

Synthesis and Bioactivity of Analogs of Maculosin, a Host-Specific Phytotoxin Produced by *Alternaria alternata* on Spotted Knapweed (*Centaurea maculosa*)

Mikhail M. Bobylev,* Ludmila I. Bobyleva, and Gary A. Strobel

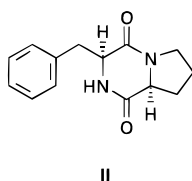
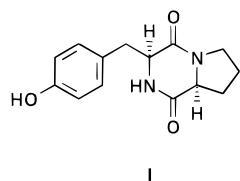
Department of Plant Pathology, Montana State University, Bozeman, Montana 59717

Maculosin (cyclo-Pro-Tyr), a host-specific phytotoxin produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*), is an ideal prototype for creating a safe and an environmentally friendly antiknapweed herbicide. To evaluate this possibility, a series of 18 maculosin analogs was synthesized and tested in the greenhouse on whole knapweed plants by spray or brush application. Interestingly, many of the maculosin analogs have significant potential as natural herbicides against spotted knapweed. Even the simplest analog, cyclo-Pro-Phe, destroys two thirds of the spotted knapweed foliage at a concentration of 6×10^{-2} mol/L within 15 days. Structure–activity relationships are discussed.

Keywords: Cyclodipeptides; herbicides; maculosin; spotted knapweed

INTRODUCTION

Spotted knapweed is one of North America's most noxious weeds (Harris and Cranston, 1979). Its invasion of rangeland, roadsides, and pastures has caused untold millions of dollars in loss in the northwestern United States and southwestern Canada. Currently, synthetic herbicides, especially Tordon, are successfully used to combat spotted knapweed (Davis, 1990). However, it seems beneficial to look for safer and more environmentally friendly herbicides among naturally occurring phytotoxins. As we suggested earlier (Strobel et al., 1987), weed pathogens are a very promising source of bioactive natural products for weed control. Maculosin [(**I**), (3*S*-*cis*)-hexahydro-3-[(4-hydroxyphenyl)-methyl]pyrrolo[1,2-*a*]pyrazine-1,4-dione] is one of the products found as a result of this novel approach.



Maculosin is a host-specific fungal toxin produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*; Stierle et al., 1988). Its unique selectivity, apparent safety, and simple structure, make maculosin an ideal chemical lead for developing a safe and an environmentally friendly antiknapweed herbicide. However, not much is known about structure–activity relationships among maculosin analogs. Although maculosin was originally isolated along with a series of six closely related cyclic dipeptides, only one (**II**) appeared to be somewhat toxic to knapweed. Furthermore, there does not seem to be literature examples of herbicide activity among cyclic dipeptides as a whole (for the most comprehensive and updated review on bioactive cyclic dipeptides see Prasad, 1995). In addition, virtually

nothing is known about the influence of maculosin and its analogs on whole knapweed plants because all previous tests were performed on the detached and punctured knapweed leaves according to the method developed by Sugawara et al. (1985).

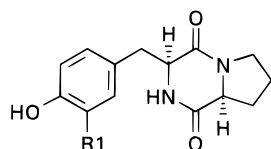
For those reasons, we began a systematic investigation of the bioactivity of maculosin and its analogs against spotted knapweed. In this report we examine the influence of different substituents and certain modifications of the structure in the part of the molecule adjacent to the hydroxy group. This group is definitely one of the most important structural features of maculosin, first because its presence results in a hundredfold increase in activity from **II** to **I**, and second because it is the primary site of the detoxification of maculosin by the host plant (Park et al., 1994). Analogs studied in the present work include compounds with various substituents placed near the free hydroxy group (**II-Ia,b,c**); compounds with the protected hydroxy group (**IVa–f**); compounds with the hydroxy group replaced with other substituents, primarily halogens (**Va–e**); and a series of compounds with no substituents at all, but with increased size of aromatic rings (**VI**, **VII**) or with more rigid plain structure (**VIII**). All synthesized compounds, except **I**, **II**, and **IIIc**, are new. Both maculosin and all its analogs were tested on whole knapweed plants in the greenhouse.

