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Alkaloid from *Thermoactinomyces* Species

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The alkaloid (1) obtained from *Thermoactinomyces* strain TM-64 is proven to be N-3′-β-indolylethyl-2-α-aminoethylthiazole-4-carboxamide on the basis of its chemical reactions and the physico-chemical method.

Attempts to obtain biologically active alkaloids from Actinomycetales from soil have been carried out in our laboratories. Several alkaloids were isolated and their structures were established.[2)] This paper is concerned with the structure elucidation of the alkaloid[3)] from a thermophilic actinomycete, *Thermoactinomyces* strain TM-64.

The strain TM-64 produced an optically active alkaloid (**1**), $C_{16}H_{18}ON_4S$, possessing local anesthetic action. Its Ehrlich's and Vohl's reactions were positive, suggesting the presence of the indole skeleton and sulfur atom, respectively, in the molecule. The ultraviolet (UV) spectrum is similar to that of indole.[4)] From the molecular formula and the infrared (IR) spectrum (KBr) showing the amide carbonyl band at 1630 cm^{-1}, it is clear that the oxygen atom in **1** only consists in the amide group. The nuclear magnetic resonance (NMR) spectrum is recorded in Fig. 1. Double irradiation experiments display spin-spin interactions of the signals at δ 4.25 (q, 1H) and 1.45 (d, 3×H) with J=6.3 Hz, and 3.74 (q, 2×H) and 3.05 (t, 2×H) with J=7.0 Hz, and 7.52 (bt, 1H) and 3.74 (q, 2×H) with J=7.0 Hz. The signals at δ 8.62 (s, 1H), 7.52 (bt, 1H), and 1.81 (s, 2×H) disappeared on addition of deuterium oxide and disappearance of the second signal turned the quartet at δ 3.74 to a triplet (J=7.0 Hz). These NMR properties show the likely presence of the CH_3CH and CH_2CH_2NH groups in **1**. As shown in Fig. 2, the chemical shifts and splitting pattern of the aromatic protons are similar to those of 3-substituted indoles.[5)] The ^{13}CNMR spectrum exhibits one methyl, two methylene, and one methine groups (Fig. 3). Also, six quarternary carbons and six olefinic carbons with one hydrogen atom are observed in lower field.

The alkaloid (**1**) was readily acetylated to give a monoacetate (**2**) and a diacetate (**3**). The monoacetate (**2**) shows the amide carbonyl band at 1660 cm^{-1} in addition to the one originally existed in **1** in the IR spectrum ($CHCl_3$). The NMR spectrum of **2** shows that among the four protons exchangeable with deuterium in **1**, the two protons remain unchanged at δ 8.32 and 7.46, and the two protons singlet at δ 1.81 in **1** shifts to the one proton doublet at δ 6.18 (J=7.3 Hz). Also, down-field shift of the one proton quartet at δ 4.25 in **1** to the one proton quintet at δ 5.32 (J=7.3 Hz) is observed. These data suggest the presence of the CH_3CHNH_2 group in **1**. The diacetate (**3**) exhibits, further, an additional amide carbonyl band corresponding to that of 1-acetylindole[6)] at 1700 cm^{-1} in the IR spectrum ($CHCl_3$).

1) Location: *Minato-ku, Tokyo 108, Japan.*
2) M. Onda, Y. Konda, Y. Narimatsu, S. Ōmura, and T. Hata, *Chem. Pharm. Bull.* (Tokyo), **21**, 2048 (1973); S. Ōmura, H. Tanaka, J. Awaya, Y. Narimatsu, Y. Konda, and T. Hata, *Agr. Biol. Chem.*, **38**, 899 (1974); M. Onda, Y. Konda, Y. Narimatsu, H. Tanaka, J. Awaya, and S. Ōmura, *Chem. Pharm. Bull.* (Tokyo), **22**, 2916 (1974).
3) S. Ōmura, Y. Suzuki, C. Kitao, Y. Takahashi, and Y. Konda, *J. Antibiotics*, **28**, 609 (1975).
4) A.I. Scott, "Interpretation of the Ultra-Violet Spectra of Natural Products", Pergamon Press, New York, 1964, p. 174.
5) L.A. Cohen, J.W. Daly, H. Kny, and B. Witkop, *J. Amer. Chem. Soc.*, **82**, 2184 (1960).
6) J. Derkosch and E. Rieger, *Monatsch. Chem.*, **90**, 389 (1959).

