Journal of Natural Products
Vol. 53, No. 6, pp. 1508-1513, Nov-Dec 1990

ISOLATION, STRUCTURE, AND SYNTHESIS OF NOVEL 4-QUINOLINONE ALKALOIDS FROM *ESENBECKIA LEIOCARPA*

TETSUO NAKATSU, TIMOTHY JOHNS, ISAO KUBO,*

Division of Entomology and Parasitology, College of Natural Resources

KATHARINE MILTON,

Department of Anthropology, University of California, Berkeley, California 94720

MASAMI SAKAI, KATSUHIRO CHATANI, KEN SAITO, YOSHIRO YAMAGIWA, and TADAO KAMIKAWA*

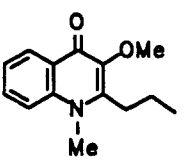
Department of Chemistry, Faculty of Science and Technology, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577, Japan

ABSTRACT.—Two new biocidal quinolinone alkaloids, 3-methoxy-1-methyl-2-propyl-4-quinolone [**1**] and 2(1′-ethylpropyl)-1-methyl-4-quinolone [**2**], were efficiently isolated using reversed-phase recycling hplc from the leaves of *Esenbeckia leiocarpa*. The structures were determined through spectroscopic data and confirmed by total synthesis. These alkaloids have antifeedant activities against the pink bollworm, *Pectinophora gossypiella*.

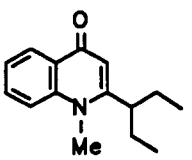
A study of the feeding ecology of the Brazilian woolly spider monkey *Brachyteles arachnoides* showed that these animals avoided the leaves of *Esenbeckia leiocarpa* Engl. (Rutaceae) as a food, even though this was one of the two most abundant tree species in the habitat (1). We tested the hypothesis that this species was avoided in the diet as a result of the presence of toxic secondary metabolites. Because we could not test the biological activity against the monkey, several simple bioassays were chosen to isolate the biologically active compounds.

The $CHCl_3$ extract of the leaves of *E. leiocarpa* showed antifeedant activities against the pink bollworm, *Pectinophora gossypiella*, in our artificial-diet feeding (ADF) assay (2). The $CHCl_3$ extract was fractionated into acidic and basic fractions. The basic fraction showed antifeedant activity. Further purification of its active principles was performed by reversed-phase chromatography on recycling hplc (r-hplc). Two novel alkaloids, leiokinine A [**1**] and leiokinine B [**2**], were completely separated after two cycles on the r-hplc in 0.01% and 0.008% yields, respectively, from the dried leaves. Leiokinines A and B showed moderate antifeedant activities against the pink bollworm *P. gossypiella* in ADF assay.

Compounds **1** and **2** had related nmr spectra as shown in Table 1. Both structures were determined through analysis of spectroscopic data and finally confirmed by synthesis. The hrms of leiokinine A [**1**] gave the molecular formula $C_{14}H_{17}NO_2$. The ir (1615 and 1590 cm^{-1}), the uv [λ max 343 (ϵ 12,700), 335 (ϵ 12,000), and 242 nm (ϵ 12,900)], two deshielded methyl groups at δ 3.79 and 3.93 in the ^1H nmr, and a carbonyl at δ 172.08 in the ^{13}C nmr indicated that a γ-pyridone moiety was present. The four aromatic protons in the ^1H nmr at δ 7.35 (dd, $J = 8.4$ and 1.0 Hz), 7.48 (bd, $J = 8.4$ Hz), 7.64 (dd, $J = 8.4$ and 2.1 Hz), and 8.52 (dd, $J = 8.4$ and 2.1 Hz) were assigned to the 1,2-disubstituted benzene. Thus, leiokinine A is a 4-quinolinone derivative.



1



2

TABLE 1. ^{1}H- and ^{13}C-nmr Data of Leiokinine A [**1**] and Leiokinine B [**2**].