EXPERIMENTAL PROCEDURES

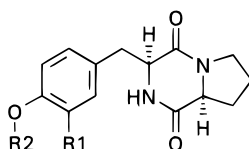
Melting points were determined on a Fisher-Johns melting point apparatus between two micro cover glasses and are uncorrected. Elemental analysis was performed by Atlantic Microlab Inc., Atlanta, GA. Infrared (IR) spectra on 3M disposable polyethylene cards (#61) were recorded on a Bruker IFS 25 spectrophotometer (ν , cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker AC300 spectrometer in CDCl_3 , with TMS as an internal reference; chemical shifts are expressed as δ values in ppm. Optical rotation was measured on a Perkin Elmer 241 MC polarimeter in a 1-cm cell in a mixed solvent ($\text{CHCl}_3/\text{MeOH}$, 1:1) at the concentration of 1 g/dL at room temperature.

Most of the compounds **I–VIII** were obtained by the standard coupling procedure from commercially available BOC-protected amino acids and amino acids methyl esters, followed by deprotection with formic acid and further cycliza-

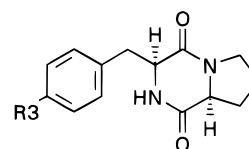
* Author to whom correspondence should be addressed [telephone (406) 994-5147; fax (406) 994-1848; e-mail uplmb@msu.oscs.montana.edu].



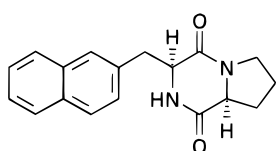
III
a: R1 = OH;
b: R1 = Cl;
c: R1 = NO₂;



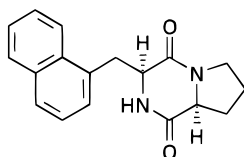
IV
a: R1 = H, R2 = Me;
b: R1 = H, R2 = Et;
c: R1 = H, R2 = Pr;
d: R1 = H, R2 = Bu;
e: R1 = H, R2 = Bn;
f: R1 = Cl, R2 = Et



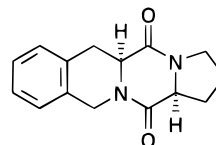
V
a: R3 = F;
b: R3 = Cl;
c: R3 = Br;
d: R3 = I;
e: R3 = NO₂



VI



VII



VIII

tion of the obtained formates in boiling 2-butanol (Nitecki et al., 1968). *O*-Propyl- and *O*-butyl-L-tyrosine used in the synthesis of compounds **IVc** and **d** were obtained by direct alkylation of L-tyrosine with propyl and butyl bromides in DMSO (Solar and Schumaker, 1966) and then transferred to their BOC-derivatives by a standard reaction with BOC-ON (Itoh et al., 1975). Compound **IVf** was obtained by alkylation of **IIIb** with ethyl sulfate, as a further development of the method originally designed for alkylation of BOC-protected esters of tyrosine (Kolodziejczyk and Manning, 1980). Compounds **I** and **IIIa** and **b** were obtained as molecular complexes with benzene (1:1), as was first described for **I** (Tatsuno et al., 1971). The intermediate linear dipeptide for the compound **IIIc** appeared to be *extremely sensitive* to 1 N sodium bicarbonate solution and, for this reason, the reaction mixture after coupling was washed only with water and 1 N citric acid solution. This preventive measure might explain the fact that the melting point of **IIIc** (194–196 °C) was significantly higher than that reported in the literature (183–184 °C; Sviridov et al., 1990; 159–164 °C; Bavykina et al., 1990).

Yields, melting points, optical rotation, molecular formulas, and elemental analysis of compounds **I–VIII** are recorded in Table 1 and spectral data are in Table 2.

Herbicide Tests. Plants. The spotted knapweed plants used in this study were individually grown in plastic pots in pasteurized AquaGro soil mixture in a greenhouse (16 h day/8 h night). For preliminary tests, plants were used in the stage of six to eight rosette leaves (~8 weeks old). For phytotoxicity evaluation at higher concentrations, plants were used in the stage of 10 to 12 rosette leaves (~12 weeks old).

Formulation. For preliminary tests, compounds were dissolved in a small amount of an auxiliary organic solution of Tween 80 in organic solvent [ethanol or dimethylformamide (DMF)], and then diluted with water to create 10⁻² M concentration. The resulting concentrations of the organic solvent and the surfactant in the final solution were 5 vol % and 5 mg/mL, respectively. For phytotoxicity evaluation at higher concentrations, compounds were dissolved in an auxiliary solution of SilWet L-77 in DMF and diluted with water to create 1 × 10⁻² to 6 × 10⁻² M concentrations. The resulting concentrations of SilWet L-77 and DMF in the final solutions are 2 mg/mL and 2 vol %, respectively. The same solvent/surfactant solutions but without analogs were applied to control plants.

