EMODIN IN BUCKTHORN: A FEEDING DETERRENT TO PHYTOPHAGOUS INSECTS[1]

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Abstract

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Emodin, a mixture of anthraquinones, occurs in the Rhamnaceae and functions as a deterrent to foliage-feeding insects. There was little attack on foliage of *Rhamnus alnifolia* in wild stands compared with associated species of woody plants. Anthraquinones extracted from foliage of *R. alnifolia* were similar to purchased emodin in thin-layer chromatographic and infra-red spectroscopic analyses. Quantities of emodin in foliage samples were high in May, declining to low levels in August. Leaf powders, leaf extracts, and purchased emodin all showed feeding deterrent activity when mixed with artificial diets and assayed with gypsy moth larvae. Purchased emodin showed similar activity when sprayed on foliage of small cherry trees and assayed with eastern tent caterpillar larvae.

Introduction

Emodin is a secondary plant substance occurring naturally in several plants, and notably in the Rhamnaceae (Kingsbury 1964; Hegnauer 1973). These list its presence in roots, fruits, seeds, bark, and foliage. Sherburne (1972) found that presence of emodin in green fruits of *Rhamnus cathartica* prevents premature predation of these structures by birds. Emodin has also seen use as a purgative drug in medicine for many years. We describe here investigations of emodin content of leaves of alder-leaved buckthorn, *R. alnifolia*, and its probably function as a feeding deterrent to herbivorous insects.

Emodin is an anthraquinone. Structure and chemistry of this and related secondary plant substances are reviewed by Whittaker and Feeny (1971), Robinson (1975), and Labadie and Svendsen (1967*a*, *b*, 1968). The exact chemical identity of the active agent in the purgative drug, and the poisoning agent involved in the death of cattle feeding on *Rhamnus* has been the subject of some controversy (Sherburne 1972; Robinson 1975).

Methods

The experimentation involved the following phases: (1) field observations on numbers of insects feeding and herbivore damage on wild stands of *R. alnifolia* and on other woody-plant associates, (2) extraction, and partial characterization and quantification of anthraquinones from *R. alnifolia* foliage, and (3) laboratory and field bioassay of the extracts and of purchased emodin on candidate herbivorous insects.

Field observations. Two wild stands of *R. alnifolia* were visited on six occasions between 26 May and 20 August 1972. Sweep samples were taken to characterize the fauna living on *R. alnifolia*. To index the degree of insect feeding, 500 leaves, 50 from each of 10 plants, were sampled from *Rhamnus* and from each of the associated plants: sugar maple, *Acer saccharum*, shad bush, *Amelanchier arborea*, pin cherry, *Prunus pennsylvanica*, quaking aspen, *Populus tremuloides*, balsam poplar, *P. balsamifera*, and speckled alder, *Alnus rugosa*.

Each leaf was ranked on a scale of 0–5 where 0 represented no feeding and 1 represented no more than a trace of feeding. Class 2 indicated more than a trace of feeding but less than 10% of the leaf surface missing; class 3, 10–25% of leaf area missing; classes 4 and 5, 25–50% and more than 50% missing, respectively. Mean damage class was calculated for each plant species and collection date, and multiplied by 100 to reduce fractions.

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Extraction of secondary plant substances. *R. alnifolia* foliage was collected on the same six occasions in 1972 and once in 1973 in the same manner as above. The 1972 foliage was frozen within 2–3 h of collection and extracted several months later. Foliage collected in 1973 was extracted in the fresh state, immediately upon return from the field. The purpose of the 1973 extraction was to determine whether freezer storage had modified the 1972 collections.

Extraction was a modification of the methods of Labadie and Svendsen (1967*a, b*). Frozen leaves were dried for 24 h at 80°C, and then ground with a mortar and pestle. One gram of powdered leaf was added to 5 ml of chloroform and heated for 2 min in an 80°C water bath; then filtered under vacuum. Fresh leaves were extracted as above without drying. Leaf extracts were compared with purchased emodin (K. and K. Industries, Plainview, N.Y.) on Gelman type SA thin layer chromatographic plates within a Gelman chamber. Two anthraquinone – specific solvent systems were used:

I. Benzene : ethyl formate : formic acid (75:24:1)

II. Petroleum ether : ethyl acetate: glacial acetic acid (90:5:5)

Following separation, the medium was dried and developed with a 5% solution of NaOH in methanol applied in a spray. Anthraquinones were seen as pink spots. Appropriate sections of undeveloped chromatograms were also isolated, eluted with chloroform, and compared with purchased emodin by infra-red spectroscopy.

