Phytochemistry, Vol. 28, No. 9, pp. 2315–2317, 1989.
Printed in Great Britain.

0031–9422/89 $3.00+0.00
Maxwell Pergamon Macmillan plc.

DIHYDROPARTHENOLIDE AND OTHER SESQUITERPENE LACTONES STIMULATE WITCHWEED GERMINATION

NIKOLAUS H. FISCHER,* JEFFREY D. WEIDENHAMER and JUDITH M. BRADOW†

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, U.S.A.; †USDA, ARS, Southern Regional Research Center, New Orleans, Louisiana 70179, U.S.A.

(*Received* 17 *January* 1989)

Key Word Index—*Striga asiatica*; Scrophulariaceae; witchweed; germination stimulation; sesquiterpene lactones; dihydroparthenolide; confertiflorin; desacetylconfertiflorin; parthenin.

Abstract—Four sesquiterpene lactones which share structural features of the lactone rings of strigol were tested as witchweed germination stimulants. Confertiflorin and parthenin significantly increased witchweed germination at 10^{-4} M, and parthenin and desacetylconfertiflorin increased germination at 10^{-5} M. Dihydroparthenolide induced 70% germination of witchweed across a concentration range of 10^{-7} to 10^{-9} M. This activity is comparable to the activity of strigol and its synthetic analogues.

INTRODUCTION

Witchweed is an obligate root parasite of major food crops in the Gramineae family, including sorghum [*Sorghum bicolor* (L.) Moench], corn (*Zea mays* L.), and sugarcane (*Saccharum officinarum* L.) [1]. Yield reductions due to witchweed infestations can reach 100% [2]. Dormant witchweed seeds germinate only in response to chemical signals emitted by the roots of suitable hosts [3, 4] or to suitable germination stimulants such as strigol (Fig. 1), a sesquiterpene isolated from the root exudate of a non-host plant, cotton (*Gossypium hirsutum* L.) [5]. Several synthetic analogues of strigol also stimulate witchweed germination [6, 7]. Unlike the germination stimulants recently isolated from the natural host sorghum, which are highly unstable and rapidly oxidized [3, 4], strigol and its analogues are relatively stable in soils [8, 9]. Strigol analogues have reduced witchweed seed populations in field tests by stimulating germination in the absence of a suitable host, causing death of the obligately parasitic seedling [6].

Sesquiterpene lactones exhibit a wide range of biological activities including cytotoxic, anti-neoplastic, insecticidal and molluscicidal effects [10, 11]. Certain sesquiterpene lactones selectively promote and inhibit seed germination at concentrations as low as 10^{-6} M [12]. Despite the obvious structural similarities of many naturally occurring sesquiterpene lactones to the lactone rings of strigol (Fig. 1), these compounds have not been

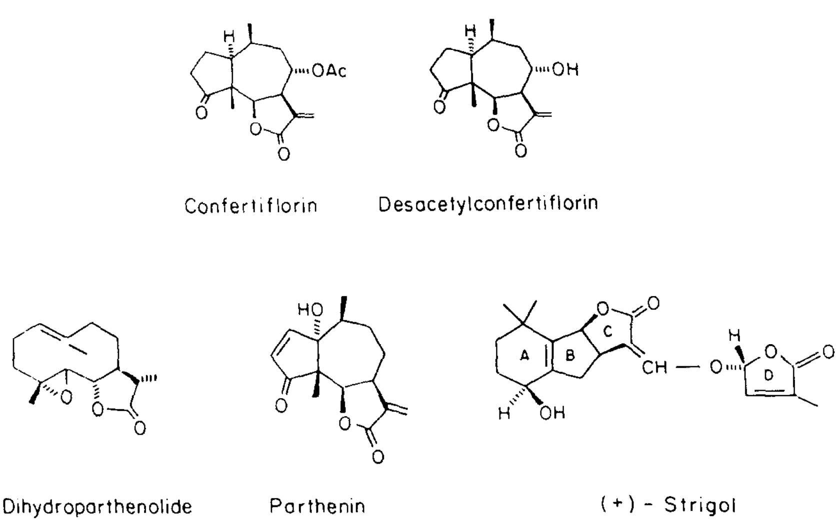

Fig. 1. Chemical structures of strigol and the sesquiterpene lactones used in this study.