Application. In preliminary tests, solutions were applied to leaves by spraying. Two plants were taken per each compound and sprayed simultaneously with 10 mL of the solution. Some of the solutions were not stable enough and precipitated in several minutes after preparation, so all

compounds were dissolved in an auxiliary organic solution in advance and diluted with water immediately before spraying. For phytotoxicity evaluation at higher concentrations, only the compounds with stable formulations and sufficient solubility were used. All solutions were prepared in advance and used within 2–3 h. Solutions were applied by wetting plants with cotton swabs to provide complete and uniform coverage of both leaf surfaces. This technique also allowed us to conserve compounds. Five plants were used per each concentration.

Evaluation of Results. In the preliminary tests series, the number and size of the necrotic lesions were not high enough to allow for quantitative measurements. The results were evaluated qualitatively in accordance with the following scale: “+”, necrotic lesions present; “–”, no lesions.

In higher concentration tests, results were read three times, on the 5th, 10th, and 15th days after treatment, and evaluated visually based on the following scale: 10%, any damage area <10% of the leaf surface; 30%, damage area within the range of 10–50% of the leaf surface; 70%, damage area within 50–90% of the leaf surface; 90%, any damage area >90% of the leaf surface.

RESULTS AND DISCUSSION

In all tests, the only observed leaf damage consisted of necrotic lesions of varying intensities. Symptoms appeared within 2–5 days after treatment and the lesions continued to develop for up to 15 days. In the most successful cases, the lesions eventually covered the whole leaf surface and killed the leaf completely. Some leaves turned yellow, then dried, and died soon after appearance of the first lesions, so symptom development generally appeared as an acceleration of aging. No other symptoms, such as retarded or accelerated growth, wilting, or deformation were observed. The results of the tests are recorded in Table 3.

To our great surprise, maculosin itself appeared to be completely inactive on whole intact plants even at the concentration of 4 × 10⁻² mol/L. This result, however, is quite consistent with the general picture of natural product research on weeds: “a myriad of assays have been utilized in the discovery of herbicidal natural products”, and only “few of these compounds have proven to have activity on intact weeds” (Stonard and Miller-Wideman, 1994). All other compounds with the free hydroxy group (**IIIa–c**) were inactive at the initial concentration of 1 × 10⁻² mol/L as well. Unfortunately,

Table 1. Analytical Data of Newly Prepared Analogs of Maculosin

compd	yield (%)	mp (°C)	[α] ²⁵ _D (c 1.0)	mol formula	found (calculated) (%)		
					C	H	N
I	58	133–8	–132°	C ₂₀ H ₂₂ N ₂ O ₃	70.26	6.56	8.37
					70.20	6.59	8.34
					(70.98)	(6.55)	(8.28)
II	82	133–5	–199°	C ₁₄ H ₁₆ N ₂ O ₂	68.74	6.58	11.49
					68.69	6.62	11.40
					(68.83)	(6.60)	(11.47)
IIIa	54	131–6	–103°	C ₂₀ H ₂₂ N ₂ O ₄	67.54	6.25	7.98
					67.52	6.27	7.92
					(67.78)	(6.26)	(7.90)
IIIb	37	123–133	–79°	C ₂₀ H ₂₁ ClN ₂ O ₃	63.80	5.75	7.61
					63.70	5.80	7.60
					(64.43)	(5.68)	(7.51)
IIIc	44	194–6	–162°	C ₁₄ H ₁₅ N ₃ O ₅	55.13	4.94	13.72
					55.04	4.96	13.69
					(55.08)	(4.95)	(13.76)
IVa	51	162–7	–111°	C ₁₅ H ₁₆ N ₂ O ₃	65.72	6.58	10.24
					65.66	6.63	10.18
					(65.68)	(6.61)	(10.21)
IVb	61	136–8	–101°	C ₁₆ H ₂₀ N ₂ O ₃	66.62	7.03	9.77
					66.69	7.04	9.69
					(66.65)	(6.99)	(9.72)
IVc	69	143–6	–96°	C ₁₇ H ₂₂ N ₂ O ₃	67.58	7.33	9.32
					67.50	7.36	9.28
					(67.53)	(7.33)	(9.26)
IVd	59	125–8	–79°	C ₁₈ H ₂₄ N ₂ O ₃	67.63	7.58	8.95
					67.57	7.61	8.88
					(68.33)	(7.64)	(8.85)
IVe	54	143–5	–72°	C ₂₀ H ₂₀ N ₂ O ₃	72.22	6.44	7.95
					72.19	6.46	7.93
					(71.41)	(5.99)	(8.33)
IVf		135–40	–74°	C ₁₆ H ₁₉ ClN ₂ O ₃	59.60	5.96	8.59
					59.51	5.98	8.58
					(59.53)	(5.93)	(8.68)
Va	66	154–6	–112°	C ₁₄ H ₁₅ FN ₂ O ₂	63.08	5.83	10.59
					62.98	5.86	10.54
					(64.11)	(5.76)	(10.68)
Vb	69	172–4	–98°	C ₁₄ H ₁₅ ClN ₂ O ₂	60.41	5.45	10.02
					60.39	5.46	9.98
					(60.33)	(5.42)	(10.05)
Vc	71	184–5	–82°	C ₁₄ H ₁₅ BrN ₂ O ₂	52.14	4.69	8.62
					52.05	4.71	8.61
					(52.03)	(4.68)	(8.67)
Vd	59	197–200	–79°	C ₁₄ H ₁₅ IN ₂ O ₂	45.51	4.06	7.54
					45.45	4.09	7.51
					(45.38)	(4.08)	(7.56)
Ve	52	212–23	–96°	C ₁₄ H ₁₅ N ₃ O ₄	58.18	5.26	14.57
					58.12	5.28	14.50
					(58.12)	(5.23)	(14.52)
VI	46	208–12	–149°	C ₁₈ H ₁₈ N ₂ O ₂	73.49	6.18	9.53
					73.40	6.23	9.45
					(73.45)	(6.16)	(9.52)
VII	41	191–4	–79°	C ₁₈ H ₁₈ N ₂ O ₂	73.61	6.21	9.40
					73.57	6.26	9.36
					(73.45)	(6.16)	(9.53)
VIII	60	151–3	–126°	C ₁₅ H ₁₆ N ₂ O ₂	70.17	6.31	10.93
					70.10	6.32	10.85
					(70.29)	(6.29)	(10.93)

