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Synthesis and Biological Evaluation of the Four Racemic Stereoisomers of the Structure Proposed for Sorgolactone, the Germination Stimulant from *Sorghum bicolor*

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Abstract: The synthesis of the racemates of the structure **1** proposed for sorgolactone and its three stereoisomers was achieved by confirming the stereostructures of the intermediate (±)-**6** and the final product (±)-**1** by X-ray analyses. Biological evaluation of the products employing clover broomrape (*Orobanche minor*) seeds revealed that the order of activity as a germination stimulant was (±)-strigol ≈ (±)-**9** ≥ (±)-**12** > (±)-**1** > (±)-**11**. © 1997 Elsevier Science Ltd.

Parasitic weeds of the genera *Orobanche* and *Striga* are known to cause severe yield losses in grains and legumes in Africa, Asia and the U. S. A.[1, 2]. The seeds of such weeds remain dormant in soil until exudates from their host plant induce germination. (+)-Strigol was first isolated from cotton root exudates and shown to be a strong stimulant for the germination of such seeds[3]. It was later isolated from the host plants such as maize, proso millet and sorghum[4]. Synthesis of (+)-strigol by Sih[5] and that of (±)-strigol by Raphael[6] evoked much interest in the chemistry of germination stimulants, which culminated in the determination of the absolute configuration of the naturally occurring (+)-strigol by X-ray analysis[7]. The synthetic endeavor in this area is still remarkable[8, 9].

In 1992 Hauck et al. isolated sorgolactone from *Sorghum bicolor*, a genuine host plant, as the germination stimulant for parasitic weeds[10]. They proposed **1** as the structure of sorgolactone based on its ^{1}H NMR analysis and CD comparison with strigol[10]. We herein report the first synthesis of (±)-**1**. Two attempted syntheses of **1** have been reported recently[11, 12].

Our synthesis of (±)-**1** is summarized in Figure 1. The sodium salt of the known diketo acid **2** (a racemic and diastereomeric mixture)[13] was reduced with sodium borohydride in the presence of cerium(III) chloride[14] to give, after acidification, a racemic and diastereomeric mixture of the hydroxy lactone **3**[15]. Treatment of **3** with carbon tetrabromide and triphenylphosphine yielded a racemic and diastereomeric mixture of the unstable bromolactone **4**, which was reduced with zinc-copper couple (Zn:Cu = 91:5, Kanto Chemical Co.)[16] and acetic acid in THF to furnish the crude reduction product. This was dissolved in chloroform and stirred for 60 hr at room temperature to cause the double bond migration to the more stable tetrasubstituted position[16]. The product was further purified by MPLC [Lobar LiChroprep® Si 60 (40-63 μm)] to give an oily fast moving

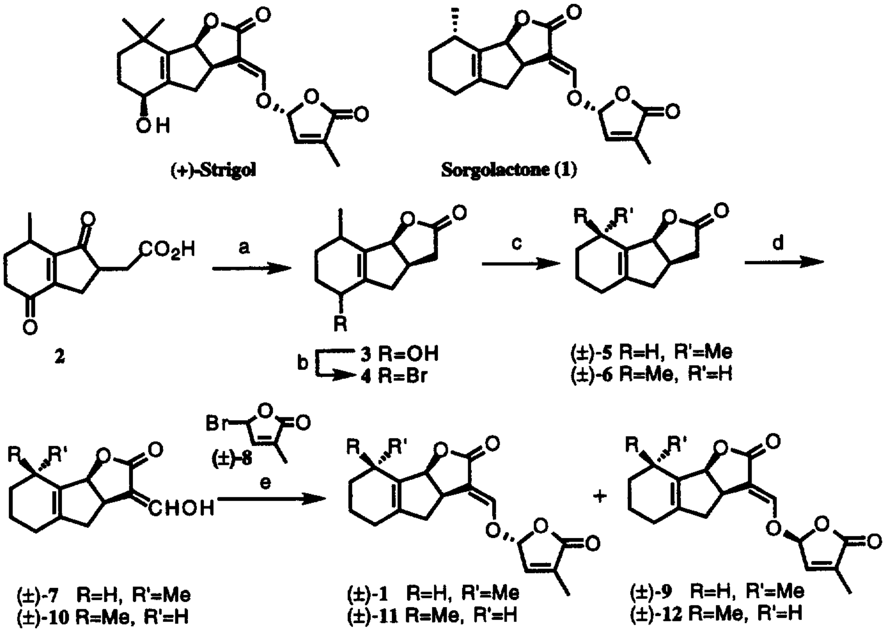
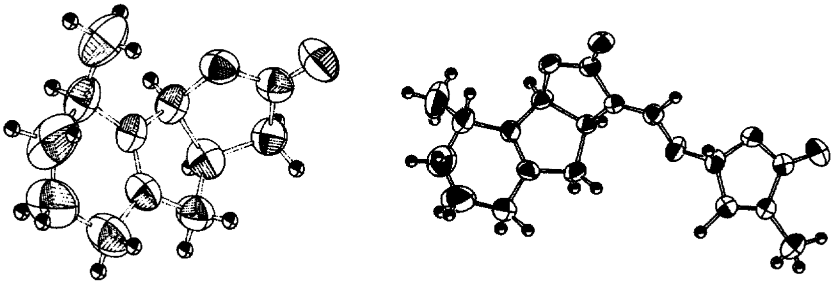


