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Structure and Synthesis of Orobanchol, the Germination Stimulant for *Orobanche minor*

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Abstract: The structure of orobanchol, a new germination stimulant isolated from red clover (*Trifolium pratense*), was proposed as **2a** (tentative absolute configuration) on the basis of GC-MS comparison of the natural product with synthetic (±)-**2a**.

Keywords: Biologically active compound; Lactones; Mass spectra; X-Ray crystal structures

Yokota and his coworkers recently isolated three seed germination stimulants for a parasitic weed called clover broomrape (*Orobanche minor*) from root exudates of its host, red clover (*Trifolium pratense*).[1, 2] One of them was a new isomer of strigol (Figure 1),[3] and named orobanchol.[2] The scarcity of the isolated orobanchol did not allow its NMR study, and only its GC-MS data were available.[2] Even with this limitation its structure was proposed as **A** (=**1**),[1] although the positions of the hydroxy group at C-5 and the 4(4a)-double bond were uncertain. As detailed below, we synthesized various stereoisomers of (±)-**A**(=**1**) and (±)-**B**(=**2**), and executed their GC-MS comparison with orobanchol, which enabled us to propose **2a** as the structure of orobanchol.

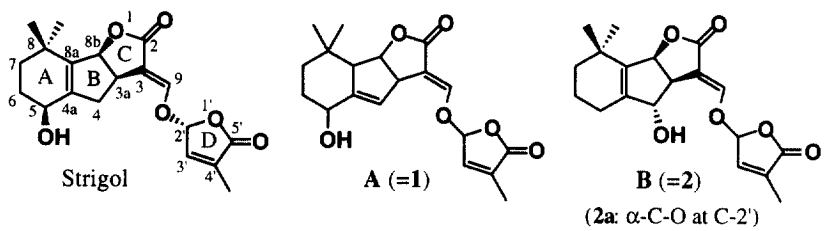

Figure 1. Structures proposed for orobanchol

Figure 2 summarizes the synthesis of the eight stereoisomers (±)-**1a** ~ (±)-**1h** of the proposed structure **A**. Citral was converted to the known hydroxy lactone (±)-**3** according to Sih,[4] Brooks,[5] and their respective coworkers. This was oxidized to the keto lactone (±)-**4**. Reduction of (±)-**4** *via* its tosylhydrazone gave (±)-**5** and (±)-**6** in moderate yields. These were separately epoxidized to give (±)-**7** and (±)-**8**, respectively. They were treated with aluminum isopropoxide to give (±)-**9** and (±)-**10**. The stereochemistry assigned to (±)-**5**, (±)-**7** and (±)-**9** was supported by the later X-ray analysis of (±)-**1a** derived from (±)-**9**. The stereochemistry of (±)-**6**, (±)-**8** and (±)-**10** was based on the ^{1}H NMR comparison with their stereoisomers (±)-**5**, (±)-**7** and (±)-**9**. The hydroxy lactone (±)-**9** yielded (±)-**1a** and (±)-**1b** after two steps. The structure of (±)-**1a**, mp 229-231 °C, was solved by its X-ray analysis, and its perspective view is shown in Figure 3.[6] Mitsunobu inversion[7] of the configuration of the hydroxy group of (±)-**9** gave (±)-**11**, which furnished (±)-**1c** and (±)-