Position	Compound			
	1		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
2	N/A[a]	147.63	N/A	158.39
3	N/A	140.25	6.31	108.94
4	N/A	172.08	N/A	177.81
5	N/A	141.21	N/A	142.30
6	8.52	126.76	8.47	126.64
7	7.35	122.75	7.38	123.35
8	7.64	131.47	7.67	131.93
9	7.48	115.19	7.57	115.83
10	N/A	126.92	N/A	126.64
OMe	3.93	60.04	N/A	N/A
NMe	3.79	34.69	3.00	34.21
1′	2.94	29.40	2.83	44.30
2′	1.68	22.26	1.73	27.66
			1.74	27.66
3′	1.10	11.46	0.92	11.66

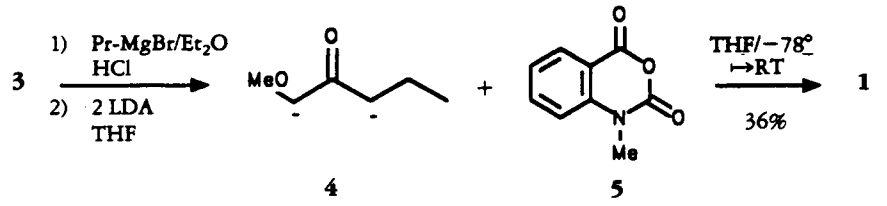
[a]N/A = not available.

The complete assignments of chemical shifts were significant in determining the whole structure using a ^{1}H-nmr nOe experiment. The ^{1}H-^{13}C heteronuclear COSY nmr spectra clarified which methyl group could be assigned to N-Me (δ 3.79 in the ^{1}H nmr and 34.69 in the ^{13}C nmr) and O-Me (δ 3.93 and 60.04). Also the protons of the propyl group (δ 1.10, 3H and 11.46; δ 1.68, 2H and 22.26; and δ 2.94, 2H and 29.40) could be assigned. The regiochemistry of the O-Me and the propyl group were determined through the nOe difference ^{1}H-nmr spectra based on the above assignment. Upon irradiation at δ 3.79 (N-Me), the enhancements were observed at three signals, δ 7.48 (H-9 in the benzene ring) and 2.94 (H-1′) and 1.68 (H-2′) of the propyl group, which meant the nitrogen of N-Me was located at the α position of benzene and adjacent to the propyl group. Thus, the structure of leiokinine A was determined as 3-methoxy-1-methyl-2-propyl-4-quinolone [**1**].

The hrms spectra of leiokinine B [**2**] gave the molecular formula $C_{15}H_{19}NO$. The ir and uv spectra of leiokinine B were closely related to those of leiokinine A, which suggested that leiokinine B was also a 4-quinolinone derivative. The ^{1}H nmr of **2** indicated that there was an olefinic proton (δ 6.31, s) instead of the methoxy group of **1**, and an ethylpropyl group (^{1}H nmr δ 0.92, 6H, t, J = 6 Hz; 1.73, 2H, quint, J = 6 Hz; 1.74, 2H, quint, J = 6 Hz; and 2.83, 1H, quint, J = 6 Hz) instead of the propyl group of **1**. The regiochemistry of **2** was determined by nmr comparison to **1**. Thus, the structure of leiokinine B was determined as 2(1′-ethylpropyl)-1-methyl-4-quinolone [**2**].

Leiokinine A had an unusual 3-oxygenated substitution of quinoline (3). Leiokinine B had an ethylpropyl group, which is an unusual group for a natural product. The confirmation of these structures and further biological studies demanded a total synthesis. According to further bioassay with pure compounds, these two alkaloids inhibit the growth of various kinds of organisms including animals, plants, and microorganisms. These compounds are, in fact, general growth inhibitors or biological poisons.