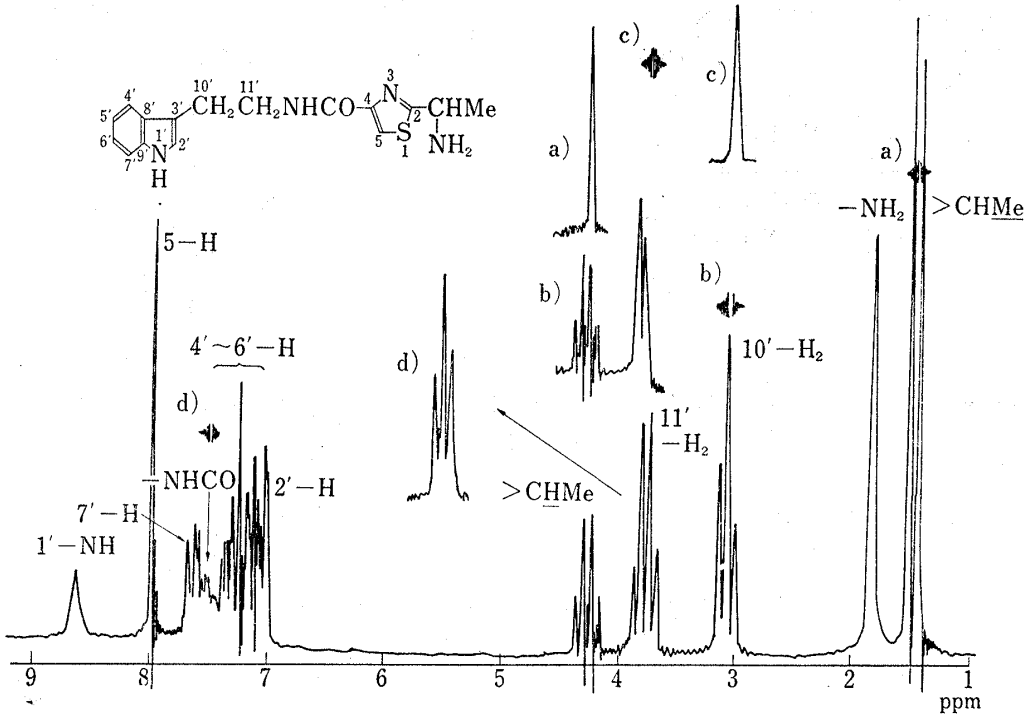


Fig. 1. NMR Spectrum of **1** ($CDCl_3$, 100 MHz)

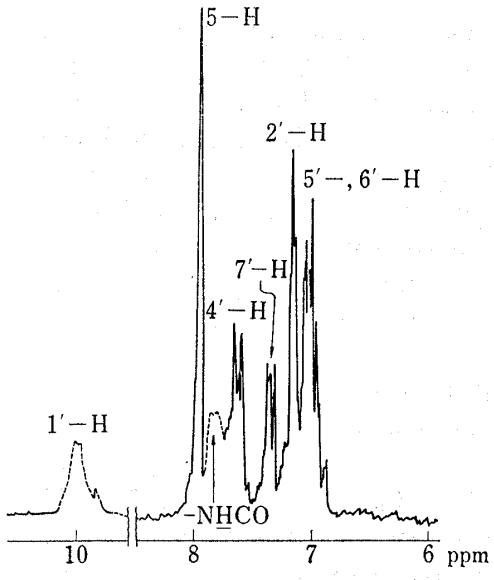


Fig. 2. NMR Spectrum of **1** (100 MHz, Acetone-d_6)

