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Article

Synthesis of a New Strigol Analogue from Natural Safrole

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O alil-benzeno natural safrol, isolado do óleo de Sassafraz (*Ocotea pretiosa*, Benth) foi utilizado como matéria-prima na síntese de um novo análogo aromático do estrigol que é um potente estimulante de germinação. O novo análogo foi obtido como uma mistura de epímeros em C2' que foram quantificados e separados por cromatografia líquida de alta eficiência.

Safrole, isolated from Sassafraz Oil (*Ocotea pretiosa*, Benth), has been used as starting material in the synthesis of a new strigol analogue which is active as germination stimulant. The new analogue was obtained as a mixture of epimers at C2' which could be separated by high-performance liquid chromatography.

Keywords: strigol analogue, germination stimulant, safrole

Introduction

Strigol (**1**, Figure 1) is a highly potent stimulant for the germination of seeds of parasitic weeds belonging to the genera *Striga* and *Orobanche* (the application of 10^{-11} mol L^{-1} solutions of strigol is reported to result in over 50% of germination). Strigol was isolated in 1966 by Cook and co-workers[1] from the root exudates of cotton (*Gossypium hirsutum*, L.) and its chemical structure was elucidated in 1972.[2] Considerable effort has been put into the development of strategies for the total synthesis of strigol and for the preparation of new analogues which have a simpler structure but which have retained an apreciable biological activity. Recently, several reports appeared in the literature involving studies on chemical, spectrometric and stereochemical aspects of strigol as well as of its analogues, which evidence the great interest arisen by this substance.[3]

Compounds **2-4** (Figure 1) were synthesized and biologically evaluated by Johnson and co-workers[4] and the results obtained suggest that the portion of the strigol molecule which is primarily responsible for the bioactivity resides in the CD moiety. Mangnus et al.[5] have carried out a systematic structure-activity relationship study on strigol analogues and the results obtained suggest that when additional rotational freedom is introduced in the analogues, by opening of ring C (**5**) or at a lesser extent by opening ring B (**6**), the bioactivity is negatively influenced.

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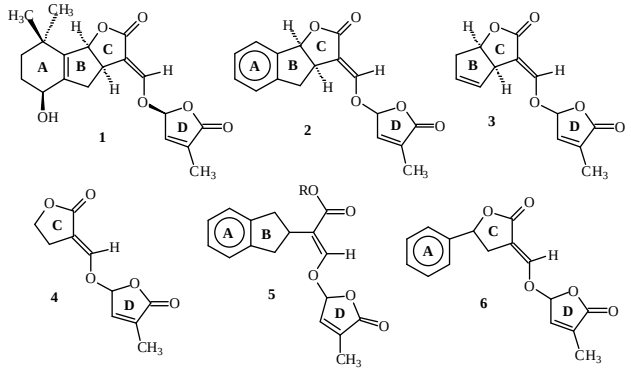
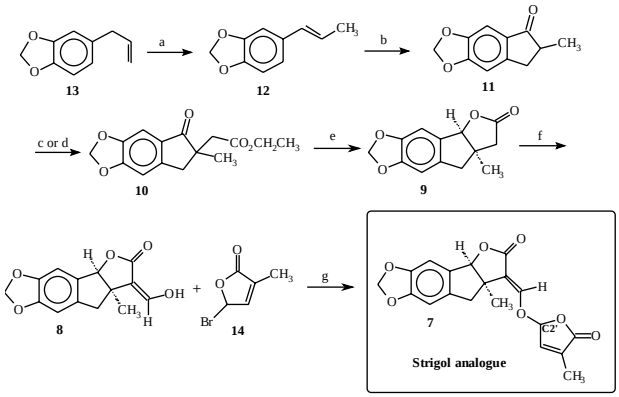


Figure 1. Structures of strigol 1 and synthetic analogues **2-6**.