Leiokinine A [**1**] was first synthesized from *N*-methylisatoic anhydride [**5**] (4) (Scheme 1). The Grignard reaction on $MeOCH_2CN$ [**3**] with C_3H_7MgBr followed by

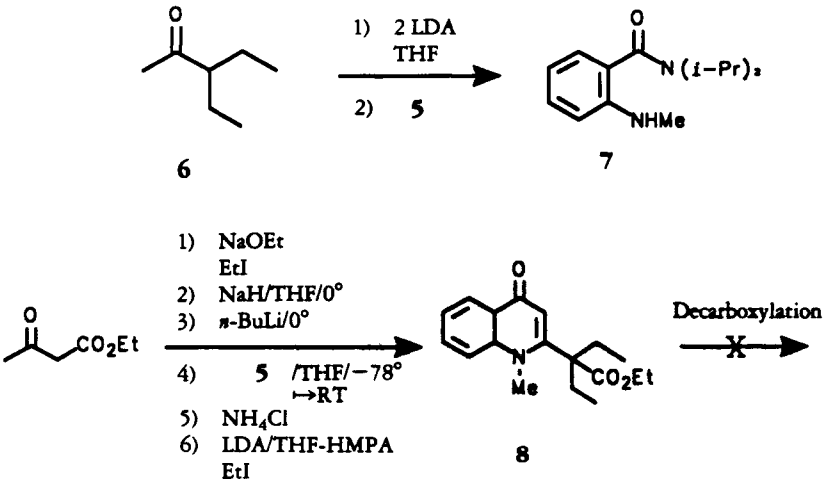


4 5

SCHEME 1. Synthesis of leiokinine A [1].

acid hydrolysis gave methoxymethylpropyl ketone in 62% yield. Treatment of **5** and dianion **4** which was derived from the above ketone with two equivalents of LDA afforded leiokinine A [**1**] in 36% yield. The synthesized **1** was identical to the leiokinine A [**1**] isolated from the leaves of *E. leiocarpa* in all data including mp, ir, uv, and nmr.

A similar synthetic strategy was not successful for the synthesis of leiokinine B [**2**]. For example (Scheme 2), treatment of **5** with a dianion derived from 3-ethyl-2-pentanone [**6**] gave a diisopropylamide **7** as a sole isolable product, and decarboxylation of compound **8** synthesized through an alternate route from carboethoxyacetone was not successful due to the large steric hindrance of the two ethyl groups.



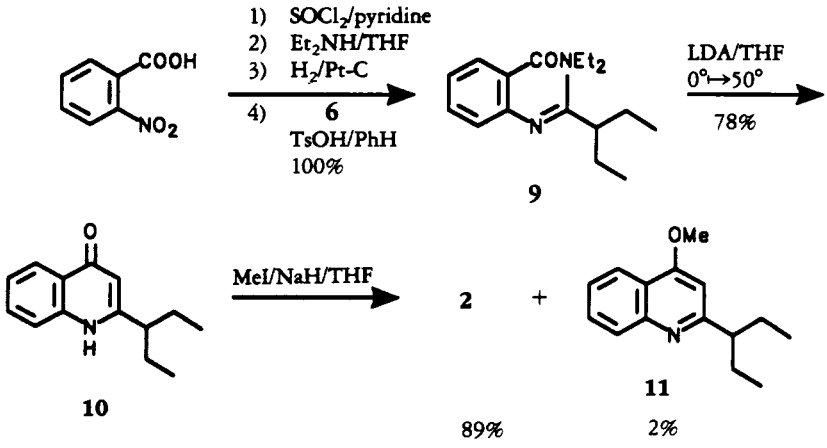
6 7 8

SCHEME 2. Synthesis of leiokinine B [**2**]. This method was not successful because of the steric hindrance of ethyl groups.

