

New Helminthosporal Analogues with Plant-Growth Regulatory Properties Synthesized *via* Oxyallyl Cation

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Z. Naturforsch. **61b**, 1287 – 1294 (2006); received March 7, 2006

Microorganisms are a rich source of bioactive molecules which serve as models for the synthesis of new commercial agrochemicals. In this work we present the synthesis of eleven new bioactive analogues of helminthosporal, a fungal secondary metabolite with plant-growth regulatory activity. The compounds were prepared *via* a [4 + 3] cycloaddition reaction between the oxyallyl carbocation generated from 2,4-dibromo-5-methylpentan-3-one and several substituted furans. The reactions yields revealed to be highly influenced by the bulkiness and electron withdrawing capacities of the substituents in the furans. The selective effect of all compounds **10a–f**, **11–14** on the radicle growth of *Sorghum bicolor* and *Cucumis sativus* were evaluated at the concentration of 10^{-3} M. All test compounds inhibited the sorghum roots growth (22–82%) and most of them stimulated the cucumber radicle growth (up to 127%).

Key words: Oxyallyl Carbocation, Herbicides, *Helminthosporium sativum*, Oxabicyclo, Growth Inhibition

Introduction

Natural products have been increasingly used in the development of new herbicides with new modes of action. Natural phytotoxins include a wide diversity of skeletal types, and among them are sesquiterpenes, phenolic compounds, heterocyclic structures, *etc.* [1–3].

The fungus *Helminthosporium sativum*, reclassified as *Bipolaris sorokiana*, is the causal agent of root rot, leaf spot disease, seedling blight of barley and wheat, causing significant yield losses [4, 5]. The sesquiterpene helminthosporol **1** (Fig. 1), a metabolite isolated from this pathogenic fungus is a plant growth regulator that promotes the development of rice seedlings at concentrations ranging from 10 to 300 ppm. This activity is similar to the one of gibberellins which constitute another class of fungal metabolites with applications in agriculture. At 50 ppm helminthosporol causes root growth inhibition and at 300 ppm it prevents root emergence. This compound also inhibits shoot growth of wheat seedlings above 30 ppm [6, 7]. The aldehyde helminthosporal **2**, isolated from the same fungus, is a crop destroying toxin that inhibits

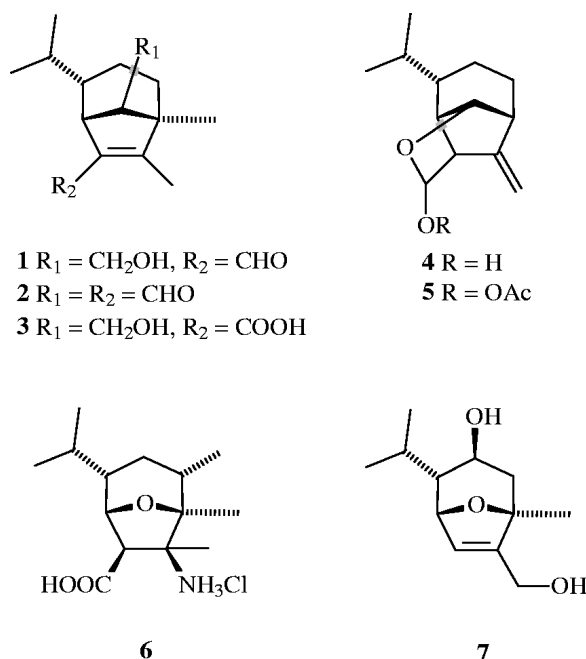


Fig. 1. Structures of natural helminthosporins (**1**, **2**, **4**) and synthetic analogues (**3**, **5–7**).

the respiration of roots and coleoptiles of barley and wheat, and of the roots and storage tissues from lettuce, turnip, potato, bean and radish [8]. It has also been shown that helminthosporal **1** and its oxidation product helminthosporic acid **3** inhibit the cholesterol acyltransferase (ACAT) activity in rat liver microsoms, but it was not established if this inhibitory activity is related to the growth-regulating effects in plants [9].

Further studies using barley endosperm bioassays have demonstrated that helminthosporic acid **3** and some analogues have considerable bioactivity. Structural comparisons between active and inactive compounds indicated that the hydroxymethyl group moiety present in the natural helminthosporins is not necessary for the biological activity [10].

Studies carried out by Cutler *et al.* [11] suggested that the phytotoxicity of *H. sativum* may be due, in part, to the hemiacetal called prehelminthosporol **4**, a compound that causes chlorosis and necrosis in beans and corn. They have also shown that **4** and its acetate **5** inhibited wheat coleoptile growth at 10^{-3} M and 10^{-4} M, and at 10^{-5} M both promoted growth. Motivated by evidences of plant growth-regulating activity from these compounds, Mann and Overton [12] designed a route to prepare analogues of helminthosporic acid which lacked the hydroxymethyl group and would also have good water solubility. In their work they prepared compound **6**, that showed modest phytotoxic activity against a varied of weeds.

In our continuous effort to develop new herbicides using natural products as models [13–19], we have also prepared some analogues of helminthosporic acid using methodology that involves a [3 + 4] cycloaddition reaction *via* oxyallyl cations and furan derivatives [19–21]. Among the most active compounds prepared is the diol **7** which caused 57% and 76% root growth inhibition of important and aggressive weeds *Brachiaria decumbens* and *Desmodium tortuosum* at the concentration of 5.5 ppm, respectively. Current positive results prompted us to report the synthesis and biological activity of new oxa analogues of helminthosporic acid.

Materials and Methods

General procedures

Reagents and solvents were purified, when necessary, according to the usual procedures described in the literature [22]. Flash column chromatography was performed using Crosfield Sorbil C60 (32–63 μ m). Melting points were

determined on an electrothermal digital apparatus with correction. Infrared spectra were recorded on a Mattson Instruments FTIR 3000 grating spectrometer, using potassium bromide disk or sodium chloride liquid film, scanning from 625 to 4000 cm^{-1} . ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 instrument (300 MHz and 75 MHz respectively), using deuterated chloroform as a solvent and tetramethylsilane (TMS) as a reference ($\delta = 0$). Mass spectra were recorded under electron impact (70 eV) conditions, using a VG ANALYTICAL ZAB-IF spectrometer. 2,4-dibromo-5-methylhexan-3-one (**8**) was obtained by bromination of 5-methylhexan-3-one according to the procedure described in the literature [23]. 3-Hydroxymethyl-2-methylfuran was prepared as previously described [19]. The other substituted furans were obtained from Aldrich Chemical Co. Inc.

