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Synthesis and biological evaluation of potential substrates for the isolation of the strigol receptor

J an W illem J. F. Thuring, Rolf Keltjens, Gerard H. L. Nefkens and Binne Z. wanenburg

NSR-Centre for Molecular Structure, Design and Synthesis, Department of Organic Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

A series of analogues derived from (+)-strigol, which is a germination stimulant for seeds of the parasitic weeds Striga and Orobanche, has been prepared. For the isolation and characterization of the strigol receptor, labelled analogues are required in which a photoreactive function may be incorporated. The synthetic strategy allows for the synthesis of a range of A-ring substituted analogues of GR24 (which is a strigol analogue), including fluorescent dansyl GR24. Bioassays reveal that the stimulatory activity of these analogues in the seed germination of Striga hermonthica is retained.

Introduction

Parasitic weeds belonging to the genera Striga and Orobanche severely reduce yields of economically important crops in tropical and semitropical areas of the eastern hemisphere. The parasitic process begins with the seed germination of the weeds, induced by a stimulant which is present in the root exudate of the host plant. Following the isolation and identification of the naturally occurring germination stimulant (+)-strigol (Fig. 1), several structure-bioactivity studies have been conducted, which revealed that the bioactiphore resides in the CD-fragment and the vinyl ether moiety.

In particular, GR24 (Fig. 1) turned out to be a highly potent synthetic strigol analogue. Based on the structural requirements for retaining stimulatory activity a tentative molecular mechanism for germination has been proposed which is depicted in Scheme 1.

According to this mechanism a nucleophilic site in the receptor cavity reacts with the Michael acceptor unit, followed by elimination of the D-ring. The C- and D-rings as well as the connecting vinyl ether unit play an important role in inducing the germination. It is thus suggested that the chemical reaction at the receptor site is of crucial importance at the very beginning of the signal-transduction chain. However, nothing is known about the protein structure nor of its localization within the seeds. Detailed knowledge of the receptor protein would enable the design of a perfectly fitting substrate. Current strategies in the purification of plant proteins involve several types of affinity chromatography and photoaffinity labelling. In photoaffinity labelling the substrate of the protein is converted into a photoaffinity ligand by covalently attaching a photoreactive moiety, such as an azido group, to the natural ligand. After exposure to a cell extract, a very short-living, highly reactive intermediate is generated upon irradiation, which will be covalently bonded onto the protein in the vicinity of the putative ligand-binding site. A radioactive or fluorescent tag can be introduced in the photoreactive ligand in order to allow detection during isolation and enable the characterization of the protein. It has been demonstrated that this technique is a useful tool in plant hormone research, exemplified by the photoaffinity labelling of auxin binding proteins and gibberellin binding proteins. In the case of the strigol receptor, incorporation of a photoreactive moiety may not strictly be necessary. If the molecular mechanism (Scheme 1) is correct, treatment of the seeds with a radioactive or fluorescent strigol analogue may directly lead to covalent attachment to the receptor protein without the need to generate a highly reactive species by irradiation. When the bioactiphore of the ligand is known, incorporation of the tag and eventually a photolabile moiety should be such that the bioactivity is retained. Structure-activity relationship studies, which have previously been performed will, therefore, provide a firm basis for the design of suitable compounds for the isolation of the strigol receptor.

Here synthetic approaches are described for the preparation of biologically active, labelled strigol analogues, suitable for, at least in principle, the identification of the strigol receptor. The synthetic concept is a general approach to a wide range of substrates containing a tag and eventually an additional photolabile moiety. In addition, the activity of the stimulation of seed germination of Striga hermonthica (Del.) Benth. and Orobanche crenata Forsk. of some of the thus obtained strigol analogues is evaluated.

Results and discussion

Strategy

In designing potential labelled germination stimulants, GR24 (Fig. 1) was used as the lead molecule. The bioactivity of GR24 is very high and its preparation is well documented. Since the CD-part as well as the connecting enol ether unit are
essential for full biological activity,\textsuperscript{8,17} it was suggested that the tag and/or photoreactive group can best be incorporated into the A-ring of the stimulant molecule. Amino tricyclic lactone 1 and amino GR24 2 were selected as appropriate compounds to serve this purpose as a wide range of transformations are feasible with these synths, such as acylation, sulfonylation or alkylation, whereby coupling with an external tag can be achieved. The strategy for the synthesis of labelled GR24 analogues is outlined in Scheme 2. The label may be introduced either before (route I) or after (route II) coupling with the D-ring.

