Synthesis and Evaluation of the Plant Growth Regulatory Activity of 8-oxabicyclo[3.2.1]oct-6-en-3-one Derivatives

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Received: December 7, 1995; September 18, 1996

A síntese de vários análogos do 8-oxabicyclo[3.2.1]oct-6-en-3-ona é relatada. O efeito desses compostos e do ácido 4-oxoexanoico sobre a germinação e crescimento radicular do *Sorghum bicolor* foi avaliado. Na concentração de 100 ppm os compostos 3-(metoxycarbonilmetil)-8-oxabicyclo[5.3.0]dec-4-eno-2,9-diona (13) e ácido 4-oxoexanoico (17) apresentaram efeito estimulador do crescimento radicular de 33-35% e a 1000 ppm um efeito inibitório foi observado em ambos os casos (29% (13) e 80.2% (17)). Todos os outros compostos inibiram o crescimento radicular a 100 e 1000 ppm. Nenhum efeito significativo foi observado sobre a taxa de germinação.

The synthesis of several analogues of 8-oxabicyclo[3.2.1]oct-6-en-3-one is reported. The effect of these compounds and 4-oxohexanoic acid on the germination and radicle growth of *Sorghum bicolor* was evaluated. At 100 ppm compounds 3-(methoxycarbonylmethyl)-8-oxabicyclo[5.3.0]dec-4-eno-2,9-dione (13) and 4-oxohexanoic acid (17) showed 33-35% stimulatory radicle growth, and at 1000 ppm a 29% (13) and 80.2% (17) inhibition was observed. All the other compounds showed an inhibitory effect on the radicle growth at 100 and 1000 ppm. None of the compound had a clear effect on the germination rate.

**Keywords:** germination, growth inhibition, lactones, [3+4] cycloaddition

**Introduction**

A number of sesquiterpene lactones affect plant growth, although the nature and extent of the effects produced depend on a number of factors, including the lactone tested, its concentration, and the species on which it acts\(^1\). Some sesquiterpene lactones have been reported to be responsible for the allelopathic properties of certain plants by affecting the germination and growth of other species\(^2\). The potential allelopathic activity of several natural and synthetic sesquiterpene lactones has been investigated and the presence of an α-methylene-β-butyrolactone has been shown to be important for the biological activity\(^3\). The presence of other reactive centres such as α,β-unsaturated ketone, chlorohydrins, epoxide, hemiacetal, and also the molecules spatial arrangement is normally important for the biological activity presented by those lactones\(^4\).\(^5\).\(^6\).

As part of our research on the synthesis of new compounds with herbicidal and/or plant growth regulatory activity, derived from the easily available 8-oxabicyclo[3.2.1]oct-6-en-3-ones \(^1\), we devised a plan that would allow the preparation of several lactones 3-6 for biological evaluation\(^3\) (Scheme 1).

**Experimental**

**Synthesis**

IR spectra were recorded on a Perkin-Elmer 881 double beam grating spectrophotometer. NMR spectra were recorded on a Perkin-Elmer R34 (220 MHz) instrument, a Bruker WH 400 spectrometer (400 MHz) or on a Varian...
T-60 (60 MHz) instrument, using tetramethylsilane as internal standard. Mass spectra were obtained on a VG ZAB-E high resolution mass spectrometer. Flash chromatography was performed using Crofield Sorbsil C60 (40-60 µm). Solvents were purified according to Perrin and Armarego, and petroleum refers to the fraction with b.p. 40-60 °C, ether refers to diethyl ether.

8-Oxabicyclo[3.2.1]oct-6-en-3-one (7)