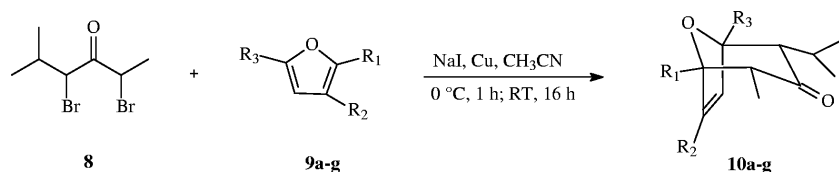
Synthesis

7-Hydroxymethyl-4 α -isopropyl-1,2 α -dimethyl-8-oxa-bicyclo[3.2.1]oct-6-en-3-one (**10a**)

A two neck round bottom flask (100 ml) was fitted with a 10 ml dropping funnel. Dry acetonitrile (4 ml) and sodium iodide (600 mg, 4 mmol) were added during vigorous stirring under a slow stream of nitrogen. Then powdered copper (190.5 mg, 3 mmol) was added, followed by 3-hydroxymethyl-2-methylfuran (145.6 mg, 1.3 mmol). A solution of 2,4-dibromo-5-methylhexan-3-one (271.8 mg, 1 mmol) in dry acetonitrile (3 ml) was added, by means of a dropping funnel, during 1 h at 0 °C. The reaction mixture was allowed to warm up to r.t. and kept stirring for 16 h. After that time the flask was cooled down to 0 °C and dichloromethane (10 ml) and water (10 ml) were added. The mixture was extracted with dichloromethane (2 \times 40 ml) and filtered through a Celite pad. The mother liquor was washed with NH_4OH 35% (50 ml), brine (30 ml), dried over anhydrous MgSO_4 , and concentrated under reduced pressure. Purification by flash chromatography (2 : 1 hexane : ether) gave the required product **10a** (53.0 mg, 0.24 mmol, 18.2%).

The same procedure was used for preparation of compounds **10b–g** and yields are presented in Scheme 1. Structures of the synthesized compounds were supported by the following spectroscopic data:

Data for 10a. Colorless solid, m.p. 83–83.2 °C. – IR (KBr): $\tilde{\nu}_{\text{max}} = 3600\text{--}3100$, 2968, 2873, 1701, 1642, 1450, 1378, 1364, 1252, 1196, 1178, 1046, 929, 877, 697 cm^{-1} . – ^1H NMR (CDCl_3 , 300 MHz): $\delta = 0.90$ (d, $J = 6.9$ Hz, Me-13), 1.01 (d, $J = 6.9$ Hz, Me-10), 1.04 (d, $J = 6.9$ Hz, Me-12), 1.47 (s, Me-9), 1.76 (s, 15-OH), 1.97 (oct, $J = 6.9$ Hz, 11-H), 2.58 (dd, $^1J = 4.5$ Hz, $^2J = 6.9$ Hz, 4-H), 2.61 (q, $J = 6.9$ Hz, 2-H), 4.20 (dm, $^1J = 1.8$ Hz, $^2J = 16.8$ Hz, 14-Ha and 14-Hb), 4.93–4.96 (m, 5-H), 6.06 (dd, $^1J = 1.8$ Hz, $^2J = 2.1$ Hz, 6-H). – ^{13}C NMR (CDCl_3 , 75 MHz): $\delta = 208.3$ (C-3), 149.5 (C-7), 127.4 (C-6), 88.4



| Compound | R groups | Product | % yield |
|-----------|--|--------------|---------|
| 9a | R ₁ = CH ₃ ; R ₂ = CH ₂ OH; R ₃ = H | 10a | 18 |
| 9b | R ₁ = R ₂ = R ₃ = H | 10b | 56 |
| 9c | R ₁ = CH ₃ ; R ₂ = CO ₂ CH ₃ ; R ₃ = H | 10c | 8 |
| 9d | R ₁ = R ₃ = CH ₃ ; R ₂ = H | 10d | 10 |
| 9e | R ₁ = CH ₃ ; R ₂ = R ₃ = H | 10e | 29 |
| | | 10e* | 10 |
| 9f | R ₁ = CH ₂ CH ₃ ; R ₂ = R ₃ = H | 10f | 17 |
| | | 10f** | 6 |
| 9g | R ₁ = R ₃ = CH ₃ ; R ₂ = CO ₂ CH ₃ | 10g | 0 |

Scheme 1. Reagents and conditions: NaI, Cu, CH₃CN, 0 °C, 1 h, r. t. 16 h.

* Compound **10e'** is the regioisomer of **10e** where R₁ = R₂ = H; R₃ = CH₃. ** Compound **10f'** is the regioisomer of **10f** where R₁ = R₂ = H; R₃ = CH₂CH₃.

(C-1), 79.4 (C-5), 62.1 (C-4), 60.1 (C-14), 56.7 (C-2), 25.1 (C-11), 23.2 (C-12), 21.1 (C-9), 20.5 (C-13), 9.9 (C-10). – MS (EI, 70 eV): *m/z* (%) = 224 (M⁺, 1), 206 (3), 181 (25), 163 (5), 137 (6), 125 (9), 111 (11), 95 (9), 83 (8), 69 (19), 65 (6), 55 (25), 43 (100). – C₁₃H₂₀O₃: calcd. C 69.61, H 8.89; found C 69.49, H 8.90.

