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The chemistry and allelopathy of *Euphorbia* esula

GARY D. MANNERS

USDA/ARS, Western Regional Research Laboratory, Albany, California

Euphorbia esula L. (Leafy spurge) infests 2.5 million acres of range and pasture land in the upper great plains of the United States. The plant is toxic to livestock [1], allelopathic to desirable forage plants [5] and poses a serious threat to livestock production on open range lands. Leafy spurge can be controlled by herbicides. However, the cost of control is high and continuous since the weed cannot be eradicated chemically [15]. Spurge is controlled naturally in Europe by indigenous insect predators, however attempts to utilize these predators as biological control agents in North America have proven unsuccessful [10]. One plant (*Antennaria microphylla*) has been reported to be allelopathic to *Euphorbia esula* [5]. Individual *Euphorbia esula* biotypes have been previously examined chemically with the reported occurrence of n-alkanes (C_{25} through C_{32}) [4], long chain alcohols (C_{26} and C_{28}) [4,6], long chain aldehydes (C_{26} , C_{28} , C_{30}) [4], Bsitosterol [2], triterpenes (24-methylenecycloartenol, cycloartenol, lupeol) [2,7], flavanoid glycosides (kaempherol-3-glucuronide) [4] and phorbol esters (ingenol derivatives) [8,9,13].

We now report the results of a chemical investigation of leaf wax constituents of five *E. esula* biotypes relative to the potential chemical taxonomic differentiation of spurge biotypes for the efficient application of biological control methods. This presentation also reports an initial biological evaluation of *E. esula* water soluble chemical constituents as allelopathic agents against favored forage species. An initial biological assay of the extractives *Antennaria microphylla* as allelochemics toward leafy spurge is also described.

Euphorbia esula leaf wax constituents

Several biotypes of *E. esula* have been identified [11,14], suggesting the possible occurrence of separate North American and European Leafy spurge species which can not be differentiated morphologically or the existence, within a single species, of intraspecies physiological or chemical differences between the biotypes. These differences may have significant effects on insects which are predators to this weed.

Intraspecies chemical and/or biochemical comparisons of the recognized *Euphorbia* esula biotypes may provide chemical taxonomic information relative to biological control methods. This investigation is the first chemical comparison of North American and

European *E. esula* biotypes to evaluate the feasibility of using epicuticular wax constituents as chemical taxonomic indicators.

Leaf wax samples were obtained from four North American and one European field selected, greenhouse grown *E. esula* plants displaying similar floral characteristics but distinctively different leaf characteristics. Leaf material from these plants was dipped in chloroform to obtain raw leaf wax samples. Chloroform samples were dissolved in methylene chloride and concentrated with acetone to yield a pseudo-crystalline material. The solid material was filtered and the solid and filtrate were each examined individually utilizing gas chromatography and gas chromatography/mass spectrometry. Specific chemical compounds were identified by comparison of chromatographic and/or mass spectral data with recorded values or with standard compounds available in the laboratory. The results of the analyses are summarized in Tables 1-5.

The analysis of the leaf wax constituents of five separate *Euphorbia esula* biotypes showed, with minor variations, that all of the Leafy spurge biotypes contained similar hydrocarbon compounds, had high yields of the same long chain alcohols (particularly hexacosanol) and were similar in both aldehyde and acid composition. These data may be chemotaxonomically characteristic of the genus *Euphorbia* and are comparable to the suggested chemotaxonomic criteria for separating the panicoid and festucoid grasses at the genus level [12].

The dramatic differences observed in the yields and occurrence of the triterpenes -amyrin, -amyrin and -amyrenone among the five *E. esula* biotypes provides evidence supporting the suggestion that North American leafy spurge may be an interspecies hybrid of *Euphorbia esula* and *Euphorbia virgata* [11] and further suggests the potential importance of the wax triterpenes as chemotaxonomic indicators in leafy spurge. A more detailed examination of the nature and distribution of the epicuticular wax triterpenes among *Euphorbia esula* could provide important information relative to the chemotaxonomic differentiation of leafy spurge.

	Biotype							
-	North American Av							
Component	5	13	14	17	10			
Hydrocarbons	12	18	16	14	25			
Free Alcohols	54	52	53	57	29			
Aldehydes*	1	1	2	1	4			
Free Acids	3	2	3	2	4			
Esters	17	13	7	10	18			
Triterpenes	3	5	10	7	11			
Triterpene Esters	2	2	4	3	2			
Unidentified	8	7	5	6	7			
Yield; % dry wt.(mg)	0.9 (86)	1.1 (256)	1.2 (183)	1.4 (47)	0.8 (139)			
Acetone sol.(%)	5.8	7.8	13.7	10.6	11.5			
Acetone insol.(%)	94.2	91.2	86.3	89.4	88.5			

Table 1. Percent composition and yield of epicuticular waxes of five biotypes of *Euphorbia* esula.

*Aldehyde yields of 5,13,14,17 rounded to next higher %

	Biotype								
		. North American							
	5	13	14	17	10				
Carbon No.									
29	13	10	10	16	12				
30	2	2	1	2	2				
31	51	60	51	55	47				
32	3	5	5	4	4				
33	18	11	11	10	23				
34	2	1	1	1	3				
35	6	5	9	5	4				
36			2						
37		1	4	1	1				
38									
39			2						
Unident.	5	5	4	6	4				

Table 2. Percent composition of hydrocarbons of epicuticular wax of five biotypes of *Euphorbia esula*.