The signals which are depicted with dotted line disappear on addition of D_2O

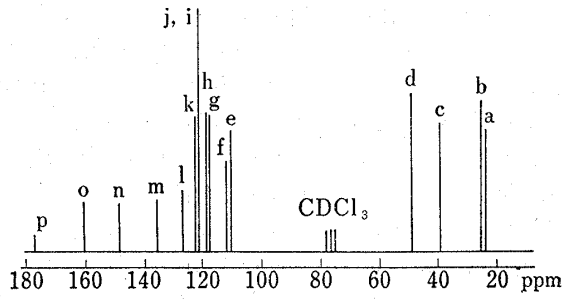


Fig. 3. ^{13}CNMR Spectrum of **1** (25.1 MHz, $CDCl_3$)

a:	24.60	q	CHMe	i: }	121.90	d	5′-C
b:	25.50	t	10′-C	j: }		d	5-C
c:	39.79	t	11′-C	k:	122.37	d	2′-C
d:	49.58	d	CHMe	l:	127.29	s	8′-C
e:	111.11	d	7′-C	m:	136.26	s	9′-C
f:	112.85	s	3′-C	n:	149.81	s	4-C
g:	118.60	d	6′-C[a)]	o:	161.12	s	2-C
h:	119.20	d	4′-C[a)]	p:	178.00	s	CO

a) These signals may be reversed.

Thus, **1** is considered to be a 3-substituted indole containing the CH_3CHNH_2, CH_2CH_2NHCO, and C_3HNS functions.

Hydrolysis of **1** with methanolic potassium hydroxide gave two fragments, $C_{10}H_{12}N_2$ (**4**) and $C_6H_8O_2N_2S$ (**5**). The compound (**4**) was proven to be tryptamine by the NMR spectroscopy and mixed melting point. The compound (**5**) is a carboxylic acid possessing the nitrogen and sulfur atoms. Its NMR spectrum (D_2O) shows the signals at δ 8.28 (s, 1H), 5.14 (1H),[7)]

7) This signal is hidden in the HOD signal.