**Synthesis**

In our strategy the main issue is the introduction of an amino function into the A-ring of tricyclic lactone 3\textsuperscript{11} in a regiocontrolled manner (Scheme 3).

This was achieved via nitration of 3 under relatively mild conditions (Scheme 3),\textsuperscript{18} to give two regioisomeric lactones 4a and 4b in a ratio of 9:1 in excellent yield, which could readily be separated. The structure of 4a was deduced unambiguously from a 2D-NOESY experiment. The preferred formation of 4a can be explained by the fact that C-7 is the least electron-deficient carbon atom in the aromatic ring. The nitration under similar conditions took place also starting from GR24 to give 7-nitro GR24 5a and its 5-nitro isomer 5b in a ratio of 9:1 (Scheme 4).

However, several attempts to obtain amino GR24 2 by reductive amination of 5 were unsuccessful. In contrast, reduction of the nitro group in 4a was accomplished employing aqueous Cu(OAc)\textsubscript{2} and NaBH\textsubscript{3} in methanol\textsuperscript{19} in yields in the range 34-95%. A more reliable procedure (Scheme 3) involves the use of Sn–HCl under reflux,\textsuperscript{20} which gave the 7-amino tricyclic lactone 1a in a reproducible yield of 99% (77% after recrystallization). The preparation of amino GR24 2a from amino tricyclic lactone 1a involves a four-step procedure as is depicted in Scheme 5.

Scheme 4 Reagents: a, TFA, NaNO\textsubscript{3}; b, Sn–HCl (aq.), heat; c, PhI(OC(O)CF\textsubscript{3})\textsubscript{2}; d, 95% c.c.

The amino group in 1a was first protected as a Schiff base 6 by reaction with benzaldehyde, then followed by formylation, coupling with bromo butenolide 7, similar to that described for GR24,\textsuperscript{11} and finally deprotection. Crude 7-benzalimino GR24 8 was isolated in an overall yield of 78%, based on 1a. Deprotection was not as straightforward as expected. Several conventional methods, such as 5% oxalic acid, failed to give the desired result and only starting imine was recovered. However, during purification of 7-benzalimino GR24 by flash chromatography, the deprotection took place on the silica gel column and amino GR24 2a was isolated in an overall yield of 27%. The diastereoisomers could not be separated, neither by flash chromatography nor by recrystallization, in contrast to GR24.\textsuperscript{11}

The key intermediate amino GR24 2a was then utilized for further derivatization (Scheme 6), especially for the purpose of receptor identification. Diazotation and substitution\textsuperscript{21} in the presence of NaN\textsubscript{3} gave 8, which is thermally rather unstable in high yield. A applying the appropriate sulfonyl chloride, mesyl GR24 9 and dansyl GR24 10 were synthesized in high yields\textsuperscript{22} The alternative procedure for the preparation of the labelled GR24 analogues 8, 9 and 10, involving modification of the amino function of 1a into the azido, mesylamino and dansylamino group, respectively, fol-
lowed by coupling with the D-ring (route I in Scheme 2), is less attractive, as the yields are low to moderate\textsuperscript{23} and the purific-
ation of the compounds prepared \textit{via} route I is much simpler than by route I (Scheme 2). The synthesis of 7-iodo GR 24\textsubscript{11} could not be accomplished starting from 2a, since the substitution of the diazo moiety by iodide, in a similar procedure to that used for the preparation of azido GR 24\textsubscript{8} (vide supra), was unsuccessful. Therefore, the tricyclic lactone 3 was iodinated (cf. route I in Scheme 2) employing iodine in the presence of [bis(trifluoroacetoxy)iodo- benzene] (Scheme 3) by adopting the procedure of Merkushev et al.\textsuperscript{24} A 1:1 mixture of the regioisomeric 7-iodo lactone 12a and the 5-iodo lactone 12b was obtained in an excellent yield, which could readily be separated. Structural assignments were performed by comparison with the\textsuperscript{41} NMR spectra of the 7-nitro and 5-nitro counterparts 4a and 4b respectively. Coupling reactions of 4a and 12a to give the desired GR 24 analogues \textit{via} route I (Scheme 2) involve a two-step procedure (Scheme 5), similar to that described for the synthesis of GR 24\textsuperscript{13} For the preparation of 7-nitro GR 24\textsubscript{a} this sequence was carried out in a one-pot procedure to give 5a as an approximately 1:1 mixture of diastereoisomers in a moderate overall yield of 35%. How-
ever, for the preparation of 7-iodo GR 24\textsubscript{11} it was found more appropriate to isolate the intermediate hydroxymethylene lactone, which could readily be purified by washing with diethyl ether to remove unchanged starting material, although some loss of material had to be accepted. Akylation with the bromo butenolide 7 provides 7-iodo GR 24\textsubscript{11} as an approximately 1:1 mixture of diastereoisomers, which could readily be separated by flash chromatography.

