Isolation of the New (−)-(3 *R*,4 *S*)-4-Hydroxymellein from the Fungus *Septoria nodorum* Berk

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The new (−)-(3 *R*,4 *S*)-4-hydroxymellein (**6**) is isolated from the fungus *Septoria (Phaeosphaeria) nodorum* Berk, together with the previously reported isomer **5.** The results so far obtained in this series of metabolites are discussed in relationship with biosynthetic considerations.

Introduction

The fungus *Septoria (Phaeosphaeria) nodorum* Berk is a common parasite of wheat. It is responsible for a fungal disease which seriously affects crops (septoriosis). This fungus produces in cultures a series of highly potent phytotoxins which were previously investigated. Thus, we reported on the isolation of (−)-(3 *R*)-mellein (ochracin) (**4**) [1], (3 *R*)-O-methylmellein, (−)-(3 *R*,4 *R*)-4-hydroxymellein (**5**), (−)-(3 *R*)-7-hydroxymellein, mycophenolic acid [2], septorine [3], N-methoxyseptorine [4], and N-methoxyseptorinol [5]. Undoubtedly, the observed phytotoxic activity of this fungus is due to the summation of these various metabolites, some of them being endowed of a particularly strong biological activity. Mellein was shown to produce an important reduction in CO_2 net assimilation and an increase in stomatal resistance in seedling leaves of wheat [6–10].

In the present publication, the isolation of the new isomer (−)-(3 *R*,4 *S*) (**6**) from the culture medium of this fungus is reported. The results so far observed are discussed in relationship with the fact that all the isocoumarins up to now isolated from *Septoria nodorum* are derived from (−)-mellein (**4**).

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Results and Discussion

The culture medium of the fungus *Septoria nodorum* Berk was extracted by ethyl acetate according to a previously [11] reported method. The concentrated extract was submitted to HPLC fractionation (UV detection), leading to the known (−)-(3 *R*,4 *R*)-4-hydroxymellein (**5**) [2], plus the new compound (−)-(3 *S*,4 *R*)-4-hydroxymellein (**6**).

The structures of these substances **5** and **6** were determined on the basis of physicochemical data, by comparing with previously reported results (m.p. and $(\alpha)_D$ as represented in Fig. 1). In this series, the coupling constants of the protons at C-3 and C-4 are determinant for the attribution of the stereochemistry. As the absolute configurations were established for (−)-mellein (**1**) and (+)-(3 *S*,4 *S*)-4-hydroxymellein (**2**) [12, 14], it was possible to fix the configurations of the products isolated from *Septoria nodorum*. The stereochemistry of the new product **6** was confirmed by irradiation at the signal of the methyl group at 1.55 ppm, resulting in two coalescent signals (2 H) for the two vicinal protons at C-3 and C-4 (one peak). Hence, it is of course deduced that the coupling constant is not noticeable between these two protons. The dihedral angle determined from a molecular model gives a value of 70° which corresponds to a coupling constant of *ca.* 0.3 Hz [16]. By now, three of the four possible isomers of 4-hydroxymellein are known [2, 12, 14, 15]. To our knowledge, the (+)-isomer (3 *S*,4 *R*) **3** still remains to be found and could exist in strains producing the corresponding (+)-mellein [14]. A diacetate of **6** was prepared which exhibited MS and ^{1}H NMR spectra in agreement with the proposed stereochemistry. In particular, the vicinal ^3J coupling constant observed in the ^{1}H NMR of this diacetate is of approximately 1 Hz. It appears from the molecular models that two conformers are possible for substance **6,** each requiring some energy for transformation. The dihedral angle 3-C–H 4-C–H is of *ca.* 70° when the methyl group is β-axial which leads to the observed coupling constant according to the Karplus equation [16]. But in the conformer having this methyl group β-equatorial, this angle is