because of their insufficient solubility, **III** could not be tested at higher concentrations.

In contrast to **I**, **II** retained its activity on whole plants. This result is of special interest for several important reasons. First, **II** has already been described in the literature as phytotoxic to whole plants (Chen, 1960). Second, it is already the third *Alternaria alternata* toxin [after AK toxins **I** and **II** (Nakashima et al., 1982, 1985)] containing phenylalanine, raising a question about phytotoxicity of phenylalanine itself. Finally, because **II** comprises a minor natural constituent of different food products, for example, cheese (Roudot-Algaron et al., 1993), cocoa (Pickenhagen et al., 1975), or beer (Sakamura et al., 1978), that might tell about its potential safety. It seems also that the absence of substituents in the phenyl ring is beneficial for activity

as a whole: all other unsubstituted analogs **VI–VIII** were also active in the preliminary test. However, only **II** and **VIII** were soluble enough to allow for increased concentrations. Compounds **II** appeared to be the most potent among all tested compounds, and **VIII** was half as active.

Among the compounds with protected hydroxy groups (**IV**), only lower alkyl members (**IVa–c, f**; R² = Me, Et, and Pr) were active in the preliminary test, and only two (R² = Me, Et) were soluble enough to test them at higher concentrations. Both **IVa** and **IVb** were less active than either of unsubstituted analogs **II** and **VIII**.

Similar to **IV**, from all five compounds with the replaced hydroxy group (**V**), only two with the smaller substituents (**Va** and **Vb**, R³ = F, Cl) were active and only the smallest one (**Va**, R³ = F) was soluble enough