0040-4039/99/$ - see front matter
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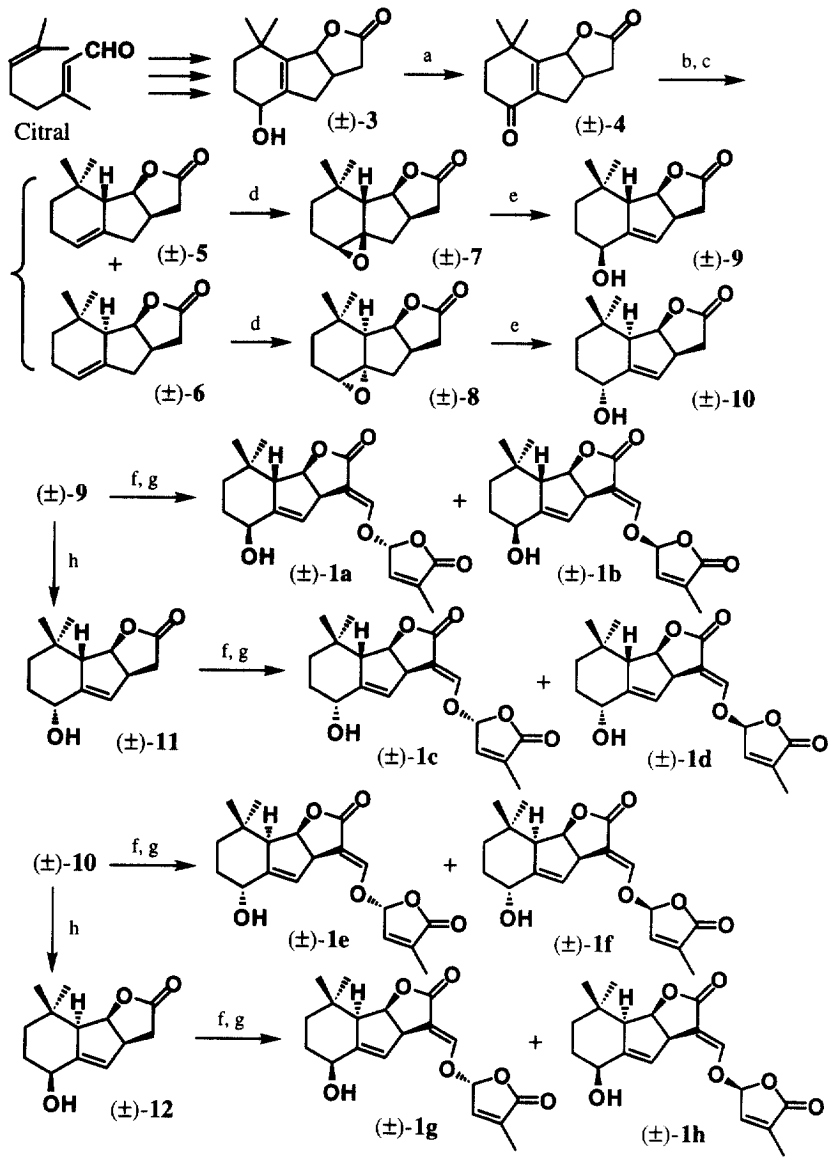
1d. Similarly, (±)-**10** was converted into (±)-**1e**, (±)-**1f**, (±)-**1g** and (±)-**1h**. Their mass spectra as their trimethylsilyl (TMS) ethers were similar to that of strigol TMS ether. All of these compounds [(±)-**1a** ~ (±)-**1h**], however, were different from orobanchol on the basis of their GC-MS analysis as their TMS ethers.[8]



Reagents: (a) PCC, CH_2Cl_2 (87%).- (b) *p*-$TsNHNH_2$, EtOH.- (c) $NaBH_3CN$, *p*-TsOH, DMF [13% for (±)-**5**; 11% for (±)-**6**].- (d) MCPBA, CH_2Cl_2 [69% for (±)-**7**; 72% for (±)-**8**].- (e) $Al(OPr^i)_3$, toluene, heat [75% for (±)-**9**; 38% for (±)-**10**].- (f) NaH, HCO_2Et, Et_2O.- (g) K_2CO_3, 4-bromo-2-methyl-2-buten-4-olide, *N*-methylpyrrolidone; SiO_2 chromatog. (65~70%; α-isomer : β-isomer = 1 : 1).- (h) i) $EtO_2CN{=}NCO_2Et$, Ph_3P, $PhCO_2H$, THF; ii) K_2CO_3, MeOH [61% for (±)-**11**; 49% for (±)-**12**].