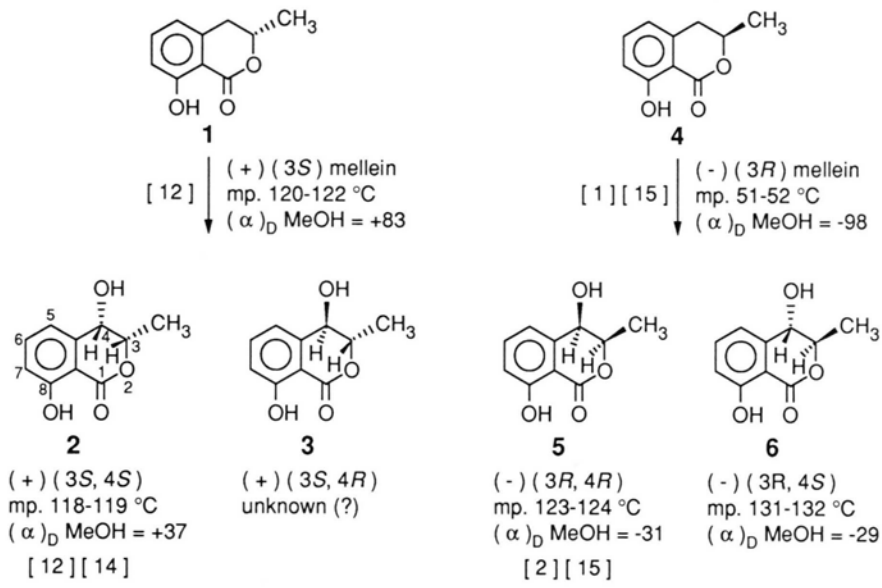

Fig. 1. Absolute configurations of the two melleins (ochracins) and of the four possible 4-hydroxymelleins.

of 180°, a value which must give a higher J (between 6 and 15 Hz). Hence, two conclusions may be drawn: 1) the compound **6** isolated from *Septoria nodorum* has the 3-β-axial conformation, 2) the possibility that two conformers exist for each represented stereochemistry of **2, 3, 5, 6,** can not be excluded. The observed differences in the $J_{3,4}$-values between conformers of the *cis*-series **2** and **5** must be small (or negligible), whereas such differences should be much bigger in the *trans*-series **3** and **6.**

The presence of the two isomers **5** and **6** in the culture medium of *Septoria nodorum* is in agreement with the demonstration that the biosynthesis of (+)-(3*S*,4*S*)-4-hydroxymellein (**2**) proceeds through oxidation of (+)-mellein (**1**) by molecular oxygen [14]. The previously found (−)-O-methylmellein and (−)-7-hydroxymellein, together present in *Septoria nodorum* with (−)-mellein [2] and the (−)-4-hydroxymelleins, demonstrate of course the same biogenetic family.

Experimental

Melting points have been determined with a Kofler apparatus under the microscope and are corrected. MS were carried out on an AEI MS 50 spectrometer, ^{1}H NMR on a Bruker 200 MHz apparatus ($CDCl_3$, δ ppm). Rotatory power was determined on an electronic Perkin-Elmer polarimeter 241. TLC's on Schleicher-Schüll SiO_2 fluorescent films, 1 mm thickness for preparative purposes, UV observation with a Desaga lamp at 254 nm, or $FeCl_3$ spray for visualization.

The 4-hydroxymelleins **5** and **6** were extracted from the culture medium of *Septoria nodorum* according to the reported [11] method. The EtOAc concentration was submitted to HPLC (elution by ethanol−water, UV detection) and the two substances **5** and **6** recovered according to the absorption curve. SiO_2 TLC (CH_3Cl−EtOAc 5:1), **5** R_f 0.60, colour reaction with $FeCl_3$: blue, **6** R_f 0.65, $FeCl_3$ pink, relative proportions 5:1. The physicochemical data of substance **5** are identical to the reported values [2] except for small differences in the m.p. and $(\alpha)_D$ due to more accurate technical facilities (such as electronic polarimeter). These values are reported on the Figure. **6:** MS m/z (%), 194 M^+ (100), 150 (70), 121 (80), 122 (80); ^{1}H NMR: 1.55 (d, 3H, CH_3), 4.50 (enlarged signal, 2H, H-3 and H-4), giving a singlet by irradiation of the methyl signal at 1.55; aromatic protons: 7.04 (d, 1H, H-5, J = 8 Hz), 7.55 (dd, 1H, H-6, J = 8 Hz), 6.95 (d, 1H, H-7, J = 8 Hz), 11 (s, 1H, 8-OH). The diacetate of **6** was prepared by treatment with acetic anhydride in the presence of anhydrous pyridine (10 mg **6,** 6 drops of each reagent, 1 h at 37 °C, drying under *in vacuo* overnight at room tempera-

ture). SiO_2 TLC in pentane–ethyl acetate 1:1, R_f 0.70, MS *m/z* (%) 278 M^+ (2), 236 $M-42^+$ (100), 194 $M-42-42^+$ (45), 176 M−42−60 (100), 150 (100), 149 (75), 43 CH_3CO^+ (95). ^1H NMR ($CDCl_3$): 1.35 (d, 3H, CH_3), 2.13 and 2.40 (s, 3H each, CH_3COO), 4.80 (m, 1H, H-3), 5.83 (d, 1H, H-4, J = 1 Hz), 7.18 (d, 1H, H-5, J = 8 Hz), 7.36 (d, 1H, H-7, J = 8 Hz), 7.66 (dd, 1H, H-6, J = 8 Hz).

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