Table 2. IR and ¹H NMR Spectral Data of Newly Prepared Analogs of Maculosin

compd	IR (3M card 61), $\nu_{\max}$ (cm ⁻¹); ¹ H NMR (CDCl ₃), δ (J, Hz)
I	IR, 3255, 1667, 1614, 1593, 1516, 1473, 1463, 730, 719; ¹ H NMR, 7.05 (2Hd, 8.4), 6.78 (2Hd, 8.4), 5.94 (1Hs), 4.22 (1Hdd, 2.7, 9.9), 4.09 (1Ht, 7.25), 3.60 (2Hm), 3.47 (1Hdd, 4.0, 14.5), 2.78 (1Hdd, 9.9, 14.5), 2.34 (1Hm), 1.96 (3Hm)
II	IR, 3190, 1683, 1659, 1496, 1481, 1454, 753, 699; ¹ H NMR, 7.29 (5H), 5.61 (1Hs), 4.28 (1Hdd, 3.4, 10.6), 4.09 (1Ht, 7.6), 3.62 (3Hm), 2.78 (1Hdd, 10.8, 14.5), 2.33 (1Hm), 1.98 (3Hm)
IIIa	IR, 3268, 1654, 1518, 1473, 1463, 730, 719; ¹ H NMR, 7.36 (6Hs), 6.81 (1Hd, 8.0), 6.72 (1Hd, 1.8), 6.61 (1Hdd, 1.8, 8.0), 6.42 (1Hs), 6.22 (1Hs), 5.86 (1Hs), 4.20 (1Hdd, 3.4, 10.27), 4.09 (1Ht, 7.6), 3.61 (2Hm), 3.45 (1Hdd, 3.8, 14.5), 2.70 (1Hdd, 10.27, 14.5), 2.33 (1Hm), 1.97 (3Hm)
IIIb	IR, 3231, 1664, 1512, 1473, 1463, 730, 719; ¹ H NMR, 7.36 (6Hs), 7.21 (1Hd, 1.9), 7.04 (1Hdd, 1.9, 8.3), 6.98 (1Hd, 8.3), 5.61 (1Hs), 4.23 (1Hdd, 3.3, 9.5), 4.09 (1Ht, 7.3), 3.58 (2Hm), 3.50 (1Hdd, 4.0, 14.7), 2.76 (1Hdd, 10.0, 14.7), 2.33 (1Hm), 1.99 (3Hm)
IIIc	IR, 3231, 1664, 1512, 1473, 1463, 730, 719; ¹ H NMR, 10.49 (1Hs), 8.00 (1Hd, 2.1), 7.55 (1Hdd, 2.1, 8.6), 7.10 (1Hd, 8.6), 6.92 (1Hs), 4.33 (1Ht, 5.7), 4.10 (1Ht, 7.3), 3.58 (2Hm), 3.38 (1Hdd, 4.5, 14.7), 3.09 (1Hdd, 7.4, 14.7), 2.32 (1Hm), 1.99 (3Hm)
IVa	IR, 1671, 1513, 1473, 1463, 1249, 730, 719; ¹ H NMR, 7.14 (2Hd, 8.5), 6.90 (2Hd, 8.6), 5.63 (1Hs), 4.22 (1Hdd, 3.2, 10.6), 4.08 (1Ht, 7.4), 3.80 (3Hs), 3.63 (2Hm), 3.55 (1Hdd, 3.6, 14.5), 2.74 (1Hdd, 10.6, 14.6), 2.32 (1Hm), 1.96 (3Hm)
IVb	IR, 1674, 1611, 1512, 1473, 1463, 1246, 730, 719; ¹ H NMR, 7.12 (2Hd, 8.6), 6.87 (2Hd, 8.6), 5.60 (1Hs), 4.22 (1Hdd, 3.0, 10.7), 4.08 (1Ht, 7.0), 4.02 (2Hq, 7.0), 3.62 (2Hm), 3.55 (1Hdd, 3.6, 14.7), 2.72 (1Hdd, 10.7, 14.6), 2.33 (1Hm), 1.97 (3Hm), 1.42 (3Ht, 7.0)
IVc	IR, 1674, 1611, 1512, 1473, 1463, 1247, 730, 719; ¹ H NMR, 7.12 (2Hd, 8.6), 6.87 (2Hd, 8.6), 5.60 (1Hs), 4.22 (1Hdd, 3.3, 10.7), 4.08 (1Ht, 7.6), 3.89 (2Ht, 6.6), 3.59 (2Hm), 3.55 (1Hdd, 3.5, 14.7), 2.72 (1Hdd, 10.7, 14.5), 2.33 (1Hm), 1.98 (3Hm), 1.83 (2Hm), 1.04 (3Ht, 7.4)
IVd	IR, 1676, 1512, 1473, 1463, 1247, 730, 719; ¹ H NMR, 7.12 (2Hd, 8.6), 6.87 (2Hd, 8.6), 5.59 (1Hbr s), 4.22 (1Hbd), 4.08 (1Ht, 7.6), 3.95 (2Ht, 6.5), 3.59 (3Hm), 2.72 (1Hdd, 10.7, 14.5), 2.34 (1Hm), 1.97 (3Hm), 1.77 (2Hm), 1.49 (2Hm), 0.98 (3Ht, 7.4)
