New 8-Oxabicyclo[3.2.1]oct-6-en-3-one Derivatives with Plant Growth Regulatory Activity†

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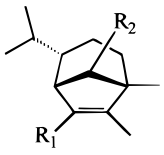
Several 8-oxabicyclo[3.2.1]oct-6-en-3-one derivatives have been prepared via the [3 + 4] cycloaddition between the oxyallyl carbocation generated from polybromoketones and alkylfurans. The selective effects of these compounds on the radicle growth of a monocotyledon [sorghum (*Sorghum bicolor* L.)] and a dicotyledon [cucumber (*Cucumis sativus* L.)] were evaluated. All of the test compounds, at the concentration of 100 μg mL^{-1}, stimulated cucumber root growth (5−30%), but they inhibited sorghum root growth (23−56%). None of the compounds had any effect on the percent of germination of the species tested.

Keywords: *Plant growth regulators; oxyallyl cation; cucumber; sorghum*

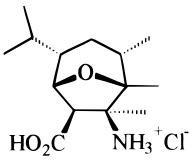
INTRODUCTION

Synthetic chemical herbicides have had a major impact in reducing crop losses and are largely reponsible for the increase in crop productivity during the past five decades (Godfrey, 1995; Copping, 1996). Although most of the new herbicides and plant growth regulators developed recently are analogues obtained as a result of QSAR studies of existing commercial products (Fairclough, 1994), plants and microorganisms continue to be a rich source of compounds with new modes of action. Some of these natural products have been used as model compounds for the development of more active synthetic molecules (Godfrey, 1995; Copping, 1996).

In this regard, helminthosporal **1** and helminthosporic acid **2** are sesquiterpenes produced by the cereal phytopatogenic fungus *Helminthosporium sativum* (De Mayo et al., 1961) which cause plant growth effects



1 R_1=R_2= CHO
2 R_1= COOH, R_2= CH_2OH



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similar to those produced by the gibberellins (Briggs, 1966). It has been shown that the hydroxymethyl group is not required for biological activity (Mander et al., 1974). In an attempt to prepare simpler analogues of helminthosporic acid, Mann and Overton (1985) prepared the hydrochloride **3** and reported that it showed modest phytotoxic activity against a variety of weeds.

Following our continued efforts to discover new molecules with herbicidal and/or plant growth regulatory activity (Demuner et al., 1996; Demuner, 1996; Barbosa et al., 1997), we describe, in this paper, the preparation and biological activity of several other simple analogues of compound **1**.

RESULTS AND DISCUSSION

The [3 + 4] cycloaddition methodology has largely been used for the preparation of seven-membered ring compounds (Demuner et al., 1997; Mann, 1986). In the present work all of the analogues were prepared using this methodology. The required 1,1,3,3-tetrabromo-4-methylpentan-2-one (**4**) was obtained in 90% yield through the treatment of 4-methylpentan-2-one with 4 equiv of bromine in the presence of a catalytic amount of PBr_3 (Barbosa et al., 1993). Compounds **11**−**14** were synthesized as previously described (Mann and Barbosa, 1992; Barbosa et al., 1993).

Treatment of tetrabromoketone **4** with diethylzinc in benzene (Barbosa and Mann, 1996) resulted in the formation of an oxyallyl carbocation that was trapped with 2-methylfuran. The crude product obtained from this reaction consisted of an isomeric mixture of 2,4-dibromo-4-isopropyl-1-methyl-8-oxabicyclo[3.2.1]oct-6-en-3-one, which was treated with Zn/Cu couple to produce the corresponding reduced oxabicycle **7** in 74% overall yield. The same procedure was used to prepare the hydroxyketone **8** in 54% yield (Scheme 1). In both cycloadditions, TLC analysis of the reaction mixture revealed formation of minor products that could be the regioisomers of **7** and **8**. The high regioselectivity for these reactions is probably due to steric factors (Mann and Holland, 1987).

To produce more hydrophilic compounds, and also to evaluate the significance of the ketone group for biological activity, compounds **7** and **8** were treated with $NaBH_4$ in methanol and the corresponding alcohols (**9** and **10**) were formed in 82 and 90% yields, respectively (Scheme 1). In both cases the minor 3β isomers were formed as indicated by the TLC analysis, but they were not isolated.

The effects of compounds **7**−**14**, at 100 μg mL^{-1}, on the radicle growth of sorghum (*Sorghum bicolor* L.) and cucumber (*Cucumis sativus* L.) are shown in Table 1.