Results and Discussion

Based on the results presented above, we proposed the synthesis of a new strigol analogue **7** (Scheme 1) using safrole, the natural allylbenzene **13** (isolated from *Ocotea pretiosa* Benth)[6] as starting material. The key step in our synthetic approach is the cyclization reaction of isosafrole **12** (obtained quantitatively from natural safrole by a base mediated isomerization reaction).[7] This cyclization takes place with concomitant homologation of one carbon atom and was originally reported by Witiak[8] and modified by Barreiro and Lima[7] giving the substituted indanone **11** in 70% overall yield from **13** (Scheme 1). The alkylated intermediate **10** was obtained by formation and reaction of the enolate of ketone **11** with ethyl bromoacetate. Although we[9] and others[10] described the use of LDA as a base for enolisation of **11**, best results were obtained employng NaH

as the base which implies in 70-80% yield and simpler manipulations. The next step in the planned synthetic route involves the reduction with sodium borohydride[11] of the sodium salt obtained by hydrolysis of keto-ester **10**, followed by treatment of the reaction mixture with acid (pH=2), affording lactone **9** in 80% yield. The benzylic alcohol obtained from **10** is a mixture of *cis* and *trans* isomers, but the only product detected after acidification is **9** which arises from usual lactonization of the *cis* isomer in acidic medium and from *trans* isomer *via* the intramolecular displacement of the protonated hydroxyl group by the carboxyl oxygen[12]. Lactone **9** was converted to its potassium enolate by treatment with *t*-BuOK in THF, and formylated using ethyl formate yielding the hydroxymethylene lactone **8** in 60% yield, after acidification of the mixture. Reaction of the potassium salt of **8** with bromobutenolide **14** in tetrahydrofuran afforded the desired product **7** as a mixture of epimers (45:50) at C2', in 50% yield (Scheme 1). Bromobutenolide **14** was easily prepared though allylic bromination[13] of the corresponding butenolide wich is commercially available. The *E* stereochemistry for the enol ether double bond of **7** is supported by the presence of signals of proton resonances in the NMR spectrum at δ 7.38 and 7.40 ppm. These are essentially the same as those reported for strigol (δ 7.42 ppm)[2] and different from those of analogues having ***Z*** stereochemistry (δ 6.6-6.8ppm)[13].



a) KOH, *n*-butanol, Δ, 3h; b) $POCl_3$, DMF, 0º C, then 110ºC, isosafrole 12, 110º C, 3h; then NaOH aq., 12h, 25ºC c) LDA, THF, - 78ºC, then $BrCH_2CO_2CH_2CH_3$, -78 → 20ºC, 8h; d) NaH, DMF, 60ºC, 20h; then $BrCH_2CO_2CH_2CH_3$ e) $NaOH/H_2O$, 3h, 25ºC, then $NaBH_4$, 65 h; then HCl, overnight f) *t*-BuOK, THF, 25ºC, 1h, then $HCO_2CH_2CH_3$, one day; g) *t*-BuOK, THF, then bromobutenolide **14**, 25ºC, 2 days.

Scheme 1. Synthetic route for the new strigol analogue **7**.

Although the spectral characteristics of isomers **7** at C2' (Scheme 1) are essentially identical, their retention times in HPLC are different. A method for the quantitation and separation of epimers **7** using normal-phase high performance liquid chromatography was developed. The quantitative determination (45:50) of the isomers was achieved on a silica analytical column using a elution gradient system at room temperature. For complete separation of the diastereoisomers a semi-preparative silica column was used under the same conditions. The relative stereochemistry of two epimers at C2' was not assigned.

Experimental

The ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 200. The IR spectra were obtained with a Perkin-Elmer 1420 spectrometer. The mass spectra were were obtained with a HP-QP-2000A computer system and the high resolution analysis in an Autospec Micromass-EBE-High resolution. The purity of compounds was observed by ^1H and ^{13}C NMR spectral analysis by gas chromatography and by TLC (Merck 60F 254) visualised with UV lamp (254 nm). The melting points were obtained with a Büchi-Tottoli apparatus and are uncorrected. The HPLC analysis were carried out on a Shimadzu LC-10 AS Chromatograph, with a SPD-10 A UV detector and the CG analysis with a Varian chromatograph mod. 2400.