Chromatographic separation and spectrophometric analysis were used to determine the relative quantities of emodin in the six collections of *R. alnifolia* leaves made in 1972. Dried leaves were extracted in chloroform and filtered as above. Samples (10 μl) were spotted on chromatographic media and separated with solvent system I. After separation and drying, the principal emodin spot (R_f 0.78) was cut out and eluted from the medium by soaking in 5 ml of 1 *N* NaOH. The resulting pink solution was centrifuged and measured for optical density at 520 nm with a spectrophotometer. Five replicates of each leaf collection were run.

Bioassays. Powdered *R. alnifolia* foliage, foliage extracts, and purchased emodin were added to artificial diets (Leonard and Doane 1966) and bioassayed with gypsy moth larvae, *Lymantria dispar* (L.). Each additive was assayed for frass production, development rates, and survival.

Upon hatching, the larvae were placed on diet in 7.5×12.5 cm clear plastic boxes and allowed to develop to the first molt before testing. Leaf samples were dried and powdered, with 1 g samples added to 100 g of diet in 30 ml plastic cups. Control media were prepared with no additives or substituting pin cherry or red oak leaf powder or powdered cellulose (Alpha Cel), for the *Rhamnus* leaf powder. Five newly molted larvae were placed in each of 12 cups per treatment and held in an environmental chamber at 25°C and a 14:10 h, light:dark regime. Frass was removed and weighed, and observations were made on mortality and molts each day. Cups were changed every third day.

Purchased emodin was assayed at several concentrations by dissolving it in chloroform, adding 1 g of Alpha Cel, and then adding the Alpha Cel to the diet after evaporation of the solvent. Controls were prepared with Alpha Cel alone or with Alpha Cel plus chloroform. Assays were run as with leaf powders.

Emodin extracts from *R. alnifolia* were also assayed as above. Half gram quantities of dried leaves were extracted in chloroform, and the entire extracts streaked across chromatographic media. After separation with solvent system I, the entire band produced at R_f 0.78 was cut out and eluted with $CHCl_3$. This was reduced in volume to 0.5 ml and mixed with 65 g of artificial diet.

Field assays involved pin cherry as a host plant and eastern tent caterpillar, *Malacosoma americana* (F.), as a test insect. Single branches or small trees were sprayed with water, or with chloroform, or with a solution of 4 mg purchased emodin in 1 ml chloroform.

In one test, 3 branches of a small tree were sprayed with each of the 3 treatments, after extra branches were removed. This test was replicated on 4 trees. In a second test, 3 small trees were sprayed in their entirety, one for each treatment. Test insects were introduced by tying 2 webs of caterpillars to each tree at a point below the lowest test branch. Webs contained predominantly fourth instar larvae. Defoliation was measured daily as described earlier for *R. alnifolia* foliage.

Results

Field observations. Sweep-samples and observations on insects associated with wild stands of *R. alnifolia* showed only one common insect throughout the season. This insect was an armored scale of the family Diaspididae. Of 24 taxa of insects identified, all but four were aquatic insects, predators, or blood-sucking flies. Other than the scale, the herbivorous insects were Bruce's span worm, *Operophtera bruceata* (Hulst), 1 individual; large aspen tortrix, *Choristoneura conflictana* (Walk.), 3 individuals; and the chrysomelid *Orsodacne atra* Ahr., 8 individuals. The two lepidopterous species were abundant in the collection area and caused noticeable defoliation on most deciduous plants present. The few individuals observed on *R. alnifolia* did not appear to be feeding. The chrysomelid was collected only during the period of the flowering of *R. alnifolia* and was reported by Blatchley (1910) to feed on buds and flowers of several plants.

