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**Naematolin, a New Biologically Active Substance produced
by *Naematoloma fasciculare* (Fr.) KARST**

We have recently isolated a biologically active substance from the fermentation broth of *Naematoloma fasciculare* (Fr.) KARST,¹⁾ *Naematoloma*, *Strophariaceae*, which is one of bitter and poisonous mushrooms. The active substance was found new and named naematolin after its producing organism. We now communicate on the isolation, characterization and biological properties of naematolin.

A piece of the mycelium successively cultured on the potato agar was aseptically inoculated in a liquid medium composed of glucose 4% and cotton seed meal (Proflo) 0.4% (pH 6.0) and cultivated for two weeks at 27° on a reciprocating shaker. The production of naematolin during fermentation was noticed by its bitter taste. Naematolin produced was extracted from the filtered broth with AcOEt. Evaporation of the solvent *in vacuo* gave a crude crystalline residue, which, on repeated recrystallization from AcOEt-C₆H₆ (1:1), followed by preparative thin-layer chromatography on Kieselgel GF using ether as solvent, gave naematolin as bitter white needles, m.p. 145.5°, $[\alpha]_D^{25} -360^\circ$ (c=1.0, CHCl₃).

Naematolin is a neutral substance and the molecular formula was determined to be C₁₇H₂₄O₅ by elementary analysis and mass spectrometry (*Anal. Calcd.*: C, 66.21; H, 7.85. M.W. 308.38. Found: C, 66.66; H, 7.64. M.W. 308 (mass)). UV $\lambda_{\max}^{\text{MeOH}}$ m μ (ϵ): 212.5 (8400), 242.5 (shoulder, 4280) and 326 (96.4). IR and NMR spectra were shown in Fig. 1 and 2 respectively. Negative Cotton effect was observed in its ORD and CD curves. ORD in MeOH: $[\phi]_{800} -1230^\circ$, $[\phi]_{350} -10150^\circ$ (trough), $[\phi]_{293} +2438^\circ$ (peak), $[\phi]_{250} -7080^\circ$. CD in MeOH: $[\theta]_{374} 0$, $[\theta]_{330} -10170$, $[\theta]_{273} 0$, (*F*/2 25 m μ).

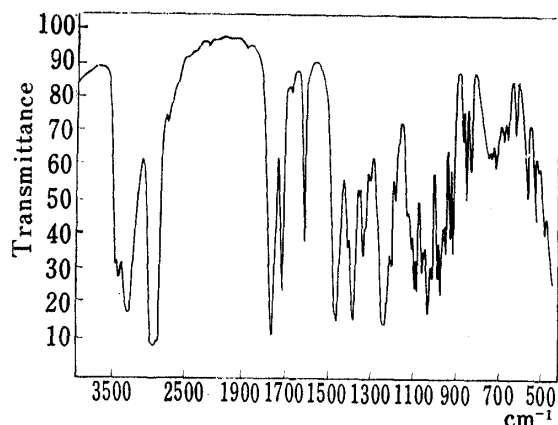


Fig. 1. Infrared Spectrum of Naematolin
(Nujol)

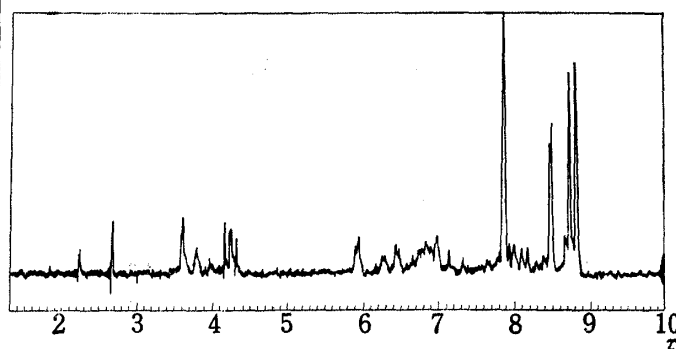


Fig. 2. Nuclear Magnetic Resonance Spectrum
of Naematolin 60 Mc. in (CD₃)₂CO/CDCl₃ (50%)

