Fluorescent Constituents and Cultivation of *Lampteromyces japonicus*

By M. ENDO, M. KAJIWARA, and K. NAKANISHI*†
(*Department of Chemistry, Tohoku University, Sendai, Japan*)

Summary Fluorescent compounds which may be related to the bioluminescence of *L. japonicus, i.e.*, illudin S and ergostatetra-4,6,8(14),22-en-3-one, have been isolated from the fruiting bodies and mycelia; conditions for growing the mushrooms in a bioluminescent state are also described.

CELL-FREE light emission from fungi extracts was first observed in 1958 by Airth and McElroy,[1] and subsequent studies[2] have led to the concept that light is emitted through the following steps in the case of fungi: $X + NADH \xrightarrow{E_s} XH_2 + NAD$; $XH_2 + O_2 \xrightarrow{E_p} X'^* + H_2O$; $X'^* \longrightarrow X' +$ light (528 nm), where X = electron acceptor, E_s = soluble enzyme, XH_2 = luciferin, E_p = particulate enzyme, X'^* = excited state of oxyluciferin, and X' = oxyluciferin. However, the structure(s) of the fungal luciferin(s) still remains to be clarified.

The luminous mycelia and fruiting bodies of *Lampteromyces japonicus* (Kawam.) Sing. ("tsukiyotake" in Japanese), a mushroom growing on rotten beech trees, emit light centred at 528 nm, which is identical with the bioluminescent peak of other fungi,[3] *e.g.*, *Armillaria mellea, Collybia velutipes, Mycena citricolor* (*Omphalia flavida*). We report the isolation, characterization, and emission spectra of several compounds from *L. japonicus*, the biological transformation of which may be related to the bioluminescence; it is hoped that further studies using Airth's enzyme system[4] will clarify the nature of the luciferin. Laboratory cultivation of bioluminescent mycelia (and fruiting bodies) is also described. This will greatly facilitate future investigations as the natural mushrooms only grow for a period of one week in mountainous areas.

(i) *Illudin S* (*Lampterol*) (I): This is the compound responsible for the anti-tumour activity[5] and toxicity of the mushroom, and can be represented by structure (I)[6] including its absolute configuration.[7] Repeated silica-gel chromatography (elution with 10 : 1 mixture of chloroform and ethanol) of the methanol extract of 80 kg of fresh fruiting bodies gave 4·4 g of illudin S, u.v. (MeOH), 235 (log ϵ 4·10), 320 nm (3·54); the fluorescence peak is at 550 nm. One of the u.v. peaks is identical with that reported by Kuwabara and Wassink[8] for fungal luciferin from *M. citricolor* (*O. flavida*) *i.e.*, 270 and 320 nm (in pH 6·5 water). The mycelia of *L. japonicus* also contain illudin S, as checked by t.l.c.

(ii) *Ergosterol*: Silica-gel chromatography (elution with 93 : 7 mixture of methylene chloride and methanol) of the methanol extract of 10 kg of fruiting bodies gave 261 mg of ergosterol.

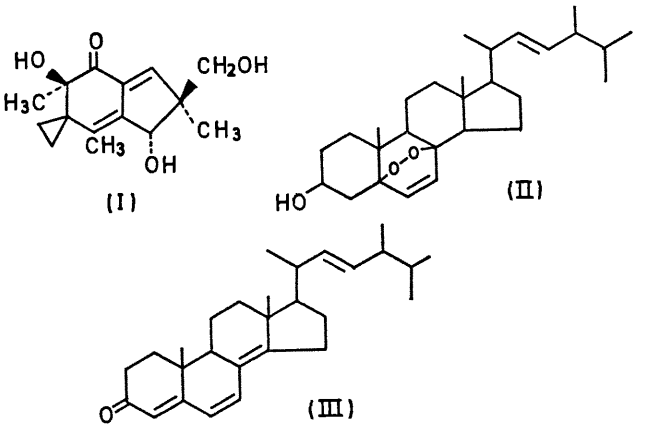
(iii) *Ergosterol peroxide* (II): Repeated silica-gel chromatography (elutions with chloroform and chloroform–methanol mixtures) and preparative t.l.c. of 10 kg of the methanol extract yielded 255 mg of ergosterol peroxide (structure based on spectral and analytical data), which was first prepared by photosensitized oxidation of ergosterol[9] and subsequently isolated from the mycelia of the mould *Asperigillus fumigatus*.[10]

(iv) *Ergostatetra-4,6,8(14),22-en-3-one* (III): Silica-gel chromatography (benzene elution), preparative t.l.c. (benzene–acetone, 9 : 1), and t.l.c. with infinite development (n-hexane–benzene, 2 : 1) gave from 52 kg of fresh fruiting

† Present address: Department of Chemistry, Columbia University, New York, N.Y. 10027.

bodies 13·5 mg of a strongly fluorescent substance. Spectral and analytical data characterized it as ergostatetra-4,6,8-(14),22-en-3-one (III); this compound has been obtained by oxidation of 14-dehydroergosterol[11] and more recently isolated from the yeast *Candida utilis*[12] and a nonluminous fungus *Fomes officinalis*.[13] U.v. (EtOH) at 348 nm (log ϵ 4·41); it is to be noted that the fluorescence emission peak at 530 nm is identical with the bioluminescent peak of luminous fungi.

Cultivation of mycelia (and fruiting bodies). The following media were inoculated with mycelial mats collected from agar slants, which in turn had been inoculated with spores collected from the fruiting bodies and inoculated at 23·5°.



(i) *Liquid medium*: To 4000 ml of a first solution (consisting of 8 g of yeast extract, 80 g of peptone, 200 g of glucose, 3·48 g of KH_2PO_4, 2 g of $MgSO_4,7H_2O$, 1·2 g of $CaCl_2,2H_2O$, and 4000 ml of water) there were added 80 ml of a second solution (consisting of 0·36 g of $MnCl_2$, 0·5 g of $FeCl_3$, 0·2 g of $ZnCl_2$, 0·05 g of $CuSO_4$, and 1000 ml of water) and 20 ml of cornsteep liquor. The pH was adjusted to 5·5 and, after inoculation, the mycelia were cultivated for 2—4 weeks at 23·5°. Formation of bioluminescent mycelial mats was observed.

(ii) *Sawdust medium*: A 3 : 1 mixture of sawdust and rice-bran was treated with 60% of its weight of water, inoculated with the mycelia, and incubated. Growth of fruiting bodies took place after 2 months; both mycelia and fruiting bodies emitted light detectable with dark-adapted eyes.

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