## Communications to the Editor

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# THE TOXIC PRINCIPLES OF NAEMATOLOMA FASCICULARE

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Fasciculol E and F were identified as the toxic principles of Naematoloma fasciculare (Nigakuritake), a poisonous mushroom. These compounds caused paralysis and death in mice. The $LD_{50}$ values of fasciculol E and F to mice were determined as 50 mg/kg and 168 mg/kg (i.p.), respectively.

KEYWORDS —— poisonous mushroom; Naematoloma fasciculare; fasciculol E; fasciculol F; lanostane-type triterpenes; $LD_{50}$ value

Naematoloma fasciculare (Fr.) Karst (Japanese name: Nigakuritake, Strophariaceae) has been known as a poisonous mushroom, and intoxication in man by ingestion of this mushroom has frequently been reported.[1,2] This mushroom has also been proved, by feeding the aqueous ethanolic extract to rats, to contain some pharmacologically active substances.[3] However, the toxic principle of this mushroom is still obscure.

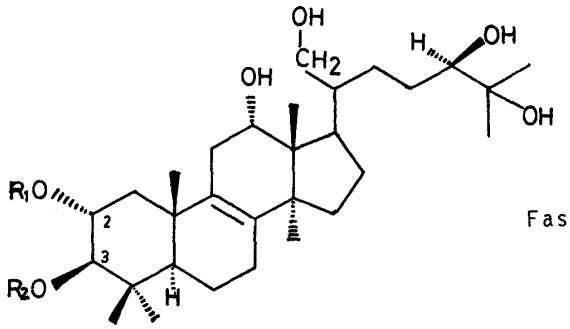
The aqueous methanolic extract (55.0 g) obtained from the fresh fruit-bodies of N. fasciculare (fresh weight 1873 g, collected in Kyoto, autumn, 1981) caused paralysis and death in mice when 500 mg/kg of the extract was administered intraperitoneally to mice. After fractionation with benzene, aqueous n-butanol and water, the toxic components were gathered in the n-butanol-soluble fraction (13.84 g). Elaborate separation of the acetone-soluble part (11.81 g) of the n-butanol-soluble fraction with a preparative high performance liquid chromatography (HPLC)[4] afforded two colorless amorphous compounds, tentatively named NFT-1 (670 mg) and NFT-2 (500 mg), as toxic principles.

Intraperitoneal administration of 250 mg/kg of NFT-1 or 100 mg/kg of NFT-2 caused similar symptoms in mice to those observed by the administration of the aqueous methanolic extract. On post-mortem examination of the mice administered NFT-1 or -2, congestions in liver and kidney were observed; however, no degeneration of tissues was recognized in these organs. The paralysis of the respiratory center might be considered to cause the mice to die. NFT-1 and -2 gave $LD_{50}$ values of 168 mg/kg and 50 mg/Kg, respectively, in intraperitoneal administration in mice[5] by the Litchfield-Wilcoxon method.

From this mushroom, some characteristic triterpenoids, named fasciculols,[6] have been isolated together with the ordinary metabolites such as amines, amino acids, purines, sugars and sterols.[7] Some of these fasciculols have been shown to have an inhibitory effect on plant growth and antimicrobial activity.

The chemical and spectral data on NFT-1 and -2 indicate that these compounds are triterpenoid derivatives, isomeric to each other, and comparison of the data shows that their structures closely resemble those of fasciculols F and E.

Fasciculol E




$R_1$ = H

$R_2$ = CO-$CH_2$-C(OH)($CH_3$)-$CH_2$-CONH-$CH_2$-$COOCH_3$

Fasciculol F

$R_1$ = CO-$CH_2$-C(OH)($CH_3$)-$CH_2$-CONH-$CH_2$-$COOCH_3$

$R_2$ = H

The difference between NFT-1 and -2 in the $^1$H-NMR spectra appeared in the signals of the acyloxy methine proton which appeared at $\delta$5.02 ppm (doublet of triplet $J_1$=10, $J_2$=4 Hz) in NFT-1 and at 4.57 ppm (doublet, J=10 Hz) in NFT-2. The above observation strongly suggested that NFT-1 and -2 are identical to fasciculol F and E, respectively. The assumption was supported by the fact that the signals corresponding to C-2 and C-3 appeared at 74.0 and 79.6 ppm in the $^{13}$C-NMR spectrum of NFT-1 and at 66.9 and 85.3 ppm in that of NFT-2. On treatment with p-toluene-sulfonic acid monohydrate and dry acetone, NFT-1 and -2 afforded NFT-1 monoacetonide, mp 122-124°C, and NFT-2 monoacetonide, mp 148-150°C, whose melting points corresponded with those reported for fasciculol F monoacetonide, mp 120°C, and fasciculol E monoacetonide, mp 147°C.[6c] As a conclusion from the results, NFT-1 and - 2, which we isolated from N. fasciculare as toxic principles, are identified as fasciculol F and E, respectively, formerly isolated from the same mushroom.

In addition, twelve samples of N. fasciculare from different places in Japan were collected. Among the samples, the aqueous methanolic extracts of seven samples exhibited toxicity to mice, but the other five were not toxic. On thin layer chromatography, the extracts of the toxic seven samples afforded large spots corresponding to fasciculols F and E, but the nontoxic five afforded only traces of these compounds.

We have very recently isolated new toxic metabolites, named hebevinosides, from Hebeloma vinosophyllum, a poisonous mushroom belonging to Cortinariaceae, which have a fatal paralytic effect on mice and whose structures seem to be glycosides of lanostane-type triterpene.[8] It is interesting that both fasciculols and hebevinosides have similar lanostane-type structures and similar biological activities.

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