
Effects of Synthetic Congeners of the Natural Product Phytotoxins Maculosins-1 and -2 on Growth of Wheat Coleoptile (*Triticum aestivum* L. cv. Wakeland)

MIKHAIL M. BOBYLEV *¹, LUDMILA I. BOBYLEVA ¹, HORACE G. CUTLER ¹,
STEPHEN J. CUTLER ¹, and GARY A. STROBEL ²

¹ Natural Products Discovery Group, Department of Pharmaceutical Sciences,
Southern School of Pharmacy, Mercer University, 3001 Mercer University Drive,
Atlanta, Georgia 30341-4155, USA

² Department of Plant Pathology, Montana State University,
Bozeman, Montana 59717, USA

Abstract

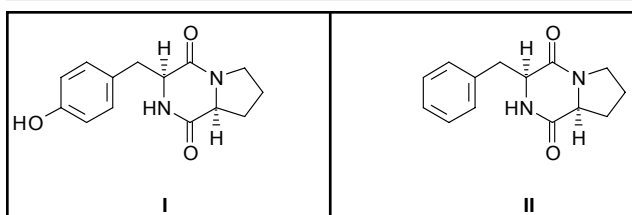
Of two natural product phytotoxins, maculosins-1 and -2, produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*), only maculosin-2 appeared active against intact knapweed plants. Furthermore, unlike host specific maculosin-1, maculosin-2 exhibited phytotoxicity to a wide range of weedy plants including Canada thistle (*Cirsium arvense*), hound's tongue (*Cynoglossum officinale*), yellow starthistle (*Centaurea solstitialis*), Russian knapweed (*Centaurea repens*), rush skeletonweed (*Chondrilla juncea*), dandelion (*Taraxacum officinale*), broad-leaved plantain (*Plantago major*), lamb's-quarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), whitetop or hoary cress (*Cardaria draba*), common mallow (*Malva neglecta*), sulfur (erect) cinquefoil (*Potentilla recta*), and leafy spurge (*Euphorbia esula*). As such, maculosin-2 and its synthetic analogs are good candidates for development as target specific post-emergence herbicides. One measure of herbicidal activity is detection in the etiolated wheat coleoptile (*Triticum aestivum* L. cv Wakeland) bioassay. In common with greenhouse tests on whole knapweed plants, maculosin-1 (cyclo-TyrPro) and pyriculamide (cyclo-3-NO₂-TyrPro) were inactive. Similarly, analogs with protected hydroxyl groups: cyclo-Tyr(Me)Pro; cyclo-Tyr(Et)Pro; cyclo-Tyr(Pr)Pro, and cyclo Tyr(Bu)Pro, significantly inhibited ($P < 0.01$) wheat coleoptiles. The heavy atom derivatives: cyclo-4-Cl-PhePro; cyclo-4-Br-PhePro and cyclo-4-I-PhePro were also inhibitory. The maculosin-2 (cyclo-PhePro); cyclo-TICPro, cyclo-1-NALPro and cyclo-2-NALPro were significantly active ($P < 0.01$) against coleoptiles. The wheat coleoptile bioassay is a fast, reliable, convenient method for screening herbicidal activity of maculosin analogs.

Keywords: cyclic dipeptides; herbicides; maculosin; spotted knapweed

Maculosin-1 {(I), (3S-cis)-hexahydro-3-[(4-hydroxyphenyl)methyl]pyrrolo[1,2-a]pyrazine-1,4-dione} and maculosin-2 {(II), (3S-cis)-hexahydro-3-phenylmethylpyrrolo[1,2-a]pyrazine-1,4-dione} are fungal phytotoxins produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*) (Stierle *et al.* 1988). They were discovered in the