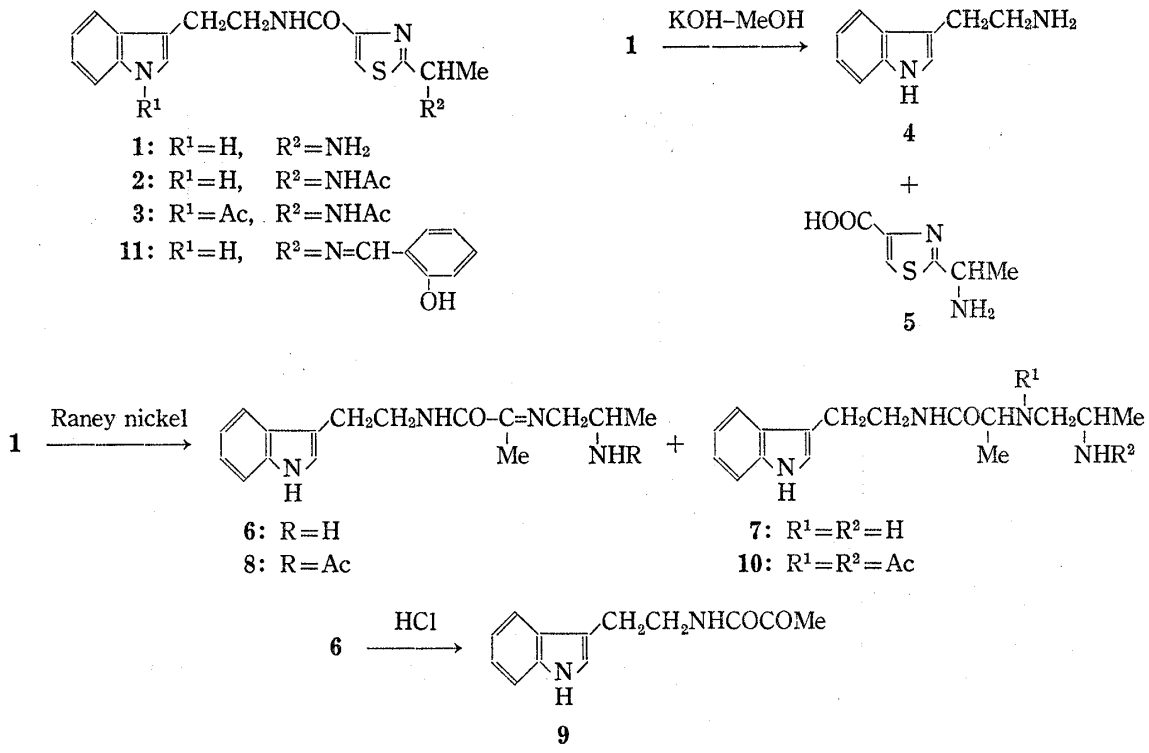


Chart 1

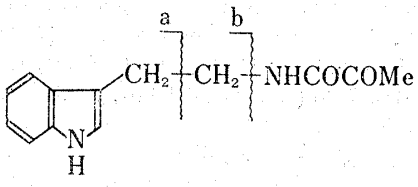
and 1.94 (d, J=5.5 Hz, 3×H). The former signal can be assigned to a proton of a hetero-aromatic compound. The latter two were found to couple on the basis of decoupling studies, indicating to correspond to the $C\underline{H}_3C\underline{H}NH_2$ which originally existed in **1**. From the molecular formula and the NMR data, **5** seems probably to be a thiazole or isothiazole with the CO_2H and CH_3CHNH_2 groups as substituents. Isothiazole carboxylic acids are known to reveal bands at 225—230 and 250—270 mμ in the UV spectrum (EtOH).[8] Since **5** shows only one band at 235 mμ (1870) (EtOH), the isothiazole derivative for **5** can be denied. The ^{13}CNMR spectrum (D_2O) shows three quarternary carbons (δ 171.28, 169.04, and 155.50) and one tertiary carbon (δ 128.69) in addition to one methine (δ 51.60) and one methyl (δ 21.49). On comparison with the ^{13}CNMR spectrum of thiazole,[9] it is clear that the signal at δ 128.69 corresponds to the C-5. Accordingly, **5** is a 2,4-disubstituted thiazole and **1** is a compound in which the amino group in **4** combines with the carboxylic acid group in **5** by forming the amide group.

Treatment of **1** with Raney nickel gave two compounds (**6**), $C_{16}H_{22}ON_4$, and (**7**), $C_{16}H_{24}ON_4$. Acetylation of **6** afforded an acetate (**8**) which showed the amide carbonyl bands at 1660 and 1650 cm^{-1} in the IR spectrum ($CHCl_3$). The latter corresponds to that originally observed for **6**. Acidic hydrolysis of **6** gave a keto amide (**9**). The keto amide (**9**) displays the carbonyl bands at 1720 and 1684 cm^{-1} for the ketone and amide groups, respectively, in the IR spectrum ($CHCl_3$). The NMR spectrum of **9** shows the signal due to one methyl group at δ 2.42 (s) in addition to those corresponding to tryptamine moiety. The mass spectrum of **9** exhibits a simple fragmentation as shown in Table I. From the above spectral data, **9** is characterized as N-3-β-indolylethylpyruvamide. Identification of **9** was achieved by comparison with the spectral data of an authentic sample. Formation of **9** leads to that **6** may be a Schiff base

8) D. Buttimore, D.H. Jones, R. Slack, and K.R.H. Wooldrige, *J. Chem. Soc.*, **1963**, 2032; M.P.L. Caton, D.H. Jones, R. Slack, and K.R.H. Wooldrige, *ibid.*, **1964**, 446; D.H. Jones, R. Slack, and K.R.H. Wooldrige, *ibid.*, **1964**, 3112.

9) C-2, δ 152.0; C-4, 141.8; C-5, 118.3. E.-J. Vincent, R. Phan-Tan-Lun, and J. Metzger, *Compt. Rend.*, **270**, 666 (1970).