As an alternate route (Scheme 3), Niementowski cyclization (5) was used for the synthesis of **2**. Condensation of **6** under azeotropic dehydration with *N,N*-diethylanthranilamide derived from *O*-nitrobenzoic acid afforded **9** quantitatively. Raising the temperature of **9** with LDA from 0 to 50° yielded 4-quinolone [**10**] in 78% yield. *N*-Methylation of **10** with NaH and MeI in THF provided leiokinine B in 89% yield and O-Me ether [**11**] in less than 2% yield. The physical measurements including mp, ir, uv, and nmr of synthetic **2**, were identical to those of natural leiokinine B.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's and bp's were uncorrected. Uv spectra were acquired on a Hitachi model 100-80 spectrometer in MeOH. Ir were acquired on either a Perkin-Elmer model 1310 ir or Nippon Bunko model A-100 ir spectrometer. All nmr spectra were acquired on either a JEOL model GSX 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C), a JEOL model GSX-270 (270 MHz for ^{1}H),



SCHEME 3. Synthesis of leiokinine B [**2**].

or a Hitachi model R-600L (60 MHz for ^1H) spectrometer with signals reported in ppm from internal TMS or solvent used. Hrms and lrms were acquired on either a JEOL model LMS-HX 100 or DX 303. The cc adsorbent used was E. Merck Si gel (Type 60, particle size 0.04–0.063 mm). All reactions were monitored by tlc on 0.25 mm E. Merck Si gel plates (60 F254). Uv light, 7% phosphomolybdic acid in EtOH, or 1.5% vanillin solution in EtOH/concentrated H_2SO_4 were used as visualizing agents. All solvents used for chromatography were hplc grade. THF, C_6H_6, and hexane used for synthesis were distilled from sodium benzophenone ketyl, and CH_2Cl_2 was distilled from P_2O_5. Recycling hplc was performed on a model JAI LC-09 (Japan Analytical Industry, Tokyo). The column used on the r-hplc was JAI ODS GI-15 (ϕ 2.5 cm × ℓ 25 cm).

ANTIFEEDANT ASSAY.—The artificial-diet feeding assay applied followed the literature (2).

EXTRACTION AND ISOLATION.—The leaves of *E. leiocarpa* were collected in the State of Sâo Paulo, Brazil and air-dried before the extraction. The dried leaves (185 g), extracted with $CHCl_3$, gave 34 g of residue, which showed weak antifeedant activity against the pink bollworm *P. gossypiella*. The extract was separated into basic and acidic fractions using routine procedures. The active basic fraction was separated on the recycling hplc with ODS column. The solvent was MeOH-H_2O-MeCN (50:25:10), and the flow rate was 4.4 ml/min. Baseline separation was achieved after two cycles.

LEIOKININE A [**1**].—Mp 86°; hrms *m/z* 231.1265 (calcd for $C_{14}H_{17}NO_2$, 231.1259); uv λ max (MeOH) nm 343 (ε 12,700), 335 (ε 1200), 280 (ε 1700), 269 (ε 1300), 242 (ε 19,200); ir ν max (KBr) cm^{-1} 1615, 1590; ^1H nmr ($CDCl_3$, 400 MHz) δ ppm 8.52 (1H, dd, J = 8.4 and 2.1 Hz), 7.64 (1H, dd, J = 8.4 and 2.1 Hz), 7.48 (1H, bd, J = 8.4), 7.35 (1H, dd, J = 8.4 and 1.0 Hz), 3.93 (3H, s, OMe), 3.79 (3H, s, NMe), 2.94 (2H, m), 1.68 (2H, hex, J = 6), 1.10 (3H, t, J = 6), see Table 1 for additional data; ^{13}C nmr ($CDCl_3$, 100 MHz) see Table 1.

LEIOKININE B [**2**].—Mp 138°; hrms *m/z* 229.1477 (calcd for $C_{15}H_{19}NO$, 229.1467); uv λ max (MeOH) nm 343 (ε 10,800), 322 (ε 10,300), 282 (ε 1500), 268 (ε 1100), 241 (ε 16,700); ir ν max (KBr) cm^{-1} 1615, 1590; ^1H nmr (400 MHz) δ ppm 8.47 (1H, dd, J = 8.4 and 2.1 Hz), 7.67 (1H, dd, J = 8.4 and 2.1 Hz), 7.57 (1H, bd, J = 8.4 Hz), 7.38 (1H, dd, J = 8.4 and 1.0 Hz), 6.31 (1H, s), 3.00 (3H, s), 2.83 (1H, quint, J = 6 Hz), 1.74 (2H, quint, J = 6 Hz), 1.73 (2H, quint, J = 6 Hz), 0.72 (6H, t, J = 6 Hz), see Table 1 for additional data; ^{13}C nmr (100 MHz) see Table 1.