A two litre round bottomed flask was charged with freshly prepared Zn/Ag couple (48.83 g, 0.75 mol), furan (350 mL, 5 mol), and dry THF (150 mL). The flask was cooled down to -10 °C and a solution of 1,1,3,3-tetrabromoacetone (172 g, 460 mmol in 150 mL of THF) was added dropwise during 2 h under nitrogen atmosphere. The resulting solution was stirred at room temperature for 17 h. After this period of time the insoluble material was removed by filtration and the solution concentrated to a brown oily residue. This residue (62.3 g) was dissolved in a saturated methanolic solution of NH4Cl (1000 mL) and freshly prepared Zn/Cu couple (200.5 g, 3.1 mol) was added. This mixture was stirred at room temperature for 2 h, and then the solid was removed by filtration. The filtrate was divided in three portions and each one was diluted with a saturated solution of Na2EDTA (300 mL), followed by extraction with dichloromethane (4 x 300 mL). The combined extracts were dried (MgSO4) and concentrated to a brown oil, which was purified by flash chromatography (1:2 petrol:ether) to afford 46.5% (26.50 g, 213.7 mmol) of the required oxabicyclo-octane 7 as a pale yellow oil. This oil crystallized on standing, m.p.37-39 °C. IR (CHCl3) νmax: 3080, 2960, 2905, 1710, 1340, 1180, 945 and 710 cm−1; 1H-NMR (60 MHz, CDCl3) δ: 2.30 (d, 2H, J = 17 Hz, H-2α and H-4α), 2.80 (dd, 2H, J = 17 and J = 5 Hz, H-2β and H-4β), 5.10 (d, 2H, J = 5 Hz, H-1 and H-5) 6.30 (s, 2H, H-6 and H-7); MS m/z(%): 124(M+, 80), 95(10), 82(90), 81(100), 68(10), 54(10).

8-Oxabicyclo[3.2.1]octan-3-one (8)

8-oxabicyclo[3.2.1]oct-6-en-3-one 7 (26 g, 209 mmol) was dissolved in ethyl acetate (150 mL), in a Parr hydrogenation bottle and 10% Pd-C (1.5 g) was added as a catalyst. The reaction was carried out under 3.0 x 105 Pa of hydrogen pressure, for 5 h. When the hydrogen uptake ceased, the catalyst was filtered off through a Celite pad and the solvent evaporated under reduced pressure. The reduced product 8 was obtained as a yellow oil, in quantitative yield (26.33 g, 209 mmol). IR (thin film) νmax: 2940, 2840, 1720, 1470, 1410 and 1205 cm−1; 1H-NMR (220 MHz, CDCl3) δ: 1.72-1.84 (bd, 2H, J = 5 Hz, H-6endo and H-7endo), 2.00-2.18 (m, 2H, H-6exo and H-7exo), 2.29 (dm, 2H, J = 16 Hz, H-2α and H-4α), 2.72 (dd, 2H, J1 = 16 and J2 = 5 Hz, H-2β and H-4β), 4.75 (m, 2H, H-1 and H-5); MS m/z: 126.0672 (M+, C7H10O2 requires 126.0678).
3-Trimethylsilyloxy-8-oxabicyclo[3.2.1]oct-2-ene (9)

Trimethylsilylchloride (1.02 mL, 0.87 g, 8.06 mmol) was added via syringe to a stirred solution of ketone 8 (604.8 mg, 4.8 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1.24 g, 8.16 mmol) in DCM (15 mL), under nitrogen atmosphere. The stirred reaction mixture was refluxed at 55-60 °C for 1.5 h. After that period of time, the solution was cooled down to 0 °C, taken up in petroleum (100 mL), and washed with sat. aq. NaHCO₃ (3 x 30 mL), and with water (30 mL). The organic phase was dried (MgSO₄) and concentrated to leave a pale yellow oil. This oil was purified by distillation (1:1 ethyl acetate: petrol) to afford 9 as a pale yellow oil (896 mg, 4.52 mmol, 94% yield). This compound was used without any further purification. IR (thin film) νₘₐₓ: 3060, 2960, 2840, and 1660 cm⁻¹; ¹H-NMR (60 MHz, CDCl₃) δ: 0.95 (s, 9H, OSiMe₃), 2.70 (ddd, 1H, J₁₀',₁₀ = 16.5, J₁₀',₁ = 5.0 Hz, H-10'), 3.70-4.70 (m, 2H, H-1 and H-5), 5.00 (d, 1H, J₁ = 5.0 Hz, H-2).