The defoliation ratings for *R. alnifolia* and plant associates taken on six dates through the summer showed that two species, *R. alnifolia* and *P. balsamifera*, suffered much less foliar damage than the other species present (Table I), particularly through July.

Extraction of secondary plant substances. Thin layer chromatography of purchased emodin produced 3 spots (Table II), with both solvent systems. The middle spot was much larger than the other two in both cases, and we assume it to be the major active component of emodin. Extracts of *R. alnifolia* foliage collected in May showed the same 3 spots in solvent system I plus 2 additional very faint spots. Only 2 spots appeared in solvent system II. The same pattern remained in June and July leaf extracts except that the uppermost emodin spot disappeared in all, and all spots became weak and poorly defined in August leaves. The 1973 leaves, which had been neither frozen nor dried, showed the same spots as 1972 leaves but weaker due to the dilution effect of water.

Infra-red spectral curves of purchased emodin and eluted chromatographic spots of leaf extracts were identical and had the same absorbence peaks. When eluted in 1 *N* NaOH, spots from purchased emodin had peaks at 522–524 and 520–521 nm. Corresponding R_f spots for leaf extracts peaked at 522–525 and 520–521 nm.

Relative concentrations of emodin in *R. alnifolia*, in terms of spectraphotometric optical density (OD), were significantly higher in May leaves (OD = 0.065) and significantly lower in August leaves (OD = 0.020). Levels in June and July leaves were intermediate (OD = 0.034 – 0.042) and not significantly different from each other. We did not determine absolute concentrations of emodin in extracts since we did not know what proportions of the total emodin was obtained in the extraction process.

Bioassays. The results of the laboratory feeding tests on frass production in gypsy moth larvae are shown in Table III and effects on development rates and on percentage mortality in Table IV. These data support the spectral studies on quantities of emodin in various leaf stages. While both leaf powders and leaf extracts were active in suppressing feeding, August foliage was much less active than the earlier leaf stages. Purchased emodin in graded dosages showed a dosage response.

Table I. Defoliation rating on alder-leaved buckthorn and associated trees and shrubs in the study plots

Species	Observation date					
	5/26	6/3	6/12	6/22	7/22	8/20
Alder-leaved buckthorn	0.2	1.8	8.6	1.8	22.4	89.0
Pin cherry	31.0	98.8	166.0	88.2	–	260.8
Shad bush	27.0	62.2	–	93.2	–	184.2
Sugar maple	13.4	–	19.8	162.4	–	167.2
Trembling aspen	23.6	–	–	200.4	–	233.8
Balsam poplar	8.0	16.6	19.2	24.2	–	49.8
Speckled alder	28.3	104.3	–	196.1	–	200.4

Table II. Chromatographic separation of purchased emodin and anthracene derivatives in extracts of *R. alnifolia* leaves

	R_f values	
	Solvent system I	Solvent system II
Purchased emodin	0.87	0.88
	0.78	0.55
	0.07	0.03
May leaves 1972	0.87	0.88
	0.78	0.56
	0.43 very faint	
	0.22 " "	
	0.07 " "	

Table III. The effect of purchased emodin, leaf powders, and extracts of *R. alnifolia* leaves added to diets on frass production in larval gypsy moth

Treatment	Mean frass production per day, mg	
	Day 2	Day 3
Control — no additives	55.2 a*	52.5 a
Control — 1 g Alpha Cel	56.1 a	55.9 a
Control — 1 g Alpha Cel + $CHCl_3$	58.3 a	57.2 a
Control — cherry leaf powder	55.4 a	56.4 a
Control — oak leaf powder	57.1 a	–
Rhamnus leaf powder 5/26†	26.6 c	27.2 c
6/22	29.7 c	30.0 c
8/20	39.9 b	36.9 b
Rhamnus leaf extracts 5/26	25.2 c	24.0 c
6/22	35.8 c	35.6 b
8/20	45.0 b	44.0 b
Purchased emodin — ppm 15	36.6 b	33.8 b
ppm 30	25.4 c	27.3 c
ppm 45	21.4 cd	31.8 bc
ppm 150	17.7 cd	22.6 cd
ppm 450	10.1 d	11.7 d

*Lower case letter indicates result of Duncan's Multiple Range Test.
Means with the same letter are not significantly different, $P > 0.05$.
†Foliage collection date.

