R(−)-Glycerol Monolinolate, a Minor Sporogenic Substance of *Sclerotinia fructicola*

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Sclerotinia fructicola, a pathogenic brown rot fungus of stone fruits, produced abundant arthrospores when grown in darkness, whereas poorly or not at all under illumination of light.¹⁾ Quite recently, we have isolated a sporogenic substance from this fungus cultured in darkness, and showed that this active compound, named sclerosporin, was able to induce sporulation on the mycelia grown under illumination of light.²⁾ Sclerosporin, $C_{18}H_{22}O_2$, was preliminarily characterized as a new fungal sesquiterpene with the guaiane-type skeleton.²⁾ During the course of these studies, we found that the acetone extracts of the mycelia cultured in darkness contained a minor sporogenic substance besides sclerosporin. We wish now to describe the isolation and structural characterization of this minor metabolite as R(−)-glycerol monolinolate, and its sporogenic activity also. Quite interestingly, the fungus produced its both enantiomers, of which only the R enantiomer exhibited the sporogenic activity.

S. fructicola was cultured on potato-sucrose medium in darkness as described in the previous paper.²⁾ When the ethyl acetate extracts of the full-grown mycelia as well as the cultured nutrient agar were made in an alkaline aqueous solution (pH 10), in order to separate them into a neutral and an acidic fraction by the conventional method, the major sporogenic substance, sclerosporin, has decomposed owing to its alkaline instability. However, a weak activity still remained in both neutral and acidic fractions, of which the former's activity was relatively stronger than the latter's. The neutral fraction was subjected to a column chromatography on alumina, from which the active compound was eluted with ethyl acetate-*n*-hexane (1:1). The active eluate was then purified by a preparative thin-layer chromatography (ptlc) on Kiesel gel 60 PF₂₆₄ (ethyl acetate: *n*-hexane = 7:3), followed by a multiply (6 times) developed ptlc, which was repeated twice with the same thin layer plate but changing the solvent composition into a 3:7. Thus, the active compound was isolated in a chromatographically single spot (yield: ca. 32 μ g from 14 liters of the cultured materials).

The active compound showed, in the mass spectrum, a molecular ion at m/e 354 ($C_{21}H_{38}O_4$: observed m/e

354.2740, and calcd. 354.2764), and the characteristic intense peak at m/e 262 ($C_{18}H_{30}O$: observed m/e 262.2308, and calcd. 262.2296). The difference between elemental composition of these two ions was $C_3H_8O_3$, which was most probably glycerol. Then, the $C_{18}H_{30}O$ fragment might be a linolyl group, thus deducing the structure as glycerol monolinolate. This assumption was confirmed by synthesizing its α isomer by the method of Osman *et al.*,³⁾ concluding that the synthetic compound was identical in a mass spectrum with that of the natural metabolite.

Since α -glycerol monolinolate has a chiral center in the molecule, the absolute stereochemistry was determined as follows. First, its R and S enantiomers were synthesized starting from D- and L-mannitol, respectively, as shown in Scheme 1. An enantiomeric purity of the synthetic products was determined by converting them into di-(+)- α -methoxy- α -trifluoromethylphenyl-acetyl (MTPA) derivative,⁴⁾ whose diastereomeric composition was analyzed by high performance liquid chromatography (HPLC) (μ -Porasil: ϕ 4 mm $\times$ 300mm, solvent: diethyl ether-*n*-hexane (1:9), a flow rate: 1.0 ml/min, detector: UV 260 nm, a retention time of (+)-MTPA ester of the R enantiomer: 28.1 min, and the one of the S enantiomer: 31.1 min). Thus, the synthetic R enantiomer, $[\alpha]_D -5.2^\circ \pm 0.3^\circ$ ($c=1.56$,

TABLE I. SPOROGENIC ACTIVITIES OF THE CHEMICALLY PREPARED BOTH ENANTIOMERS OF α -GLYCEROL MONOLINOLATE ON *S. fructicola*^a

Dose (μ g)/ Agar medium (1ml)	Light condi- tion	Numbers of arthrospores (%)	
0	dark	301 (100)	
0	light	16 (5)	
		R	S
0.01	light	31 (10)	—
0.05	"	42 (14)	13 (4)
0.1	"	57 (19)	25 (8)
0.5	"	67 (22)	23 (7)
1	"	77 (26)	26 (8)
5	"	172 (57)	17 (6)
10	"	161 (54)	24 (8)
50	"	227 (75)	18 (6)

^a A test solution dissolved in ethyl acetate (30 μ l) was added onto the slants preliminarily cultured for three days at 25°C under a fluorescence lamp (Toshiba FL 40S.D/NL, 7500 lux). Then, this slants were incubated further three days under the same conditions, and the number of arthrospores produced was counted as described in the previous paper.²⁾ As a control, the slants to which only ethyl acetate was added were incubated similarly either in darkness or under a fluorescence lamp.

SCHEME 1. The Synthesis of R- and S-Glycerol Monolinolate from D- and L-Mannitol, Respectively.

MeOH), was shown to be contaminated by 2.4% of the S enantiomer, and the synthetic S one, $[\alpha]_D +5.4^\circ \pm 0.3^\circ$ ($c=1.16$, MeOH), by 3.5% of its antipode. Under the similar conditions, the enantiomeric composition of the natural active compound was analyzed on its di-(+)-MTPA ester, revealing that the compound was the mixture of R and S-glycerol monolinolate as well as its β isomer, whose retention time was 29.4 min (their composition ratio was calculated as R:S: $\beta=32:26:42$).

In order to determine either enantiomer of α -glycerol monolinolate to be an active principle, a sporogenic activity of the chemically prepared both enantiomers was assayed with essentially the same method as described previously²⁾ but modified so even the weak activity as to be assayed enhanced. The result is shown in Table I. The R enantiomer was found only to be active, inducing sporulation on the mycelia grown under illumination of light. The activity at 0.05~0.1 $\mu\text{g/ml}$ was weak but clearly significant, and the activity was enhanced as the amount of the sample was increased. At the maximum concentration (50 $\mu\text{g/ml}$) tested, the 75% production of arthrospores compared with those in darkness was observed. These activity was much lower than that of sclerosporin. The activity of the β isomer was not determined, because its pure form could not be obtained by our synthesis.

Based on these results, R(–)-glycerol monolinolate was shown to be a minor sporogenic substance of *S. fructicola*.

The above results suggest that the acidic fraction exhibiting the weak activity may contain linolic acid. GC-MS analysis was conducted on this fraction after converting it into the methyl ester, revealing that linolic acid was actually present as a major component of it. Sporogenic activity of authentic linolic acid and its related fatty acids such as stearic and oleic acids was measured. Among them, only linolic acid exhibited the weak activity (ca. 20% production of arthrospores compared with those in darkness). Recently, Hyeon *et al.*⁹⁾ isolated linolic and linolenic acids as sporulation-inducing substance of *Alternaria tomato*, whose asexual conidia were produced only under illumination of light. Interestingly, linolic acid was commonly found in the both fungi as a sporogenic substance, although the activity was quite low.

3-Phosphate ester of the S(+)-enantiomer of 1-monoacyl glycerol has been known to be an obligatory intermediate in biosynthesis of triglycerides and phospholipids.¹⁰⁾ The present study revealed that the antipode of this important but ubiquitous metabolite was active for inducing asexual arthrospores on a fungus, *S. fructicola*.

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