2-(Methoxycarbonylmethyl)-8-oxabicyclo[3.2.1]octan-3-one (10a)

1.4 M MeLi in ether (3.4 mL, 4.76 mmol) was added to a stirred solution of 9 (895 mg, 4.52 mmol) in DME (20 mL), at -40 °C for 1.5 h, and then a solution of methyl bromoacetate (2.1 g, 13.73 mmol) in DME (10 mL), at -40 °C for 1.5 h, and then a solution of methyl 4-oxohexanoate (18) in 69% yield (3.19 g; 24.6 mmol; 90 °C/16 mmHg) and a small amount (5.2 g, 1.78 mmol; 65 °C/16 mmHg) of compound 16. Data for 17: IR (thin film) νₘₐₓ: 3500-2500, 1710, 1420, 1370, 1220, 1170, 1120, 960 and 840 cm⁻¹; ¹H-NMR (200 MHz, CDCl₃) δ: 1.20 (t, 3H, J = 7.0 Hz, CH₃), 2.50 (q, 2H, J = 7.0 Hz, CH₂), 2.60-2.80 (m, 4H, CH₂CH₂), 11.30 (bs, 1H, COOH); MS m/z (%): 131 (M + 1)⁺, 129(100), 108(45), 73(22), 57(95), 45(30).

Methyl 4-oxohexanoate (18)

To a solution of the acid 17 (2.56 g; 1.97 mmol) in dry methanol (50 mL), was added conc. H₂SO₄ (0.5 mL). The resultant solution stirred at room temperature for 18 h, before addition of an aqueous solution of NaHCO₃ (sat. 10 mL). The aqueous layer was extracted with DCM (3 x 30 mL). The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure to leave the required ester 18 in 88% yield (2.50 g; 17.4 mmol). No further purification was carried out. IR (thin film) νₘₐₓ:
Methyl 3,5-dibromo-4-oxohexanoate (19)

Bromine (2.0 g; 12.5 mmol) was added dropwise to a stirred iced-cooled solution of ketoester 18 (0.9 g; 6.25 mmol) in ether (10 mL) and HBr aq. (48%; 5 mL). After 72 h stirring at room temperature, diethyl ether (80 mL) and an aqueous solution of Na2S2O5 (10%, 30 mL) were added. The two phases were separated and the organic layer was washed with brine (2 x 30 mL), dried over MgSO4 and concentrated using a rotatory evaporator. The ketoester 19 (800 mg; 2.64 mmol) in dry acetonitrile (5 mL) was added, via a dropping funnel, during 10 min, at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 16 h. After that time the mixture was filtered and the filtrate was washed with brine (2 x 30 mL), dried over MgSO4 and concentrated under reduced pressure to leave the required product 20 as a white solid (210 mg, 1 mmol) in quantitative yield. No further purification was necessary. IR (thin film) νmax: 2953, 2840, 1738, 1711, 1440, 1360, 1270, 1200, 1180, 1160, 1050, 950 and 890 cm-1; 1H-NMR (220 MHz, CDCl3) δ: 0.96 (d, 3H, J = 6.5 Hz, CH3), 2.05 (dd, 1H, J1 = 16.9, J2 = 9.1 Hz, H-3), 2.20 (ddd, 1H, J1 = 7.0, J2 = 5.7, J3 = 4.2 Hz, H-2), 4.45 (m, 1H, H-8), 2.80 (bd, 1H, J1 = 9.1, J2 = 5.7 Hz, H-4), 4.53-4.60 (m, 2H, H-1 and H-6), 6.16 (dd, 1H, J1 = 6.2, J2 = 1.7 Hz, H-10), 6.38 (dd, 1H, J1 = 6.2, J2 = 1.8 Hz, H-8).

To a stirred solution of ketone 20a (210 mg, 1 mmol) in methanol (10 mL) was added NaBH4 (80 mg, 2 mmol). After stirring at room temperature for 4 h, the reaction was quenched with water (20 mL), and the product extracted with DCM (3 x 30 mL). The organic extract was dried over MgSO4 and concentrated under reduced pressure to an yellow oil. This oil was purified by column chromatography (petroleum/ether, 1:8) to afford the required lactone 22 in 59% yield as a white solid (210 mg, 1.2 mmol). This product was recrystallized from DCM:ether. A similar procedure was used to prepare lactone 23 from ketoester 21.