Figure 2. Synthesis of (±)-**1a** ~ (±)-**1h**

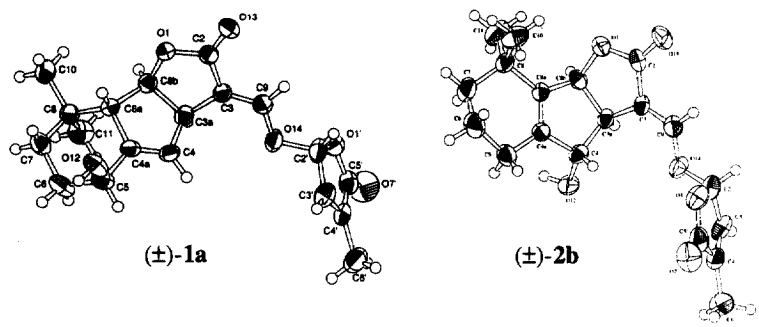
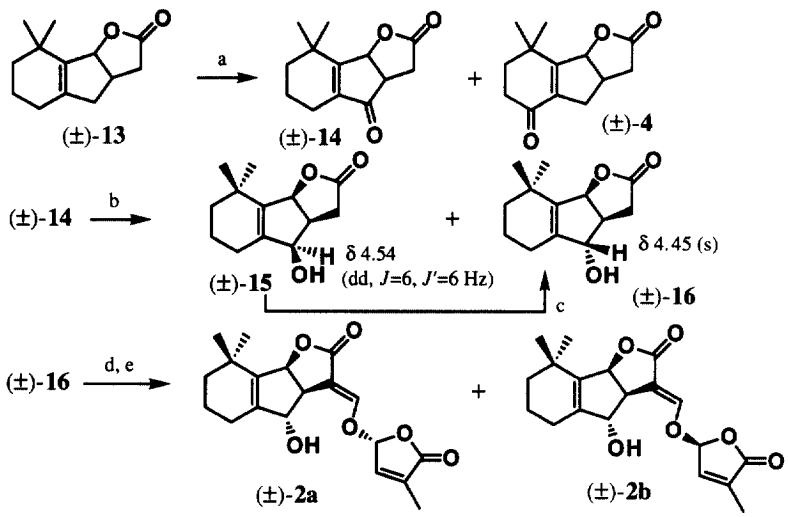


Figure 3. Perspective views of (±)-**1a** and (±)-**2b**

We then synthesized (±)-**2a** and (±)-**2b** (Figure 4) as the possible candidates for (±)-alectrol[9] and (±)-orobanchol. The known tricyclic lactone (±)-**13**[10] was oxidized to give (±)-**14** as the minor product [major product = (±)-**4**]. Reduction of (±)-**14** furnished (±)-**15** and (±)-**16**. The structure of (±)-**15** was confirmed by its X-ray analysis.[11] The minor product (±)-**16** with a 1H singlet at δ = 4.45 (**CHOH**) could be further secured by Mitsunobu inversion of the isomer (±)-**15**. Finally, (±)-**16** was converted to (±)-**2a** (mp 200-201 °C) and (±)-**2b** (mp 170-172 °C), whose structures were confirmed by the X-ray analysis of (±)-**2b**.[12] Its perspective view is also shown in Figure 3. Formylation of (±)-**15** was unsuccessful due to translactonization and other side-reactions.



Reagents: (a) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2 [23% for (±)-**14**; 75% for (±)-**4**].- (b) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, EtOH [79% for (±)-**15**; 5% for (±)-**16**].- (c) i) $EtO_2CN{=}NCO_2Et$, Ph_3P, $PhCO_2H$, THF; ii) K_2CO_3, MeOH (98%).- (d) NaH, HCO_2Et, Et_2O.- (e) K_2CO_3, 4-bromo-2-methyl-2-buten-4-olide, *N*-methylpyrrolidone; SiO_2 chromatog. [38% for (±)-**2a**; 31% for (±)-**2b**].

Figure 4. Synthesis of (±)-**2a** and (±)-**2b**

The final products, (±)-**2a** and (±)-**2b**, were then analyzed by GC-MS. The mass spectra of the TMS ethers of (±)-**2a** and (±)-**2b** were superimposable. More importantly, however, they were identical with that of orobanchol TMS ether as shown in Figure 5. The mass spectrum of (±)-**1a** TMS ether was totally different from that of (±)-**2a** TMS ether. The GC relative retention times of the TMS ethers of (±)-**2a** and (±)-**2b** were virtually the same. We thus tentatively conclude that orobanchol is **2a**, considering the known absolute configuration of strigol.[13] The importance of GC-MS analysis in structure elucidation is well established in