Table IV. The effect of purchased emodin, leaf powders, and extracts of *R. alnifolia* leaves added to diets on development time and mortality in larval gypsy moth

Treatment	Development in days by instar: II	III	IV	V	Final % mortality
Control — no additives	4.8	5.2	5.3	6.0	3.3
Control — 1 g Alpha Cel	4.9	5.4	5.4	5.9	8.8
Control — 1 g Alpha Cel + $CHCl_3$	4.9	5.2	5.8	6.1	5.0
Control — cherry leaf powder	5.0	5.5	5.1	6.0	0.0
Rhamnus leaf powder 5/26	5.9	8.2*	–	–	51.7
6/22	5.5	7.9*	–	–	50.0
8/20	5.1	5.4	5.9	6.9	15.0
Rhamnus leaf extract 5/26	5.1	7.4	9.8*	–	46.7
6/22	5.3	7.7*	–	–	40.0
8/20	5.3	5.2	6.6	7.9	8.3
Purchased emodin — ppm 15	5.6	6.4	10.6*	–	41.7
ppm 45	5.6	6.9	8.8*	–	46.7
ppm150	13.1*	–	–	–	85.0
ppm 450	12.2*	–	–	–	100.0

*Observations discontinued due to more than 30% mortality.

Effects of the field test of purchased emodin on pin cherry foliage are in Table V. Emodin produced a striking protection of foliage for up to 3 days except where rain intervened.

Discussion

Data taken on the insect population associated with *R. alnifolia* are only suggestive, but the results are consistent with the theory that the foliage of *R. alnifolia* is protected from herbivores. With the existing evidence of emodin in other species of Rhamnaceae, and with the chromatographic and spectral studies reported here, it is apparent that emodin, which may be a mixture of chemicals, is at least part of the

Table V. Relative defoliation of pin cherry by eastern tent caterpillar when sprayed with purchased emodin

Treatment		Defoliation rating: Pretreatment	Days post-treatment: 1	2	3	4	5
Whole trees							
Trial 1	Water	4	69	165	213*	258*	298
	$CHCl_3$	0	56	144	200*	250*	266
	Emodin	0	4	29	97*	188*	232
Trial 2	Water	8	76	144*	*	†	191
	$CHCl_3$	3	88	184*	*	†	207
	Emodin	4	8	24*	*	†	114
Individual branches							
Trial 1	Water	2	26†	96	136	–	–
	$CHCl_3$	2	38†	88	142	–	–
	Emodin	4	20†	72	138	–	–
Trial 2	Water	0	66	126	222	–	256
	$CHCl_3$	2	87	121	170	–	232
	Emodin	0	10	36	91	–	186

*Rain showers.
†Heavy rain.

herbivore defense system within foliage. Seasonal decline of emodin in *R. alnifolia* foliage followed that reported in fruits of *R. cathartica* (Sherburne 1972).

Malicky *et al.* (1970) investigated the insects associated with *R. cathartica* in Europe as an approach to biological control of the plant in Canada where it has been introduced. They found many insect herbivores in Europe but few in Canada. If emodin occurs in foliage of *R. cathartica*, many insects have penetrated its defensive properties through long co-evolution (Dethier 1970; Feeny 1975) in Europe. However, this has not occurred in Canada.

The effect of emodin on insects appears chiefly due to feeding deterrence. At low and moderate concentrations of emodin in diet, gypsy moth larvae survived for long periods but with reduced feeding and prolonged development. At high concentrations, pronounced mortality occurred in 2–3 days, but feeding was slight indicating that starvation could be involved.

These results conflict with those of Gupta and Thorsteinson (1960), who placed *R. crenata* among a group of plants containing relatively weak feeding inhibitors or none. They found that *R. crenata* leaf discs were readily accepted by diamond-back moth, *Plutella maculipennis* Curt., larvae when the discs were treated with sinigrin. Hegnauer (1973) reports that emodin is found in *R. crenata*, but the plant structures analyzed are not specified. We cannot explain this conflict of results.

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