Data for 22: m.p. 121-122 °C. white solid. IR (CHCl3) νmax: 3083, 2972, 2930, 2887, 1758, 1598, 1460, 1424, 1369, 1200, 1109, 1049, 989 and 816 cm-1; 1H-NMR (400 MHz, CDCl3) δ: 1.05 (d, 3H, J = 7.0 Hz, CH3), 2.05-2.10 (m, 4H, H-2 and H-6), 2.57-2.60 (m, 2H, H-1 and H-5), 4.89 (dd, 1H, J1 = 4.0, J2 = 1.8 Hz, H-2), 3.71 (s, 3H, OCH3), 4.98 (dd, 1H, J1 = 4.0, J2 = 1.8 Hz, H-1), 6.32 and 6.38 (2x dd, 2H, J1 = 6.0, J2 = 1.8 Hz, H-6 and H-7); MS m/z(%): 211.0970 ([M+1]+, C11H15O4 requires 211.0961, 121(34), 105(17), 99(15), 85(22), 81(22), 73(5), 55(20).
Results and Discussion

Synthesis

The oxabicyclic ketone 7, has already been transformed into a number of natural products and their analogues[8,9], and we thought to explore further its chemistry by using it as starting material for the synthesis of sesquiterpene lactone 4 (R1 = H or CH3), according to the strategy shown on Scheme 1.

The ketone 7 was prepared on a large scale using Sato and Noyori’s methodology[10]. Catalytic hydrogenation of 7 using 10% Pd-C afforded the oxabicyclic ketone 8 in almost quantitative yield. The enol silyl ether 9 was prepared in about 95% yield using trimethylsilylchloride (TMSCl) in the presence 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The almost quantitative conversion of the parent ketone 8 into the required enol ether 9 was confirmed by the virtual disappearance of the carbonyl stretching at around 1715 cm⁻¹ (in the infrared spectrum) with concomitant appearance of a very strong band at ~1640 cm⁻¹ corresponding to the enol trimethylsilyl group.

The enol ether 9 was treated with methyl lithium and the enolate formed was trapped with methyl bromoacetate (Scheme 2). It was observed that when the enolate was generated at -78 °C/1.5 h, the reaction was incomplete resulting in 29% recovery of the starting enol ether. Raising the temperature to -40 °C resulted in complete transformation of 9 into the corresponding enolate. In general, typical overall yields for this alkylation was 30-45% for the monoalkylated compound 10a, 15-20% for the corresponding dialkylated methyl ester 10b and 20-30% recovery of the starting ketone 8. It was found that separation of the monoalkylated compound 10a and the dialkylated methyl ester 10b was extremely difficult, and 10b was not obtained in a pure form. Some reactions were carried out with 10a con-
taminated with 15-20% of 10b, as estimated by 220 MHz 1H-NMR.

Although a high degree of exo-stereoselectivity for this alkylation has been claimed, the complexity of the signals for H-1 and H-5 between δ 4.50 and δ 4.70 associated with two signals for methoxy group at δ 3.72 and δ 3.80 (ratio 5:1) showed that a considerable amount (~20%) of the endo-alkylated product was formed (the sample analysed was not contaminated with 10b as judged by the mass spectrum).

It was envisaged that the transformation of 10a into lactone 12 could be achieved via an intermediate like 11, formed by the cleavage of the ether bridge (Scheme 3).

The ketoester 10a was then submitted to a treatment with the following reagents in order to accomplish the ether cleavage: HBr (aq. 48%), hexadecyltributylphosphonium bromide, 48 h, 60 °C; BF3, KI, CHCl3, 144 h, 55 °C; Me6Si2I, C6H6, 65 °C, 72 h, dark; Me3SiCl, NaI, CH3CN, 70 °C, 72 h, dark; t-BuMe2SiCl, NaI, CH3CN, 65 °C, 120 h, dark; Me2BBr, TEA, DCM, 0 °C, 4h. In all these attempts no reaction or partial decomposition of the starting material, accompanied by the formation of several compounds was observed as judged by TLC analysis.

Another attempt to produce lactone 12 was made by treating compound 10a with trimethylsilyltrifluoromethanesulfonate (TMSOTf) and triethylamine (TEA) for two hours at room temperature. In this case all the starting material was consumed and a very complex mixture was formed. However when a mixture of 10a+10b was treated with TMSOTf/TEA, the only product isolated was the lactone 13 (Scheme 4).

