI*

Lester A. Mitscher,* Jeffrey A. Ward, Steven Drake and Gollapudi S. Rao
Department of Medicinal Chemistry, Lawrence, Kansas 66045 USA

Abstract—Bioassay directed fractionation of extracts of the roots of *Erythrina crista-galli* produced the new pterocarpene, erycristagallin (**1**) as the main antimicrobial constituent. Structural assignment is based upon degradation and spectroscopic studies.

The roots and to a lesser extent the bark of the Bolivian coral tree, *Erythrina crista-galli* (Leguminosae) (*ceibo* in Spanish and *chilichi* in Quechua) were collected near Pairumani, Departamento de Cochabamba, Bolivia, in January, 1983. Ethanolic extracts were active *in vitro* against *Staphylococcus aureus* and *Mycobacterium smegmatis*. A total of 2.5 kg of air dried milled roots produced 105 g of active residue after exhaustive Soxhlet extraction with ethanol and evaporation. Bulk fractionation by mass transfer methods[1] produced 16.5 g of polar lipids containing all of the activity. Chromatography over silica gel with CH_2Cl_2/MeOH mixtures followed by flash chromatography of the active band (SiO_2;C_6H_6:MeOH-95:5) gave 245 mg of pure, bioactive erycristagallin (**1**), mp 179-180°C; ir ($CHCl_3$) 3630ν, 3470, 1660, 1620, 1590, 1500 and 1220; uv λmax(MeOH) 214nm (ε 29,270), 244 (15,300), 251 (15,080), 291 (inf. 6,650), and 330 (sh. 22,000), 339 (24,800), 356 (22,000); λmax(MeOH, NaOH) 306nm (ε 6,600), 354 (29,250) and 370 (25,725); ^{1}H-nmr ($CDCl_3$) 1.79δ (9H, s, 3xCH_3), 1.85 (3H, s, CH_3), 3.35 (2H, d, J=8Hz, CH_2), 3.70 (2H, d, J=8Hz, CH_2), 5.18 (2H, s, exch., OH), 5.35 (2H, m, CH=), 5.46 (2H, s, OCH_2), 6.41 (1H, s, ArH), 6.68 (1H, d, J=10Hz, ArH), 7.00 (1H, d, J=10Hz, ArH), 7.21 (1H, s, ArH); ^{13}C-nmr (^{6}d-DMSO, decoupled) 17.57ppm, 17.62, 25.59, 27.13, 64.86, 103.36, 105.63, 107.95, 114.44, 112.17, 117.70, 120.09, 120.86, 122.19, 122.83, 130.89, 131.70, 146.19, 152.41, 152.49, 154.52, and 156.02; in the coupled spectrum, 64.86ppm was a triplet; ms m/z390 ($M^+=C_{25}H_{26}O_4$, 373 (42), 335 (30), 279 (66), 69 (31), 55 (23). Two phenolic OH groups were detected by the formation of a diacetate in 92% yield on stirring with Ac_2O/NaOAc in benzene at 70°C for 4 h under argon; ^{1}H-nmr 2.21δ (3H, s, CH_3CO), 2.25 (3H, s, CH_3CO), 6.81 (1H, d, J=10Hz, ArH), 6.55 (1H, s, ArH), other signals essentially unchanged, M^+ = m/z 474. Treatment of erycristagallin with lead tetraacetate in HOAc produced an immediate scarlet red color which faded to bright orange on standing. A Gibb's test was negative.

The properties of erycristagallin indicate a biprenylated pterocarp-6a-ene (appropriate nmr signals and M^+-55 and -111). In particular, the uv spectrum was a close match for that of anhydroglycinol (2) from Tetragonolobus maritimus.[2] Placement of the phenolic hydroxyls in the common C-3 and -9 positions[3] was based upon biogenetic analogy, ease of extended quinone formation with lead tetra-acetate, and the lack of hydrogen atoms para to phenolic OH groups (negative Gibb's test). One of the prenyl groups was placed at C-10 by consideration of the common biogenetic pattern (one especially notes that the structures of the phytoalexins phaseollidin and cristacarpin from E. crista-galli[4] and sandwicin and sandwicarpin from E. sandwicensis[5] all contain C-10 prenyl groups) and one of the aromatic rings had a coupled AB pair of ortho hydrogens. The other prenyl group was placed at C-2 because of a likely biogenetic relationship to erythrabyssin-II (3), an antimicrobial pterocarpan isolated from the East African medicinal plant Erythrina abyssinica,[6] and the observation that the other aromatic ring has two aromatic hydrogens with no observable coupling at 90 MHz. One notes also that each phenolic OH has one ortho aromatic hydrogen atom which shifts downfield upon acetylation.[7] These considerations leave no doubt that erycristagallin is 3,9-dihydroxy-2,10-diprenylpterocarp-6a-ene (1) or the biogenetically less likely isomer 4. Final distinction between these possibilities will be made by synthesis. Pure 1 is active against Staphylococcus aureus and Mycobacterium smegmatis at 1.56 μg/ml in agar dilution streak assays.

