Die Vergleichssubstanzen entstammten teils dem Handel: Cholesterin, Campesterin, Stigmasterin, Sitosterin, oder wurden synthetisiert: Δ^7-Campesterin und Δ^7-Stigmasterin [8, 9], $\Delta^{7,22}$-Stigmastadienol [10]. Aus einem in seiner Zusammensetzung bekannten Steringemisch von Sonnenblumenöl wurde 24-Äthylcholesta-7,24(25)-dien-3β-ol isoliert und aus Kürbiskernöl 24-Äthylcholesta-7,25-dien-3β-ol und 24-Äthylcholesta-7,22,25-trien-3β-ol. Die gaschromatographische Zuordnung des 24-Äthylcholesta-5,25-dien-3β-ols erfolgte an Hand bekannter chromatographischer Gesetzmäßigkeiten [11].

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PRODUCTION OF METHYLATED PHENOLIC ACIDS BY SPECIES OF *LENTINUS* (BASIDIOMYCETES)

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Key Word Index—*Lentinus*; *Lentinellus*; Basidiomycetes; methylated phenolic acids; cinnamate metabolism.

Lentinus lepideus is a suitable organism for studies of cinnamate metabolism because it readily produces the methyl esters of a number of substituted cinnamic acids when grown in liquid culture [1,2]. We have examined the following species of *Lentinus* and of *Lentinellus*, a closely related genus, for their ability to produce these compounds: *Lentinus lepideus* Fr., *L. ponderosus* O.K. Miller, *L. edodes* (Berk.) Singer, *L. kauffmanii* Smith, *L. tigrinus* Bull. ex Fr., *L. sulcatus* Berk, and *Lentinellus vulpinus* (Fr.) Kuhner & Maire, *L. cochleatus* (Fr.) Karst.

Of these only *Lentinus lepideus* and *L. ponderosus* produced methyl esters of phenolic acids when grown under our conditions [3]. *L. ponderosus* yielded methyl cinnamate, methyl *p*-methoxycinnamate, methyl isoferulate and methyl anisate. *L. lepideus* produced methyl *p*-coumarate in addition to these compounds. The other fungi also produced phenolic compounds but they have not been identified. Neither *L. lepideus* nor *L. ponderosus* produced detectable amounts of free *p*-coumaric, caffeic or isoferulic acids and there were no qualitative or quantitative differences between light and dark grown cultures of *L. ponderosus*. *L. lepideus* has been previously reported to produce methyl *p*-methoxycinnamate when cultured in dark [4]. Light, therefore, does not seem to play a role in the regulation of cinnamate metabolism in these two species. Increased production of phenylalanine ammonia lyase, the enzyme which catalyzes the synthesis of cinnamate from L-phenylalanine, occurs when certain Basidiomycetes are cultured in light, but this is not a general phenomenon [5,6].

We have previously reported the occurrence, in *L. lepideus*, of a *p*-specific *O*-methyltransferase which can only methylate methyl esters of *p*-hydroxycinnamic acids [3]. This enzyme also occurs in *L. ponderosus*. We also have indirect evidence that a similar enzyme is produced by the rust, *Uromyces phaseoli*, the uredospores of which produce a germination inhibitor, methyl *cis*-3,4-dimethoxycinnamate [7]. Leaves of the Mexican bean, when infected by this fungus, yield an *O*-methyltransferase which catalyzes the methylation of methyl *p*-coumarate but not *p*-coumaric acid. Preparations from uninfected leaves are unable to methylate either methyl *p*-coumarate or *p*-coumaric acid [8].

'Woody' bracket fungi do not produce typical lignins of higher plants. This has been shown with *Polyporus* and *Fomes* [9], and more recently with *Phellinus igniarius* [10]. These results appear to confirm the prediction of Swain who stated in 1971 that "it appears, therefore that the fungi possess all the necessary enzymic system to produce lignin precursors, except one. This is the enzyme which catalyzes the methylation of the hydroxyl group oriented *meta* to the side chain, which would yield ferulic acid from caffeic acid . . ." [11].