Table I. Mass Fragments of 9[a]

	m/e	Formula	Fragmentation
	230(13)[b]	$C_{13}H_{14}O_2N_2$	(M^+)
	144(15)	$C_{10}H_{10}N$	b
	143(26)	$C_{10}H_9N$	b−H[c]
	131(13)	C_9H_9N	a+H[c]
	130(100)	C_9H_8N	a

a) 75 eV
b) relative intensity (%)
c) This is accompanied by a rearrangement of a hydrogen atom.

shown in Chart 1. Desulfurisation of thiazoles with Raney nickel is known to give Schiff bases which are hydrolyzed to afford ketones.[10] If the thiazole moiety in **1** possesses the 2-$CH(NH_2)CH_3$ and 4-CONH groups, formation of **9** from **1** *via* **6** can be reasonably explained. Since it was observed by monitoring with thin-layer chromatography (TLC) that during desulfurisation **6** was formed initially and converted into **7**, **6** is an intermediate for **7**. From the molecular formula, it can be seen that the imino group in **6** was further hydrogenated to give **7**. Actually, the fact that acetylation of **7** gave a diacetate (**10**) is in accord with the above deduction.

Conclusively, the alkaloid (**1**) is, now, established as N-3′-β-indolylethyl-2-α-aminoethyl-thiazole-4-carboxamide.

As can be seen easily, **1** possesses an asymmetric carbon atom on the α-aminoethyl group. The N-salicylidene derivative (**11**) of **1** displays positive Cotton effects at 252 and 316 mμ in the circular dichroism (CD) curve (EtOH). The N-salicylidene derivatives of the (S)-α-aryl-alkylamines examined in ethanol have been known to show the positive CD maxima near 255 and 315 mμ, by which the absolute configuration of α-arylalkylamines were determined.[11] These CD maxima are thought to be due to an inherently dissymmetric chromophore arising from an interaction of the π-electrons of the aryl group and salicylidenimino moiety. If the thiazole group can be assumed to interact with the salicylidenimino moiety in the similar manner to the aryl group, tentative assignment of the S configuration for **1** would be made on the basis of the CD curve of **11**. In order to solve this problem the X-ray analysis is in progress in our laboratories.

It is of particular interest that the alkaloid possessing the thiazole ring was obtained from Actinomycetales. The biogenetic formation of the thiazole ring may be considered to be resulted from alanine and cysteine.

Experimental

Melting points were determined on a micro hot-stage and are uncorrected. UV spectra were measured with a Hitachi EPS-2U. CD curves were taken on a JASCO J-20. IR spectra were taken on a JASCO IR-G. NMR spectra were measured with a JEOL JNM-4H-100 in a $CDCl_3$ solution. ^{13}CNMR spectra were taken on a JEOL JNM PS-100/PFT-100 at 25.1 MHz using tetramethyl silane as reference and assignments of each carbon atom were determined by employing off-resonance decoupling technique. Mass Spectra were recorded on a JEOL JMS-OlS.

Physical Properties of the Alkaloid (1)——Light yellow needles of mp 120—122° (from benzene). $[\alpha]_D^{20}=-6.0$ ($c=1$, MeOH). UV λ_{max}^{MeOH} (ε): 291 (8350), 282 (10500), 275 (10500), 223 (57150). IR ν_{max}^{KBr} cm^{-1}: ca. 3300 (NHs), 1630 (CON). CD ($c=0.013$, EtOH) $[\theta]^{27}$ (mμ): +29 (283) (positive maximum), −114 (253) (negative maximum), +643 (223). Mass Spectrum: M^+, *m/e* 314.118. Calcd. for $C_{16}H_{18}ON_4S$: M. 314.119. *Anal.* Calcd. for $C_{16}H_{18}ON_4S$: C, 61.12; H, 5.77; N, 17.82; S, 10.19. Found: C, 61.37; H, 5.89; N, 18.04; S, 9.70.