* Author to whom correspondence should be addressed.

evaluated as witchweed germination stimulants. Therefore, experiments were conducted to study the promotion of witchweed germination by several naturally occurring sesquiterpene lactones: confertiflorin, desacetylconfertiflorin, dihydroparthenolide and parthenin.

RESULTS AND DISCUSSION

Witchweed controls incubated in 0.1% DMSO germinated at a rate of 6.5% (Table 1). Millimolar levels of the test sesquiterpenes had no effect on witchweed seed germination. Decreasing concentrations of dihydroparthenolide led to increasing witchweed germination (Table 1). Stimulation of witchweed germination by dihydroparthenolide reached a plateau of *ca* 70% between 10^{-7} and 10^{-9} M. This is comparable to the observed activities of strigol and some of its synthetic analogues, which exhibit reproducible activity to 10^{-9} M [6, 7].

The other sesquiterpene lactones tested were less active than dihydroparthenolide (Table 1). Confertiflorin and parthenin significantly increased witchweed germination at 10^{-4} M, and parthenin and desacetylconfertiflorin increased witchweed germination at 10^{-5} M. At lower concentrations, these compounds no longer induced witchweed germination.

The observed stimulation of witchweed germination at low concentrations with decreasing effects at higher concentrations has also been reported for other witchweed germination stimulants [13, 14]. It is well-known that at higher concentrations a stimulant can become less effective or even inhibitory [15].

The structure of the lactone ring of dihydroparthenolide is similar to the butenolide ('D') ring of strigol, except that it lacks the double bond (Fig. 1). Most strigol analogues which have shown activity share the butenolide ring [7]. The lactone rings of the less active parthenin, confertiflorin and desacetylconfertiflorin, on the other hand, are structurally analogous to the 'C' ring of strigol.

Dihydroparthenolide occurs in significant concentrations in local populations of *Ambrosia artemisiifolia* (0.15% of dry weight), and potentially could be isolated in kg quantities. Our results suggest that dihydroparthenolide could be used in witchweed control to stimulate witchweed germination in the absence of a host, as has been done with ethylene [16] and strigol analogues [6]. The strong activity of dihydroparthenolide over a broad range of concentrations indicates that its activity should not disappear immediately as the compound breaks down or be overly dependent on the application of precise rates.

Table 1. Effects of sesquiterpene lactones on witchweed germination

Concentration	Germination*†			
	Conf‡	Desa	Dihy	Part
(M)		(%)		
10^{-3}	4.2	1.5	0.7	2.1
10^{-4}	17.1*	2.4	7.3	28.8*
10^{-5}	7.1	34.5*	24.5*	36.1*
10^{-6}	12.3	10.8	25.4*	14.6
10^{-7}			76.6*	
10^{-8}			75.2*	
10^{-9}			69.9*	
Control	6.5			

Values followed by significantly different from controls germinated in 0.1% DMSO ($P = 0.01$).

†Data from two or more experiments were pooled to make percentages shown the mean of eight or more replicates.

‡Conf = confertiflorin, Desa = desacetylconfertiflorin, Dihy = dihydroparthenolide, Part = parthenin.

EXPERIMENTAL

Isolation of sesquiterpene lactones. The isolation of the sesquiterpene lactones used in these experiments has previously been described. Confertiflorin and desacetylconfertiflorin were isolated from slimleaf bursage, *Ambrosia confertiflora* DC. [17] and 11,13-dihydroparthenolide from common ragweed, *Ambrosia artemisiifolia* L. [18]. Parthenin was isolated from ragweed parthenium, *Parthenium hysterophorus* L., by normal procedures for sesquiterpenes [19].