**Biological activity**

The stimulatory activity of nitro GR 24\textsubscript{5a}, amino GR 24\textsubscript{2a} and dansyl GR 24\textsubscript{10} was determined using seeds of \textit{Striga hermonthica} and \textit{Orobanche crenata} in an aqueous control containing 0.1, 0.01 and 0.001% (v/v) acetone. The germination percentages given are the mean of two replicate tests. In each test the percentage was determined 12 times by counting the number of germination seeds. The values in parentheses are the mean germination percentages for seeds tested under the same conditions and at the same time, with GR 24 as stimulant. A quiescent control containing 0.1, 0.01 and 0.001% (v/v) acetone.

### Table 1

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<th>Entry</th>
<th>Compound</th>
<th>1 mg l\textsuperscript{-1}</th>
<th>0.01 mg l\textsuperscript{-1}</th>
<th>0.001 mg l\textsuperscript{-1}</th>
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<td>57.8 ± 3.0</td>
<td>19.4 ± 1.3</td>
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<td>(40.3 ± 2.4)\textsuperscript{a}</td>
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<td>(21.4 ± 1.7)\textsuperscript{a}</td>
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<td>2</td>
<td>5a</td>
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<td>59.3 ± 4.6</td>
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<td>(51.1 ± 2.4)\textsuperscript{a}</td>
<td>(60.1 ± 3.0)\textsuperscript{a}</td>
<td>(32.4 ± 2.0)\textsuperscript{a}</td>
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<tr>
<td>3</td>
<td>2a</td>
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<td>17.5 ± 2.2</td>
<td>7.1 ± 1.9</td>
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<tr>
<td></td>
<td></td>
<td>(50.5 ± 1.2)\textsuperscript{a}</td>
<td>(54.8 ± 1.4)\textsuperscript{b}</td>
<td>(29.0 ± 5.6)\textsuperscript{b}</td>
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<tr>
<td>4</td>
<td>Control\textsuperscript{c}</td>
<td>9.8 ± 0.5</td>
<td>11.2 ± 1.3</td>
<td>9.8 ± 0.8</td>
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</table>

*Germination percentages given are the mean of two replicate tests. In each test the percentage was determined 12 times by counting the number of germination seeds. The values in parentheses are the mean germination percentages for seeds tested under the same conditions and at the same time, with GR 24 as stimulant.

### Table 2

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<th>Entry</th>
<th>Compound</th>
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<th>0.1 mg l\textsuperscript{-1}</th>
<th>0.01 mg l\textsuperscript{-1}</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1.5 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.2</td>
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<td></td>
<td>(60.7 ± 2.8)\textsuperscript{a}</td>
<td>(27.3 ± 3.6)\textsuperscript{a}</td>
<td>(1.2 ± 0.9)\textsuperscript{a}</td>
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<td>2</td>
<td>5a</td>
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<td>9.3 ± 1.6</td>
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<td>(60.0 ± 3.1)\textsuperscript{a}</td>
<td>(27.3 ± 3.6)\textsuperscript{a}</td>
<td>(1.6 ± 0.4)\textsuperscript{a}</td>
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<tr>
<td>3</td>
<td>2a</td>
<td>33.6 ± 3.5</td>
<td>2.7 ± 1.0</td>
<td>0.0 ± 0.0</td>
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<tr>
<td></td>
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<td>(58.1 ± 3.8)\textsuperscript{b}</td>
<td>(24.7 ± 2.4)\textsuperscript{b}</td>
<td>(0.1 ± 0.1)\textsuperscript{b}</td>
</tr>
<tr>
<td>4</td>
<td>Control\textsuperscript{c}</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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</table>

*Germination percentages given are the mean of two replicate tests. In each test the percentage was determined 12 times by counting the number of germination seeds.