The structure of the lactone 13 was deduced by spectroscopic means. In the high resolution mass spectrum, there was a peak at m/z 238.0827 corresponding to the proposed formula C12H14O5. The infrared spectrum showed a very strong absorption at 1781 cm⁻¹ due to the γ-butyrolactone, and another band at 1717 cm⁻¹ for the ketone superimposed with the ester group. Special features in the 13C-NMR spectrum are the absorptions at δ 172, 175 (lactone and ester), and δ 195 (ketone). Signals corresponding to three CH2, one CH3, and five CH were observed. The 220 MHz 1H-NMR spectrum showed a singlet at δ 3.72 for the methoxy group, and a multiplet at δ 5.80-5.90 for alkene protons (Fig. 1).

The formation of lactone 13 probably involves the intermediate 14, and it shows the feasibility of our initial synthetic proposal (Scheme 1).

The formation of a complex mixture of products from this reaction is probably due to the fact that keto ester 10a was a mixture of α and β-alkyl isomers and also because the cleavage of the ether bridge was not regioselective. A further investigation on the preparation of the 10b and its reaction under the conditions described should be carried out, since one can envisage the transformation of lactone 13 into a pseudoguaianolid skeleton 4.

Due to the problems with the stereoselective monoalkylation of 8 and purification of 10a, an alternative route leading to lactones 4 and 6 was investigated (Schemes 5 and 6).

Succinic anhydride was converted into the keto acid 17 in 69% yield. After methylation with CH3OH/H2SO4, the ester 18 formed was brominated with Br2/HBr to afford the required dibromoketone 19 in 42% yield.

The cycloaddition between the dibromoketoester 19 and furan was carried out in the presence of Cu/NaI. The re-
quired cycloadduct 20 was formed in 27% yield, and the ratio between αα- and ββ-isomers was around 4:1.

In order to accomplish the strategy presented on route 1, compound 20b was required, and since this was the minor isomer formed, we used the major isomer 20a to follow the synthesis according to route 2 (Scheme 1).

The bicyclic ketone 20a was treated with NaBH₄/MeOH and the intermediate formed by the reduction of the keto group reacted in an intramolecular fashion with the carbomethoxy group resulting in the formation of lactone 22 in 59% yield (Scheme 6).

The hydrogenation of the oxabicyclo 20a followed by similar treatment with NaBH₄/MeOH led to the isolation of the lactone 23 in 50% yield.

Work is now in progress to transform compounds 22 and 23 into more complex and functionalized lactones.

**Herbicidal Activity**

The discovery of new herbicides usually involves the following approaches: i) the rational design of specific inhibitors of key metabolic processes; ii) analogue synthesis of compounds with known herbicidal activity and iii) the random screening of new chemicals.

Although in this work we planned to make use of strategy ii), by developing a synthetic route for the preparation of several sesquiterpene lactones, having an α,β-unsaturated carbonyl group, we decided to carry out a random screening on several synthetic intermediates (strategy iii).

For this screening the in vivo effect of compounds 7, 13, 17, 20a, 22 and 23 on the germination and radicle growth of Sorghum bicolor was evaluated according to the methodology proposed by Enhelling et al. Two concentrations (100 and 1000 ppm) of each compound were tested, since it has already been shown that some compounds exhibited both stimulatory and inhibitory effects on seedling growth, depending on the concentration.

Figure 2 shows the radicle length (mm) of Sorghum after 3 days incubation at 25 °C and the percentage of radicle growth (inhibition or stimulation) in relation to the control is presented in Table 1. At 100 ppm all compounds showed a considerable inhibitory effect on the radicle growth, especially the ketoacid 17, that caused a 80% inhibition. As compound 17 showed a remarkably different effect on plant development at lower and higher concentration and since it can be easily prepared, it becomes an interesting
starting material for the preparation of other products for biological evaluation. Although the lactone 13 showed a similar effect on radicle growth as 17, its preparation is more laborious and this makes further biological evaluation less appealing. Compound 7 showed no clear effect at 100 ppm and 34% inhibition at a 1000 ppm.

Compound 20a (at 100 ppm) was 26 times more active than its simple analogue 7, and this effect can be attributed to the presence of the substituents at the 2 and 4 positions.

In view of these results and due to the versatility of the [3+4] cycloaddition methodology used for the preparation of compounds 7 and 20a, the synthesis of other oxabicyclic compounds like 7 having different substituents at various positions is now our next goal. Also an investigation of the herbicidal selectivity of the compounds already discussed towards a wide range of crops and weeds is underway and will be published elsewhere.

Acknowledgments

We thank the Brazilian Agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Su-
perior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

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