SYNTHESIS OF METHOXYMETHYLPROPYL KETONE.—A solution of methoxyacetonitrile (0.05 mol, 3.0 ml in 15 ml Et_2O) was added at ambient temperature to a Grignard reagent prepared from C_3H_7Br (0.07 mol, 6.4 ml) and Mg (0.09 mol, 2.19 g) under Ar. The mixture was stirred for 18 h, and 2 N HCl was added. The extract was washed with aqueous Na_2CO_3 and brine followed by drying with $MgSO_4$ and evaporation. The residual oil was purified by distillation in vacuo to afford methoxymethylpropyl ketone as a colorless oil (3.59 g, 62%): bp 60°/25 mm Hg; ^1H nmr ($CDCl_3$, 60 MHz) δ ppm 0.93 (3H, t, J = 6.4 Hz), 1.56 (2H, m), 2.41 (2H, t, J = 6.8 Hz), 3.41 (3H, s), 3.97. *Anal.* found C 61.43, H 10.54; calcd for $C_6H_{12}O_2$, C 62.04, H 10.41.

SYNTHESIS OF LEIOKININE A [**1**].—A solution of methoxymethylpropyl ketone (0.01 mol, 1.16 g) in THF (10 ml) was added dropwise to a stirred solution of LDA (0.03 mol) in THF (30 ml) at 0° under Ar.

After stirring the mixture at 0° for 2 h, it was cooled to −65° and a solution of **5** (5 mmol, 0.88 g) in THF (50 ml) was added dropwise. The mixture was stirred at −65° for 30 min and allowed to warm to 0°; it was then quenched with NH_4Cl and extracted with CH_2Cl_2. The extracts were washed with brine followed by drying with $MgSO_4$ and evaporation. The residue was purified by SiO_2 cc eluting with $CHCl_3$ to afford **1** as colorless needles, mp 89–91.2°. The spectroscopic data were identical to natural **1**. *Anal.* found C 72.25, H 7.43, N 5.98; calcd for $C_{13}H_{17}NO_2$, C 72.70, H 7.41, N 6.01.

SYNTHESIS OF ETHYL-2,2-DIETHYL-2-(1-METHYL-4-QUINOLONYL) ACETATE [**8**].—A solution of 3-carboethoxypentanone (0.02 mol, 2.92 g) in THF (3 ml) was added to a stirred suspension of NaH (0.026 mol, 1.04 g of 60% oil) prewashed twice with hexane in THF (20 ml) at 0° under Ar. After the mixture was stirred at 0° for 15 min, *n*-BuLi (0.02 mol, 1.5 mol) solution in hexane was added and stirred for another 20 min at 0°. The mixture was cooled to −70°, and a solution of **5** (0.01 mol, 1.77 g) in THF (40 ml) was added. The mixture was stirred at −70° for 20 min, allowed to warm to ambient temperature, and quenched with a saturated NH_4Cl solution. The aqueous mixture was extracted with CH_2Cl_2 and washed with brine, followed by drying with $MgSO_4$ and evaporation. The residue was purified using SiO_2 cc eluting with $CHCl_3$-MeOH (98:2) to afford ethyl 2,2-ethyl-2-(1-methyl-4-quinolonyl)acetate (1.96 g, 72%): mp 101–102.8°; ir ν max (Nujol) cm^{-1} 1730, 1620, 1190, 750; ^1H nmr ($CDCl_3$, 60 MHz) δ ppm 1.04 (3H, t, J = 6.4 Hz), 1.24 (3H, t, J = 6.4 Hz), 2.0 (2H, m), 3.73 (1H, t, J = 7 Hz), 3.80 (3H, s), 4.20 (2H, q, J = 6.4 Hz), 6.36 (1H, s), 7.58 (2H, m), 8.48 (1H, d, J = 7.8 Hz).

