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BIOSYNTHESIS OF OOSPONOL AND OOSPOGLYCOL ELUCIDATED BY ^{13}C NMR

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Key Word Index—*Gloeophyllum abietinum*; fungal metabolites; oosponol; oospoglycol; biosyntheses; ^{13}C NMR.

Abstract—The biosynthesis of the antibiotic metabolities oosponol and oospoglycol by the fungus *Gloeophyllum abietinum* has been analysed using ^{13}C-labelled acetate, malonate, serine and formate as precursors and ^{13}C NMR as the analytical method. The polyketide pathway could be confirmed. One carbon of the isocoumarin ring originates from serine. Copyright © 1996 Elsevier Science

INTRODUCTION

Oosponol (**1**) is a secondary fungal metabolite which has been isolated from the following basidiomycetes; *Oospora adstringens* [1], *Lenzites subferoginea* [2], *Gloeophyllum sepiarium* [3], *L. trabea* [4] and *L. thermophila* [5]. We isolated oosponol and the reduction product oospoglycol (**2**) from *G. abietinum* (Bull. ex FR.) P. Karst. [6]. Oosponol is a prominent toxin with antibiotic activities against plants and Gram-positive bacteria, whereas the reduced oospoglycol is a precursor metabolite within the mycelia [7]. The biosynthesis of oosponol is elicited and stimulated 1000-fold if *Gloeophyllum* is growing in the vicinity of an antagonistic fungus or is attacking conifer host cells [8].

In 1966, Nitta *et al.* [9] investigated the biogenesis of oosponol in the fungus *O. adstringens* by radioactive labelling with [1-^{14}C]-, [b-^{14}C] and [u-^{14}C]- glucose, ethyl [2-^{14}C] malonate and sodium [^{14}C] formate. They proposed that the compound is formed by condensation of five C-2 units following the polyketide pathway and via incorporation of a C-1 unit (C-3) followed by a cyclization between this C-1 element and a terminal carboxyl group. However, the degradation route used in the localization of the labelled C atoms was very complicated and the origin of the C-1 unit remained doubtful.

RESULTS AND DISCUSSION

Fourteen-day-old, dual cultures of *G. abietinum* and *Heterobasidion annosum* (as an inducer of metabolite production) were incubated for 48 hr with [1-^{13}C] acetate, [2-^{13}C] acetate, [1-^{13}C] malonate, [2-^{13}C] malonate or [3-^{13}C] serine.

Oosponol (**I**) and oospoglycol (**II**) were then isolated from the cultures and the positions of the ^{13}C-labelled carbons in these two compounds located by ^{13}C NMR spectroscopy.

The incorporation of ^{13}C was investigated mainly in oospoglycol, since it can be isolated in higher yields from the fungal cultures.

The ppm values and the relative signal intensities of the carbons of unlabelled oospoglycol are listed in Table 1. After labelling with ^{13}C-precursors, the signals from the labelled carbon moieties are increased.

In Table 1, this increase of the signal intensities is compared to the normal oospoglycol values and standardized by setting the intensities of C-9 or C-10, respectively, to 1.0. On quantitative analysis of the corresponding oosponol resonances, we observed very similar changes of the standardized signal intensities for the dehydrogenated metabolite.

The data for the labelled acetate experiments make it easy to derive which carbon comes from which C of acetate. The polyketide pathway can be thus confirmed. As expected, malonate as the committed intermediate in polyketide synthesis is incorporated in an analogous mode. Control experiments with labelled mevalono-

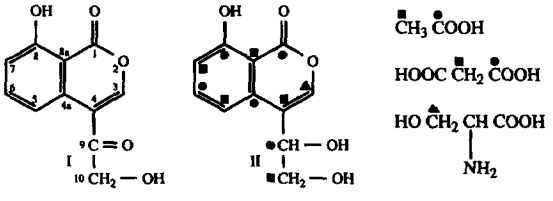

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Table 1. ^{13}C signals from oospoglycol and relative intensities in comparison with the signals of unlabelled oospoglycol