Figure 1. Synthesis of (±)-1

Reagents: (a) NaOH, $NaBH_4$, $CeCl_3 \cdot 7H_2O$, CH_2Cl_2/H_2O (67%).– (b) CBr_4, Ph_3P, CH_2Cl_2 (87%).– (c) 1) Zn-Cu, AcOH, THF; 2) $CHCl_3$, stirring (40% as a mixture of **5** and **6**); 3) MPLC separation.– (d) NaH, HCO_2Et, Et_2O (quant.).– (e) K_2CO_3, *N*-methylpyrrolidone; SiO_2 chromatog. separation [42% for (±)-**1**, 41% for (±)-**9**, 39% for (±)-**11** and 45% for (±)-**12**].



6 **1**

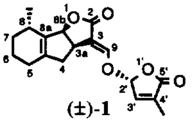
Figure 2. Perspective views of (±)-**6** and (±)-**1**

diastereomer and a crystalline slowly moving one. The latter (m.p. 43-46 °C) was submitted to the X-ray analysis[17] to reveal its structure as (±)-**6**. Its computer-generated stereoview is shown in Figure 2. The oily lactone must therefore be (±)-**5**, which leads to (±)-**1**. These two stereoisomeric lactones were separately processed to give (±)-**1** and its three stereoisomers.

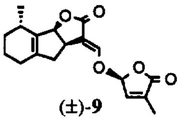
Formylation of (±)-**5** with sodium hydride and ethyl formate gave (±)-**7**, m.p. 114-116 °C. Treatment of (±)-**7** with (±)-4-bromo-2-methyl-2-buten-4-olide [(±)-**8**] in the presence of potassium carbonate gave a mixture of (±)-**1** and (±)-**9**, which could be separated by silica gel chromatography to give two crystalline products. The structure of one of the products with m.p. 127-129 °C was solved by X-ray analysis[18], and its stereoview is shown in Figure 2. This product was thus (±)-**1**[19], and the other with m.p. 117-119 °C was (±)-**9**. Similarly, the stereoisomeric lactone (±)-**6** was converted to (±)-**11**, m.p. 131-133 °C, and (±)-**12**, m.p. 116-118 °C. These structures were tentatively assigned on the basis of their R*f* values.

The ^{1}H NMR spectra of (±)-**1**, (±)-**9**, (±)-**11** and (±)-**12** in $CDCl_3$ or C_6D_6 were measured and compared with the copies (sent by Dr. C. Hauck) of the spectra of the natural sorgolactone. As shown in Table 1, although they were all very similar to each other, the spectrum of (±)-**1** was almost identical with that of the natural product. Unfortunately, Dr Hauck's spectra of sorgolactone were of poor quality due to the scarcity of the sample and the presence of impurities. Because the natural product itself is no more available, we conclude that the structure of sorgolactone is most probably **1** and its reisolation must be attempted to completely settle the problem by direct TLC comparison of (±)-**1** with the reisolated sample.