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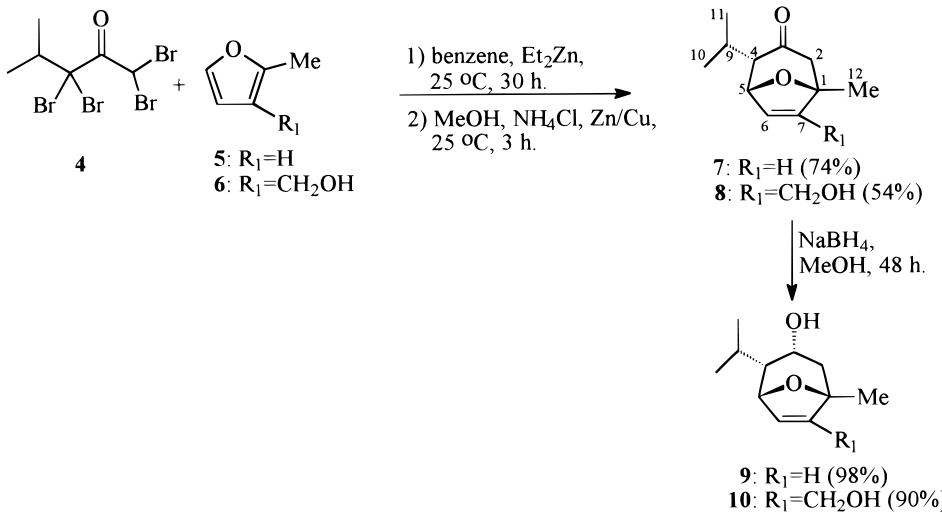
Scheme 1



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5: R_1=H
6: R_1=CH_2OH

7: R_1=H (74%)
8: R_1=CH_2OH (54%)

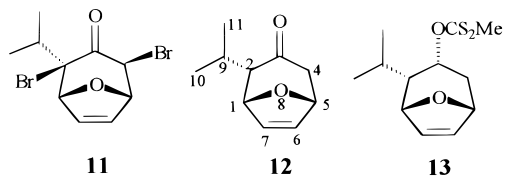
9: R_1=H (98%)
10: R_1=CH_2OH (90%)

Table 1. Effect of 8-Oxabicyclic Compounds 7−14, at 100 μg mL^{-1}, on the Germination and Radicle Growth of *S. bicolor* L. and *C. sativus* L. after 3 Days of Incubation at 25 °C[a]

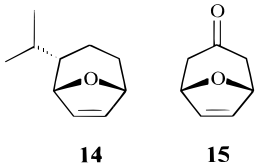
	S. bicolor L.			*C. sativus* L.		
substance	radicle length ± SD (cm)	inhibition (%)	germination[b] (%)	radicle length (cm)	promotion (%)	germination (%)
water	2.69 ± 0.20 a		100 a,b*	3.72 ± 0.30 a*		100 a
7	1.28 ± 0.07 b	53	105 a	4.67 ± 0.28 a,b	26	100 a
8	1.75 ± 0.31 a,b	35	101 a,b	4.22 ± 0.31 a,b	14	98 a
9	1.64 ± 0.05 b	39	107 a	4.41 ± 0.31 a,b	19	100 a
10	1.19 ± 0.20 b	56	95 a,b	4.29 ± 0.26 a,b	15	99 a
11	2.08 ± 0.41 a,b	23	78 b	3.89 ± 0.29 a,b	5	100 a
12	1.86 ± 0.40 a,b	33	84 a,b	4.09 ± 0.30 a,b	10	96 a
13	1.63 ± 0.34 b	40	102 a,b	3.93 ± 0.23 a,b	6	99 a
14	1.56 ± 0.26 b	42	96 a,b	4.84 ± 0.07 b	30	95 a

[a] Means in the same column with the same letter are not significantly different at $P = 0.05\%$ by Tukey's test. [b] The germination data for sorghum were normalized with the control at 100%, as in water it was ~85%. For cucumber, the germination rate in water was 100%.