]	Biotype	e						
		North American										A	Austria	n	
		5			13			14			17			10	
	ALC	ALD	AC	ALC	ALD	AC	ALC	ALD	AC	ALC	ALD	AC	ALC	ALD	AC
Carb.No.															
26	91	24	24	91	14	24	91	34	22	88	34	37	71	27	18
28	8	69	30	7	62	40	8	61	22	11	65	26	14	64	31
30	1	7	30	2	24	22	1	5	37	1	10	16	15	9	35
32			12			8			12			12			11
34			4			6			7			9			5

Table 3. Percent composition of free alcohols, aldehydes and acids in five biotypes of *Euphorbia esula*

Table 4. Percent composition of esters of five biotypes of *Euphorbia esula*.

	Car Cas Alcohol							Lao Cao A	lcohol	
	BIOTYPE				BIOTYPE					
	N. American				Aust.	N. American				Aust.
	5	13	14	17	10	5	13	14	17	10
Acids										
C ₁₆ , C ₁₈	14	13	12	7	18					
C ₁₈ , C ₂₀	16	16	17	14	29					
C ₂₂ , C ₂₄	38	42	35	28	42	26	24	27	30	21
Unidentified	6	5	9	6	4					

Table 5. Percent composition of free triterpenes of five biotypes of *Euphorbia esula*

	BIOTYPE								
		North American							
	5		14	17	10				
Terpene									
Amyrin	5	4	9	7	44				
Amyrenone	11	7	7	8	_				
Amyrin	37	41	67	55	22				
24-Me Cycloartenol	28	31	5	19	23				
Lupeol OAc	4	5	1	4	2				
Unidentified	15	12	11	7	9				

Allelopathic evaluation of *E. esula*

In an effort to confirm the reported allelopathic activity of *Euphorbia esula* [5], a chemical separation of an aqueous extract of Leafy spurge was undertaken with the purpose of identifying biologically active constituents in spurge which act as allelochemical agents. Individual chemical constituents obtained from the water soluble extract were assessed for biological activity using a lettuce seed germination bioassay system.

The extraction and separation of Leafy spurge chemicals was accomplished according to Scheme 1. Lettuce seed germination bioassay of the fully differentiated water soluble extract of the plant showed significant biological activity to occur in the sodium bicarbonate soluble portion of the ethyl acetate extract of the aqueous plant extract. The flavanoid compound kaempherol-3-glucuronide (I) was isolated (0.06% of dry plant wt.) and characterized from this extract and subsequently evaluated in the lettuce seed bioassay. The yield of the glucuronide from the aqueous extract was approximately 100 times larger than previously reported in *E. esula*.

Lettuce seed bioassay results of kaempherol and kaempherol-3-glucuronide are summarized in Table 6. The bioassay results show significant reduction (49%) of lettuce seed root length at a concentration of 500 ppm and contrast with an observed root length elongation (5%) for kaempherol at the same concentration. The observed biological activity of the glucuronide, the high concentration of the compound in the plant and the expected slow degradation of the compound in the soil suggests the potential important contribution of this compound to the reported allelopathy of Leafy spurge toward other plant species.

Table 6. Lettuce seed bioassay of Kaempherol and Kaempherol-3-glucuronide from *Euphorbia esula*.

Compound	Root	Length Reduc	% Yield (Dry wt.)	
	500ppm	250ppm	125ppm	
Kaempherol	(5%)	(17%)	2%	1.9 x 10 ⁻⁴
Kaempherol-3-Glucuronide	49%	11%	13%	5.9 x 10 ⁻³

Biological activity of Antennaria microphylla extractives.

Antennaria microphylla (small everlasting) is the only plant reported to be allelopathic to E. esula [5]. This report strongly suggests the source of this allelopathy to be chemical in nature. The determination of the chemical of chemicals responsible for the phytotoxicity of this plant toward spurge could provide important information pursuant to the development of new herbicides with improved efficiency for the control of eradication of E. esula.

A biological assay (lettuce seed germination) was administered to four sequential solvent extracts of *Antennaria microphylla*. The results of this bioassay are summarized in Table 7.

Examination of the bioassay data for the *A. microphylla* extracts shows most biological activity to reside in the ether and water extracts of the plant. Although the water extract showed the greatest activity, the activity of the ether extract is probably of more significance because of the low solubility of this extract in the test system. The activity is also in sharp contrast to that observed for the preceding hexane extract and the following acetone extracts.

The preliminary biological evaluation of *A. microphylla* extracts confirms the presence of biologically active chemical constituents in this plant. Isolation, characterization and further biological evaluation of specific chemical constituents from the biologically active extracts is presently underway.

	Root Length Reduction	
Extract	500ppm	% Yield (Dry wt.)
Hexane	6%	3.6%
Ether	21%	0.5%
Acetone	(5%)	1.5%
Methanol	12%	6.8%
Water	35%*	10.1%

Table 7. Lettuce seed bioassay of Antennaria microphylla extracts.

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