Data for 10b. Colorless solid, m.p. 56.7–56.8 °C. – IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3088, 2988, 2967, 2892, 2871, 1702, 1452, 1381, 1365, 1347, 1158, 1057, 1012, 945, 931, 734 cm⁻¹. – ¹H NMR (CDCl₃, 300 MHz): δ = 0.88 (d, *J* = 6.9 Hz, Me-12), 0.92 (d, *J* = 6.9 Hz, Me-9), 1.03 (d, *J* = 6.9 Hz, Me-11), 1.99 (oct, *J* = 6.9 Hz, 10-H), 2.58 (dd, ¹*J* = 4.2 Hz, ²*J* = 6.9 Hz, 4-H), 2.74 (qd, 1H, ¹*J* = 4.5 Hz, ²*J* = 6.9 Hz, 2-H), 4.81 (d, *J* = 4.5 Hz, 1-H), 5.01 (d, *J* = 4.2 Hz, 5-H), 6.24 (s, 6-H and 7-H). – ¹³C NMR (CDCl₃, 75 MHz): δ = 207.8 (C-3), 133.9 (C-6), 132.5 (C-7), 83.1 (C-1), 80.8 (C-5), 63.1 (C-4), 50.7 (C-2), 24.9 (C-11), 23.2 (C-10), 20.5 (C-12), 10.6 (C-9). – MS (EI, 70 eV): *m/z* (%) = 180 (M⁺, 3), 165 (3), 149 (4), 137 (23), 123 (5), 109 (16), 95 (24), 85 (25), 81 (77), 69 (100), 60 (57), 55 (68), 48 (58). – C₁₁H₁₆O₂: calcd. C 73.30, H 8.95; found C 73.29, H 8.97.

Data for 10c. Colorless solid, m.p. 51.5–52.5 °C. – IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3086, 2962, 2873, 1725, 1611, 1438, 1385, 1345, 1269, 1231, 1094, 1041, 881, 765 cm⁻¹. – ¹H NMR (CDCl₃, 300 MHz): δ = 0.90 (d, *J* = 6.9 Hz, Me-13), 1.01 (d, *J* = 6.9 Hz, Me-10), 1.03 (d, *J* = 6.9 Hz, Me-12), 1.68 (s, Me-9), 2.01 (oct, *J* = 6.9 Hz, 11-H), 2.59–2.68 (m, 2-H and 4-H), 3.71 (s, Me-15), 5.03 (dd, ¹*J* = 2.0 Hz, ²*J* = 4.7 Hz, 5-H), 7.07 (d, *J* = 2.0 Hz, 6-H). – ¹³C NMR (CDCl₃, 75 MHz): δ = 207.2 (C-3), 163.0 (C-14), 144.8 (C-6), 140.1 (C-7), 88.6 (C-1), 78.8 (C-5), 62.1 (C-4), 56.8 (C-2), 52.2 (C-15), 25.0 (C-11), 23.0 (C-12), 21.6 (C-9), 20.6 (C-13), 10.0 (C-10). – MS (EI, 70 eV): *m/z* (%) = 252 (M⁺, 1), 209 (38), 177 (41), 153 (20), 149 (15), 135 (19), 109 (14), 83 (14), 69 (28), 55 (24), 43 (100). – C₁₄H₂₀O₄: calcd. C 66.65, H 7.99; found C 66.52, H 7.92.

Data for 10d. Yellow oil. – IR (film): $\tilde{\nu}_{\text{max}}$ = 3082, 2971, 2933, 2873, 1709, 1461, 1378, 1339, 1168, 1082, 946, 878, 754 cm⁻¹. – ¹H NMR (CDCl₃, 300 MHz): δ = 0.97 (d, *J* = 7.2 Hz, Me-12 and Me-13), 1.04 (d, *J* = 7.2 Hz, Me-10), 1.46 (s, Me-14), 1.51 (s, Me-9), 2.15–2.33 (m, 11-H), 2.45 (q, *J* = 7.2 Hz, 2-H), 2.59 (d, *J* = 2.1 Hz, 4-H), 5.95 (d, *J* = 5.9 Hz, 6-H), 6.02 (d, *J* = 5.9 Hz, 7-H). – ¹³C NMR (CDCl₃, 75 MHz): δ = 208.2 (C-3), 137.5 (C-7), 134.8 (C-6), 87.9 (C-1), 87.6 (C-5), 66.6 (C-4), 55.1 (C-2), 25.9 (C-9), 23.6 (C-14), 22.6 (C-13), 22.1 (C-12), 20.2 (C-11), 10.8 (C-10). – MS (EI, 70 eV): *m/z* (%) = 208 (M⁺, 1), 193 (1), 167 (4), 149 (11), 123 (11), 95 (26), 81 (71), 69 (100), 55 (45). – C₁₃H₂₀O₂: calcd. C 74.96, H 9.68; found C 75.00, H 9.70.

Data for 10e. Yellow oil. – IR (film): $\tilde{\nu}_{\text{max}}$ = 3076, 2963, 2935, 2874, 1709, 1600, 1463, 1381, 1337, 1163, 1076, 1029, 1003, 878, 743 cm⁻¹. – ¹H NMR (CDCl₃, 300 MHz): δ = 0.87 (d, *J* = 6.6 Hz, Me-13), 0.96 (d, *J* = 6.9 Hz, Me-10), 1.02 (d, *J* = 6.6 Hz, Me-12), 1.47 (s, Me-9), 1.98 (oct, *J* = 6.6 Hz, 11-H), 2.50 (q, *J* = 6.9 Hz, 2-H), 2.55 (dd, ¹*J* = 4.4 Hz, ²*J* = 6.6 Hz, 5-H), 4.99 (dd, ¹*J* = 1.8 Hz, ²*J* = 4.4 Hz, 5-H), 6.02 (d, 1H, *J* = 6.0 Hz, 7-H), 6.15 (dd, ¹*J* = 1.8 Hz, ²*J* = 6.0 Hz, 6-H). – ¹³C NMR (CDCl₃, 75 MHz): δ = 208.0 (C-3), 135.6 (C-6), 133.6 (C-7), 88.2 (C-1), 80.7 (C-5), 62.2 (C-4), 55.9 (C-2), 25.2 (C-11), 23.1 (C-12), 22.1 (C-9), 20.5 (C-13), 10.5 (C-10).