^{13}C-spectrum	C-1	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	C-9	C-10
Unlabelled oospoglycol											
Found (ppm)	167.3	143.5	119.2	137.0	116.0	138.2	114.4	162.7	107.4	69.7	66.1
Calculated (ppm)	163.0	122.5	123.3	137.5	118.7	134.1	114.8	158.4	115.4	76.5	67.7
Rel. natural signal intens.	16.9	98.5	32.3	15.4	107.7	86.1	109.2	27.7	11.5	100	100
Labelled oospoglycol (standardized)*											
$^{13}CH_3$-CO_2H	0.8	1.2	10.2	0.9	10.9	1.2	10.8	1.1	13.0	1.0	11.8
CH_3-$^{13}CO_2H$	8.9	1.3	1.0	12.3	0.8	13.1	0.8	7.9	1.4	9.6	1.0
$^{13}CH_3$-$(CO_2H)_2$	1.0	1.0	4.6	1.6	2.8	1.1	2.8	1.0	4.3	1.0	3.1
CH_2-$(^{13}CO_2H)_2$	5.9	1.6	0.7	12.9	0.7	7.5	1.0	4.3	0.9	6.6	1.0
$HO^{13}CH_2 \cdot CH(NH_2)CO_2H$	not visible	7.0	n.v	n.v	1.0	0.6	1.0	0.1	n.v	0.5	1.0
$H^{13}CO_2H$	not visible	4.8	n.v	n.v	0.9	1.0	0.9	0.1	n.v.	0.8	1.0

*C-9 or C-10 set to 1.

lactone demonstrated no influence on the original signal intensities of oospoglycol or oosponol.

The above data showed that C-3 is not derived from either an acetate or a malonate unit, but by a C-1 transfer from serine which is an important C_1-donor to tetrahydrofolic acid (THF) in which process it also gives rise to the simple amino acid glycine. THF can bind this carbon in different oxidation states, i.e. $-CH_3$, CH_2OH or $-CHO$, and acts as a one-carbon donor in many biosynthesis pathways [10, 11]. Therefore, formic acid could also be used as a C-1 donor for this position.

EXPERIMENTAL

Cultures. Gloeophyllum abietinum (Bull. ex FR.) P. Karst. was grown in 2.4% potato dextrose (Difco, Detroit), 2% Bacto-Agar (Difco), pH 5.5, sterilized for 20 min at 120°, 3 atm. in Petri dishes with 9 cm diameter, agar 5 mm. Dual cultures with *H. annosum* (Fr.) Bref. P. Karst. were used in order to stimulate oosponol and oospoglycol synthesis, as mentioned before. Incubation was performed at 26° in the dark.

Labelling with ^{13}C. Fourteen days after inoculation of the fungi, the following compounds were solubilized in 100 μl water and added to the cultures, at 20 mg each per agar dish: [1-^{13}C] acetate, [2-^{13}C] acetate (from MSD Isotopes, Merck, Montreal, Canada, 93% isotopic abundance), [1-^{13}C] malonate, [2-^{13}C] malonate, DL-[3-^{13}C] serine, ^{13}C-formate (from Ambridge Isotope Laboratories, Andover, MA, U.S.A, 99% isotopic abundance).

Fractionation and isolation of the metabolites. 48 hr after addition of the ^{13}C precursors, the fungal metabolites were isolated. The agar medium of the *Gloeophyllum* area with fungal mycelium including the growth inhibition zones between the fungi, was extracted with MeOH (×3) using a Warring Blendor. After evapn at 30°, the extract residues were redissolved in ethyl acetate, and the solutions were then centrifuged and concd. A methanolic solution of the isolated metabolites was fractionated by semipreparative and analytic HPLC on reverse phase columns: Hypersil, RP 18, 250 mm × 5 mm, size 5 μm and Kontrosorb, RP18, 250mm × 10mm, size μm (Kontron), respectively. The elution was preformed using a linear gradient 30% methanol/water to 100% methanol in 50 min. Flow rates of 0.5 ml min^{-1} or 2.0 ml min^{-1}, respectively, with detection at 238 nm.

NMR data. The ^{13}C NMR spectra of the isolated oosponol and oospoglycol were measured with a Bruker AMX 500 using broadband decoupling. The signals were assigned with C/H correlation spectra and also using computer calculations according to Pretsch [12].

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