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OPEN AND RING FORMS OF MYCOSPORIN-2 FROM THE ASCOMYCETE *GNOMONIA LEPTOSTYLA*

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Key Word Index—*Gnomonia leptostyla*; ascomycete; mycosporin-2; glutamic acid derivative.

Abstract—The three reproductive forms of *Gnomonia leptostyla* synthesize mycosporin-2 ring and open forms. The chemical structures were determined from NMR and MS data.

INTRODUCTION

Gnomonia leptostyla (Fr.) Ces. et de Not. (Ascomycetes) is responsible for anthracnose in the walnut tree and has a natural cycle characterized by the succession of three reproductive forms: acervula with both macroconidia or with microconidia and, finally, perithecia. As one of us has clearly demonstrated [1], these different reproductive forms can be selectively induced *in vitro* under adequate culture conditions by appropriately regulating light and temperature. In darkness, perithecia ripening at low temperature (10°) are formed; in light, conidia are produced, macroconidia requiring higher temperatures than microconidia [1]. This morphogenetic variability linked to environmental conditions clearly reflects biochemical modifications in the organism and some of these (e.g. organic acids [1], lipids [1] and steroids [2]) have already been studied.

The ethanolic or aqueous extracts from mycelia of *Gnomonia leptostyla*, producing either conidia or perithecia, exhibited a more or less intense absorption at 310 nm, and it was thus interesting to investigate (a) the nature of the mycosporins [3] involved in this fungus and (b) if there were qualitative differences in addition to quantitative ones observed, by UV spectra, in the mycosporin content with regard to the dry weight.

RESULTS AND DISCUSSION

After ion exchange chromatography on cationic and then anionic resins of the aqueous extracts obtained from irradiated mycelia (producing conidia), we obtained two fractions absorbing at 310 nm: one was eluted with water and the other with 0.05 N formic acid.

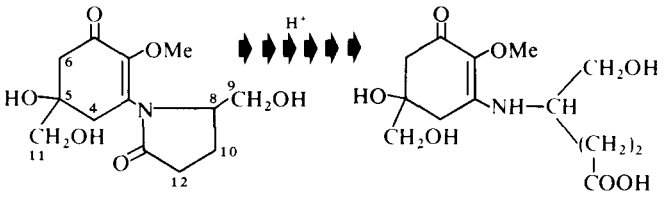
However, HPLC and MS analysis showed that these two fractions were identical. In any case, the ions of highest mass of natural compounds at *m/z* 285 corresponded to dehydration of molecular ions (M^+: 303), because all the M^+ of TMSI derivatives occurred at *m/z* 591 (303 + 4 TMSi). In D/CI, with NH_3 as reactant gas, we observed the ion of the highest mass at *m/z* 286 corresponding to MH^+ of dehydrated product, while, by HPLC/MS, the MH^+ ion occurred at *m/z*: 304 (100%).

The ^1H NMR spectrum of the most highly purified fraction was very similar to that of mycosporin-2 (ring form) isolated from *Botrytis cinerea* [4] in which cyclic and reduced glutamic acid substitutes the cyclohexenone ring. The single significant difference concerned the presence of a doublet at 6.80 ppm (J = 10 Hz), absent in the spectrum of mycosporin-2 from *B. cinerea*. This doublet has been attributed to NH [5], following spin decoupling experiments: after irradiation of the 3.9–4.2 ppm multiplet (–NH–CH) the doublet gave a

Table 1. ^1H NMR of mycosporins-2 (open and ring forms), isolated from mycelia of *Botrytis cinerea* and *Gnomonia leptostyla* (C_5D_5N, δ, ppm/TMS, 250 MHz)

	CH_2 (4 and 6)	OMe	CH (8)	CH_2OH (9)	CH_2 (10)	CH_2 (11)	CH_2 (12)	NH
Mycosporin-2	3.00							
open form	*d*, J = 17 Hz (1 H)							
(*G. leptostyla*)	3.20	3.80	3.9–4.20*		2.73	4.00*	2.3	6.8
	d, J = 17 Hz (2 H)	*s* (3 H)		3.9–4.20*	*sextuplet* (2 H)		*m* (2 H)	*d*
	3.50							
	d, J = 17 Hz (1 H)							
Mycosporin-2 ring form	3.00							
(*B. cinerea*)	3.20	3.80	3.9–4.20*		2.73	4.00*	2.3	—
	3.50			3.9–4.20*			*m*	

* Into a multiplet of 5 H.



Mycosporin-2

Ring form
Botrytis cinerea
Gnomonia leptostyla

Open form
Botrytis cinerea
Gnomonia leptostyla

singlet and, reciprocally, irradiation at 6.8 ppm produced a clear modification into the multiplet (Table 1).

These MS and NMR data were entirely consistent with an open form of mycosporin-2. The remaining question was to determine if this compound was genuine, or if it was an artefact obtained, from the ring form, during purification on ion exchange chromatography. To answer this question, we analysed several mycelia from the following cultures: C, irradiated conidial cultures at 20°; P1, dark grown cultures at 20° giving young abortive perithecia and P2, dark grown cultures at 10°, giving maturating perithecia.

HPLC showed the presence in all three cases of a main mycosporin, *ca* 95%, corresponding to the ring form of mycosporin-2, and also, in the three cases, of an open form of this mycosporin-2, *ca* 5%. We also found similar results with *B. cinerea* in which we observed a little of the open form of mycosporin-2, in larger amount in the aged cultures than in young cultures on the same medium.

Finally, we concluded that the open form of mycosporin-2 cannot be considered an artefact; this compound is really present in the mycelia. Table 2 summarizes the absolute and relative concentrations of mycosporins produced by the two morphogenetic types C and P: the amount of mycosporins increases during the maturation of perithecia, at 10°, but remains at a low level compared with that synthesized by irradiated conidial mycelia; that is in agreement with others [6].

In summary, mycelia of *G. leptostyla* synthesize the same mycosporins, ring (95%) and open (5%) forms of mycosporin-2 in both types of morphogenesis, but conidiogenesis is accompanied by a greater synthesis of these compounds than in dark perithecial development.

Table 2. Absolute and relative quantities of mycosporins-2 into mycelia of *Gnomonia leptostyla* producing either conidia (C) or abortive (P1) or maturating (P2) perithecia

Mycelia and conditions	Quantities of mycosporins-2 (%) Relative UV	Relative HPLC	Absolute (% d.w.)
C (light, 20°)	100	100	0.6
P1 (dark, 20°)	22	24	0.14
P2 (dark, 10°)	34	33	0.20

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