Data for 10e'. ¹H NMR (CDCl₃, 300 MHz): δ = 0.91 (d, *J* = 6.6 Hz, Me-13), 0.96 (d, *J* = 6.9 Hz, Me-10), 1.02 (d, *J* = 6.6 Hz, Me-12), 1.50 (s, Me-9), 2.20 (sepd, ²*J* = 2.4 Hz, ²*J* = 6.9 Hz, 11-H), 2.62 (d, *J* = 2.4 Hz, 2-H), 2.69 (qd, ¹*J* = 4.8 Hz, ²*J* = 6.9 Hz, 4-H), 4.78 (dd, ¹*J* = 1.8 Hz, ²*J* = 4.8 Hz, 5-H), 6.09 (d, *J* = 6.0 Hz, 7-H), 6.15 (dd, ¹*J* = 1.8 Hz, ²*J* = 6.0 Hz, 6-H). – ¹³C NMR (CDCl₃, 75 MHz): δ = 208.0 (C-3), 137.9 (C-7), 131.8 (C-6), 88.3 (C-5), 82.8 (C-1), 67.5 (C-2), 49.8 (C-4), 25.7 (C-11), 23.2 (C-12), 22.1 (C-9), 20.1 (C-13), 10.7 (C-10). – MS (EI, 70 eV):

m/z (%) = 194 (M^+ , 1), 179 (1), 129 (9), 97 (34), 81 (63), 69 (87), 55 (98), 45 (100). – $C_{12}H_{18}O_2$: calcd. C 74.19, H 9.34; found C 74.33, H 9.40.

Data for 10f. Yellow oil. – IR (film): $\tilde{\nu}_{\max}$ = 3077, 2963, 2937, 2875, 1709, 1596, 1463, 1377, 1336, 1342, 1253, 1175, 1082, 1041, 1010, 975, 937, 856, 744 cm^{-1} . – 1H NMR ($CDCl_3$, 300 MHz): δ = 0.87 (d, J = 6.9 Hz, Me-14), 0.93 (d, J = 6.6 Hz, Me-11 and Me-10), 1.03 (d, J = 6.9 Hz, Me-13), 1.70–1.90 (m, Me-9), 1.98 (oct, J = 6.9 Hz, 12-H), 2.52–2.58 (m, 2-H and 4-H), 5.02 (dd, 1J = 1.2 Hz, 2J = 4.2 Hz, 5-H), 5.97 (d, J = 6.0 Hz, 7-H), 6.16 (dd, 1J = 1.2 Hz, 2J = 5.7 Hz, 6-H). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 208.5 (C-3), 134.9 (C-6), 133.8 (C-7), 91.1 (C-1), 80.6 (C-5), 62.3 (C-4), 53.9 (C-2), 27.3 (C-12), 25.2 (C-9), 22.5 (C-13), 20.5 (C-10), 10.1 (C-14), 8.3 (C-11).

Data for 10f'. 1H NMR ($CDCl_3$, 300 MHz): δ = 0.91 (d, J = 7.2 Hz, Me-14), 0.96 (d, 1J = 2J = 7.2 Hz, Me-11 and Me-10), 1.06 (d, J = 7.2 Hz, Me-13), 1.70–1.89 (m, Me-9), 2.00–2.16 (m, 12-H), 2.64–2.68 (m, 2-H and 4-H), 4.79 (dd, 1J = 1.2 Hz, 2J = 4.5 Hz, 5-H), 6.05 (d, J = 6.0 Hz, 7-H), 6.16 (dd, 1J = 1.2 Hz, 2J = 6.0 Hz, 6-H). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 208.5 (C-3), 137.1 (C-7), 131.9 (C-6), 91.1 (C-5), 82.7 (C-1), 65.5 (C-2), 50.0 (C-4), 28.0 (C-12), 25.7 (C-9), 23.1 (C-13), 19.6 (C-10), 10.8 (C-14), 8.2 (C-11). – MS (EI, 70 eV): m/z (%) = 208 (M^+ , 4), 166 (13), 165 (100), 151 (7), 137 (14), 123 (20), 109 (53), 95 (35), 69 (31), 57 (46), 49 (35). – $C_{13}H_{20}O_2$: calcd. C 74.96, H 9.68; found C 74.90, H 9.65.

Oxidation of alcohol 10a to 7-formyl-4 α -isopropyl-1,2 α -dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one (11)

In a two neck round bottom flask under nitrogen atmosphere and magnetic stirring dichloromethane (10 ml) and oxalyl chloride (0.1 ml, 1.1 mmol) were added. The mixture was cooled to $-78^\circ C$ and DMSO was added (0.17 ml, 2.2 mmol) dissolved in dichloromethane (3 ml). After 45 min the alcohol **10a** (224 mg, 1 mmol) also dissolved in dichloromethane (3 ml) was added. The temperature was maintained at $-78^\circ C$ for more 45 min and triethylamine was added (0.7 ml, 5 mmol) and the temperature maintained for 30 more min. The solution was kept under magnetic stirring for 16 h. After this time H_2O was added (15 ml), and the solution was extracted with dichloromethane (4×15 ml). The organic phase was washed with HCl 1% (7 ml), $NaHCO_3$ 5% (7 ml) and brine (10 ml). The organic phase was dried over anhydrous $MgSO_4$ and concentrated in rotary evaporator. Compound **11** was obtained pure, after flash chromatography (hexane : ether, 4 : 1) and recrystallization, in the form of white crystals (90% yield, 199.8 mg, 0.9 mmol).