10) G.M. Badger and N. Kowanko, *J. Chem. Soc.*, **1957**, 1652.
11) H.E. Smith and R. Records, *Tetrahedron*, **1966**, 813; H. Ripperger, K. Schreiber, G. Snatzke, and K. Ponsold, *ibid.*, **1969**, 827; H.E. Smith and T.C. Willis, *ibid.*, **1970**, 107.

The picrate: orange needles of mp *ca.* 210° (decomp.) (from ethyl acetate). *Anal.* Calcd. for $C_{22}H_{21}O_8N_7S$: C, 48.62; H, 3.89; N, 18.04. Found: C, 48.61; H, 3.95; N, 18.18. The hydrochloride: colorless needles of mp *ca.* 215° (from EtOH).

Acetylation of the Alkaloid (1)——A mixture of **1** (20 mg), Ac_2O (0.2 ml), and pyridine (1 ml) was refluxed for 5 hr. After work-up, the resulting residue was purified by preparative TLC on silica gel plates (0.25 mm) using $CHCl_3$–MeOH (10: 1, v/v) as solvent. The zone with *Rf* 0.25 afforded the monoacetate (**2**) (10 mg) as colorless needles of mp 100—102° (from $CHCl_3$–*n*-hexane). NMR: δ 8.32 (s, 1′-H), 7.95 (s, 5-H), 7.65 (q, *J* 7 and 2 Hz, 7′-H), ca. 7.46 (11′-NH), 7.10—7.54 (m, 4′-, 5′-, and 6′-H), 7.00 (s, 2′-H), 6.18 (d, *J* 7.3 Hz, 1″-NH), 5.32 (qui, *J* 7.3 Hz, 1″-H), 3.73 (q, *J* 6.5 Hz, 11′-H_2), 3.05 (t, *J* 6.5 Hz, 10′-H_2), 2.02 (s, 1″-NHAc), 1.45 (d, *J* 7.3 Hz, 1″-Me). Mass Spectrum: M^+, *m/e* 356.135. Calcd. for $C_{18}H_{20}O_2N_4S$: M, 356.130. The zone with *Rf* 0.50 gave the diacetate (**3**) (3 mg) as colorless needles of mp 69—70° (from $CHCl_3$–*n*-hexane). Mass Spectrum: M^+, *m/e* 398.141. Calcd. for $C_{20}H_{22}O_3N_4S$: M, 398.141.

Hydrolysis of the Alkaloid (1)——To a solution of **1** (50 mg) in MeOH (2 ml) was added 20% KOH–MeOH (0.5 ml) and the resulting mixture was refluxed for 4 hr. After removal of solvent *in vacuo*, the residue was treated with $CHCl_3$ and H_2O (1 ml). The residue obtained from the $CHCl_3$ layer was recrystallized from benzene to give the compound (**4**) (20 mg) as light yellow needles of mp 112° which was identified with an authentic sample of tryptamine by mixed mp. NMR: δ 8.68 (bs, 1-H), 7.57 (q, *J* 8 and 3.5 Hz, 7-H), 7.00—7.36 (m, 4-, 5-, and 6-H), 6.95 (s, 2-H), 3.02 (oct, $2\times CH_2$), 2.10 (bs, NH_2). Mass Spectrum: M^+, *m/e* 160.099. Calcd. for $C_{10}H_{12}N_2$: M, 160.100. The H_2O layer described above was passed through a column of Amberlite IR-120 (7 ml). Elution with 3% NH_4OH solution (150 ml) gave the compound (**5**) (13 mg) as colorless needles of mp 220° (decomp.) (from EtOH–H_2O). IR ν_{max}^{KBr} cm^{-1}: 1560 (COO̅). Ninhydrin test: light violet. TLC: *Rf* 0.50 (Avicel, BuOH–AcOH–H_2O=2: 1: 1, v/v). Mass Spectrum: M^+, *m/e* 172.028. Calcd. for $C_6H_8O_2N_2S$: M, 172.028.

