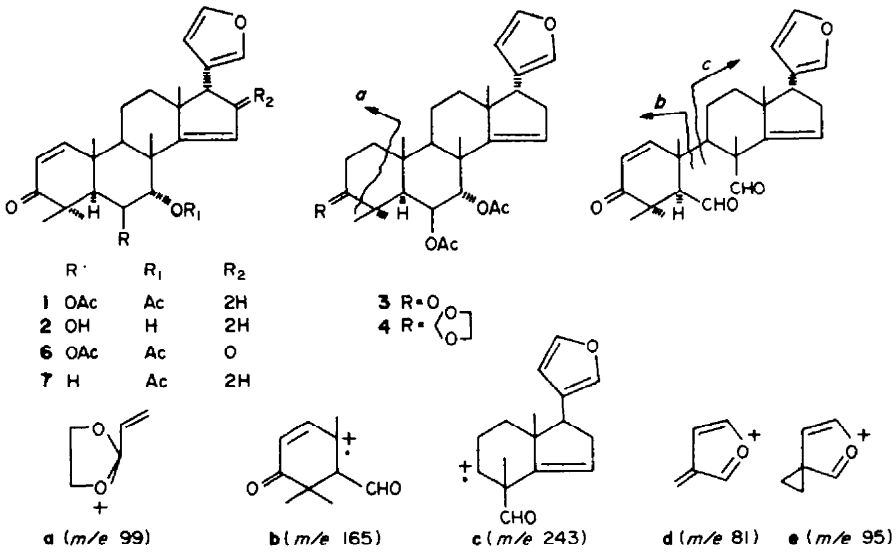

deshielded and appeared as a doublet at δ 2.7 (J = 4 Hz) ppm, coupled with the newly generated aldehyde proton.

Dysobindiol failed to yield an isopropylidine derivative, indicating that the two hydroxyl groups were *trans* to each other. Further, there was no evidence of axial, axial couplings of the C-5, C-6 and C-7 protons in the NMR spectra of **1** and **2** and the hydroxyls at C-6 and C-7 could possibly have the β-axial and α-axial configurations respectively. Dysobindiol on reacetylation (Ac_2O–Py) yielded dysobinin, indicating that no change in configuration took place during alkaline hydrolysis of dysobinin to dysobindiol.

The mass spectra of **1**, **2** and **3** gave rise to a fragment *d* (*m/e* 81) due to a β-substituted furan ring at C-17 and another peak at *m/e* 95 due to fragment *e* which could arise by the cleavage of C-15:C-16 bond facilitated by the presence of a C-14 double bond. This also confirmed the absence of a carbonyl group in ring-D and was in agreement with the cracking pattern of other meliacins [5,6]. The presence of the trisubstituted double bond at C-14 was confirmed by SeO_2 oxidation of **1** when a new diketone (**6**), $C_{30}H_{36}O_7$ mp > 300° was obtained, which had a carbonyl absorption at 1710 cm^{-1} for cyclopentenone [6].

Dysobinin is an example of the growing family of meliacins [5–7] and is of chemotaxonomic importance as it is a 6-acetoxy derivative of azadirone (**7**) occurring in *Melia azadirachta* [6] a plant of the same family.

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STEROLS OF *ALTERNARIA KIKUCHIANA*

ALVIN N. STARRATT
Agriculture Canada, Research Institute, University Sub P. O., London, Ontario, Canada, N6A 5B7

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Key Word Index—*Alternaria kikuchiana*; Dematiaceae; fungi; sterols; ergosterol; ergosterol peroxide; lanosterol; 24-methylene-24,25-dihydrolanosterol.

Although sterols have been isolated from many fungi, there are only two reports of their identification in *Alternaria* species. Aizina and Zlatoust [1] reported that ergosterol was the main sterol of *A. brassicicola* and *A. tenuis* and Brown and Jacobs [2] obtained ergosterol peroxide from *A. dianthicola*. An unidentified steroid, mp 180°, was isolated by Sugiyama *et al.* [3] from culture filtrates of the phytopathogenic fungus *A. kikuchiana*.

The results of our investigation of the sterols of *A. kikuchiana* are presented in this report.

Ergosterol was the main sterol isolated from the mycelium of *A. kikuchiana* when cultures were grown in the dark. In addition, four minor sterol constituents were obtained. Two of these were identified as lanosterol and 24-methylene-24,25-dihydrolanosterol by direct comparison with authentic samples. The third sterol was tentatively identified as 4,4-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol. The purest sample that was obtained crystallized as plates, mp 160–168° (lit. [4] 167–170°). IR, NMR and MS data as well as the result obtained with the Liebermann–Burchard reagent were consistent with this structure. Goulston *et al.* [5] have proposed the same structure for a 4,4-dimethyl sterol which they isolated from *Phycomyces blakesleeanus* and *Agaricus campestris*. The fourth compound, a 4-monomethyl sterol, was probably 4α-methyl-5α-ergosta-7,24(28)-dien-3β-ol (24-methylenelophenol). Its MS was in general agreement with that reported for 24-methylenelophenol acetate [6,7]. The observation that a peak at *m/e* 285 was the base peak supports the presence of a Δ^7 rather than a $\Delta^{8,9}$ double bond [8]. Goulston *et al.* [9] have tentatively identified 24-methylenelophenol as a metabolite of *Aspergillus fumigatus*. They have also discussed the significance of these 4,4-dimethyl and 4α-methyl sterols in ergosterol biosynthesis by fungi [5,9].