All compounds stimulated radicle growth of cucumber, but this effect was small and varied from 5 to 30%. Interestingly, all compounds had an inhibitory effect on the radicle growth of sorghum, and no injury symptoms (necrosis, chlorosis, or secondary root inhibition) were observed in either of the tested plants. The most active compound was the diol **10**, which gave 56% inhibition. From these results (Table 1) it is evident that structure−activity relationships are not straightforward. The presence of a methyl group on carbon 1 increased the activity of compound **7** (33% for compound **12** versus 53% for compound **7**). Reduction of the keto group of **7** decreased the activity (53% inhibition for **7** versus 39% for **9**), but in the case of **8** an increase in activity was observed (35% for **8** versus 56% for **10**). The presence of bromine (**11**) and xanthate (**13**) had no significant effect on the activity of these oxabicyclic compounds. The 8-oxabicyclo[3.2.1]oct-6-en-3-one (**15**), at the concentration of 100 μg mL^{-1}, had no effect on root development or on the germination rate of sorghum (Barbosa et al., 1997), which suggests that the isopropyl group is essential for biological activity. None of the compounds tested had any significant effect on the seed germination rate of the two test species. Although the activities presented by the compounds tested were weak, the selective effect observed on the root growth of monocotyledon and dicotyledon should be further investigated. In conclusion, the chemistry described could be explored for the preparation of other bicyclic compounds closely related to helminthosporic acid.



11 **12** **13**



14 **15**

EXPERIMENTAL METHODS

Mass spectra were recorded under electron-impact (70 eV) and chemical ionization (NH_3) conditions using a G ZAB-E high-resolution spectrometer. Infrared spectra were obtained on a Mattson Instruments FTIR 3000. NMR spectra were recorded with a Bruker ACP 400 (400 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard. Coupling constants (J) are given in hertz. Flash chromatography was performed using Crosfield Sorbil C60 (40−60 μm), and the solvents used were purified according to the method of Perrin and Armarego (1988).

Synthesis. *3-Hydroxymethyl-2-methylfuran (**6**).* To a round-bottom flask containing THF (40 mL) and $LiAlH_4$ (1.92 g, 50 mmol) kept at 0 °C with and stirring was added methyl 2-methyl-3-furoate (Aldrich Chemical Co. Inc.) (7 g, 50 mmol) in THF (10 mL). The reaction mixture was stirred for 16 h,

water (4 mL) was then added, and the solid formed was removed by filtering through a Celite pad. The organic phase was extracted with dichloromethane (5 × 30 mL), dried over $MgSO_4$, and concentrated in a rotary evaporator. The yellow oil obtained was purified by flash chromatography (hexane/ethyl ether, 1:1) to afford the required product (**6**) as a yellow oil in 72% yield (4 g, 35,7 mmol): IR (film, cm^{-1}) ν_{max} 3600−3200, 3000, 2940, 2910, 2860, 1620, 1510, 1435, 1415, 1240, 1210, 1140, 1125, 1047, 1000, 940, 895, 760, 730; ^1H NMR ($CDCl_3$) δ 7.20 (d, 1H, $J_{5,4} = 2.0$, H5), 6.30 (d, 1H, $J_{4,5} = 2.0$, H4), 4.38 (s, 2H, C**H**$_2$OH), 2.60 (bs, 1H, CH$_2$O**H**), 2.25 (s, 3H, C**H**$_3$-2).

*4α-Isopropyl-1-methyl-8-oxabicyclo[3.2.1]oct-6-en-3-one (**7**) and 7-(Hydroxymethyl)-4α-isopropyl-1-methyl-8-oxabicyclo-[3.2.1]oct-6-en-3-one (**8**).* Diethylzinc (1 M in hexane, 6 mL, 6 mmol) was added to a solution of 1,1,3,3-tetrabromo-4-methylpentan-2-one (**4**) (2.08 g, 5 mmol) and 2-methylfuran (410 mg, 5 mmol) in dry benzene (60 mL), maintained under nitrogen at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and then at room temperature for 28 h. The reaction was quenched by addition of aqueous saturated ammonium chloride (50 mL). The solid formed was removed by filtration through a Celite pad, and the filtrate was extracted with dichloromethane (3 × 30 mL). The organic phase was dried over $MgSO_4$, and the solvent was removed in a rotary evaporator. The oil obtained was dissolved in a saturated methanolic solution of ammonium chloride (50 mL), and freshly prepared Zn/Cu (see below) couple (2 g, 31 mmol) was added (Barbosa, 1991). After 3 h of stirring at room temperature, the solid was removed by filtration through a Celite pad. Saturated Na_2EDTA (40 mL) was added to the filtrate, and the product was extracted with dichloromethane (3 × 40 mL). The organic phase was dried over $MgSO_4$ and concentrated in a rotary evaporator to yield a brown oil. This oil was purified by flash chromatography (hexane/ethyl ether, 6:1) to afford the required cycloadduct (**7**) as a pale yellow oil in 74% yield (670 mg, 3.7 mmol).