FOOTNOTE

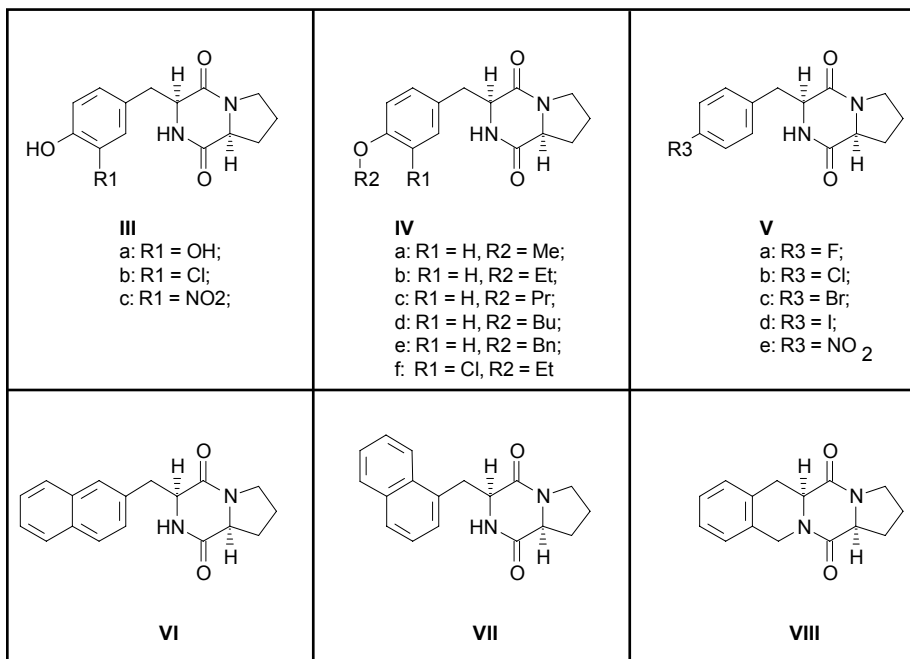
* Corresponding Author: Mikhail M. Bobylev



course of a novel systematic search for bioactive natural products for weed control among weed pathogens (Strobel *et al.* 1987).

In the primary screening test on

detached and perforated leaves, maculosin-1 was shown to be a host specific fungal toxin, active at the concentration of 10^{-6} M [1], while maculosin-2 was active only at 10^{-3} M on spotted knapweed. However, on whole and intact knapweed plants, maculosin-1 was inactive, and maculosin-2 was active at 10^{-2} M (Bobylev *et al.* 1996). Among the synthetic analogs, none of the free hydroxy compounds (IIIa, b, c) were active on whole plants,



while several derivatives with protected (IVa-c, f), substituted (Va, b), or completely removed (VI, VII, VIII) hydroxy groups were active. Unlike host specific maculosin-1, maculosin-2 exhibited phytotoxicity to a wide range of weedy plants including Canada thistle (*Cirsium arvense*), hound's tongue (*Cynoglossum officinale*), yellow starthistle (*Centaurea solstitialis*), Russian knapweed (*Centaurea repens*), rush skeletonweed (*Chondrilla juncea*), dandelion (*Taraxacum officinale*), broad-leaved plantain (*Plantago major*), lamb's-quarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), whitetop or hoary cress (*Cardaria draba*), common mallow (*Malva neglecta*), sulfur (erect) cinquefoil (*Potentilla recta*), and leafy spurge (*Euphorbia esula*) (Bobylev *et al.* 1999).

All these results showed that maculosin analogs did have a significant potential as

natural herbicides, especially with regard to noxious weeds of the West. However, further synthetic development of these promising compounds was hampered by the lack of a suitable bioassay. Indeed, tests on detached and perforated leaves, although relatively simple and fast, did not correlate well with tests on whole plants, while the assay on whole plants was slow, laborious, and required high concentrations of the test compounds. Therefore, the necessity to have a bioassay that would allow for accessing the biological activity of all maculosin derivatives through the whole series was paramount.

One measure of herbicidal activity detection is the etiolated wheat coleoptile (*Triticum aestivum* L. cv Wakeland) bioassay. This assay has been used successfully for the discovery of numerous natural products (Cutler 1984). It was proven to be highly reliable and sensitive to herbicides, thus requiring small quantities of the test substance (Jacyno and Cutler 1993). In this report we examine structure-activity relationships of the compounds I - VIII in the wheat coleoptile bioassay.

Materials and Methods

Synthesis of the compounds I - VIII is described in (Bobylev *et al.* 1996).

Etiolated Wheat Coleoptile Bioassay.