3,4-methylenedioxy-propenylbenzene (**12**)[7]

To 9.1 mL (10g, 62 mmol) of natural allyl benzene **13** were added 50 mL (150 mmol) of a 3 mol L^{-1} solution of potassium hydroxide in *n*-butanol and the reaction mixture was stirred under reflux for 3 h. After cooling, the mixture was poured into a solution of 3 mL of concentrated hydrochloric acid and 20 mL of ice water. After neutralization the organic layer was washed with water and dried with anydrous Na_2SO_4. After removal of the excess *n*-butanol, the residue obtained was distilled under reduced pressure giving 9.5 g (95%) of the styrene derivative **12** as a colorless oil. The spectroscopic data obtained from an analytical sample are identical to those previously reported[7].

(2-Methyl-5,6-methylenedioxy-1-oxa)-indane (**11**)[7]

To 5.2 mL (4.9g, 66.6 mmol) of dry dimethylformamide 1.2 mL (1.97g, 12.9 mmol) of $POCl_3$ was added in a dropwise manner with stirring at 0ºC and under N_2 atmosphere. The ice bath was removed and the mixture was stirred for 15 min at room temperature. The mixture was then stirred in an oil bath at 110-120ºC when 1.5 mL of the styrilbenzene **12** (1.37g, 9.26 mmol) was added dropwise and the reaction mixture was stirred at 110-120ºC for 3 h. The resulting black mixture was poured into ice water and extracted twice with 10 mL of diethyl ether. The aqueous layer was basified by addition of a 7 mol L^{-1} aqueous NaOH solution and stirred over-night at room

temperature. The reaction mixture was extracted three times with 15 mL of chloroform. The organic layer was washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The residue obtained was purified by column chromatography [eluent: hexane-ethyl acetate (9:1)] giving 1.2g (70%) of ketone **11** as an yellow pale solid. mp: 64-65ºC (lit.: 63-63.5ºC).[7,8] IR $\nu_{max}(cm^{-1})$ (KBr): 1688.8, 936.2, 853.0 and 807.0. ^1H NMR ($CDCl_3$, 200 MHz): δ 6.95 (s,1H), 6.69 (s,1H), 5.96 (s, 2H), 3.24 (dd, 1H, *J* 16.57, 7.13 Hz), 2.61-2.58 (m, 2H) and 1.18 (d, 3H, *J* 7.13 Hz). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 207.16 (C=O), 154.01 (C), 150.70 (C), 147.95 (C), 130.45 (C), 105.33 (CH), 103.75 (CH), 101.95 (CH_2), 42.15 (C), 34.64 (CH_2), 16.33(CH_3). MS (70 eV): 190 ($M^{+\cdot}$ 70%), 175 (100%), 162 (8%), 147 (17%), 131 (11%), 103 (17%), 89 (7%), 77 (19%), 63 (16%) and 51 (17%).

Ethyl (5,6-methylenedioxy-2-methylindan-2-yl) acetate (**10**)

To a suspension of 0.172g (3.57 mmol) of NaH (50% in mineral oil) in 10 mL of DMF at room temperature was added dropwise a solution of 0.452g (2.37 mmol) of ketone **11** with stirring. The resulting mixture was stirred in an oil bath at 65ºC for 1h, followed by addition of 0.53 mL (0.079g, 4.74 mmol) of ethyl bromoacetate. The mixture was stirred at 65ºC for an additional 1h and then poured into 20 mL of ice water; it was then extracted with diethyl ether (3 x 10 mL). The organic layer was washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The residue obtained was purified by flash-chromatography [eluent: hexane-ethyl acetate (9:1)] giving 0.38g (70%) of the keto ester **10** as a viscous yellow oil. IR $\nu_{max}(cm^{-1})$ (film): 1729.7, 1701.2, 936.4, 869.9 and 805.6. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.01 (s, 1H), 6.77 (s, 1H), 6.03 (s, 2H), 3.98 (q, 2H, *J* 6.9 Hz), 3.17 (d,1H, *J* 17.0 Hz), 2.80 (d, 1H, *J* 17.0 Hz), 2.75 (d, 1H, *J* 16.16 Hz), 2.57 (d, 1H, *J* 16.16 Hz); 1.18 (s, 3H) and 1.10 (t, 3H, *J* 6.9 Hz). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 207.13 (C=O), 171.20 (C=O), 154.34 (C), 149.58 (C), 148.19 (C), 129.64 (C), 105.67 (CH), 102.77 (CH), 102.07 (CH_2), 60.36 (CH_2), 47.13 (C), 41.64 (CH_2), 40.10 (CH_2), 24.78 (CH_3) and 13,98 (CH_3). MS (70 eV): 276 ($M^{+\cdot}$ 36%), 231 (14%), 188 (100%), 175 (5%), 145 (11%) and 115 (13%).