The same procedure was used to produce compound **8** as a pale yellow oil in 54% yield (570 mg, 2.75 mmol).

Preparation of Zn/Cu Couple. To a hot solution of cupric acetate monohydrate (0.5 g) in glacial acetic acid (50 mL) was added granular zinc (30 g). The mixture was shaken for 3 min. The acetic acid was removed, and the Zn/Cu couple was washed with glacial acetic acid (50 mL) and with diethyl ether (3 × 50 mL).

*Data for **7**:* IR (film, cm^{-1}) ν_{max} 3080, 2960, 2870, 1715, 1465, 1380, 1370, 1340, 1180, 1083, 1023, 880, 840, 790, 745; ^1H NMR ($CDCl_3$) δ 6.15 (dd, 1H, $J_{6,7} = 6.0$, $J_{6,5} = 1.6$, H6), 6.00 (d, 1H, $J_{7,6} = 6.0$, H7), 5.05 (dd, 1H, $J_{5,4} = 4.4$, $J_{5,6} = 1.6$, H5), 2.53 (dd, 1H, $J_{4,9} = 7.0$, $J_{4,5} = 4.4$, H4), 2.50 (d, 1H, $J_{2\beta,2\alpha} = 14.1$, H2$\beta$), 2.32 (d, 1H, $J_{2\alpha,2\beta} = 14.1$, H2α), 2.04 (oct, 1H, $J_{9,4} = J_{9,10} = J_{9,11} = 7.0$, H9), 1.48 (s, 3H, C**H**$_3$-12), 1.04 (d, 3H, $J_{11,9} = 7.0$, C**H**$_3$-11), 0.89 (d, 3H, $J_{10,9} = 7.0$, C**H**$_3$-10); ^{13}C NMR ($CDCl_3$) δ 206.9 (C3), 136.8 (C7), 132.3 (C6), 84.4 (C1), 80.0 (C5), 62.0 (C4), 52.1 (C2), 24.5 (C9), 22.9 (C12), 22.5 (C11), 20.0 (C10); HRMS, *m/z* (%) 180.1146 (M^+, $C_{11}H_{16}O_2$ requires 180.1146, 11), 178 (13), 151 (12), 123 (19), 111 (39), 95 (35), 85 (23), 69 (11), 57 (22), 43 (100), 41 (29).

*Data for **8**:* IR (film, cm^{-1}) ν_{max} 3600−3200, 2958, 2930, 2865, 1700, 1470, 1390, 1370, 1330, 1198, 1065, 1040, 965, 948, 878, 855, 800; ^1H NMR ($CDCl_3$) δ 5.98 (bs, 1H, H6), 4.97 (bd, 1H, $J_{5,4} = 4.8$, H5), 4.13 (d, 1H, $J_{13',13} = 14.9$, H13′), 4.24 (d, 1H, $J_{13,13'} = 14.9$, H13), 3.00 (bs, 1H, O**H**), 2.60−2.40 (m, 1H, H4), 2.54 (d, 1H, $J_{2\beta,2\alpha} = 14.9$, H2$\beta$), 2.45 (d, 1H, $J_{2\alpha,2\beta} = 14.9$, H2α), 2.00 (oct, 1H, $J_{9,4} = J_{9,10} = J_{9,11} = 7.0$, H9), 1.46 (s, 3H, C**H**$_3$-12), 1.03 (d, 3H, $J_{11,9} = 7.0$, C**H**$_3$-11), 0.87 (d, 3H, $J_{10,9} = 7.0$, C**H**$_3$-10); ^{13}C NMR ($CDCl_3$) δ 208.0 (C3), 149.2 (C7), 126.5 (C6), 84.8 (C1), 78.6 (C5), 61.3 (C13), 58.0 (C4), 52.4 (C2), 24.5 (C9), 22.6 (C12), 21.6 (C11), 19.9 (C10); HRMS, *m/z* (%) 210.1251 (M^+, $C_{12}H_{18}O_3$ requires 210.1254, 25), 192 (7), 167 (14), 139 (9), 111 (100), 95 (22), 69 (44), 43 (36).

