An Investigation of Anthelmintic Secondary Metabolites of *Dalea parryi* (Fabaceae)

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some General Points

There has been little work done on new treatments for hookworm disease, and for a better understanding of the types of molecules that are active toward these helminths.

Plant metabolites, or combinations of them, could be as safe and effective as current treatments like mebendazole **13**. They may also overcome increasing resistance to azoles.

We use an *ex vivo* bioassay for testing against the hookworm *Ancylostoma ceylanicum*. *In vivo* testing is done in hamsters…the host/source animals.

Phenolic metabolites of *Dalea* spp. are very structurally diverse, offer characterization challenges, and exhibit wide-ranging bioactivities. This fuels speculation about the (essentially) unknown natural functions of these compounds.

Dalea parryi
Torr. & Gray

Whole plants, in flower, were collected in south-central Arizona. Roots (112 g) and aerial portions (1149 g) were extracted separately in MeOH leading to 15 g and 135 g of crude extract, respectively.

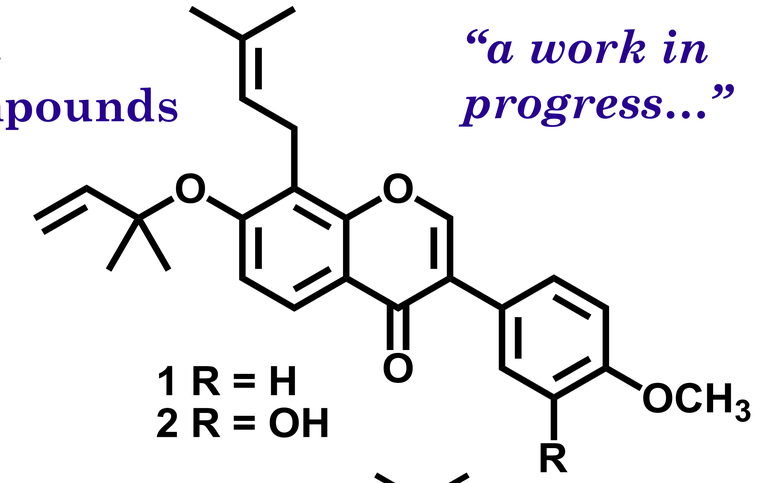
key steps in the Isolation & Characterization

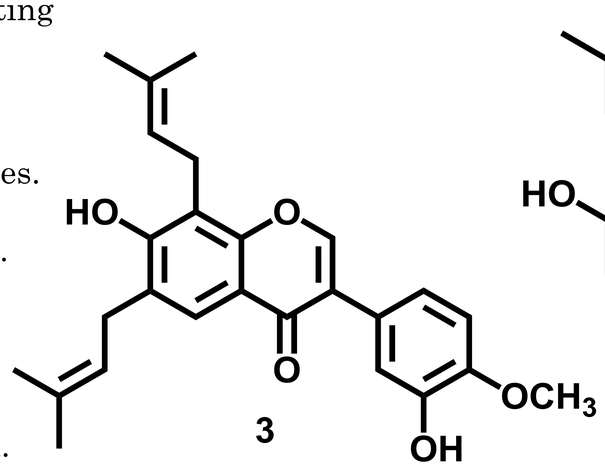
Initial fractionation of the crude extract of the root portions by vacuum liquid chromatography (over silica gel with mixtures of hexane-EtOAc and CH_2Cl_2-MeOH) led to eleven fractions; those eluting with 40-80% EtOAc in hexane were of the highest interest. Advanced fractionation was performed by Sephadex LH-20 chromatography in 3:1:1 hexane-toluene-MeOH...later switching to 100% MeOH.