Wheat seedlings (*Triticum aestivum* L., cv Wakeland) were grown in the dark for 4 days on moist vermiculite at $21 \pm 1^\circ\text{C}$, then harvested. The apical 2 mm was cut, in a Van der Weij guillotine, and discarded. The next 4 mm was cut and retained for bioassay. Ten 4 mm sections were placed into test tubes containing dilutions of the compounds to be tested at 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} M formulated in acetone and phosphate-citrate buffer supplemented with 2% sucrose. Assays were incubated for 24 hours at 22°C in the dark with a roller tube apparatus (0.25 rpm). At termination of the bioassay, coleoptile sections were placed on a glass plate, put into a photographic enlarger, to give a x3 image, and the

Table 1.
Influence of maculosin analogs on wheat coleoptiles length, mmx3*

Compound	Concentration of the test compound, M				
	10-3	10-4	10-5	10-6	Control
I	17.0	17.0	17.0	17.0	17.0
II	15.3	17.0	17.1	17.1	17.0
IIIa	17.0	17.1	17.0	17.1	17.0
IIIb	15.0	17.2	17.2	17.2	17.2
IIIc	17.0	17.0	17.0	17.0	17.0
IVa	15.0	17.0	17.1	17.0	17.0
IVb	15.0	17.1	17.0	17.1	17.0
IVc	14.0	17.1	17.2	17.2	17.2

Compound	Concentration of the test compound, M				
	10-3	10-4	10-5	10-6	Control
IVd	12.0	15.2	17.0	17.2	17.2
IVe	13.4	17.1	17.2	17.2	17.2
IVf	14.0	17.2	17.0	17.2	17.2
Va	17.1	17.0	17.1	17.0	17.1
Vb	15.0	17.2	17.2	17.2	17.2
Vc	15.0	17.2	17.2	17.2	17.2
Vd	15.0	16.2	17.1	17.0	17.2
Ve	15.2	17.1	17.0	17.2	17.2
VI	15.2	17.1	17.0	17.2	17.0
VII	14.6	16.4	17.2	17.1	17.2
VIII	15.0	17.0	17.0	17.1	17.0

* Arabic figures in bold type are significant inhibition ($P < 0.01$)

length of each coleoptile recorded. The data were statistically analyzed (Kurtz *et al.* 1965). All assays were duplicated. The results of the tests are recorded in Table 1.

Results and Discussion

Of the two natural maculosins, maculosin-1 appeared totally inactive in the etiolated wheat coleoptile (*Triticum aestivum* L. cv Wakeland) bioassay, while maculosin-2 was active at the concentration of 10^{-3} M. Both the inactivity of I, and the activity of II, correlate well with the results of the whole plant test.

Similarly, two other compounds with free hydroxy groups, IIIa (L-DOPA derivative), and IIIc (pyriculamide) were inactive. Surprisingly, however, the 3-chlorinated derivative IIIb was active at the concentration of 10^{-3} M, with the inhibition level (42%) close to that of II (35%).

All of the analogs with protected (alkylated) hydroxy group IVa-IVf were active at the concentration of 10^{-3} M, showing a steady increase of inhibition from IVa ($R^2 = \text{Me}$, inhibition 40%) to IVd ($R^2 = \text{Bu}$, inhibition 100%). The butylated analog IVd appeared to be the most active compound in the whole series, and the only one in the group of the alkylated derivatives with some activity (inhibition 38%) at the concentration of 10^{-4} M. The compound with the larger benzyl group (IVe) was less active (inhibition 73%). Of the two ethylated analogs IVb and IVf the one with the chloro substituent (IVf) was significantly more active (inhibition 62% versus 40%). Interestingly, the most active compounds IVd and IVE did not show any activity in the test on whole plants. This inactivity could possi-

bly be explained by their poor solubility in the aqueous system: even their solutions at the initial concentration of 10^{-2} M were unstable and precipitated very fast. This explanation is supported by the fact, that the much better soluble IVb was the most active compound of the group in the whole plant test.