Desulfurisation of the Alkaloid (1)——To a solution of **1** (50 mg) in MeOH (2 ml) was added 1/3 amount of Raney Ni (W-7) obtained from nickel alloy (1 g) and the reaction mixture was refluxed for 2 hr with stirring. Then, the remaining catalyst was added and refluxing and stirring were continued for 1 hr. After work-up, the residue obtained from the MeOH solution was treated with $CHCl_3$. The portion soluble in $CHCl_3$ gave the compound (**6**) (13 mg) as oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650 (CON). Mass Spectrum: M^+, *m/e* 286.179. Calcd. for $C_{16}H_{22}ON_4$: M, 286.179. The acetate (**8**): colorless needles of mp 70—72° (from benzene–*n*-hexane). Mass Spectrum: M^+, *m/e* 328.183. Calcd. for $C_{18}H_{24}O_2N_4$: M, 328.189. The portion insoluble in $CHCl_3$ was taken up in EtOH. The residue obtained from the EtOH solution was recrystallized from ether–EtOH to give the compound (**7**) (10 mg) as colorless needles of mp 200° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 1640 (CON). Mass Spectrum: M^+, *m/e* 288.196. Calcd. for $C_{16}H_{24}ON_4$: M, 288.195. The diacetate (**10**): colorless needles of mp 90—92° (from $CHCl_3$–*n*-hexane). Mass Spectrum: M^+, *m/e* 372.219. Calcd. for $C_{20}H_{28}O_3N_4$: M, 372.216.

N-3-β-Indolylethylpyruvamide (9)——a) A solution of **6** (11 mg) and 20% HCl (one drop) in EtOH (3 ml) was allowed to stand at room temperature for 2 hr. After removal of solvent *in vacuo*, the resulting residue was dissolved in $CHCl_3$. The residue obtained from the $CHCl_3$ solution was purified by preparative TLC (silica gel plates, 0.25 mm; $CHCl_3$: MeOH=10: 1, v/v) to give **9** (3 mg) as oil. NMR: δ 8.05 (s, 1-H), 7.60 (q, *J* 7 and 3Hz, 7-H), 7.50 (bt. *J* 7 Hz, CONH), 7.45—7.05 (m, 4-, 5-, and 6-H), 7.05 (s, 2-H), 3.61 (q, *J* 7 Hz, CH_2N), 2.98 (t, *J* 7 Hz, 3-CH_2), 2.42 (s, Me). Mass Spectrum: M^+, *m/e* 230.110. Calcd. for $C_{13}H_{14}O_2N_2$: M, 230.105.

b) A mixture of tryptamine (50 mg), crude pyruvyl chloride (100 mg) obtained from pyruvic acid and $SOCl_2$, and K_2CO_3 (200 mg) in dry benzene (5 ml) was stirred at room temperature for 2 hr. After work-up, the remaining residue was purified by preparative TLC (silica gel plates, 0.25 mm; $CHCl_3$: MeOH=40: 1, v/v) to give **9** (10 mg) as oil. IR and NMR spectra were superimposed on those of the compound prepared by the procedure described above.

N-Salicylidene Derivative (11)——A solution of **1** (20 mg) and salicylaldehyde (9.4 mg) in dry EtOH (10 ml) was refluxed for 1 hr. After evaporation *in vacuo*, the resulting residue was purified by preparative TLC (silica gel plates, 0.25 mm; $CHCl_3$: MeOH=10: 1, v/v) to afford **11** (20 mg) as light yellow needles of mp 155—156° (from benzene–*n*-hexane). UV λ_{max}^{EtOH} mμ (ε): 322 (9100), 292 (12000), 283 (13000), 259 (28700), 223 (105000). CD (c=0.001, EtOH) $[\theta]^{23}$ (mμ): +19228 (316) (positive maximum), −1881 (276) (negative maximum), +94050 (252) (positive maximum), +31350 (237) (negative maximum), +52250 (230). Mass Spectrum: M^+, *m/e* 418.145. Calcd. for $C_{23}H_{22}O_2N_4S$: M, 418.146.