IVe	IR, 1670, 1513, 1473, 1463, 1248, 730, 719; ¹ H NMR, 7.39 (5Hm), 7.14 (2Hd, 8.6), 6.96 (2Hd, 8.6), 5.65 (1Hs), 5.06 (2Hs), 4.22 (1Hdd, 3.1, 10.5), 4.07 (1Ht, 7.34), 3.64 (2Hm), 3.54 (1Hdd, 3.6, 14.6), 2.74 (1Hdd, 10.5, 14.6), 2.33 (1Hm), 1.94 (3Hm)
IVf	IR, 3,221 1673, 1502, 1475, 1426, 1284, 1257, 1111, 1063, 1040, 805, 754; ¹ H NMR, 7.38 (1Hdd, 2.2, 8.4), 7.25 (1Hd, 2.2), 6.88 (1Hd, 8.4), 5.78 (1Hbs), 4.23 (1Hdd, 3.4, 10.2), 4.08 (3Hq, 6.7), 3.61 (2Hm), 3.49 (1Hdd, 3.7, 14.7), 2.75 (1Hdd, 10.0, 14.6), 2.33 (1Hm), 1.97 (3Hm), 1.47 (3Ht, 6.7)
Va	IR, 3223, 1674, 1602, 1510, 1418, 1336, 1305, 1222, 1160, 1111, 825; ¹ H NMR, 7.21 (2Hdd, 5.4, 8.5), 7.03 (2Ht, 8.6), 5.89 (1Hs), 4.25 (1Hdd, 3.7, 9.5), 4.08 (1Ht, 7.5), 3.59 (3Hm), 2.85 (1Hdd, 9.7, 14.6), 2.34 (1Hm), 1.96 (3Hm)
Vb	IR, 3241, 1670, 1491, 1424, 1336, 1303, 1276, 1115, 1090, 1016, 813; ¹ H NMR, 7.32 (2Hd, 8.4), 7.18 (2Hd, 8.4), 5.64 (1Hs), 4.26 (1Hdd, 3.7, 10.0), 4.08 (1Ht, 7.5), 3.58 (2Hm), 3.54 (1Hdd, 3.7, 14.5), 2.82 (1Hdd, 10.0, 14.6), 2.34 (1Hm), 1.93 (3Hm)
Vc	IR, 3223, 1684, 1670, 1488, 1420, 1336, 1303, 1115, 1071, 1011, 808; ¹ H NMR, 7.47 (2Hd, 8.3), 7.12 (2Hd, 8.3), 5.9 (1Hbs), 4.26 (1Hdd, 3.6, 9.7), 4.08 (1Hbs), 3.57 (3Hm), 2.83 (1Hdd, 9.5, 14.7), 2.33 (1Hm), 1.96 (3Hm)
Vd	IR, 3216, 1683, 1668, 1485, 1423, 1338, 1304, 1116, 1007, 804; ¹ H NMR, 7.66 (2Hbd), 6.99 (2Hd, 8.3), 5.9 (1Hbs), 4.25 (1Hdd, 3.1, 9.9), 4.07 (1Hbd, 5.98), 3.55 (3Hm), 2.80 (1Hdd, 9.5, 14.5), 2.33 (1Hbs), 1.97 (3Hm)
Ve	IR, 3220, 1684, 1604, 1514, 1437, 1347, 1314, 1109, 856, 737, 703; ¹ H NMR, 8.21 (2Hd, 8.6), 7.46 (2Hd, 8.6), 5.90 (1Hs), 4.37 (1Hdd, 4.1, 8.7), 4.10 (1Ht, 7.4), 3.61 (3Hm), 3.06 (1Hdd, 8.8, 14.6), 2.34 (1Hm), 1.97 (3Hm)
VI	IR, 1667, 1664, 1634, 1473, 1462, 1428, 794, 768; ¹ H NMR, 8.03 (1Hm), 7.90 (1Hm), 7.83 (1Hd, 8.1), 7.55 (2Hm), 7.44 (1Ht, 8.0), 7.36 (1Hd, 6.9), 5.53 (1Hs), 4.42 (1Hd, 11.5), 4.33 (1Hdd, 3.2, 14.7), 4.05 (1Ht, 7.8), 3.66 (2Hm), 3.04 (1Hdd, 11.4, 14.6), 2.34 (1Hm), 2.08 (2Hm), 1.94 (1Hm)
VII	IR, 1668, 1473, 1462, 1418, 730, 719; ¹ H NMR, 7.81 (3Hm), 7.68 (1Hs), 7.50 (2Hm), 7.34 (1Hdd, 1.5, 8.4), 5.82 (1Hs), 4.37 (1Hdd, 2.8, 10.4), 4.08 (1Ht, 7.4), 3.77 (1Hdd, 3.6, 14.4), 3.61 (2Hm), 2.95 (1Hdd, 10.5, 14.4), 2.30 (1Hm), 1.95 (3Hm)
VIII	IR, 1667, 1473, 1462, 1417, 752, 730, 719; ¹ H NMR, 7.24 (4Hm), 4.93 (1Hd, 16.0), 4.45 (1Hd, 16.0), 4.08 (2Hm), 3.61 (2Hm), 3.41 (1Hdd, 4.3, 15.6), 3.11 (1Hdd, 11.1, 15.5), 2.41 (1Hm), 2.34 (1Hm), 1.98 (2Hm)