Four of the five analogs with replaced hydroxy group: Vb, Vc, Vd, and Ve were active at the concentration of 10^{-3} M, all showing a very close level of inhibition, ranging from 38 to 42%. The compound with the largest halogen substituent Vd ($R^3 = I$) was the only one with some activity (inhibition 19%) at the concentration of 10^{-4} M, that is similar to the results of the previous group. The compound with the smallest halogen substituent Va ($R^3 = F$) was not active at all. Interestingly, this compound was the most active of the group in the whole plant test, and also the most soluble. Compounds Vc, Vd, and Ve were inactive in the whole plant test, and all had very poor solubility in the aqueous system.

All three compounds with removed hydroxy group: VI, VII, and VIII were active (at the concentration of 10^{-3} M), that correlates well with the results of the whole plant test. VIII is the only compound in this group with good solubility in the aqueous system, and therefore seemed to be the most active in the test on whole plants. In the wheat coleoptile bioassay, however, its activity was close to that of the 1-naphthyl analog VII (inhibition, respectively, 40 and 36%), while 2-naphthyl VI showed 50% inhibition and was the only compound in the group with some activity (inhibition 15%) at 10^{-4} M.

The etiolated wheat coleoptile (*Triticum aestivum* L. cv Wakeland) bioassay proved to be a valuable resource in studying the biological activity of maculosin analogs. It showed very good correlation with whole plant tests with regard to natural maculosins-1 and -2. It also showed that maculosin analogs not only can produce necrotic lesions and destroy foliage by deccication, but also possess significant plant growth regulating activity. High sensitivity of the bioassay provided an opportunity to study even the worst soluble compounds and thus create a complete picture of structure-activity relationships among maculosin analogs. Contrary to the previous results, larger and more lipophilic substituents, like butoxy or iodo, or fused rings like 2-naphthyl, improved the activity significantly, and even extended it to the concentration of 10^{-4} M. Significantly higher activity of the 2-naphthyl analog VI compared to 1-naphthyl VII indicates, possibly, that 3,4-substitution is better, than 2,3. The importance of certain substituents in the 3-position is also supported by higher activity of 3-chloro substituted compounds IIIb and IVf compared to I and IVb, respectively. The combined effect of the substitution in positions 3 and 4 is yet to be studied.

The wheat coleoptile bioassay is a fast, reliable, and convenient method for screening herbicidal activity of maculosin analogs. It proved that maculosin analogs have a significant potential as weed biocontrol agents and facilitate structural changes in order to improve their activity. While it may be argued that 10^{-3} M is a high concentration for biological activity it has been our experience, over the past thirty years, that the expression of activity is greatly amplified when the compound is placed in its correct biological niche. That is, the bioassay is a good indicator of biological activity.

References

- Bobylev, M.M., L.I. Bobyleva, and G.A. Strobel. 1996.** Synthesis and bioactivity of analogs of maculosin, a host specific phytotoxin produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*). *J. Ag. Food Chem.*, 44(12), 3960-3964.
- Bobylev, M.M., L.I. Bobyleva, and G.A. Strobel. 1999.** Natural products containing

phenylalanine as potential bioherbicides. *In* Biologically Active Natural Products: Agrochemicals and Pharmaceuticals. CRC Press, Boca Raton, pp. 169-174. (Book Chapter).

- Cutler, H.G. 1984.** A fresh look at the wheat coleoptile bioassay. *In* Proceedings of the Eleventh annual meeting of the Plant growth regulator society of America. Boston, Massachusetts, July 29 - August 1, pp. 1- 9.
- Jacyno, J.M., and H.G. Cutler. 1993.** Detection of herbicidal properties: scope and limitations of the etiolated wheat coleoptile bioassay. *PGSRA Quaterly*. 21: 15-24.
- Kurtz, T.E., R.F. Link, J.W. Tukey, and D.L. Wallace. 1965.** Short-cut multiple comparisons for balanced single and double classifications: Part 1, Results. *Technometrics*, 7, 96-161.
- Stierle, A., J.H. Cardellina, and G.A. Strobel. 1988.** Maculosin, a host-specific phytotoxin for spotted knapweed from *Alternaria alternata*. *Proc. Natl. Acad. Sci. USA*, 85, 8008-8013.
- Strobel, G., F. Sugawara, and J. Clardy. 1987.** Phytotoxins from plant pathogens of weedy plants. *In* Allelochemicals: Role in Agriculture and Forestry. American Chemical Society, Washington, DC, pp. 516-523