Data for 11. Colorless solid, m.p. 66.2–66.7 $^\circ C$. – IR (KBr): $\tilde{\nu}_{\max}$ = 3072, 2961, 2872, 2736, 1708, 1686, 1602, 1572, 1465, 1383, 1325, 1254, 1198, 1100, 1043, 1005, 933, 881, 860, 705 cm^{-1} . – 1H NMR ($CDCl_3$, 300 MHz):

δ = 0.92 (d, J = 6.9 Hz, Me-13), 0.98 (d, J = 6.9 Hz, Me-10), 1.06 (d, J = 6.9 Hz, Me-12), 1.68 (s, Me-9), 2.06 (oct, 1J = 2J = 3J = 6.9 Hz, 11-H), 2.62 (q, J = 6.9 Hz, 2-H), 2.72 (dd, 1J = 4.8 Hz, 2J = 6.9 Hz, 4-H), 5.14 (dd, 1J = 2.4 Hz, 2J = 4.8 Hz, 5-H), 7.13 (d, J = 2.4 Hz, 6-H), 9.73 (s, 14-H). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 206.8 (C-3), 186.5 (C-14), 152.3 (C-6), 148.7 (C-7), 88.5 (C-1), 79.3 (C-5), 62.3 (C-4), 56.5 (C-2), 25.0 (C-11), 23.0 (C-12), 21.1 (C-9), 20.7 (C-13), 9.8 (C-10). – MS (EI, 70 eV): m/z (%) = 222 (M^+ , 5), 193 (6), 180 (12), 179 (99), 151 (27), 137 (51), 123 (51), 109 (50), 95 (33), 69 (100), 55 (80). – $C_{13}H_{18}O_3$: calcd. C 70.24, H 8.16; found C 70.09, H 8.11.

Reduction of compound 10a to 7-hydroxymethyl-4 α -isopropyl-1,2 α -dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3 α -ol (12)

In a two neck round bottom flask were added the compound **10a** (96.3 mg, 0.43 mmol), $NaBH_4$ (50 mg, 1.3 mmol) and ethanol (10 ml). The mixture was maintained under atmosphere of N_2 and reflux at $55^\circ C$ by 5 h. At the end of the reaction H_2O was added (10 ml), and the mixture was extracted with dichloromethane (3×10 ml). The organic phase was dried over anhydrous $MgSO_4$ and concentrated in rotary evaporator. After purification by flash chromatography (hexane : ether, 1 : 1) the compound **12** was obtained as a crystalline solid 65.1% yield (63.28 mg, 0.28 mmol).

Data for 12. Colorless solid, m.p. 90.2–91.3 $^\circ C$. – IR (KBr): $\tilde{\nu}_{\max}$ = 3650–3100, 2960, 2878, 1676, 1459, 1383, 1369, 1197, 1157, 1093, 1029, 999, 970, 889, 738 cm^{-1} . – 1H NMR ($CDCl_3$, 300 MHz): δ = 0.96 (d, J = 6.0 Hz, Me-13), 0.99 (d, J = 6.0 Hz, Me-12), 1.04 (d, J = 7.5 Hz, Me-10), 1.27 (s, Me-9), 1.68 (oct, 1J = 2J = 3J = 6.0 Hz, 11-H), 1.58–1.77 (m, 4-H), 1.97 (dq, 1J = 4.3 Hz, 2J = 7.5 Hz, 2-H), 2.95 (s, 15-OH and 16-OH), 3.87 (td, 1J = 1.0 Hz, 2J = 3J = 4.3 Hz, 3-H), 4.09 (dd, 1J = 0.9 Hz, 2J = 14.4 Hz, 14-Ha), 4.25 (dt, 1J = 1.2 Hz, 2J = 14.4 Hz, 14-Hb), 4.72 (s, 5-H), 6.20 (d, J = 1.2 Hz, 6-H). – ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 150.4 (C-6), 130.0 (C-7), 86.0 (C-1), 79.6 (C-5), 71.3 (C-3), 59.2 (C-14), 51.4 (C-4), 44.6 (C-2), 26.2 (C-11), 21.7 (C-12), 21.1 (C-9), 20.9 (C-13), 13.2 (C-10). – MS (EI, 70 eV): m/z (%) = 226 (M^+ , 1), 190 (1), 175 (1), 167 (6), 125 (7), 109 (7), 95 (10), 69 (9), 55 (20), 43 (100). – $C_{13}H_{22}O_3$: calcd. C 68.99, H 9.80; found C 69.00, H 9.83.

Hydrogenation of compound 10a to 7-hydroxymethyl-4 α -isopropyl-1,2 α -dimethyl-8-oxabicyclo[3.2.1]octan-3-one (13)

A solution of **10a** (111 mg, 0.5 mmol) in ethyl acetate (11 ml), was placed in a Parr hydrogenation bottle with 10% Pd-C (20 mg), and kept under hydrogen atmosphere (1.0×10^5 Pa) for 8 h. After this time the catalyst was filtered off through a Celite pad and the solvent evaporated under reduced pressure. The reduced product **13** was obtained as a

white solid after column chromatography purification on silica gel, using hexane-ethyl acetate (2 : 1) as eluent, to yield 110.7 mg (0.49 mmol, 98%).

Data for 13. Colorless solid, m.p. 110.9–111.4 °C. – IR (KBr): $\tilde{\nu}_{\max}$ = 3600–3100, 2968, 2872, 1703, 1636, 1476, 1463, 1379, 1094, 1039, 1024, 934, 867, 663 cm^{-1} . – ^1H NMR (CDCl_3 , 300 MHz): δ = 0.84 (d, J = 6.6 Hz, Me-13), 1.01 (d, J = 6.6 Hz, Me-12), 1.06 (d, J = 7.4 Hz, Me-10), 1.24–1.36 (m, 7-H), 1.50 (s, Me-9), 1.54 (s, 15-OH), 1.88–2.00 (m, $^1J = ^2J = 6.6$ Hz, 11-H), 2.17–2.33 (m, 6-H), 2.45 (dd, 1J = 3.9 Hz, 2J = 9.6 Hz, 4-H), 2.68 (q, J = 7.4 Hz, 2-H), 3.57 (dd, 1J = 7.8 Hz, 2J = 10.3 Hz, 14-Ha), 3.66 (dd, 1J = 6.8 Hz, 2J = 10.3 Hz, 14-Hb), 4.64–4.68 (m, 5-H). – ^{13}C NMR (CDCl_3 , 75 MHz): δ = 209.7 (C-3), 87.4 (C-1), 77.3 (C-5), 63.0 (C-4), 62.5 (C-14), 56.3 (C-2), 52.9 (C-7), 31.9 (C-6), 25.9 (C-12), 24.6 (C-11), 23.4 (C-9), 19.9 (C-13), 9.5 (C-10). – MS (EI, 70 eV): m/z (%) = 226 (M^+ , 1), 208 (1), 139 (4), 123 (4), 109 (8), 95 (12), 83 (18), 69 (28), 55 (32), 43 (100) – $\text{C}_{13}\text{H}_{22}\text{O}_3$: calcd. C 68.99, H 9.80; found C 68.89, H 9.78.