EXPERIMENTAL

Culture conditions. All cultures were incubated in 200 ml medium in 500 ml Erlenmeyer flasks as described previously [3].

TLC, PC and GLC. TLC and PC have also been described previously [3]. GLC was carried out with a Tracor 500 Gas

Chromatograph: 5% OV-1 on Chromosorb 60/80 column, temp programmed from 100° to 250° at 10°/min, FID.

Isolation of methyl esters from L. ponderosus. Mycelial mats from 4 flasks of a 5-week-old culture were separated from the medium by decanting and ground in a Waring blender with MeOH. After filtering, MeOH was evaporated and residue extracted with Et_2O (mycelial dry wt: 3.2 g). The medium (pH 4.3) was extracted with Et_2O and pooled together with the Et_2O extract from the mycelial mat. A small sample of this extract and the Et_2O extract of the medium acidified to pH 2 were used for TLC, PC and GLC in the detection of phenolic compounds. The remaining total Et_2O extract was passed through a silicic acid column with C_6H_6–Me_2CO (19:1) as eluting solvent to isolate a mixture of the methyl esters of cinnamic and benzoic acids. These methyl esters were then separated on a Sephadex LH-20 column. The gel was swollen in MeOH satd with *n*-heptane and the eluting solvent was *n*-heptane satd with MeOH. The appearance of each compound in the eluate was monitored by UV. Methyl cinnamate was the first compound collected (0.9 mg). Its identity was proved by UV, GLC and TLC. UV. λ_{max}^{MeOH} (log ε) 217, 222,275 nm (4.14, 4.13, 4.16). The second fraction consisted of a very small amount of methyl *p*-methoxybenzoate (λ_{max}^{MeOH} 254 nm) and *cis* and *trans* methyl *p*-methoxycinnamate as determined by UV and GC. The third fraction contained *trans* methyl *p*-methoxycinnamate (108 mg), mp 87–88°, λ_{max}^{MeOH} (log ε) 225,291 *sh*, 298 *sh*, 309 nm (4.13, 4.16, 4.16, 4.17). Its *trans* configuration was determined by NMR which gave a coupling constant value of 16 Hz for the methylene protons. It also showed the same retention time as a standard on GLC. Methyl isoferulate (8 mg) was eluted from the column with MeOH satd with *n*-heptane, and identified by UV, TLC and NMR. UV λ_{max}^{MeOH} (log ε) 220, 242, 294, 324 nm (4.13, 4.13, 4.14, 4.14).

Enzyme assay. Enzyme assay for phenolic *O*-methyltransferase was carried out as described for *L. lepideus* [3].

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TWO NEW COUMARINS IN *BOENNINGHAUSENIA ALBIFLORA*

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Key Word Index—*Boenninghausenia albiflora*; Rutaceae; (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one; (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one.

Abstract—Two new isomeric coumarins were isolated from leaves of *Boenninghausenia albiflora* Reichb. Their structures were elucidated as (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one and (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one.

INTRODUCTION

The occurrence of many coumarins in *Boenninghausenia albiflora* has been reported [1–7], among which, a dimeric coumarin, matsukaze lactone [2], nodakenetin acetate [3] and 3-(1,1-dimethyl allyl)-xanthyletin [4] were reported to be novel.

In the course of our studies on the fragrant components in the essential oil of *B. albiflora*, two compounds were conspicuous because of their strong fluorescence under UV irradiation and higher polarities on TLC. The isolation and structural elucidation of these compounds has now revealed them to be (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one and (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one. Some other coumarin derivatives which were new in this essential oil were also identified.

RESULTS AND DISCUSSION

Structure of compound 1

From the ether extract of the steam distillate of the plant a crystalline precipitate separated out on evaporation of the solvent. It had a mp 159–160° after recrystallization from acetone–benzene (compound **1**). It gave a