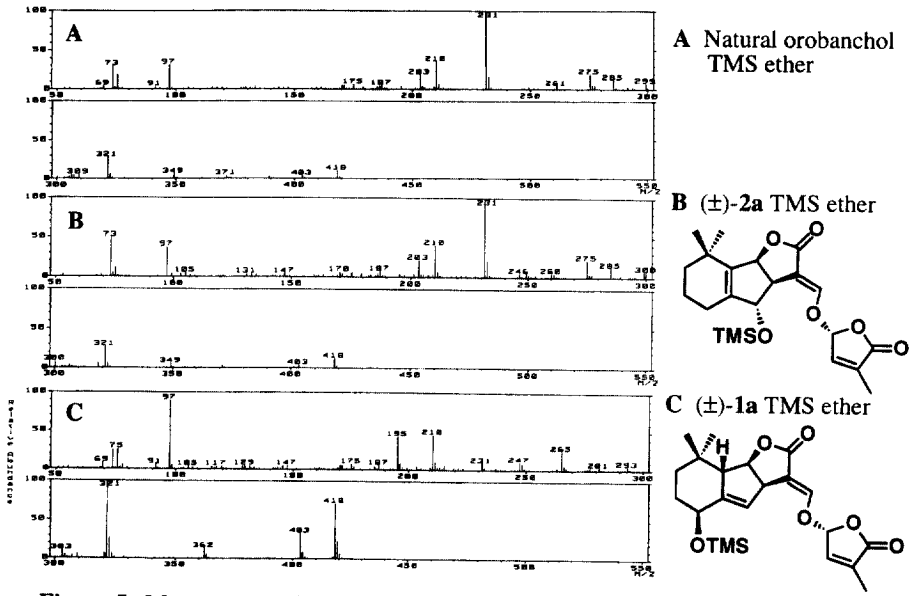


Figure 5. Mass spectra of TMS ethers of orobanchol (A), (±)-**2a** (B), and (±)-**1a** (C)

pheromone chemistry, and it has also been proved in the present phytochemical work dealing with a scarce bioregulator. Synthesis of the enantiomer **2a** of orobanchol is now in progress in our laboratory. It should finally be added that (±)-**2a** and (±)-**2b** showed strong bioactivity against the seeds of *Orobanche minor* and *Striga asiatica*, although they were less active than (+)-strigol. The stereoisomers of **1** were also bioactive. Accordingly, the bioactivity seems to reside in the C/D ring part of strigolactones.

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References and Notes

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6. X-Ray analysis of (±)-**1a**: Crystal size, 0.5 x 0.3 x 0.3 mm. The crystal data and the intensity data were obtained on Rigaku AFC-5S automated four-circle diffractometer with graphite-monochromated Mo $K\alpha$ radiation. Crystal data: $C_{19}H_{22}O_6$, Mr = 346.38, triclinic, space group $P\bar{1}$, a = 9.822(7), b = 12.247(3), c = 8.979(2) Å, α = 97.94(2), β = 115.40(2), γ = 108.10(3)°, V = 879.8(8) Å^3, Z = 2, Dc = 1.307 Mgm^{-3}, $F(000)$ = 368 and μ(Mo $K\alpha$) = 0.968 cm^{-1}. Of the 3116 independent reflections which collected, 2305 reflections with $I > 3.0\sigma(I)$ were used for structure determination and refinement. The final refinement converged with R = 0.045 and Rw = 0.047 for 230 parameters. Atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre.
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8. GC-EIMS analysis (ionization voltage = 70 eV) of strigolactones was conducted on a short DB-5 capillary column (4 m x 0.25 mm) with He as the carrier gas at a flow rate of 1 ml/min.[2] Samples were introduced (splitless mode) after trimethylsilylation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide for 10 min at room temp. The column temp was kept at 130 °C for the first 1.5 min, elevated to 220 °C by a gradient of 32 °C/min, then to 270 °C by a gradient of 16 °C/min.
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11. Details of the X-ray analysis of (±)-**15** will be reported in the full paper.
12. X-Ray analysis of (±)-**2b**: Crystal size, 0.5 x 0.2 x 0.2 mm. All data were obtained as for (±)-**1a**. Crystal data: $C_{19}H_{22}O_6$, Mr = 346.38, monoclinic, space group $P2_1/n$, a = 7.799(10), b = 10.337(6), c = 21.240(5) Å, β = 95.77(4)°, V = 1704(2) Å^3, Z = 4, Dc = 1.350 Mgm^{-3}, $F(000)$ = 736 and μ(Mo $K\alpha$) = 1.001 cm^{-1}. Of the 3216 independent reflections which collected, 1674 reflections with $I > 2.0\sigma(I)$ were used for structure determination and refinement. The final refinement converged with R = 0.078 and Rw = 0.089 for 230 parameters. Atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre.
13. Another possible structure is a stereoisomer of **2a** with a β-OH at C-4 of **2a**. Attempted Mitsunobu inversion of (±)-**2a** failed.