When *A. kikuchiana* was cultured under normal light conditions, the main sterol isolated from extracts of the mycelium was ergosterol peroxide. This compound is possibly identical to the steroid isolated by Sugiyama *et al.* [3] from culture filtrates of this fungus. Although there were apparent differences in other respects, the mps of the steroid and its monoacetyl drivative were in close agreement with that of ergosterol peroxide and its acetate. A recent paper reported that ergosterol is converted into ergosterol peroxide in two fungi by simultaneous photo-oxidation and enzymic pathways and also that this conversion can take place during the work-up phase unless low-level illumination is used [10]. Although we did not study its production in detail, our results indicate that the ergosterol peroxide which we isolated was not an artifact of extraction but was formed in part at least by a photo-oxidative process during growth of the fungus.

EXPERIMENTAL

Mp's were determined on a Kofler hot stage and are uncorrected. NMR spectra were obtained for solns in $CDCl_3$ at 60 MHz; for small samples a Varian C-1024 time averaging computer was used. 20% $AgNO_3$–Kieselgel (Camag) plates were used for TLC. GLC analyses were carried out on a 1.8 m × 3.5 mm glass column packed with 3% SE-30 on 100–120 mesh Gas Chrom Q at 280° with 22 ml/min N_2.

Cultural conditions. Alternaria kikuchiana Tanaka, obtained from American Type Culture Collection (number 11570), was grown at 30° with aeration for 6–8 days in a 10 l. bottle containing 6.5 l. of the medium used by Sugiyama *et al.* [3] for cultivation of this organism. Foaming was controlled by the addition of Antifoam B Emulsion (Dow Corning). Mycelium was separated from the culture filtrate, air dried, and ground in a Waring blender.

Extraction and isolation of sterols. Mycelium (45.8 g) from a culture grown in the dark was Soxhlet extracted with $CHCl_3$. Extracted material (4.6 g) was chromatographed over neutral alumina (Woelm, Grade V). Elution with C_6H_6–petrol (1:1) gave fractions containing 4,4-dimethyl and 4α-methyl sterols (20 mg) and C_6H_6 yielded fractions containing 4-demethyl sterols (188 mg). Recrystallization of the latter from Et_2O–Me_2CO gave material, mp 155–159°, λ_{max}^{EtOH} nm (log ε): 293(3.84), 282(4.08), 272(4.05), $[\alpha]_D$ − 124° (*c*, 1.2), which was identified as ergosterol by comparison (TLC, IR, NMR) with an authentic sample. Material from 3 similar chromatograms was bulked for the investigation of the 4,4-dimethyl and 4α-methyl sterols. PLC on $AgNO_3$–Kieselgel with $CHCl_3$ (developed twice) yielded four sterol-containing fractions designated 1–4 in order of increasing polarity. Fraction 1 (5 mg) was crystallized from Et_2O–MeOH to give crystals, mp 130–132°. This compound, M^+ 426, was identified as lanosterol by comparison (mmp, IR, TLC, GLC, reaction with Liebermann–Burchard reagent) with authentic material. Fraction 2 (7 mg) yielded fine needles, mp 154–155°, from Et_2O–MeOH. This compound, M^+ 440, ν_{max}^{KBr} cm^{-1}: 890 (>C═CH_2), was identified as 24-methylene-24,25-dihydrolanosterol by comparison (mmp, IR, TLC, GLC, reaction with Liebermann–Burchard reagent) with material synthesized from lanosterol acetate [11]. Fraction 3 (8 mg) yielded a small quantity of plates mp 160–168°, $\nu_{max}^{CHCl_3}$ cm^{-1}: 890 (>C═CH_2), δ 4.72 (broad; >C═CH_2), from Et_2O–MeOH. Treatment with the Liebermann–Burchard reagent gave a rapid greenish–blue coloration. This compound had a longer retention time (10.1 min) on GLC than lanosterol (8.7 min) and 24-methylene-24,25-dihydrolanosterol (9.8 min). The MS had a molecular ion at *m/e* 426 and fragment ions at *m/e* values of 411 (M^+-Me), 393 (M^+-[Me + H_2O]), 342 (M^+-84), 327 (M^+-[Me + 84]), 299 (M^+-[side chain + 2H]), 259 (M^+-[side chain + 42]), 241 (M^+-[side chain + 42 + H_2O]). Fraction 4 (3 mg) yielded fine needles, mp 146–154°, δ 4.72 (broad; >C═CH_2), from Et_2O–MeOH. The MS had a molecular ion at *m/e* 412 and fragment ions at *m/e* values of 397 (M^+-Me), 379 (M^+-[Me + H_2O]), 328 (M^+-84), 313 (M^+-[Me + 84]), 285 (M^+-[side chain + 2H]), 269 (M^+-[side chain + H_2O]), 245 (M^+-[side chain + 42]), 227 (M^+-[side chain + 42 + H_2O]). This compound was indistinguishable from 24-methylenelophenol by GLC, TLC, and reaction with the Liebermann–Burchard reagent but lack of material prevented definite establishment of identity. Mycelium (25 g) from a culture grown under normal light conditions was extracted with $CHCl_3$ and the extract chromatographed over neutral alumina (Woelm; Grade I). Elution with C_6H_6–Et_2O (3:1) yielded material (68 mg) which crystallized (EtOH) as needles, mp 179–181°, $[\alpha]_D$ −30° (*c*, 1.0), identified as ergosterol peroxide (mmp, TLC, IR, NMR); acetate mp 201–202° (mmp, TLC, IR, NMR).

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