4 α -Isopropyl-7-(methoxymethyl)-1,2 α -dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one (14)

In a two neck round bottom flask were added sodium hydride in mineral oil (42 mg, 1.75 mmol) and imidazole (6.8 mg, 0.1 mmol) and the compound **10a** (224 mg, 1 mmol) diluted in THF (4 ml). The mixture was maintained under magnetic stirring and reflux at 70 °C under N_2 atmosphere for 2 h. A solution of methyl iodide was added (284 mg, 2 mmol, 2.5 ml in THF) and the mixture was kept under the same conditions for four more hours. The THF was eliminated in rotary evaporator and H_2O was added (8 ml). The solution was extracted with diethyl ether (4 \times 8 ml) and the organic phase was washed with brine, and dried over anhydrous MgSO_4 . The organic phase was concentrated under reduced pressure in rotary evaporator. The product **15** was obtained after purification with flash chromatography (16.1% yield 38.1 mg, 0.16 mmol).

Data for 14. Yellow oil. – IR (film): $\tilde{\nu}_{\max}$ = 2959, 2929, 1709, 1463, 1380, 1097, 1044, 1009, 926, 877, 808, 699 cm^{-1} . – ^1H NMR (CDCl_3 , 300 MHz): δ = 0.89 (d, J = 6.9 Hz, Me-13), 1.02 (d, J = 7.2 Hz, Me-10), 1.05 (d, J = 6.9 Hz, Me-12), 1.48 (s, Me-9), 1.99 (oct, $^1J = ^2J = ^3J = 6.9$ Hz, 11-H), 2.57 (dd, 1J = 4.4 Hz, 2J = 6.9 Hz, 4-H), 2.60 (q, J = 7.2 Hz, 2-H), 3.30 (s, 15-H), 3.91 (d, 1J = 1.8 Hz, 2J = 14.0 Hz, 14-Ha), 4.02 (d, J = 14.0 Hz, 14-Hb), 4.94 (d, J = 4.4 Hz, 5-H), 6.05 (d, J = 1.8 Hz, 6-H). – ^{13}C NMR (CDCl_3 , 75 MHz): δ = 208.0 (C-3), 145.9 (C-7), 129.2 (C-6), 88.4 (C-1), 79.3 (C-5), 69.2 (C-14), 61.9 (C-4), 58.6 (C-15), 56.7 (C-2), 25.2 (C-11), 23.2 (C-12), 21.1 (C-9), 20.5 (C-13), 9.9 (C-10). – MS (EI, 70 eV): m/z (%) = 238 (M^+ , 1), 195 (22), 163 (16), 125 (26), 95 (15), 69 (21),

48 (45), 45 (100). – $\text{C}_{14}\text{H}_{22}\text{O}_3$: calcd. C 70.56, H 9.30; found C 70.36, H 9.26.

The same procedure was used for preparation of the compound 7-(benzyloxymethyl)-4 α -isopropyl-1,2 α -dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one (**15**).

Data for 15. Yellow oil. – IR (film): $\tilde{\nu}$ = 3087, 3063, 3030, 2960, 2933, 2870, 1705, 1496, 1454, 1371, 1328, 1199, 1165, 1090, 1073, 1029, 920, 871, 808, 738, 699 cm^{-1} . – ^1H NMR (CDCl_3 , 300 MHz): δ = 0.90 (d, J = 6.9 Hz, Me-13), 0.99 (d, J = 6.9 Hz, Me-10), 1.05 (d, J = 6.9 Hz, Me-12), 1.48 (s, Me-9), 2.00 (oct, $^1J = ^2J = ^3J = 6.9$ Hz, 11-H), 2.59 (dd, 1J = 4.2 Hz, 2J = 6.9 Hz, 4-H), 2.62 (q, J = 6.9 Hz, 2-H), 4.02 (dd, 1J = 1.6 Hz, 2J = 14.3 Hz, 14-Ha), 4.10 (dt, 1J = 1.6 Hz, 2J = 14.3 Hz, 14-Hb), 4.43 (d, J = 12.2 Hz, 15-Ha), 4.52 (d, J = 12.2 Hz, 15-Hb), 4.97 (sl, 5-H), 6.11 (d, J = 1.6 Hz, 6-H), 7.27–7.37 (m, 5H, 17-H, 18-H, 19-H, 20-H and 21-H). – ^{13}C NMR (CDCl_3 , 75 MHz): δ = 208.0 (C-3), 146.0 (C-6), 137.9 (C-7), 129.5 (C-16), 128.6 (C-19), 127.9 (C-18 and C-20), 127.8 (C-17 and C-21), 88.4 (C-1), 79.4 (C-5), 72.6 (C-15), 66.7 (C-14), 62.0 (C-4), 56.7 (C-2), 25.2 (C-11), 23.2 (C-12), 21.2 (C-9), 20.5 (C-13), 10.0 (C-10). – MS (EI, 70 eV): m/z (%) = 314 (M^+ , 1), 271 (14), 163 (15), 105 (21), 91 (100), 77 (72), 65 (24), 51 (40). – $\text{C}_{20}\text{H}_{26}\text{O}_3$: calcd. C 76.40, H 8.33; found C 76.31, H 8.28.