10 behave quite differently towards seeds of \textit{Striga hermonthica} and \textit{Orobanche crenata}. In the case of \textit{Striga hermonthica}, the biological activity of the substituted GR 24 derivatives is relatively little affected as compared to GR 24. The activity of amino GR 24\textsubscript{2a} is about one order of magnitude lower than GR 24, whereas nitro GR 24\textsubscript{5a} and dansyl GR 24\textsubscript{10} possess comparable concentration-dependent activities. Interestingly, the intrinsic activity of dansyl GR 24\textsubscript{10} is considerably higher than that of GR 24, which becomes apparent at the higher con-
centrations. In contrast, dansyl GR 24\textsubscript{10} is completely inactive in the stimulation of \textit{Orobanche crenata} seeds. Evidently, sub-
stituents in the A-part of GR 24 have a negative effect on the bioactivity in the case of this parasitic species. The difference in response exerted by A-ring analogues 2a, 5a and 10 on seeds of \textit{Striga hermonthica} and \textit{Orobanche crenata} is rather unexpected, since previous studies revealed that structural modifications in the BC-part\textsuperscript{8} enol ether moiety 25 and D-ring\textsuperscript{26} generally give similar results for both parasitic species. Nevertheless, the prospects of incorporating a tag in the A-ring of GR 24 with the aim of identifying the receptor protein is very promising for \textit{Striga hermonthica}. The remarkable activity of the bulky dansyl derivative 10 suggests a large degree of struc-
tural freedom in the A-part with retention of full biological activity.

**Conclusion**

In this synthetic study the preparation of amino GR 24\textsubscript{2a} is described. This compound is a versatile synthon for the prepar-
ation of a range of A-ring analogues of GR 24. The synthesis of the fluorescent GR 24 analogue 10 has been accomplished. Application of the thus developed synthetic strategy provides a feasible approach to the incorporation of radioactive tags and

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**Scheme 6** Reagents and conditions: a, X = NHMe, c.y. = 78%; b, X = NHDNS, c.y. = 89%; c, X = N\textsubscript{2}, c.y. = 88%.
photoreactive units in the G R 24 molecule. The germination stimulatory activity of G R 24 analogues 2a, 5a and 10 is relatively little affected for seeds of Striga hermonthica, whereas it is considerably reduced for seeds of Orobanche crenata. Therefore Striga hermonthica is an attractive target to perform protein fishing experiments.

**Experimental**

**Synthesis**

**Nomenclature.** We have used the AUTONOM 1.0 program, provided by the Belstein Institute and Springer Verlag, Weinheim, BRD.

**General remarks.** 100 M Hz 2 H N M R. Spectra and 400 M Hz 1 H N M R. spectra were recorded on a Bruker AC 100 spectrometer and a Bruker AM-400 spectrometer, respectively (M e 5 Si as internal standard). All coupling constants are given as 3 in H, unless indicated otherwise. IR Spectra were recorded on Perkin-Elmer 298 IR spectrophotometer. For mass spectra a double focussing VG 7070E mass spectrometer was used. G C - M S Experiments were run on a Varian Saturn 2 GC - M S ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30 M x 0.25 mm). H elium was used as carrier gas, and electron impact (E1) was used as ionization mass spectrometry. GLC and GC/MS runs were conducted using the following methods: dichloromethane was distilled from P 2 O 5 ; tetrahydrofuran was distilled from lithium aluminium hydride; cyclohexane was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (T L C) was carried out on M erck pre-coated silica gel 60 F254 plates (0.25 mm) using the solvent was removed from the mixture by evaporation in vacuo and the residue purified by flash chromatography (SiO 2 hexane-ethyl acetate, 9:1). Collection of the pure fractions afforded the 7-iodo tricyclic lactone 12a (65 mg, 38%) and the 5-iodo tricyclic lactone 12b (48 mg, 28%) both as white solids. Analysental samples of 12a and 12b were obtained by recrystallization from dichloromethane-diisopropyl ether.

**Compound 12a,** R 0.03 (hexane-ethyl acetate, 1:1), mp 110-111°C (from dichloromethane-diisopropyl ether): 4a,(100 M Hz; CDCl 3 ) 2.34 (1 H, dd, 3, 4-H), 2.76-3.55 (4 H, m, 3-H, 4-H and 3a-H), 7.03 (1 H, d, 3a-H), 7.10 (1 H, d, 8-H), 7.45 (1 H, d, 8-H), 7.45 (1 H, d, 8-H), 7.45 (1 H, d, 8-H) and 8.35 (1 H, d, 3-H), 2.1, 8-H); m/z 219 (M +, 39%), 175 (100, C 25 H 44 N 2 O 4 ); 12b (84, C 25 H 41 N 2 O 4 ) and 115 (33, C 25 H 37 N 2 O 4 ).