*4α-Isopropyl-1-methyl-8-oxabicyclo[3.2.1]oct-6-en-3-ol (**9**) and 7-(Hydroxymethyl)-4α-isopropyl-1-methyl-8-oxabicyclo[3.2.1]-oct-6-en-3-ol (**10**).* Sodium borohydride (114 mg, 3 mmol) was added to a solution of ketone **7** (540 mg, 3 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 48 h. After this period, water (4 mL) was added and the solid formed was removed by filtration through a Celite pad. The filtrate was extracted with dichloromethane (5 × 40 mL). The organic phase was dried over $MgSO_4$ and concentrated in rotary evaporator to yield a yellow oil. This oil was purified by flash chromatography (hexane/ethyl ether, 4:1) to afford the required alcohol **9** in 82% yield (450 mg, 2.46 mmol) as a yellow oil.

The same procedure was used to produce the diol **10**, as a yellow oil in 90% yield (560 mg, 2.7 mmol).

*Data for **9**:* IR (film, cm^{-1}) ν_{max} 3550−3200, 3070, 2960, 2930, 2870, 1460, 1378, 1368, 1348, 1160, 1078, 1020, 1010, 970, 880, 745, 735, 695; ^1H NMR ($CDCl_3$) δ 6.42 (d, 1H, $J_{6,7} = 5.9$, H6), 6.22 (d, 1H, $J_{7,6} = 5.9$, H7), 4.79 (bs, 1H, H5), 4.01 (m, 1H, H3), 2.20−1.50 (m, 5H, H2α, H2β, H4, H9, O**H**), 1.37 (s, 3H, C**H**$_3$-12), 1.07 (d, 3H, $J_{11,9} = 6.3$, C**H**$_3$-11), 0.98 (d, 3H, $J_{10,9} = 6.3$, C**H**$_3$-10); ^{13}C NMR ($CDCl_3$) δ 139.8 (C7), 134.8 (C6), 82.8 (C1), 80.9 (C5), 67.0 (C3), 49.9 (C4), 42.5 (C2), 25.8 (C12), 23.1 (C9), 21.3 (C11), 20.5 (C10); HRMS, *m/z* (%) 182.0123 (M^+, $C_{11}H_{18}O_2$ requires 182.0123, 8), 149 (14), 137 (35), 124 (68), 109 (30), 95 (84), 82 (78), 71 (41), 43 (100).

*Data for **10**:* IR (film, cm^{-1}) ν_{max} 3600−3200, 3040, 2950, 2920, 2860, 1500, 1375, 1270, 1155, 1130, 1040, 1015, 895, 875, 840, 740; ^1H NMR ($CDCl_3$) δ 6.19 (s, 1H, H6), 4.74 (bs, 1H, H5), 4.30 (d, 1H, $J_{13',13} = 12.7$, H13′), 4.12 (bs, 1H, H3), 4.08 (d, 1H, $J_{13,13'} = 12.7$, H13), 2.05−1.92 (m, 2H, H2α, H2β), 1.75−1.50 (m, 2H, H4, H9), 1.32 (s, 3H, C**H**$_3$-12), 1.02 (d, 3H, $J_{11,9} = 6.5$, C**H**$_3$-11), 1.00 (bs, 2H, 2O**H**), 0.99 (d, 3H, $J_{10,9} = 6.5$, C**H**$_3$-10); ^{13}C NMR ($CDCl_3$) δ 148.4 (C7), 130.3 (C6), 82.6 (C1), 79.3 (C5), 66.6 (C3), 57.9 (C13), 48.6 (C4), 41.5 (C2), 25.6 (C9), 22.1 (C12), 21.3 (C11), 20.5 (C10); HRMS, *m/z* (%) 212.1408 (M^+, $C_{12}H_{20}O_3$ requires 212.1408, 25), 181 (15), 179 (6), 167 (15), 151 (21), 137 (16), 124 (50), 109 (35), 95 (44), 83 (23), 69 (13), 55 (27), 43 (100), 41 (49).