Final purification was by continuous linear gradient or step gradient chromatography over silica gel. Structure determination is primarily by extensive 1D and 2D NMR spectroscopy.

new compounds

"a work in progress…"





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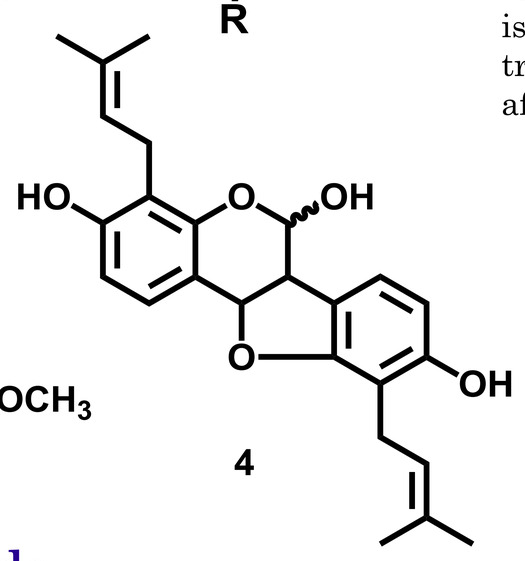

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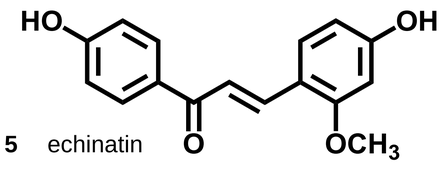
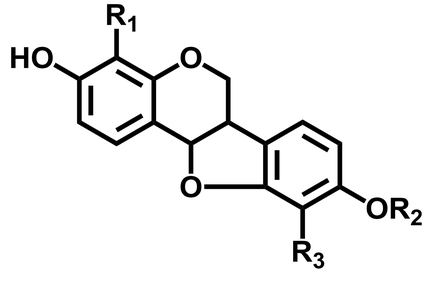
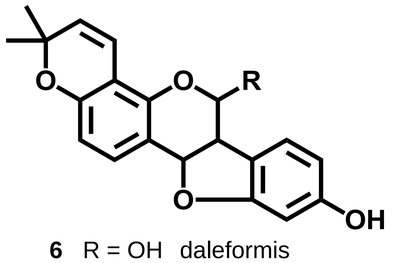
known compounds



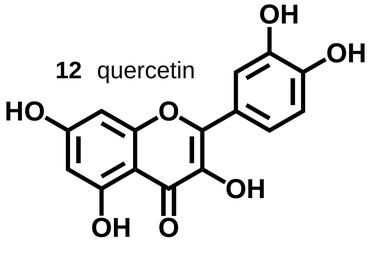
5 echinatin



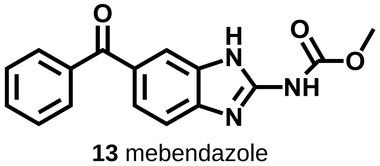
	R_1	R_2	R_3	
8		H		erybraedin A
9		H	H	3,9-dihydroxy-4-(3,3-dimethylallyl)-pterocarpan
10		CH_3	H	licoagcarpin
11	H	CH_3	H	medicarpin



6 R = OH daleformis
7 R = H shinterocarpin



12 quercetin



13 mebendazole
(prescribed in treatment)

key features of the Bioassay

Hamsters (8-10) are infected with hookworm larvae that are harvested at day 21 as mature worms. After 24 h of observation the most active worms are randomly placed in a 24-well culture plate.

Wells, typically containing 10-15 worms each, are challenged with test materials at screening concentrations of 100 µg/mL for crude extracts and at 100, 50, and 10 µg/mL for advanced fractions. The assay runs for 5 days.

Both mortality and motility (on a 1-3 scale, where 1 is fully mobile) are assessed throughout. A treatment that is otherwise inactive but strongly affects worm motility may still be worthwhile.

D. parryi* root extracts[a] are weakly active *ex vivo* toward *A. ceylanicum

	numbers indicate % worm survival			motility
day	DMSO control	tephrosin[b] 25 µg/mL	root extract 100 µg/mL	
1	100	90	100	reduced by 21% over the course of 5 days
2	100	40	100	
3	100	0	97	
4	100	0	97	
5	100	0	90	

[a]The crude extract of aerial portions was inactive. [b]Positive control revealed in earlier work with *D. ornata* [ref]

Needed to finish this work:

Completion of biological testing on all pure compounds, including *ex vivo* hookworm assay and measurement of toxicity to healthy cells.

Determination of absolute configuration of **4** by Mosher's method; and known compounds by comparison of specific rotations.

Acknowledgements

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