**Compound 12b,** R 0.40 (hexane-ethyl acetate, 1:1), mp 165-167.5°C (from dichloromethane-diisopropyl ether): 4a,(100 M Hz; CDCl 3 ) 2.42 (1 H, dd, 3, 4-H), 2.76-3.38 (4 H, m, 3-H, 4-H and 3a-H), 5.99 (1 H, d, 8-H), 7.02 (1 H, d, 8-H), 7.45 (1 H, d, 8-H) and 8.35 (1 H, d, 8-H); m/z 219 (M +, 39%), 175 (100, C 25 H 44 N 2 O 4 ); 12b (84, C 25 H 41 N 2 O 4 ) and 115 (33, C 25 H 37 N 2 O 4 ).

**7-Nitro-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one 4a**

Potassium tert-butoxide (563 mg, 5.03 mmol) was added in small quantities to a solution of the 7-nitro tricyclic lactone 4a (1.00 g, 4.57 mmol) and methyl formate (0.84 ml, 13.7 mmol) in THF (35 ml) with stirring at -78°C under nitrogen. The mixture was allowed to warm to room temperature and then stirred for 18 h. A fter the THF had been removed in vacuo, the residue was dissolved in DMF (35 ml) and the solution was cooled to
−60 °C, and treated with the bromo butenolide 7 (0.97 g, 5.5 mmol) in DMF (5 ml), added gradually under nitrogen. The mixture was brought to room temperature and stirred for 18 h after which it was treated with acetic acid (0.60 g, 10 mmol); the suspension was then concentrated in vacuo. The residue was dissolved in chloroform and water and the aqueous phase was separated and extracted with chloroform (2×). The combined organic layers were washed with water (1×), dried (MgSO₄) and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, hexane-ethyl acetate 1:1) to afford two diastereoisomers of 5a (549 mg, 35%; [R] 0.12 and 0.18 (hexane-ethyl acetate, 1:1) as pale yellow solids. Only the fast-moving diastereoisomer could be obtained in an analytically pure form by recrystallization from ethyl acetate, mp 216–219 °C (from ethanol) (Found: C₉H₇NO₂, 181.1; C, 78.9; H, 6.1; N, 10.1); the slow-moving diastereoisomer separated and extracted with chloroform (2×). The combined organic layers were washed with diethyl ether (2×) to provide pure 5a, mp 216–219 °C by recrystallization from ethyl acetate, mp 216–219 °C. The spectrum of the fast-moving diastereoisomer was in complete agreement with those reported above.

7-Amino-3-(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy-methylene)-3,3a,4,8b-tetraydroindeno[1,2-b]furane-2-one 2a

A solution of the amino tricyclic lactone 1a (440 mg, 2.33 mmol) and benzaldehyde (247 mg, 2.33 mmol) in ethyl acetate (20 ml), in the presence of molecular sieves 4Å, was stirred for 12 h at room temperature. After this MgSO₄ was added to the mixture which was then filtered over Hyflo and then concentrated by removal of the solvent in vacuo to give the imine 6 (645 mg, 100%) as a pale yellow solid, which was used immediately in the coupling reaction; [α]D (100 M H₂O; CDCl₃ 2.36 (1 H, dd, 5), 18.1 (J 5.4, 3-H), 2.70–3.56 (4 H, m, 3-H, 4-H and 5a-H) and 5.87 (1 H, d, 1.8, 8b-H), 7.24–7.27 (3 H, m, Ph), 7.43–7.53 (3 H, m, Ph), 7.64–7.94 (2 H, m, Ph) and 8.44 (1 H, s, N–CH₃).