A solution of the above acetate (0.0023 mol, 0.61 g) in THF (10 ml) was added dropwise to a stirred solution of LDA (0.0023 mol) in THF (20 ml) and HMPA (2.2 ml) at −45° under Ar. After stirring at −45° for 20 min, the red clear solution was gradually allowed to warm to 0° over 30 min and cooled to −78°. EtI (0.0069 mol, 0.72 ml) was added, and the mixture was stirred at −78° for 20 min, gradually warmed to ambient temperature, and quenched with a saturated NH_4Cl solution. The aqueous mixture was extracted with CH_2Cl_2 and washed with brine followed by drying with $MgSO_4$ and evaporation. The residue was purified using SiO_2 cc eluting with $CHCl_3$ to afford **8** (0.32 g, 46%) as colorless needles: mp 132.5–136° from CH_2Cl_2; ir ν max (Nujol) cm^{-1} 1720, 1630, 1600, 1240, 1220, 770, 720; ^1H nmr ($CDCl_3$, 60 MHz) δ ppm 0.82 (3H, t, J = 7 Hz), 1.23 (3H, t, J = 7 Hz), 2.05 (2H, m), 3.63 (3H, s), 4.23 (2H, q, J = 7 Hz), 6.48 (1H, s), 7.55 (3H, m), 8.47 (1H, dd, J = 2.7 Hz).

SYNTHESIS OF *N*-(3-ETHYL-2-PENTYLIDENE)-*N'*,*N'*-DIETHYLANTHRANILAMIDE.—A mixture of *O*-nitro-*N*,*N*-diethylbenzylamide (0.0081 mol, 1.80 g) and 5% Pd/C (0.203 g) in EtOAc (15 ml) was hydrogenated for 6 h under H_2. The Pd/C was removed by filtration, and the filtrate was evaporated to give the amine oil (1.14 g, 92%). The oil used for the next reaction was not purified further: ir ν max (neat) cm^{-1} 3450, 3340, 3230, 1610, 1585, 1490, 1290, 1100, 880, 750; ^1H nmr ($CDCl_3$, 60 MHz) δ ppm 1.17 (6H, t, J = 7.1 Hz), 3.43 (4H, q, J = 7 Hz), 3.86 (2H, brs), 6.56–7.29 (4H, m).

A mixture of the above oil (ca. 0.006 mol, 1.14 g), compound **5** (0.0063 mol, 0.72 g), and *p*-TsOH monohydrate (0.2 g) in C_6H_6 (35 ml) was azeotropically refluxed using a Dean-Stark H_2O separator for 15 h. The mixture was washed with aqueous $NaHCO_3$ followed by drying with $MgSO_4$ and evaporation. The residue was purified using SiO_2 cc eluting with $CHCl_3$-MeOH (9:1) to afford **9** as a pale yellow oil (1.30 g, 76%). The crude oil used for the next step was not purified further: ir ν max (neat) cm^{-1} 1710, 1650, 1620, 1590, 1490 (sh), 1290, 750; ^1H nmr ($CDCl_3$, 60 MHz) δ ppm 0.92 (6H, t, J = 7 Hz), 1.06–1.55 (10H, m), 1.74 (3H, s), 2.10 (1H, m), 3.35 (2H, m), 6.52–7.43 (4H, m).