Naematolin is positive to 2,4-dinitrophenylhydrazine and hydroxamic acid reactions, and decolorized KMnO₄ aq. solution and Br₂ in CHCl₃, while it is negative to ninhydrin, anthrone, Tollens, FeCl₃, and Lieberman-Burchard's reactions. Naematolin is readily soluble in most of organic solvents except hydrocarbons. It is slightly soluble in H₂O, 5% HCl and 5% NaHCO₃ aq. solution, but soluble in 5% NaOH with decomposition. On thin-layer chromatography using Kieselgel GF as a carrier, naematolin shows following R_f values: cyclohexane·CHCl₃·EtOH (2:8:1) 0.47, C₆H₆·MeOH (9:1) 0.25, *n*-hexane·Me₂CO

1) Synonym: *Hypholoma fasciculare* (Fr.) Quél. Nigakuritake (にがくりたけ) in Japanese. The living organism grown in the copse near Fujieda city was collected and afforded us by Mr. Shoji Kawamura, Member of The Mycological Society of Japan, to whom the writers are deeply indebted.

(4:1) 0.19, $C_6H_6 \cdot Me_2CO$ (4:1) 0.35, *n*-hexane·ether (1:4) 0.50, ether·AcOEt (1:1) 0.91. On acetylation with Ac_2O in pyridine, naematolin gives the diacetate as white needles, m.p. 134~135.5°, $[\alpha]_D^{20} -280^\circ$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{28}O_7$: C, 64.27; H, 7.19. M.W. 392.46. Found: C, 64.72, 64.73; H, 7.15, 6.98. M.W. 392 (mass). UV λ_{max}^{MeOH} $m\mu$ (ϵ): 210.5 (8900), 240 (shoulder, 4580), 326 (82.3). IR and NMR spectra of naematolin diacetate were illustrated in Fig. 3 and 4 respectively.

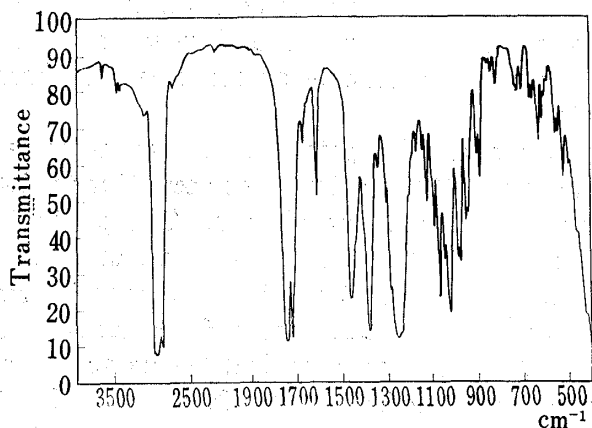


Fig. 3. Infrared Spectrum of Naematolin Diacetate (Nujol)

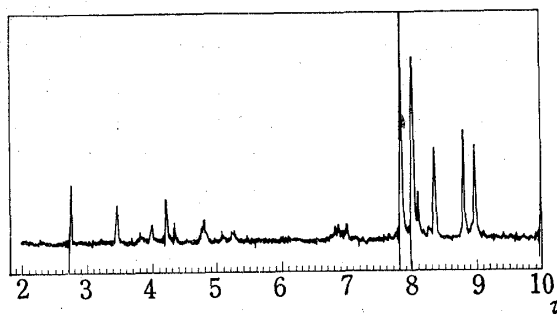


Fig. 4. Nuclear Magnetic Resonance Spectrum of Naematolin Diacetate 60 Mc. in $CDCl_3$

Naematolin showed no antimicrobial activity at 1 mg./ml., but showed cytotoxic activity against HeLa cells, 50% of the cells (HeLa S_3) being degenerated at 6.25 γ /ml. in tissue culture. By the plaque method, naematolin was found to inhibit the growth of Polio virus (host cells: HeLa) and Vesicular stomatitis virus (Host cells: CEC) at 1250 γ /ml. Besides, interestingly, it showed coronary vasodilating action to the isolated guinea-pig heart on administration of 100 γ per a heart, when examined by the Langendorff's technique using Lock's solution as perfusion fluid. LD_{50} of naematolin in mice was 400 mg./kg. (i.v.).

Among the metabolites of basidiomycetes including *Naematoloma fasciculare*, no substances were reported which are similar to naematolin in their properties. The structure elucidation and detailed evaluation of the pharmacological activity of naematolin are now under investigation.

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