Witchweed seed germination bioassay. Seeds of witchweed, *Striga asiatica* (L.) Ktze., 1982 crop, were obtained from R. E. Eplee (APHIS, Whiteville, North Carolina). The seeds were briefly (<30 sec) surface sterilized with 1% (v/v) commercial NaOCl, followed by two deionized water rinses, and were then pre-incubated in 10 ml deionized water in the dark at 28° for 10 days. Samples of pre-incubated seeds were collected on 5 μm filters and floated in 10 ml test solution or control solution. All solutions were prepared with 0.1% DMSO (v/v) as a solvent carrier [7]. For the germination incubations, seeds and test solution were transferred to 96-well plastic culture dishes (0.4 ml containing an average of 70 ± 5 seeds per well and 4 replicates of 8 wells each for a given assay). The witchweed seeds were then incubated in the dark for 3 days at 28° before evaluation of germination (radicle protrusion viewed under $\times 40$ magnification). Each experiment was repeated at least once.

Statistical analyses. Data were subjected to the G-statistic goodness of fit test and concentration dependence was determined by the R $\times$ C test of independence using the G-test [20]. A significance level of $P = 0.01$ was used.

Acknowledgements—Appreciation is expressed to Mary P. Lovisa for technical assistance with the bioassays.

REFERENCES

1. Shaw, W. C., Shepherd, D. R., Robinson, E. L. and Sand, P. F. (1962) *Weeds* **10**, 182.
2. Ramaiah, K. V. (1983) in *Striga: Biology and Control* (Ayensu, E. S., Doggett, H., Keynes, R. D., Marton-Lefevre, J., Musselman, L. J., Parker, C. and Pickering, A., eds), pp. 71–92. ICSU Press, Paris.
3. Chang, M., Netzly, D. H., Butler, L. G. and Lynn, D. G. (1986) *J. Am. Chem. Soc.* **108**, 7858.
4. Netzly, D. H., Riopel, J. L., Ejeta, G. and Butler, L. G. (1988) *Weed Sci.* **36**, 441.
5. Cook, C. E., Whichard, L. P., Turner, B., Wall, M. E. and Egley, H. H. (1966) *Science* **154**, 1189.
6. Johnson, A. W., Roseberry, G. and Parker, C. (1976) *Weed Res.* **16**, 223.
7. Pepperman, A. B., Connick, W. J. Jr., Vail, S. L., Worsham, A. D., Pavlista, A. D. and Moreland, D. E. (1982) *Weed Sci.* **30**, 561.
8. Babiker, A. G. T., Ibrahim, N. E. and Edwards, W. G. (1988) *Weed Res.* **28**, 1.

9. Hsaio, A. I., Worsham, A. D. and Moreland, D. E. (1983) *Weed Sci.* **31**, 763.
10. Rodriguez, E., Towers, G. H. N. and Mitchell, J. (1976) *Phytochemistry* **15**, 1573.
11. Picman, A. (1986) *Biochem. System. Ecol.* **14**, 255.
12. Fischer, N. H., Weidenhamer, J. D. and Bradow, J. M. (1989) *J. Chem. Ecol.* **15**, 1785.
13. Worsham, A. D., Moreland, D. E. and Klingman, G. C. (1959) *Science* **130**, 1654.
14. Worsham, A. D., Klingman, G. C. and Moreland, D. E. (1962) *Nature* **195**, 199.
15. Thimann, K. V. (1956) *Am. Nat.* **40**, 145.
16. Eplee, R. E. (1975) *Weed Sci.* **23**, 433.
17. Fischer, N. H. and Mabry, T. J. (1967) *Tetrahedron* **23**, 2529.
18. Fischer, N. H., Wu-Shih, Y. F., Chiari, G., Fronczek, F. R. and Watkins, S. F. (1981) *J. Nat. Prod.* **44**, 104.
19. Fischer, N. H., Wiley, R. A., Lin, H. N., Karimian, K. and Politz, S. M. (1975) *Phytochemistry* **14**, 2241.
20. Sokal, R. R. and Rohlf, F. J. (1981) *Biometry*, 2nd Edn, pp. 321–344, 691–731. W. H. Freeman, San Francisco.