to evaluate its phytotoxicity at higher concentrations. Compounds **Va** showed virtually the same high activity as **II**.

All these results show that some maculosin analogs do have a significant potential as natural herbicides against spotted knapweed. Even the simplest analog **II** destroys two thirds of the spotted knapweed foliage at the concentration of 6×10^{-2} mol/L. However, none of the current analogs can control knapweed completely because they do not affect younger leaves or buds and thus allow the weed to regrow its foliage within 3–4 weeks after treatment. We do not have an explanation for the resistance exhibited by younger leaves.

Penetration itself seems to be the most critical factor affecting the activity of maculosin analogs. Maculosin, which was two orders of magnitude more active than **II** when applied to *punctured leaves*, was totally inactive on whole intact plants. Evidently, the presence of free hydroxy group is the major reason of the poor penetration and subsequent loss of activity. This conclusion is supported both by inactivity of all other hydroxy analogs **III** and by the fact that the elimination of the hydroxy

group by any means (protection as in **III**, replacement as in **IV**, or total removal as in **V**) restored the activity. Any further modification of the structure or any other technique leading to improved penetration might also result in increased activity. One such technique, using a special penetrant SilWet L-77 instead of regular surfactants, was applied to knapweed plants and resulted in a threefold increase in activity.

Another limiting factor is poor translocation. Like maculosin, its analogs do not move far from the place of penetration. This lack of movement is evident by a slow growth and restricted size of necrotic lesions. These lesions do not spread to untreated parts of the plant including newly grown parts.

Both penetration and translocation seem tightly connected to solubility in aqueous systems. It is clear from the pattern of the necrotic lesions that analogs of maculosin do not penetrate inside the leaf through the cuticle but only with the test solution through opened stomata and hydathodes. It seems logical to assume that increased solubility in aqueous systems will lead to increased activity and vice versa. Solubility also

Table 3. Antiknapweed Activity of Analogs of Maculosin

compd	pre-liminary test	days after treatment	phytotoxicity evaluation at higher concentration				
			leaf damage in % at concentration (c), $c \times 10^{-2}$ M				
			0	1	2	4	6
I	—						
II	+	5	0		18	30	57
		10	2		33	48	64
		15	4		44	50	66
IIIa	—						
IIIb	—						
IIIc	—						
IVa	+	5	0	4	7		
		10	1	4	8		
		15	2	6	12		
IVb	+	5	0	6	11	11	29
		10	2	8	18	26	39
		15	2	9	20	39	41
IVc	+						
IVd	—						
IVe	—						
IVf	+						
Va	+	5	0		20	42	
		10	2		40	50	
		15	4		49	55	
Vb	+						
Vc	—						
Vd	—						
Ve	—						
VI	+						
VII	+						
VIII	+	5	4	8	15	24	
		10	9	15	22	36	
		15	10	19	26	40	

might explain the fact, that larger substituents or condensed aromatic rings that increase hydrophobicity lead to decreased activity.

Further development of analogs of maculosin, especially targeting improvements in penetration and translocation, will certainly result in creating compounds of real practical value.