To a solution of the freshly prepared imine 6 (645 mg, 2.33 mmol) and methyl formate (0.5 ml, 7 mmol) in THF (25 ml) was added potassium tert-butoxide (287 mg, 2.56 mmol) in small portions at 0 °C under nitrogen. The mixture was allowed to warm to room temperature at which point it was stirred for 18 h and then concentrated by removal of the THF in vacuo. The residue was dissolved in DMF (20 ml) and the solution was cooled to −60 °C when it was treated with the bromo butenolide 7 (494 mg, 2.70 mmol) in DMF (3 ml), added gradually under nitrogen. The mixture was brought to room temperature and stirred for 18 h after which it was concentrated by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and saturated aqueous NaHCO₃. The aqueous phase was separated and extracted with dichloromethane (2×) and the combined organic layers were washed with saturated aqueous NaHCO₃ (1×), dried (MgSO₄) and the solvent was removed in vacuo to provide crude 7-benzalimino GR24 24 (78%). Flash chromatography (SiO₂, dichloromethane, followed by ethyl acetate–dichloromethane, 3:1) gave 7-amino GR24 24a (190 mg, 27%) as a mixture of two inseparable diastereoisomers. Crystallization from butyl acetate afforded 2a as pale yellow crystals (Found: C, 65.16; H, 4.96; N, 4.31. C₉H₇NO₂ requires C, 65.17; H, 4.82, N, 4.47; [α]D (100 M H₂O; CDCl₃) 1.94 (3 H, m, Ch₃), 2.86 (1 H, dd, 5), 16.3 (J 3.0, 4-H), 3.23 (1 H, dd, 16.3, 8.4, 4-H), 3.75 (3 H, m, N–H and 3a-H), 5.77 (1 H, d, 7.6, 8b-H), 6.10 (1 H, m, COH), 6.72 (1 H, d, 7.6, 8b-H), 7.23 (2 H, m, 8.4, 4-H), 2.52 (2 H, m, 7.6, 8b-H), 6.88 (1 H, m, –CH₃), 6.93 (1 H, d, 8.4, 5a-H) and 7.33 (1 H, d, 7.6, –CHO); m/z 313 (M⁺, 57%), 216 (40, CH₃CO₂HNO₂), 188 (3, CH₃H₂NO₂) and 97 (100, CH₃O₂).

7-Azido-3-(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy-methylene)-3,3a,4,8b-tetraydroindeno[1,2-b]furane-2-one 8

Sodium nitrate (33 mg, 0.48 mmol) was added to a solution of the diastereoisomers of 7-amino GR24 24a (138 mg, 0.44 mmol) in 80% acetic acid (20 ml) at 0 °C with protection from light. After 5 min sodium azide (32 mg, 0.48 mmol) was added to the mixture and stirring was continued for 18 h. The mixture was then concentrated in vacuo and the residue was dissolved in ethyl acetate and saturated aqueous NaHCO₃. The aqueous phase was separated and extracted with ethyl acetate (2×) and the combined organic layers were washed with saturated aqueous NaHCO₃ (1×), dried (MgSO₄) and concentrated in vacuo. Purification of the residue by flash chromatography (SiO₂, hexane-ethyl acetate 1:1) afforded two diastereoisomers of 8 (131 mg, 88%) as white solids.

Fast-moving diastereoisomer of 8, R, 0.29 (hexane-ethyl acetate, 1:1), mp 151–154 °C; vmax(Cℓ–Cl) from 2120 (N₂, 1795 (C=O), 1765 (C=C), and 1685 (C=O) enol ether); [α]D (100 M H₂O; CDCl₃) 2.04 (3 H, m, Ch₃), 3.06 (1 H, d, 16.7, 3.6, 4-H), 3.41 (1 H, d, 16.7, 8, 8.8, 4-H), 3.96 (1 H, m, 3a-H), 5.91.
Preparation of test solutions. A compound (10 mg) to be tested was weighed out very accurately, dissolved in acetone p.a. (10 ml) and diluted with demineralized water to 100 ml. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0.1, 0.01 and 0.001% (v/v) of acetone, respectively.

Bioassays. For sterilization, seeds of Striga hermonthica and Orobanche crenata were exposed to an aqueous sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fibre filter paper disks (8 mm diameter; approximately 30–70 seeds per disk) in Petri dishes, moistened with water and stored in the dark for 14 days at 20 °C for Orobanche seeds and at 30 °C for Striga seeds. The conditioning water was then removed and replaced by 100 µl of test solution per disk. After incubation for 24 h (Striga) and 5 days (Orobanche) in the dark at the indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.1, 0.01, 0.001 and 0.0001% (v/v) of acetone were used as negative control. Test solutions of the stimulant GR 24 (concentrations of 1, 0.1, 0.01 and 0.001 mg l⁻¹) were used as positive controls. All tests were performed in duplicates, and in each test the germination percentages were determined on 12 disks. For full details of the bioassay, see ref. 27.

Acknowledgements

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References

14 For a recent review of chemical reagents in photoaffinity labelling, see A. S. Fleming, Tetrahedron, 1995, 51, 12, 4749.