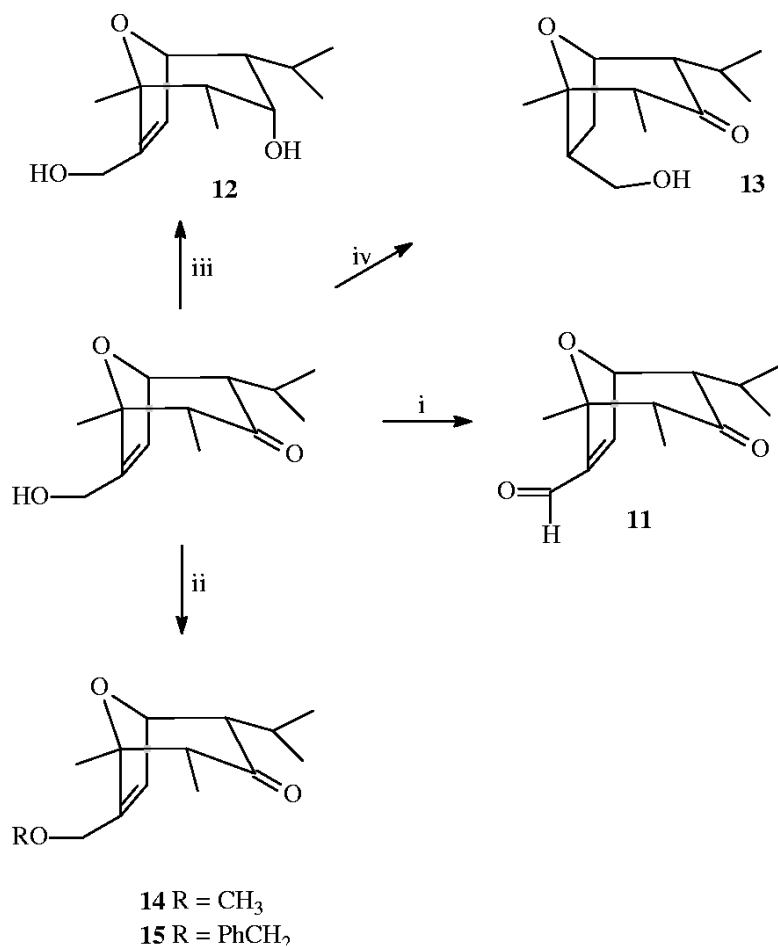
Root elongation assays

Stock solutions were prepared for the bioassays by dissolving each compound (10^{-3} mol) in xylene (3 ml). To the stock solutions were added sodium dodecylsulfate (0.12 g), Tween 40 (0.7 ml) and water (10 ml). The resultant suspension was sonicated for 3 min and then transferred to a volumetric flask (1 l), and the volume completed with water. A mixture with the same composition as described above, but without the compound to be tested, was used as a control.

Groups of 8 pre-germinated *Sorghum bicolor* L. or *Cucumis sativus* L. seeds were placed in Petri dishes (i. d. = 9 cm) with acid washed sand (150 g) and the solution (20 ml) containing the compound to be tested. The Petri dishes were sealed with Parafilm and incubated at 28 °C, in the dark and placed at a 75° angle in relation to the surface of the incubator. After 72 h, the root length was measured to the nearest millimeter. All treatments were replicated six times in a completely randomised design. The percentage of root growth inhibition was calculated in relation to the root length of the control. The data were analysed using Tukey's test at 0.05 probability level [24].

Results and Discussion

For the preparation of several oxa-analogues of helminthosporic acid we chose the [4+3] cycloaddition reaction between the oxyallyl cation generated from



Scheme 2. Reagents and conditions: i) (COCl)₂, CH₂Cl₂, DMSO, -78 °C, 45 min; TEA, -78 °C, 0.5 h, r. t., 16 h, 90%; ii) NaH, imidazole, THF, 70 °C, 2 h; CH₃I or PhCH₂Br, 70 °C, 4 h, 86% for **14** and 94% for **15**; iii) NaBH₄, EtOH, 55 °C, 4 h, 65%; iv) H₂ (1 atm), 10% Pd-C, AcOEt, 40 °C, 8 h, 98%.

2,4-dibromo-5-methylpentan-3-one and different substituted furans as indicated in Scheme 1.

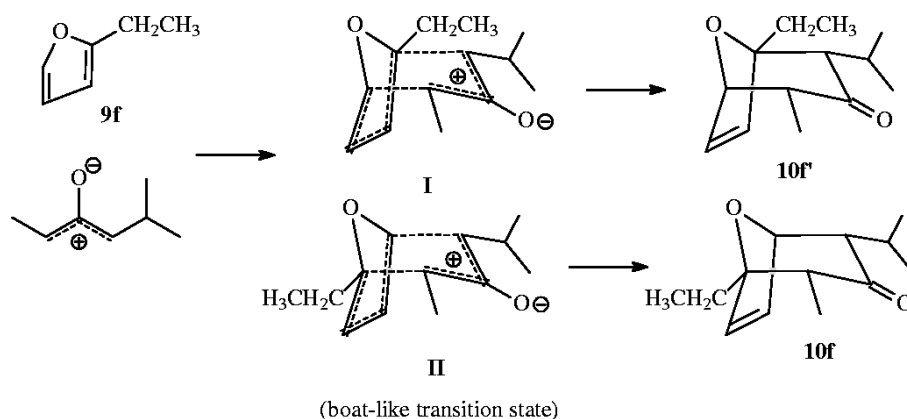
The cycloadditions were carried out on a 1 mmol scale and resulted in the formation of compounds **10a–g** in variable yields. The best result was obtained for the reaction involving furan **9b**, that resulted in 56% yield of bicycle **10b**. The other alkyl furans presented a methyl or ethyl groups at position 2, and this renders a more crowded transition state during the cycloaddition, resulting in lower yields of the required products, due to the formation of products of nucleophilic capture [21,25]. For furan **9g**, which has two methyl groups at positions 2 and 5, and also a electron withdrawing group (COOMe) the required cycloadduct was not obtained.

For all cycloadditions the major compounds isolated had the 2-methyl and 4-isopropyl groups at equatorial position, as expected for this type of reaction. This

stereochemistry was proved by detailed NMR investigations [19].

