NK374200, a Novel Insecticidal Agent from *Taralomyces*, Found by Physico-chemical Screening

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In the course of a physico-chemical screening program, a novel microbial secondary metabolite with an adenine moiety was isolated from the culture broth of the fungus *Taralomyces* sp. The compound NK374200 had a novel peptidyl adenine nucleus. NK374200 was screened in various biological assay systems, and found to have anti-mosquito larval activity. In this report we describe the screening, isolation, structural elucidation and preliminary biological activities of NK374200.

Physico-chemical screening for novel compounds with purine or pyrimidine moiety was attempted since these compounds were previously reported to have various biological activities[1]. As a first step, 50 filtrates of fungal culture broth were applied to Dowex 50W × 8 (H^+) and eluted with 1.4 M NH_4OH solution respectively, since targeted compounds were expected to be partially purified by this procedure. As the second step, each eluate was subjected to HPLC analysis under the following column conditions: Hewlett packard ODS hypersil, temperature; 40°C, carrier; 5 mM NH_4OAc solution with a linear MeCN gradient to 50%, flow rate; 0.3 ml/minute, detection; UV absorption at 260 nm. In this step, peaks which had the same retention times as known metabolites were omitted. As the third step, about 100 unidentified peaks were applied to 3 dimensional UV absorption spectrum analysis to detect the presence of a purine or pyrimidine moiety in a molecule. One peak showed the same UV spectrum as purine, and a compound corresponding to this peak was selected as a candidate and tentatively named NK374200. The producing strain had been isolated from a soil, and was identified as the fungus *Taralomyces* sp.

NK374200 was produced by rotary shaking culture in the medium containing sucrose 2%, glucose 1%, soy bean meal 2%, KH_2PO_4 0.1% and $MgSO_4$ 0.01% (pH = 6.2). The maximum productivity was attained at 4 days under 27°C. NK374200 in the filtrate of culture broth (40 liters) was adsorbed on Dowex 50W × 8 (H^+) and eluted with 1.4 M NH_4OH solution. The eluate was again adsorbed on Dowex 1 × 2 (OH^-) and eluted with a linear HCl gradient from 0.05 M to 1 M. After removal of HCl by Dowex WGR(OH^-), NK374200 was further purified by successive column chromatographies, Sephadex G-10 (developed with H_2O), carbon (eluted with a linear NH_4OH gradient from 0.05 M to 2 M) and Sephadex LH-20 (developed with 50% aqueous MeOH). NK374200 was finally concentrated to dryness and 830 mg of NK374200 was obtained as a pale brown powder.

NK374200 was soluble in H_2O but insoluble in MeOH, EtOH, Me_2CO and $CHCl_3$. NK374200 gave a positive color in Rydon Smith, toluidine-chlorine and ninhydrin reaction. On silica gel TLC (Merck Art. No. 5715) developed with EtOH - 28% NH_4OH (19 : 1) solution, NK374200 gave a single spot at Rf = 0.42. Physico-chemical properties were as follows: MP (°C) 172 ~ 175; $[\alpha]_D^{20}$ +5.9 (*c* 0.731, H_2O); HRFAB-MS *m/z* Found 308.1472 $(M+H)^+$ Calcd 308.1471 for $C_{12}H_{18}N_7O_3$; UV $\lambda_{max}^{H_2O}$ nm ($E^{1\%}$) 261.8 (441), 261.2 in 0.1 M NaOH, 258.8 in 0.1 M HCl; IR (KBr) cm^{-1} 3320 1650 1600 1390 1250 800; Amino acid analysis, alanine. ^1H NMR and ^{13}C NMR spectroscopic data of NK374200 are shown in Table 1. Based on these physico-chermical data and 2D NMR data, a structure of NK374200 was deduced as 9-(3-L-alanylamino-3-carboxypropyl)adenine (Fig. 1a). This structure including absolute configurations was finally established by synthetic study, which will be reported elsewhere. NK374200 is a novel metabolite with peptidyl adenine nucleus and is related to deoxyeritadenine (Fig. 1b) isolated from *Lentinus edodes*[2] and discadenine (Fig. 1c) isolated from *Dictyostelium discoideum*[3].

Biological activities of NK374200 were tested with various assay systems available in our laboratories, such as cytotoxicity, immunosuppressive, immunostimulative, antiviral, antibaterial and antifungal activities. NK374200 was found to have anti-mosquito larval activity (Table 2). NK374200 was shown to have low

Table 1. ^1H and ^{13}C NMR spectroscopic data of NK374200 in DMSO-d_6.

Atom No.[a]	δ_C (100 MHz)	δ_H (400 MHz)
2	141.06 (d)	8.13 (s)
4	149.30 (s)	—
5	118.69 (s)	—
6	155.78 (s)	—
6-NH_2	—	-NH_2, 7.21 (2H, s)
8	152.19 (d)	8.14 (s)
10	40.38 (t)	4.17 (2H, m)
11	32.70 (t)	2.00 (m)
		2.26 (m)
12	51.24 (d)	3.97 (m)
13	173.07 (s)	—
14	—	-NH, 8.42 (d, J = 5.5 Hz)
15	170.52 (s)	—
16	48.54 (d)	3.80 (m)
16-NH_2	—	-NH_2, 5.0 ~ 6.5 (2H, br)
17	18.12 (q)	1.28 (3H, d, J = 6.6 Hz)

[a] Numbering is illustrated in Fig. 1.

Fig. 1. Structures of NK374200 (a) and two related compounds, deoxyeritadenine (b) and discadenine (c).

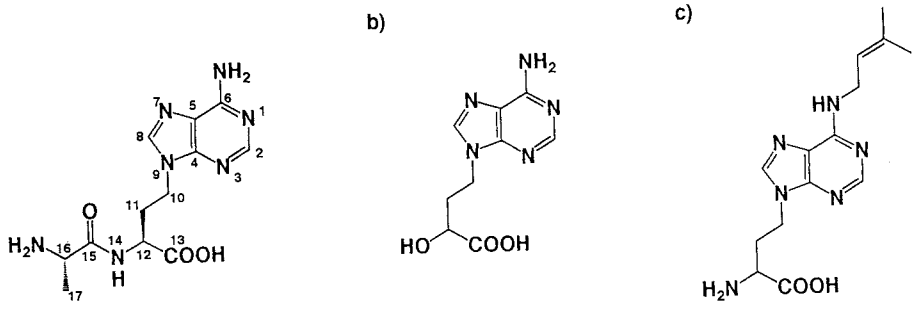

Table 2. Insecticidal activity of NK374200 against mosquito larva[a].

Dose (μg/ml)	Mortality (%)
10	100
1	90
0.1	10
0.01	0

[a] A laboratory colony of mosquito, *Culex pipiens* was used for the bioassay. Ten 3rd instar larvae were released into test sample solutions in separated plastic cup and the dead larvae were counted 48 hours after treatment.

toxicity. Cytotoxicity against HeLa cell was not observed at 100 μg/ml and no acute toxicity was observed in mice at 160 mg/kg (ip).

In this report we isolated a novel metabolite, NK374200, with anti-mosquito larval activity. Detailed insecticidal activities, and another biological activities of NK374200 will be examined in future studies. NK374200 was selected by screening focused on the physico-chemical properties of purine and pyrimidine moiety. This pointed out the usefulness of physico-chemical screening for discovery of unique microbial metabolites.

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