LITERATURE CITED

- Bavykina, N. I.; Gabrielyan, A. E.; Korshunova, G. A.; Shvachkin, Yu. P. Synthesis of diketopiperazines related to cyclo-histidyl-proline. *Zh. Obshch. Khim.* **1990**, 60, 170–175.
- Chen, Y. Studies on the metabolic products of *Rosellinia necatrix*. I. Isolation and characterization of several physiologically active neutral substances. *Bull. Agric. Chem. Soc. Jpn.* **1960**, 24, 372–81.
- Davis, E. S. Spotted knapweed (*Centaurea maculosa* L.) seed longevity, chemical control and seed morphology. Master's Thesis, Montana State University, Bozeman, MT, 1990.
- Harris, P.; Cranston, R. An economic evaluation of control methods for diffuse and spotted knapweed in Western Canada. *Can. J. Plant Sci.* **1979**, 59, 375–382.
- Itoh, M.; Hagiwara, D.; Kamiya, T. A new *tert*-butyloxycarbonylating reagent, 2-*tert*-butyloxycarbonyloxyimino-2-phenylacetonitrile. *Tetrahedron Lett.* **1975**, 49, 4393–4394.
- Kolodziejczyk, A. M.; Manning, M. A convenient method for O-alkylation of N-substituted tyrosines using a crown ether. *J. Org. Chem.* **1981**, 46, 1944–1946.
- Nakashima, T.; Ueno, T.; Fukami, H. Structure elucidation of AK toxins, host specific phytotoxic metabolites produced by *Alternaria kikuchiana* Tanaka. *Tetrahedron Lett.* **1982**, 23, 4469–4472.
- Nakashima, T.; Ueno, T.; Fukami, H.; Taga, T.; Masuda, H.; Osaki, K.; Otani, H.; Kohmoto, K.; Nishimura, S. Isolation and structures of AK toxins I and II, host specific phytotoxic metabolites produced by *Alternaria alternata* Japanese pear pathotype. *Agric. Biol. Chem.* **1985**, 49, 807–15.
- Nitecki, D. E.; Halpern, B.; Westley, J. W. A simple route to sterically pure diketopiperazines. *J. Org. Chem.* **1967**, 33, 864–866.
- Park, S. H.; Stierle, A.; Strobel, G. A. Metabolism of maculosin, a host-specific phytotoxin produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*). *Phytochemistry* **1994**, 35 (1), 101–106.
- Pickenhagen, W.; Dietrich, P.; Keil, B.; Polonsky, J.; Nouaille, F.; Lederer, E. Identification of the bitter principle of cocoa. *Helv. Chim. Acta* **1975**, 58 (4), 1078–1086.
- Prasad, Ch. Bioactive cyclic dipeptides. *Peptides* **1995**, 16 (1), 151–164.
- Roudot-Algaron, F.; Le Bars, D.; Einhorn, J.; Adda, J.; Gripon, J. C. Flavor constituents of aqueous fraction extracted from Comte cheese by liquid carbon dioxide. *J. Food Sci.* **1993**, 58 (5), 1005–1009.
- Sakamura, S.; Furukawa, K.; Kasai, T. Bitter diketopiperazines in roasted malts for beer brewing. *Agric. Biol. Chim.* **1978**, 42 (3), 607–612.
- Solar, S. L.; Schumaker, R. R. Selective O-alkylation of tyrosine. *J. Org. Chem.* **1966**, 31, 1996–1997.
- Stierle, A.; Cardellina, J. H.; Strobel, G. A. Maculosin, a host-specific phytotoxin for spotted knapweed from *Alternaria alternata*. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, 85, 8008–8013.
- Stonard, R. J.; Miller-Wideman, M. A. Herbicides and Plant Growth Regulators. In *Agrochemicals from Natural Products*; Godfrey C. R. A., Ed.; Dekker: New York, 1995; Chapter 6.
- Strobel, G.; Sugawara, F.; Clardy, J. Phytotoxins from plant pathogens of weedy plants. In *Allelochemicals: Role in Agriculture and Forestry*; American Chemical Society: Washington, DC, 1987; pp 516–523.
- Sugawara, F.; Strobel, G.; Fisher, L. E.; Van Duyne, G. D.; Clardy, J. Bipolaroxin, a selective phytotoxin produced by *Bipolaris cynodontis*. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, 82, 8291–8294.
- Sviridov, S. I.; Ermolinskiy, B. S. Secondary metabolites of *Pyricularia oryzae*. *Khim. Prir. Soedin.* **1990**, 7 (4), 811–818.
- Tatsuno, T.; Sato, M.; Kubota, Yu.; Kubota, Ya.; Tsunoda, H. Toxicological investigations of metabolic substances of *Fusarium nivale*. *Chem. Pharm. Bull.* **1971**, 19 (7), 1498–1500.

Received for review February 8, 1996. Revised manuscript received August 16, 1996. Accepted August 30, 1996.[®] We thank Montana Noxious Weed Trust Fund, the Montana Agricultural Experimental Station, and Beim Foundation for financial support.

JF960091C

[®] Abstract published in *Advance ACS Abstracts*, November 1, 1996.