As can be observed in Scheme 1, the reactions involving furans **9e** and **9f** allowed the isolation of two regioisomeric cycloadducts (**10e/10e'** and **10f/10f'**). Due to greater steric interactions involving the isopropyl and ethyl groups, shown in Fig. 2, the regioisomers **10e'** and **10f'** were formed in smaller amounts than **10e** and **10f**, respectively.

A regioisomer of **10a** was also formed, but it was not isolated in enough quantity to be fully characterized. The alcohol **10a** was then transformed into the corresponding aldehyde **11** in 90% yield by Swern oxidation [26]. The structure of **11** was proved by spectroscopic analysis. The corresponding infrared spectrum showed a strong absorption at 1686 cm⁻¹ due to the conjugated C=O stretching. In the ¹³C NMR and ¹H NMR spectra signals at δ = 186.5 and δ = 9.73

Fig. 2. Mechanism for the cycloaddition of **9f**.

were observed, confirming the presence of the carbonyl function.

Treatment of alcohol **10a** with sodium hydride and imidazole, followed by methyl iodide or benzyl bromide resulted in the formation of the corresponding ethers **14** and **15** in 86% and 94% yield, respectively. The aim to prepare such derivatives was the fact that many monoterpenes benzyl ethers prepared by Vaughn and Spencer [27] have shown a considerable phytotoxicity against several weed species and soybean.

This alcohol (**10a**) was further treated with sodium borohydride to produce the *endo* alcohol **12** in 65% yield. As we have reported previously [16,20], the attack of the hydride on this type of bicyclic compound occurs preferentially on the less hindered face, resulting in the *endo* alcohol as the major or sole product.

Based on the study of several helminthosporic acid analogues, Turner *et al.* [28] have proposed that the double bond has a minor effect on the biological activity. In order to investigate that information, we have prepared alcohol **13**, in almost quantitative yield, by catalytic hydrogenation of **10a**. The ^{13}C NMR spectrum of **13** revealed the absence of signals due to sp^2 carbons (except the signal at $\delta = 209.7$ due to the carbonyl group) and from the DEPT spectrum was observed a signal at $\delta = 52.9$ due to C-7 (CH) and another one at $\delta = 31.0$ corresponding to C-6 (CH_2).

The effects produced by compounds **10a–f**, **11–15**, at $10^{-3} \text{ mol l}^{-1}$, on the root growth of *Sorghum bicolor* and *Cucumis sativus* are shown in Table 1.

All compounds caused a significant inhibitory effect on the radicle growth of *S. bicolor*, ranging from 22 to 82%. None of the compounds evaluated

Table 1. Effect of compounds **10a–f**, **11–15** at $10^{-3} \text{ mol l}^{-1}$ on the development of *C. sativus* L. and *S. bicolor* L. 72 h at 30 °C, using sand as a substrate.

| Compound | <i>C. sativus</i> L. | | <i>S. bicolor</i> L. | |
|------------|----------------------|----------------|----------------------|----------------|
| | Roots (cm) | Inhibition (%) | Roots (cm) | Inhibition (%) |
| Control | 2.73 cd | – | 6.33 a | – |
| 10a | 0.70 f | 74 | 6.10 b | 22 |
| 10b | 2.78 cd | –2 | 4.40 cd | 44 |
| 10c | 1.58 ef | 42 | 2.93 e | 63 |
| 10d | 3.60 c | –32 | 3.95 de | 49 |
| 10e | 2.30 de | 16 | 3.38 de | 57 |
| 10f | 2.23 de | 18 | 2.93 e | 63 |
| 11 | 2.28 de | 16 | 1.38 f | 82 |
| 12 | 3.38 c | –24 | 5.45 bc | 30 |
| 13 | 6.20 a | –127 | 4.37 cd | 44 |
| 14 | 3.55 c | –30 | 3.78 de | 52 |
| 15 | 4.85 b | –78 | 3.90 de | 50 |
| CV (%) | 13.69 | – | 10.45 | – |

* Means, in the same column, with the same letter are not significantly different at $P = 0.05\%$ by Tukey's test.

have caused any effect on the germination rate, nor have they caused chlorosis or necrosis on the tested plants.

From Table 1 it is evident that the aldehyde **11** is the most active compound, causing 82% inhibition, while the corresponding alcohol **10a** is the least active (22%). In the case of compounds **10e** and **10f**, lacking the hydroxymethyl group at carbon C-7, a 63% inhibition on the radicle growth was observed. These results show that the nature of the alkyl group (methyl or ethyl) at carbon C-1 has no effect on this activity. Compound **12**, also presenting a hydroxymethyl group at C-7, was the second less active (30%), not significantly different from **10a**. The conversion of the hydroxymethyl group of **10a** into a methyl (**13**) or benzyl (**14**) ethers resulted in a significant increase in activity,

with both compounds causing around 50% root growth inhibition.

Compound **13**, although having a CH₂OH group attached at C-7 like **10a** and **12**, caused a significant 44% inhibition on the radicle growth of *S. bicolor*. This difference, we postulate, could be explained considering that the lack of the double bond in **13** alters considerably the three dimensional structure of the compound, what could be important for the biological activity, as in the case of some lactones [29]. Also, for this compound the CH₂OH group is at the *endo* face and close enough from the carbonyl group at C-3 to participate in an intramolecular hydrogen bond. This could hinder such OH group to participate in any other hydrogen bonding process limiting the activity.

In the case of *C. sativus* it was observed that compounds **10a** and **10c** caused a significant radicle growth inhibition. For this species **10a** was the most active compound (74% inhibition), contrary to the observations for *S. bicolor*. An interesting result is the fact

that compounds **13** and **15** caused significant radicle growth promotion (127% and 78%, respectively).

From the results obtained it was clear that in the case of the monocot species, the aldehyde group is important for the inhibitory activity, while the presence of the hydroxymethyl group at C-7 causes a reduction in activity. In the case of the dicot species results where the opposite, with the allylic hydroxymethyl group related to a significant root growth inhibitory effect. The removal of the carbon-carbon double bond resulted in a compound with a root growth promotion activity.

Acknowledgements

The authors are grateful to the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for research fellowships (LCAB, ADJ, AAS and FCC) and Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) for a research grant.

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