cis-5,6-methylenedioxy-2-methylindan-2-yl acetic acid γ-lactone (**9**)

A solution of 1.62 g (6.98 mmol) of the keto ester 10 in 24 mL 1 mol L^{-1} NaOH (water/methanol; 1:1) was stirred at room temperature for 3h. Then 0.36g (9.51 mmol) of $NaBH_4$ was added to the mixture and it was stirred for 65h at room temperature. The solution was cooled in ice and acidified to pH 2 with the addition of concentrated hydrochloric acid and stirred for additional 8 h. After this time white crystals were deposited and filtered giving 1.39g (80%) of γ-lactone **9** in a very pure form. mp:139-141ºC. IR $\nu_{max}(cm^{-1})$ (KBr): 1761.6, 942.2, 880.6 and 852.8. ^1H NMR ($CDCl_3$, 200 MHz): δ 6.82 (s, 1H), 6.62 (s, 1H), 5.94 (m, 2H), 5.28 (s, 1H); 2.99 (d, 1H, *J* 16.07 Hz), 2.84 (d, 1H, *J* 16.07 Hz), 2.63 (d, H, *J* 17.73 Hz), 2.52 (d, H, *J* 17.73 Hz) and 1,38 (s, 3H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 176.26 (C=O), 149.34 (C), 147.36 (C), 135.84 (C), 131.09 (C), 105.95 (CH), 105.14 (CH), 101.31 (CH_2), 92.78 (CH), 46.55 (C), 44.22 (CH_2), 42.48 (CH_2) and 24.59 (CH_3). MS (70 eV): 232 ($M^{+\cdot}$ 47%); 203 (3%); 188 (16%); 173 (100%); 143 (19%) and 129 (11%).

Preparation of ***7***

To a stirred solution of 0.35g (3.02 mmol) of potassium *tert*-butoxide in 7 mL of dry THF at 0ºC under argon atmosphere was added dropwise a solution of 0.50g (2.16 mmol) of γ-lactone **9** in 5 mL of dry THF. The mixture was stirred for 30 min at 0ºC when 0.3 mL (0.275g, 3.6 mmol) of ethyl formate was added. After 48 h, the reaction mixture was evaporated under reduced pressure and 20 mL of iced dichloromethane was added to the residue. This was filtered and washed with iced dichloromethane (2 x 5 mL) giving 0.307g (65%) of the enol intermediate **8** as a white solid. This product was used directly in the next step without further purification (mp: 172-174ºC).

To a stirred solution of 0.307g (0.17 mmol) of hydroxymethylene lactone **8** and 0.184g (1.63 mmol) of potassium *tert*-butoxide in 15 mL of dry THF under argon atmosphere was added 0.29g (1.63 mmol) of bromobutenolide **14**[11] and the reaction mixture was stirred for 48h at room temperature. Then the mixture was poured into 25 mL of water and extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The residue obtained was filtered on a silica gel column (eluent: dichlorometane) giving 0.153g (50%) of a mixture of epimers of **7** as a white solid. mp: 200-220ºC. Exact mass: 356.0896 (calculated); 356.08955 (found). IR $\nu_{max}(cm^{-1})$ (KBr): 1783.5, 1747.0, 1675.5 and 957.0. MS (70 eV): 356 ($M^{+\cdot}$ 34%), 297 (1%), 259 (15%), 213 (41%), 187 (24%), 129 (9%) and 97 (100%).