SYNTHESIS OF 2-(1'-ETHYLPROPYL)-4-QUINOLONE [**10**].—A solution of **9** (ca. 0.074 mol, 2.137 g) in THF (10 ml) was added to a stirred solution of LDA (0.015 mol) in THF (20 ml) at 0° under Ar. More THF (22 ml) was added, and the mixture was stirred at 0° for 25 min and gradually allowed to warm to ambient temperature followed by stirring for 4.3 h, heating at 55° overnight, quenching with MeOH, and evaporating under reduced pressure. The residue was purified using SiO_2 cc eluting with $CHCl_3$-MeOH (95:5) to afford **10** (1.24 g, 78%) as colorless needles. The crystals from EtOAc/MeOH were modified at 179° and gave mp 264–265°; ir ν max (Nujol) cm^{-1} 3220, 3050, 1625, 1585, 1540, 1490, 845, 760; ^1H nmr ($CDCl_3$, 270 MHz) δ ppm 0.83 (6H, t, J = 7.5 Hz), 1.69 (4H, m, J = 7.0 Hz), 2.37 (0.5H, brs, exchangeable with D_2O), 2.52 (1H, m), 6.26 (1H, s), 7.34 (1H, dt, J = 1.2 and 7.7 Hz), 7.57 (1H, dt, J = 1.2 and 8.0 Hz), 7.84 (1H, d, J = 8.0 Hz), 8.38 (1H, dd, J = 1.2 and 8.0 Hz), 11.93 (0.8H, brs, exchangeable with D_2O). *Anal.* found C 77.98, H 7.80, N 6.30; calcd for $C_{14}H_{17}NO$, C 78.10, H 7.96, N 6.51.

SYNTHESIS OF LEIOKININE B, 2-(1'-ETHYLPROPYL)-1-METHYL-4-QUINOLONE [**2**].—A solution of **10** (1.93 mmol, 0.415 g) in THF (80 ml) was added to a stirred suspension solution of NaH (2.13 mmol, 0.085 g of 60% oil prewashed twice with hexane) in THF (3 ml) at 0° for 5 min, and MeI (0.038 mol, 2.4 ml) was added to the mixture. The mixture was stirred overnight at ambient temperature and quenched with saturated NH_4Cl solution. The mixture was adjusted to pH 10 with 10% NaOH and ex-

tracted with $CHCl_3$. The extract was washed with 10% NaOH followed by drying ($MgSO_4$) and evaporation. SiO_2 cc of the residue with $CHCl_3$-MeOH (97:3) gave first **11** and then leiokinine B [**2**]. Compound **11** was obtained as colorless needles: mp 77.8–78.5°; ^1H nmr ($CDCl_3$, 60 MHz) δ ppm 0.85 (6H, t, $J = 7.2$ Hz), 1.56–2.04 (4H, m), 2.74 (1H, m, $J = 7.2$ Hz), 4.03 (3H, s), 6.58 (1H, s), 7.41–8.23 (4H, m). *Anal.* found C 78.44, H 8.33, N 6.06; calcd for $C_{15}H_{19}NO$, C 78.56, H 8.35, N 6.11. Leiokinine B [**2**] was obtained in 89% yield (0.372 g) as pale yellow prisms, mp 137–140° from Et_2O. The ir, uv, ms, and nmr were identical to those of natural leiokinine B. *Anal.* found C 78.29, H 8.40, N 6.09; calcd for $C_{15}H_{19}NO$, C 78.56, H 8.35, N 6.11.

ACKNOWLEDGMENTS

We are indebted to the late Professor H. Hikino for the cytotoxic assay, Mr. M. Himejima for the antimicrobial assay, and Mr. T. Hinomoto, JEOL, USA, for taking nmr and mass spectra.

LITERATURE CITED

1. K. Milton, *Int. J. Primatol.*, **5**, 491 (1984).
2. I. Kubo, J.F. Klocke, and S. Asano, *J. Insect Physiol.*, **29**, 307 (1983).
3. I. Mester, *Annu. Proc. Phytochem. Soc. Eur.*, **22**, 31 (1983).
4. G.M. Coppola, *Synthesis*, 939 (1983).
5. R.J. Chong, M.A. Siddiqui, and V. Snieckus, *Tetrahedron Lett.*, **27**, 5323 (1986).

Received 23 April 1990