The non-nitrogenous constituents of Erythrina species have been relatively neglected. The biological activity of leaf[4-6,10] and now root constituents suggest that this be remedied. Given the biosynthetic origin of pterocarpins from isoflavanoids, it is mildly surprising that previous studies of such constituents in E. crista-galli[8] failed to turn up any prenylated examples whereas the related species E. variegata from India contained such compounds.[9] It is also interesting that the antimicrobial flavanones from Cameroonian Erythrina sigmoidea, sigmoidins A and B,[11] are structurally rather different than the putative flavanone precursors of erycristagallin and erythrabyssin-II.

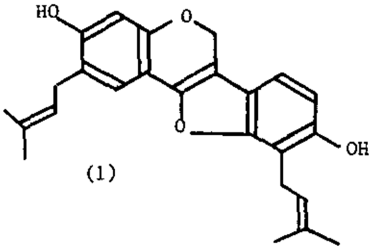
ACKNOWLEDGEMENTS

The authors are grateful to Ms. Martha Perez for assistance in collecting; to Dr. Alvaro Padilla, Dr. Walter de Groot, Ing. Simon Ortega Salamanca and Ing. Ruben M. Ibanez of the Department of Chemistry, Universidad de San Simon, Cochabamba; to Ing. Luis Arteaga and Ing. Oscar Rojas of CORDECO; and to the Jardin Botanico de Martin Cardenas, Cochabamba, for use of facilities and to the NIH, USA, for grant support under AI-13155.

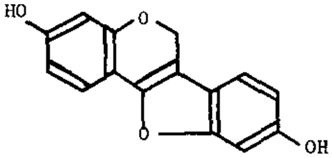
REFERENCES

1. L. A. Mitscher, "Plant-Derived Antibiotics" in Isolation, Separation and Purification of Antibiotics, G. Wagman and G. Weinstein, Eds., Elsevier, Amsterdam, 1977.

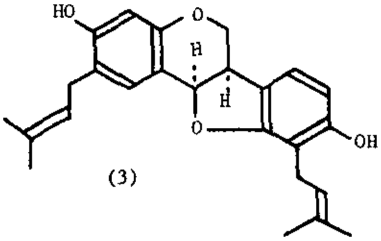
2. J. L. Ingham and P. M. Dewick, Phytochem. 1978, 17, 535.
3. J. L. Ingham, "Phytoalexins from the Leguminosae" in Phytoalexins, J. A. Bailey and J. W. Mansfield, Eds., Wiley and Sons, New York, 1982.
4. J. L. Ingham and K. R. Markham, Phytochem. 1980, 19, 1203.
5. J. L. Ingham, Z. Naturforsch. 1980, 35c, 384.
6. V. S. Kamat, F. Y. Chuo, I. Kubo and K. Nakanishi, Heterocycles 1981, 15, 1163.
7. T. Saitoh, T. Kinoshita and S. Shibata, Chem. Pharm. Bull. 1976, 24, 1242.
8. H. Imamura, M. Ito and H. Ohashi, Res. Bull. Fac. Agr. Gifu Univ. 1981, 45, 77.
9. V. H.Deshpande, A. D. Pendse and R. Pendse, Indian J. Chem. 1977, 15B, 205.
10. S. A. Ross, S. E. Magalla, O. W. Bishay and A. H. Awad, Fitoterapia 1980, 51, 303.
11. Z. T. Fomum, J. F. Ayafor and J. T. Mbafor, Tetrahedron Letters 1983, 24, 4127.



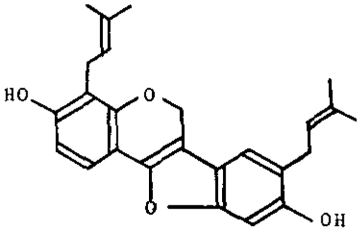
(1)



(2)



(3)



(4)

Received, 5th March, 1984