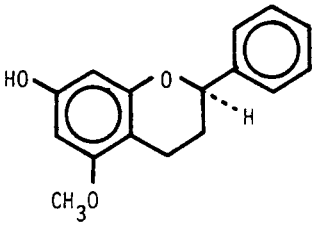
ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. DRAGON'S BLOOD RESIN

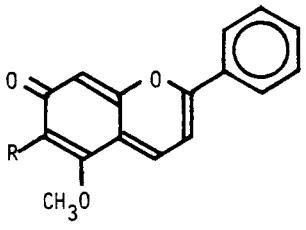
G. S. R. Rao, M. A. Gerhart,[1] R. T. Lee, III,[2] L. A. Mitscher* and S. Drake
Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

The luridly named dragon's blood resin is principly derived from the scales of fruit belonging to *Daemonorops draco* Blume (fam. Palmaceae). It has been used medicinally as a stimulant and astringent, especially in dentifrices and mouth washes. It has also found commercial use in varnishes and lacquers, imparting a mahogany stain to wood, and as a protectant for zinc in photoengraving and etching (1,2).

Previous chemical studies have dealt with the separation, structure determination (3,4), and synthesis of its pigments (5). The early work has been succinctly reviewed (2). Our interest in this material was aroused when extracts of the commercial resin showed reproducible activity *in vitro* against *Staphylococcus aureus* and *Mycobacterium smegmatis* (6). Bioassay-directed fractionation was, therefore, undertaken.



(1)

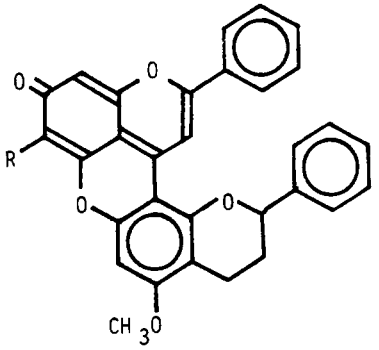


(2), R = CH_3

(5), R = H

RESULTS AND DISCUSSION

The deeply red colored resin, as obtained, was only partially soluble when treated successively with chloroform and then methanol. Removal of solvents gave a highly colored active material which was, in turn, taken up partially in acetone. Tlc examination revealed ten spots, two of which were active by bioautography against *Staph. aureus*. Silica gel chromatography resolved the mixture producing pure samples of the known substances 5-methoxy-7-hydroxyflavan (**1**), dracorhodin (**2**), dracorubin (**3**) and nordracorubin (**4**).



(3), R = CH_3

(4), R = H

Rechromatography of selected fractions on a non-commercial medium-pressure liquid chromatograph (7) produced pure nordracorhodin (**5**) and three apparently homogeneous but poorly soluble, unstable, and difficulty characterizable blue pigments. Test-

[1]National Science Foundation, Undergraduate Research Participant, 1981.
[2]Biomedical High School Research Apprentice, 1981.

TABLE. Antimicrobial activity of various constituents derived from dragon's blood resin (6). Results are in mcg/ml.

Sample	Organism					
	1	2	3	4	5	6
Dragon's Blood Resin Solubles	1000	i	i	i	100	i
1	i	i	i	i	i	i
2	50	i	i	100	25	25
3	50	i	i	i	25	12.5
4	i	i	i	i	i	i
5	i	i	i	i	i	i
Blue Pigment I	i	i	i	i	i	i
Blue Pigment II	i	i	i	i	i	i
Blue Pigment III	i	i	i	i	i	i

i = inactive at 100 mcg/ml.
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.

ing of the pure constituents (see table) showed that the antimicrobial bioactivity of the resin is due to its content of dracorhodin (2) and dracorubin (3). The apparently minor change represented by removal of the C-methyl group leads to *inactive* nordacorhodin (5) and nordracorubin (4). These constituents are all well known from previous investigations (2) to be present in dragon's blood resin, although the antimicrobial activity of 2 and 3 had not been observed before. The three blue pigments were inactive. This, coupled with their relatively intractable nature and the small quantities available have discouraged further work at this time.

EXPERIMENTAL

PLANT MATERIAL.—Commercial dragon's blood resin was a gift of the Meer Corporation. For initial examination, 62 g of the deep red product was extracted successively with 300 ml of chloroform and 200 ml of methanol. When combined and evaporated, 37 g of red resin was obtained and tested for antimicrobial potency as described (see table for results) [6]. Dissolution of a portion in acetone and examination by tlc with chloroform-methanol (9:1) revealed ten spots. Bioautography on an agar sheet seeded with a lawn of *Staphylococcus aureus* showed active zones at Rf 0.84 and 0.44.

