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MYCOSPORINS FROM *ASCOCHYTA PISI, CLADOSPORIUM HERBARUM* AND *SEPTORIA NODORUM*

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Key Word Index—*Ascochyta pisi*; *Cladosporium herbarum*; *Septoria nodorum*; *Morchella esculenta*; Deuteromycetes; ascomycete; mycosporin-2 glucoside; glutamic acid derivative.

Abstract—The chemical structure of the mycosporin isolated from *Ascochyta pisi*, *Cladosporium herbarum* and *Septoria nodorum* was established as mycosporin-2 glucoside.

INTRODUCTION

Ascochyta pisi Lib. was the first fungus from which Leach [1] isolated a characteristic 310-nm absorbing substance named P 310. Later, other compounds of this type were observed in numerous fungi and biological studies of their properties (light induction, sporogenic activity, etc.) were developed and have been reviewed [2–4].

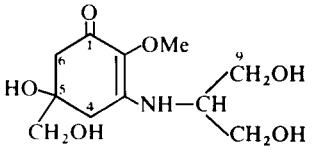
The chemical structure of this type of compound was established by Arpin *et al.* [5], who elucidated the structure of mycosporin-1 (**1**) from *Stereum hirsutum* (Basidiomycetes). The most widespread of the class is mycosporin-2, at first isolated by the same workers from *Botrytis cinerea* [6] in the ring form **2**. Later, these same authors, studying mycosporins from *Gnomonia leptostyla* [7], pointed out that the open chain form **3** could be obtained, either as an artefact of the purification process, or as a result of particular physiological or cultural conditions.

This work reports the structural identification of the 9-*O*-glucoside of the ring form of mycosporin-2 (**4**) and under the same conditions as observed with the aglycone, of its open form **5**, as the mycosporins of *Ascochyta pisi*, Lib., *Cladosporium herbarum* (Pers.) Link and *Septoria nodorum* Berk. In addition, we have demonstrated the natural occurrence of mycosporin-2 open form (**3**), in the cap of the receptacle (ascospores?) of the ascomycete *Morchella esculenta* (L.) *ex* St Amans.

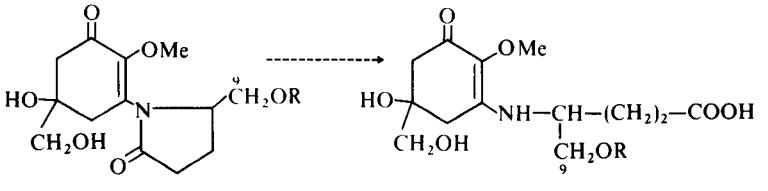
RESULTS AND DISCUSSION

The general process of extraction and purification of mycosporins was similar to that used by Trione and Leach [8]. The spores were extracted by MeOH or EtOH and the alcoholic filtrate concentrated to dryness and the residue taken up in a small volume of H_2O and applied to an ion exchange resin column and eluted with H_2O. Chromatography was monitored by UV absorption at 310 nm.

Two main fractions were obtained from *C. herbarum* cultured on malt medium and analysed by HPLC (see Table 1). The first fraction (F1) showed a single peak (R_t 6



1
Mycosporin-1



Mycosporin-2

2 R = H
4 R = Glc

3 R = H
5 R = Glc

Table 1. HPLC* data of mycosporins-2

Compound:	**4**	**5**	**2**	**3**
R_t min:	9	6	8	5.2
Crude extract of:	(% of total mycosporins)			
Botrytis cinerea				
conidia	—	—	95–100	0–5
Gnomonia leptostyla				
conidia and perithecia	—	—	95–100	0–5
Morchella esculenta				
cap (spores)	—	—	—	100
stipe	—	—	—	—
Cladosporium herbarum				
Crude extract of spores	95–100	0–5	0	0
Fraction F1	0	100	0	0
Fraction F2	80	20	0	0
Acid hydrolysis† of F2:				
5 min	24	66	3	7
12 min	13	70	0	27
30 min	0	54	0	46
40 min	0	30	0	70
Ascochyta pisi				
Crude extract	95–100	0–5	0	0
Fraction F1	0	100	0	0
Fraction F2	90	10	0	0
Fraction F2 purified on anionic exchange resin‡	100	0	0	0
Acid hydrolysis† of F2 purified:				
3 min	63	33	1	3
6 min	29	65	1	5
10 min	10	72	0.5	17
30 min	0	48	0	52
70 min	0	15	0	85
130 min	0	0	0	100
Septoria nodorum crude extract	95	5	0	0

* HPLC on a Waters apparatus, Microbondapack C_{18} column (30 × 0.4 cm); acetate buffer 0.1 M, pH 5.6; UV detection at 310 nm.

† N HCl, 100°.