The mixture of diastereoisomers **7** (epimers at C2'; Scheme 1) was separated (**7a** and **7b**) using normal-phase high-performance liquid chromatography (HPLC). Prior to the HPLC analysis, the crude isomers were preliminarly purified by flash chromatography on silica gel using CH_2Cl_2. A model LC-10AS high-performance liquid chromatograph (Shimadzu, Japan) was used with

a Rheodyne-7125 injector system and a model SPD-10A UV-VIS detector set at 298 nm. An analytical silica column (250 x 4.6 mm i.d. x 5μm, Alltech Co.) and a semipreparative column (250 x 10 mm i.d. x 5μm, Alltech Co.) were used for analysis and separation. The chromatograms and integration data were recorded with a CR-6A integrator (Shimadzu, Japan). The samples were dissolved in ethyl acetate and 10 μL aliquots were injected for HPLC analysis. The analytical separation in a non-overload condition used gradient elution system at a flow-rate of 1.1 mL min^{-1}; in a semipreparative mode in a column-overload condition the flow-rate was 6.0 mL min^{-1}. The gradient elution program was set at 1.1 mL min^{-1}, starting with a *n*-hexane-ethyl acetate mixture (65% v/v) for 15 min. The program proceeded to *n*-hexane-ethyl acetate (75% v/v) with the same flow-rate for 15 min and then returned to initial conditions. The infrared and mass spectra of two isomers (**7a** and **7b**) were virtually superimposable and this was run for the mixture.

Diastereoisomer **7a** (retention time: 21.7 min): mp: 214-215 ºC. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.40 (s, 1H), 6.95-6.93 (m, 1H), 6.79 (s, 1H), 6.55 (s, 1H), 6.15 (s, 1H), 5.92-5.91 (m, 2H), 5.32 (s, 1H), 3.27 (d, 1H, *J* 16 Hz), 2.90 (d, 1H, *J* 16 Hz), 2.01 (d, 3H, *J* 1.48 Hz) and 1.49 (s, 3H). ^{13}C RMN ($CDCl_3$, 50.3 MHz): d 171.208 (C=O), 170.202 (C=O), 151,11 (C=), 149.59 (C), 147.50 (C), 140.98 (C=), 135.99 (C=), 135.91 (C), 131.15 (C), 117.42 (C=), 105.71 (CH), 104.77 (CH), 101.40 (CH), 100.73 (CH_2), 92.20 (CH), 48.26 (C), 44.07 (CH_2), 23.92 (CH_3) and 10.72 (CH_3).

Diastereoisomer **7b** (retention time: 24.9 min): mp: 226-227ºC. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.38 (s, 1H), 6.95-6.93 (m, 1H), 6.83 (s, 1H), 6.57 (s, 1H), 6.12 (m, 1H), 5.95-5.93 (m, 2H), 5.34 (s, 1H), 3.30 (d, 1H, *J* 16.54 Hz), 2.94 (d, 1H, *J* 16.51 Hz), 2.03 (d, 3H, *J* 1.56 Hz) and 1.49 (s, 3H). ^{13}C NMR ($CDCl_3$, 50,3 MHz): δ 171.22 (C=O), 170.20 (C=O), 150.92 (C=), 149.65 (C), 147.58 (C), 140.85 (C=), 136.08 (C=), 135.94 (C), 131.36 (C), 117.49 (C=), 105.89 (CH), 104.71 (CH), 101.47 (CH), 100.64 (CH_2), 92.19 (CH), 48.39 (C), 43.98 (CH_2), 23.93 (CH_3) and 10.81 (CH_3).

Conclusion

The results obtained demonstrate the feasibility of employing natural products as starting materials in the synthesis of biologically active molecules. The new analogue **7** will be submitted to biological assays in order to investigate its potential activity as germination stimulant. Preliminary results obtained were very promising[14].

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