Biological Assay. Plant growth regulatory activity assay was carried out as described by Einhellig et al. (1983) with seeds of *S. bicolor* L. (cultivar BR303 obtained from the Centro Nacional de Pesquisa de Milho e Sorgo, at Sete Lagoas, MG, Brazil) and *C. sativus* L. (cultivar Caipira obtained from the market). Dichloromethane solutions of compounds **7**−**14** were prepared at concentrations of 100 μg mL^{-1}. The experiments were conducted in 100 × 15 mm glass Petri dishes lined with one sheet of filter paper and sealed with Parafilm. To each dish was added 2 mL of each solution, and the solvent was evaporated before addition of 2 mL of water, followed by 20 seeds, previously surface sterilized by soaking for 10 min in a 20% bleach solution of one of the two species. Assays were carried out at 25 °C under fluorescent light (8 × 40 W) in an incubator for 3 days. Radicle length was measured and total germination recorded. Seeds were considered to have germinated if a radicle protruded at least 1 mm. Controls were included under the same conditions, using only water. Each bioassay was replicated four times in a completely randomized design. The data were analyzed using Tukey's test (Gomes, 1990).

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LITERATURE CITED

Barbosa, L. C. A. A new synthetic approach to the pseudoguaianolide skeleton via oxyallyl cation chemistry. Ph.D. Thesis, University of Reading, 1991.

Barbosa, L. C. A.; Mann, J. The use of diethylzinc for the generation of oxyallyl carbocation from polybromo ketones and their reactions with substituted furans. *Synthesis* **1996**, 31−33.

Barbosa, L. C. A.; Demuner, A. J.; Mann, J.; Pilo-Veloso, D. Novel rearrangement of 2α-isopropyl-8-oxabicyclo[3.2.1]oct-

6-ene producing the monoterpene 3-hydroxyphellandral. *J. Chem. Soc., Perkin Trans. 1* **1993**, 585−587.

Barbosa, L. C. A.; Demuner, A. J.; Borges, E. E. L.; Mann, J. Synthesis and evaluation of the plant growth regulatory activity of 8-oxabicyclo[3.2.1]oct-6-en-3-one derivatives. *J. Braz. Chem. Soc.* **1997**, *8*, 19−27.

Briggs, D. E. Gibberellin-like activity of helminthosporol and helminthosporic acid. *Nature* **1966**, *210*, 418−419.

Copping, L. G. *Crop Protection Agents from Nature. Natural Products and Analogues*; Royal Society of Chemistry: Cambridge, U.K., 1996.

De Mayo, P.; Spencer, E. Y.; White, R. W. Helminthosporal, the toxin from *Helminthosporium sativum*: isolation and caracterization. *Can. J. Chem.* **1961**, *39*, 1608−1612.

Demuner, A. J. Síntese de derivados do ácido 6α,7β-dihidroxivouacapan-17β-óico e de análogos do ácido helmintospórico, visando estudos de atividades analgésica e reguladora do crescimento de plantas. Ph.D. Thesis, University of Belo Horizonte, 1996.

Demuner, A. J.; Barbosa, L. C. A.; Pilo-Veloso, D.; Ferreira-Alves, D. L.; Howarth, O. W. Structure and plant growth regulatory activity of new diterpenes from *Pterodon polygalaeflorus*. *J. Nat. Prod.* **1996**, *59*, 770−772.

Demuner, A. J.; Barbosa, L. C. A.; Pilo-Veloso, D. Cicloadições [3+4] via cátions oxialílicos: aplicações em sínteses orgânicas. *Quim. Nova* **1997**, *20*, 18−29.

Einhellig, F. A.; Schan, M. K.; Rasmunsen, J. A. Synergistic effects of four cinnamic acid compounds on grain sorghum. *Plant Growth Regul.* **1983**, *1*, 251−258.

Fairclough, B. Pesticide chemistry. *Chem. Int.* **1994**, *16*, 210−214.

Godfrey, C. R. A. *Agrochemicals from Natural Products*; Dekker: New York, 1995.

Gomes, F. P. *Curso de Estatística Experimental*, 13th ed.; Nobel: Piracicaba, 1990.

Mander, L. N.; Turner, J. V.; Coombe, B. G. Synthetic plant growth regulators. I. The synthesis of (±)-14-norhelminthosporic acid and related compounds. *Aust. J. Chem.* **1974**, *27*, 1985−2000.

Mann, J. The synthetic utility of oxyallyl cations. *Tetrahedron* **1986**, *42*, 4611−4659.

Mann, J.; Holland, H. J. Cycloaddition reactions of polysubstituted furans with oxyallyl carbocations. *Tetrahedron* **1987**, *43*, 2533−2542.

Mann, J.; Overton, H. J. Synthetic utility of oxyallyls: preparation of an analogue of helminthosporic acid. *Tetrahedron Lett.* **1985**, *26*, 6133−6136.

Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, U.K., 1988.

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