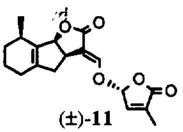
Bioactivity of strigol, its stereoisomers or structural analogues as a germination stimulant is known to be strongly dependent on its structure including relative and absolute stereochemistry[20-22]. We therefore carried out the bioassay of four final products by using seeds of clover broomrape (*Orobanche minor*) as the test parasitic weed[23]. All of four products stimulated the germination of the *Orobanche* seeds, and the order of the stimulant activity was (±)-strigol ≈ (±)-**9** ≥ (±)-**12** > (±)-**1** > (±)-**11** as shown in Table 1. The details of this work will appear in *Liebigs Ann.*



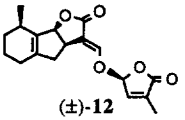
(±)-**1**



(±)-**9**



(±)-**11**



(±)-**12**

		(±)-1	(±)-9	(±)-11	(±)-12	
m.p.		127-129 °C	117-119 °C	131-133 °C	116-118 °C	
TLC(hexane-EtOAc 1:1)		R*f* 0.42	R*f* 0.34	R*f* 0.42	R*f* 0.33	
1H-NMR Data (Our data were measured at 500 MHz as $CDCl_3$ solutions.)						
	lit.[10]					
8-Me	*1.1 (3H)*	1.06 (3H, d, *J*=7.0 Hz)	1.04 (3H, d, *J*=7.0 Hz)	1.13 (3H, d, *J*=7.0 Hz)	1.13 (3H, d, *J*=7.0 Hz)	
7-H		1.24 (1H, m)	1.23 (1H, m)	1.35 (1H, m)	1.35 (1H, m)	
7-H'		1.56 (1H, m)	1.55 (1H, m)	1.55 (1H, m)	1.54 (1H, m)	
6-H		1.70, 1.78 (2H, m)	1.68, 1.77 (2H, m)	1.72 (2H, m)	1.74 (2H, m)	
5-H		1.94 (2H, m)	1.93 (2H, m)	1.96 (2H, m)	1.91, 2.00 (2H, m)	
4'-Me	*2.0 (3H)*	2.03 (3H, t, *J*=1.5 Hz)	2.01 (3H, t, *J*=1.5 Hz)	2.03 (3H, t, *J*=1.5 Hz)	2.03 (3H, t, *J*=1.5 Hz)	
4-H	*2.4 (1H)*	2.34 (1H, d, *J*=16.5 Hz)	2.33 (1H, d, *J*=16.5 Hz)	2.32 (1H, m)	2.31 (1H, m)	
8-H		2.38 (1H, m)	2.36 (1H, m)	2.37 (1H, d, *J*=16.5 Hz)	2.33 (1H, d, *J*=16.5 Hz)	
4-H'	*2.7 (1H)*	2.75 (1H, dd, *J*= 15.0, 9.0 Hz)	2.72 (1H, dd, *J*= 15.5, 9.0 Hz)	2.70 (1H, ddd, *J*= 16.5, 9.5, 3.4 Hz)	2.68 (1H, ddd, *J*= 16.5, 9.5, 3.3 Hz)	
3a-H		3.63 (1H, m)	3.60 (1H, m)	3.62 (1H, m)	3.60 (1H, m)	
8b-H	*5.5 (1H)*	5.49 (1H, d, *J*=8.0 Hz)	5.48 (1H, d, *J*=7.5 Hz)	5.35 (1H, d, *J*=7.5 Hz)	5.37 (1H, d, *J*=7.5 Hz)	
3'-H	*6.1 (1H)*	6.15 (1H, t, *J*=1.5 Hz)	6.14 (1H, s)	6.14 (1H, t, *J*=1.5 Hz)	6.13 (1H, t, *J*=1.5 Hz)	
2'-H	*6.9 (1H)*	6.92 (1H, t, *J*=1.5 Hz)	6.93 (1H, t, *J*=1.5 Hz)	6.92 (1H, t, *J*=1.5 Hz)	6.93 (1H, t, *J*=1.5 Hz)	
9-H	*7.4 (1H)*	7.41 (1H, d, *J*=3.0 Hz)	7.42 (1H, d, *J*=3.0 Hz)	7.41 (1H, d, *J*=2.5 Hz)	7.42 (1H, d, *J*=2.5 Hz)	
Concentration (M)		Relative germination of *Orobanche minor* (%)				
10^{-5}		82, 90	95, 95	70, 57	92, 95	(cf.)
10^{-6}		77, 65	93, 93	12, 18	92, 95	(±)-strigol
10^{-7}		40, 51	90, 90	7, 3	87, 78	94, 94
10^{-8}		21, 23	84, 87	3, 0	80, 70	86, 85