‡ Dowex 1 × 8, Cl^-: open form remains adsorbed.

min), whereas the next fraction (F2) was a mixture of F1 (20%) and of a second compound (80%). The crude extract of the spores (without ion exchange chromatography) showed in most cases, either only this last substance or, more rarely, this one mixed with a trace of F1.

The MS of F1 and F2 in which the molecular ions were absent, both show, as a base peak, an ion corresponding to the mycosporin-2 aglycone MS base peak: *m/z* 267 (M − 18 for the ring form and M − 2 × 18 for the open form) [6,9]. The MS of the TMSi derivatives show a hexosyl moiety but were different (in F1, 7 TMSi and 1 hexosyl groups and in the main compound of F2, 6 TMSi and 1 hexosyl groups). In F2 MS, the presence of F1 can be detected, at the same level as in HPLC. In both cases after loss of 18 amu (F1 TMSi, *m/z* 951, main compound of F2 TMSi, *m/z* 879) an intense ion was obtained by the removal of a fragment $-CH_2O$-tetraTMSi-hexosyl (+H with F1TMSi, *m/z* 470, and +2H with the other compound, *m/z* 397). By analogy with the MS of mycosporin-2 [6,9] this fragmentation allowed us to show that the *O*-hexosyl substituent was on the 9-CH_2OH, α to the N or NH group.

Acid hydrolysis of F2, followed by HPLC (see Table 1) showed that the main compound was changed, at first into F1 then, after a short time, in mycosporin-2 aglycone, mainly in the open form **3**. Moreover, some **2** was observed in the ring form which quickly opened. In the hydrolysis of F2, glucose was also identified, as TMSi derivative, using GC.

The same procedures have been used to study the mycosporins of *A. pisi*. The mycosporins from *S. nodorum* were identified from the crude extract, by comparative HPLC.

From this work, it can be concluded that the natural mycosporin of these three fungi is 9-*O*-glucosyl-mycosporin-2(**4**), eluted in F2 and in which the amino moiety is in the ring form, but is easily opened by acid treatment, for example during the transfer to a cation exchange column to give **5** eluted in F1. This result

explains at least two of the three compounds obtained by Trione and Leach from *A. pisi* [8]. The third substance should be the aglycone open form **3**.

From these results, it is tempting to think that the open forms of mycosporin-2(aglycone and glucoside) are artefactual. Nevertheless, the HPLC analysis of the crude extract of *Morchella esculenta* (L.) *ex* St Amans has shown the natural and single existence of the ring opened aglycone **3** in the cap (spore receptacle) of this fungus. It is noteworthy that there was no absorption at 310 nm (no mycosporin) in the stipe extract. The biological significance and biosynthetic pathways of these two structural forms and of their glycosylation process remain an open question and are under investigation.

EXPERIMENTAL

Rapid analysis of mycosporin from S. nodorum *and* M. esculenta. Two Petri dishes of *S. nodorum* culture or *ca* 200 mg of *M. esculenta* carpophore were extracted with EtOH or MeOH (25 ml) and the suspended material filtered. The alcoholic solns were taken to dryness at room temp. under vacuum and the residues, taken up in 20 ml of H_2O, were analysed by UV at 310 nm and by HPLC (see Table 1).

Isolation and purification of the mycosporins from C. herbarum. *C. herbarum* was cultured on malt medium: (g/l) malt extract (30), casein hydrolysate (0.7), $MgSO_4$ (0.1), KH_2PO_4 (0.2), agar (12), under 12 hr light, 12 hr dark. After 1 month, the powdery black conidia were suspended in MeOH and filtered. UV spectral control at 310 nm showed that the first washing extracted 95% of the absorbing compounds. The filtrate was concd to dryness and the residue, dissolved in H_2O (25 ml), put on a Dowex 50X 8(H^+) column (16 × 2.5 cm); elution by H_2O was followed at 310 nm. The first impure fractions were discarded (240 ml) and two main fractions were obtained and lyophilized: F1 (5 mg) and F2 (17 mg) as amorphous colourless (or pale yellow) powders.

9-O-*Glucosylmycosporin*-2 *open form* (from F1): UV $\lambda_{max}^{H_2O}$ nm 310, HPLC (see Table 1). MS of the TMSi derivative: *m/z* (%) 969 (40) M^+, 951 (100) M − 18, 935 (19), 470 (100) M − CH_2O-tetra TMSi-hexosyl + H, 380 (17), 361 (14).

9-O-*Glucosylmycosporin*-2 *ring form*: UV $\lambda_{max}^{H_2O}$ nm 310, ε = 25000, HPLC (see Table 1) (from F2 purified).

MS of the TMSi derivative (from F2): *m/z* (%) 969 (13) M^+ open form, 951 (19), M − 18 open form, 897 (71) M^+ ring form, 879 (86) M − 18 ring form, 864 (17), 397 (100) M − CH_2O-tetraTMSi-hexosyl + 2H ring form, 470 (28) corresponding peak for open form.

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