ISOLATION.—The soluble portion of the resin (37 g) was dissolved in 100 ml of hot acetone and impregnated onto silica gel (75 g). This was added to the top of a silica gel column (850 g) set with 90% hexane in ethyl acetate. The column was eluted with hexane, ethyl acetate and methanol mixtures; 10 ml fractions were collected and combined according to tlc results. Fractions 38–40 [hexane-ethyl acetate (4:1)] produced 149 mg of a residue which was further purified on 100 g of silica gel [benzene-ethyl acetate (6:1)] to give crystalline 5-methoxy-7-hydroxy-flavan, mp 83–87°; ir, uv, pmr and ms in accordance with the structure (8). Likewise, processing of fractions 60–66 (ethyl acetate) gave 150 mg which were further chromatographed on 20 g of alumina (ether) to produce 75 mg of dracorhodin, mp 164–166, spectral properties in accord with the structure. Processing fractions 75–84 (ethyl acetate) and rechromatography on 100 g silica gel [chloroform-methanol (9:1)] gave 2.42 g, mp 318–323°, identified from its spectral properties as dracorubin. Fractions 98–113 [ethyl acetate-methanol (4:1)] crystallized after concentration and dissolution into chloroform-methanol to give 570 mg, mp 253–260°, identified as above to be nordracorubin.

Fractions 66–75 (ethyl acetate) contained a complex mixture (five tlc spots) so the violet-colored residue (10 g) was dissolved in 50 ml acetone and impregnated on 20 g silica gel. This was divided into two equal lots and subjected to medium pressure liquid chromatography (mplc) (7). A precolumn (2.5 x 21 cm) and a main column (2.5 x 97 cm) of silica gel-60 (0.40–0.063 mm) were used with degassed methlene chloride-methanol (99:1) for elution at 5 psi. Fractions were collected based on visual examination; blue pigment I was present in fractions 115–125. This was further purified by precipitation from chloroform-methanol mixtures to give 32 mg, mp 136–139°; ir 2940, 2850(sh), 1720, 1610(sh), 1605(sh), 1600, 1455(sh), 1445, 1360, 1250, 1100 cm^{-1}; uv (MeOH-HCl) 262, 426, 557 nm; uv (MeOH-NaOH) 285, 326, 456, 488

and 633 nm; too insoluble for satisfactory pmr and too nonvolatile for satisfactory ms. Next, fractions 125–130 contained a mixture which was further resolved by repeated mplc with chloroform-methanol (9:1) to give 10.5 mg, mp 122–126°, was identified to be nordracorhodin from its spectral properties. Also isolated from this third column was blue pigment 11 (20 mg), mp 196–198°; ir 2960, 2880, 1710, 1610(sh), 1605, 1490, 1450, 1370, 1310, 1270(br), 1140(sh), 1100 cm^{-1}; uv (MeOH-HCl) 280, 297(sh), 324, 384 and 548 nm; uv (MeOH-NaOH) 296, 320, 385, 484(w), 592(sh) and 634 nm. Finally, fractions 131–141 contained blue pigment III, mp 144–148°, purified by repeated precipitation from chloroform-hexane and methanol; ir 2940, 2860, 1710(br), 1660, 1600, 1450, 1420(sh), 1350(br), 1270 (br), 1140(sh), 1105; uv (MeOH-HCl) 263, 272, 278, 324, 405 and 556 nm; uv (MeOH-NaOH) 268, 288, 404, 450 and 620 nm.

ANTIMICROBIAL ACTIVITY.—The various fractions were assayed quantitatively by an agar dilution-streak method as previously described [6]. The results are set out in the table.

ACKNOWLEDGMENTS

We are grateful to the NIH (USA) for grant no. AI 13155; NSF-URP (USA) for stipend support (M.A.G.) under grant SPI-8026418; NIH (USA) Biomedical Research Support Grant RR 03053 (R.T.L.), and the Meer Corporation for a gift of the Dragon's Blood Resin.

Received 8 January 1982

LITERATURE CITED

1. G. Usher, A Dictionary of Plants Used by Man, New York, 1974, Macmillan Publishing Company, Inc.
2. T. R. Seshadri in "Recent Developments in the Chemistry of Natural Carbon Compounds", Vol. 7, p. 11, Académiai Kiadò, Budapest, 1976.
3. A. A. Olaniyi, J. W. Powell and W. B. Whalley, *J. Chem. Soc., Perkin Trans. I*, 179 (1973).
4. L. Merlini and G. Nasini, *J. Chem. Soc., Perkin I*, 1570 (1976).
5. E. O. P. Agbakwuru and W. B. Whalley, *J. Chem. Soc., Perkin Trans. I*, 1392 (1976).
6. L. A. Mitscher, R.-P. Leu, M. S. Bathala, W.-N. Wu, J. L. Beal and R. White, *Lloydia*, **35**, 157 (1972).
7. A. I. Meyers, J. Slade, R. K. Smith, E. D. Mihelich, F. M. Hershenson and C. D. Liang, *J. Org. Chem.*, **44**, 2247 (1979).
8. G. Cardillo, L. Merlini, G. Nasini and P. Salvadori, *J. Chem. Soc.* (C), 3967 (1971).