Table 1. Physical and biological data of (±)-**1** and its stereoisomers

Acknowledgment: We thank Dr. C. Hauck (Novartis, Basel) for kindly sending to us the files of ^{1}H NMR spectrum of the natural **1** in C_6D_6 and a copy of his doctoral dissertation in which the ^{1}H NMR spectra of **1** in $CDCl_3$ and C_6D_6 are recorded. This work was financially supported by Kanebo Co., Ltd..

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[†] Research fellow on leave from Kanebo Co., Ltd. (1996-1998).

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17. X-ray analysis of (±)-**6**: Crystal size, 0.2 x 0.5 x 0.8 mm. All the data were obtained on Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo $K\alpha$ radiation. Crystal data: $C_{12}H_{16}O_2$, Mr = 192.26, monoclinic, space group $P2_1/a$, a = 9.020(6) Å, b = 7.494(6) Å, c = 15.673(3) Å, β = 96.23(3), V = 1053.1(8) Å^3, Z = 4, Dx = 1.213 g/cm^3, $F(000)$ = 416 and μ(Mo $K\alpha$) = 0.756 cm^{-1}. Of the 1521 independent reflections collected, 840 reflections with $I > 3.0\sigma(I)$ were used for the structure determination. The final refinement converged with R = 0.058 and Rw = 0.072 for 127 parameters. Atomic coordinates have been deposited at the Cambridge Crystallographic Data Center.
18. X-ray analysis of (±)-**1**: Crystal size, 0.3 x 0.4 x 0.5 mm. All the data were obtained on Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo $K\alpha$ radiation. Crystal data: $C_{18}H_{20}O_5$, Mr = 316.35, monoclinic, space group $P2_1/n$, a = 6.957(3) Å, b = 17.541(3) Å, c = 13.777(4) Å, β = 97.78(3), V = 1665.8(9) Å^3, Z = 4, Dx = 1.261 g/cm^3, $F(000)$ = 672 and μ(Mo $K\alpha$) = 0.86 cm^{-1}. Of the 2285 independent reflections collected, 1248 reflections with $I > 3.0\sigma(I)$ were used for the structure determination. The final refinement converged with R = 0.056 and Rw = 0.063 for 208 parameters. Atomic coordinates have been deposited at the Cambridge Crystallographic Data Center.
19. Properties of (±)-**1**: m.p. 127-129 °C; IR (KBr) 2928, 2860, 1790, 1746, 1728, 1678, 1182, 1096, 1021, 957 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 0.94 (d, J = 7.0 Hz, 3H), 1.02 (m, 1H), 1.31 (m, 1H), 1.35 (t, J = 1.5 Hz, 3H), 1.43 (m, 1H), 1.53 (m, 1H), 1.60 (m, 1H), 1.70 (m, 1H), 2,27 (d, J = 16.5 Hz, 1H), 2.33 (br s, 1H), 2.41 (dd, J = 16.5 and 8.5 Hz, 1H), 3.19 (m, 1H), 5.09 (d, J = 8.0 Hz, 1H), 5.31 (t, J = 1.5 Hz, 1H), 5.81 (t, J = 1.5 Hz, 1H), 7.48 (d, J = 2.5 Hz, 1H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 10.7, 18.5, 20.6, 26.0, 27.8, 31.1, 36.4, 41.3, 88.0, 100.4, 114.3, 135.8, 137.2, 141.0, 141.3, 150.1, 170.2, 171.3; MS (EI) *m/z* 316(M$^+$), 219, 201, 173, 145, 131, 105, 97, 91.
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