Isolation and Structure Elucidation of a New Indole Metabolite from *Aspergillus ruber*

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In the course of our screening research for insecticidal metabolites of fungi, we found the occurrence of a substance with growth inhibitory activity for silkworm larvae (*Bombyx mori* L.) by oral administration in mycelia of *Aspergillus ruber* separated from beet pulp. In this paper we wish to report the isolation and structure elucidation of a new indole metabolite as the active principle.

Asp. ruber was stationarily cultivated at 26°C for 19 days on the medium composed of sucrose 10 g, NaCl 6 g, rice 100 g and water 50 ml. Mycelia was extracted with ethyl acetate and an ethyl acetate-soluble neutral fraction was separated from the extract by conventional method. The fraction was applied to silica gel column chromatography using benzene-ethyl acetate. An eluate with benzene containing 40% ethyl acetate gave two compounds (I) and (II) by preparative thin-layer chromatography developed with benzene-ethyl acetate (1:1).

The physicochemical data of I are as follows. Mp 262~264°C. MS *m/e*: 323.1601 (M^+ , $C_{19}H_{21}N_5O_2$; calcd. 323.1631), 254 ($M-C_6H_9$). NMR $\delta_{TMS}^{DMSO-d_6}$: 11.03 (1H, s, $-NH-$, indole nucleus), 8.57 (1H, s, $-NH-CO-$), 8.31 (1H, s, $-NH-CO-$), 7.44~7.00 (4H, m), 6.92 (1H, s), 6.10 (1H, dd, $J=9.9$ Hz and 17.8 Hz, $-CH=CH_2$), 5.02 (1H, dd, $J=9.9$ Hz and

1.1 Hz; 1H, dd, $J=17.8$ Hz and 1.1 Hz, $-CH=CH_2$), 4.17 (1H, q, $J=7.0$ Hz, $-CH-CH_3$), 1.48 (6H, s, $(CH_3)_2C-$), 1.38 (3H, d, $J=7.0$ Hz, $-CH-CH_3$). IR ν_{max}^{Nujol} cm^{-1} : 1675, 1633. UV λ_{max}^{MeOH} nm (ϵ): 224 (31,600), 283 (8,300), 290 (8,050), 338 (9,340). Acid hydrolysis of I yielded alanine and alkali hydrolysis gave 2-(1,1-dimethyl-2-propenyl)-indol-3-aldehyde. On the basis of these evidences we determined the structure of I as alanyl-2-(1,1-dimethyl-2-propenyl)-dehydrotryptophan anhydride,*** which had already been reported by Pochini *et al.*¹⁾

I R = H

II R =

The molecular formula of compound II, a new metabolite, was assigned as $C_{24}H_{29}N_5O_2$ by high resolution mass spectrometry (*m/e* 391.2262; calcd. 391.2258). NMR spectrum in CD_3COCD_3 showed signals due to three aromatic protons at 7.34 (1H, d, $J=8.2$ Hz), 7.10 (1H, d, $J=1.7$ Hz) and 6.99 (1H, dd, $J=8.2$ Hz and 1.7 Hz). Signals at 5.38 (1H, broad t, $J=7.1$ Hz), 3.40 (2H, d, $J=7.1$ Hz) and 1.71 (6H, broad s) suggested the presence of $Ar-CH_2-CH=C(CH_3)_2$. Other data in NMR, IR and UV were almost identical with those of I.

Accordingly II was considered to have a side chain, 3-methyl-2-butenyl group, at 5 or 6 position of indole nucleus in I. The values of chemical shifts and coupling constants due to aromatic protons of II were very close to those of neoechinulin²⁾ and 6-(3-methyl-2-butenyl)-indole.³⁾ Thus we assigned the side chain at 6 and established the structure of the new

*** Pochini *et al.* had not mentioned the details of spectrophotometric data of this compound essential for structure determination.

metabolite as II.

II causes growth inhibition to silkworm larvae by oral administration at the concentration of 1000 ppm. The details will be reported elsewhere.

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