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Alkaloids from *Streptomyces* sp. NA-337

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Two alkaloids (I) and (II) are isolated from *Streptomyces* sp. NA-337. I is unknown and possesses fat clearing activity. Its structure is determined to be (E,E)-4-methyl-2-pentadienyl-1-pyrroline by its chemical reactions and the physico-chemical method. II is established as abikoviromycin obtained from *Streptomyces abikoensis*.

Previously, we reported isolation[2] and structure elucidation[3] of the alkaloid, pyrindicin, from *Streptomyces griseofluvus* var. *pyrindicus* and attempts to obtain biologically active and basic substances from *Streptomyces* strains have been continued in our laboratories. We now wish to report basic substances from *Streptomyces* sp. NA-337.

The characteristics of the strain NA-337 are summarized in Table I. From the results obtained, the strain NA-337 is established as a strain of section Verticillati of the genus *Streptomyces*[4] (the genus *Streptoverticillium*[5]). The strain NA-337 produced two optically active alkaloids (I) and (II) which were highly unstable and polymerized promptly at room temperature, and yet were fairly stable in the salt forms.

Table I. Characteristics of *Streptomyces* sp. NA-337

	Characteristics
Morphology: Spore chain	section verticillati. Both mono verticillate and umbellate monoverticillate (biverticillate) are found.
Spore surface	smooth.
Color of colony	Aerial mass color is yellowish white to pale yellow on yeast-malt agar, inorganic salts-starch agar, glycerolasparagine agar and sucrose-nitrate agar. Aerial mycelium is usually poorly developed on oatmeal agar.
Reverse side of colony	Color of growth is usually yellowish brown on most media, but on oatmeal agar, pale olive color is produced.
Color in medium	Melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Yellow to brown pigment is found in medium in yeast-malt agar, oatmeal agar, glycerol-asparagine agar and sucrose-nitrate agar, but no pigment is found in the medium in inorganic salts-starch agar.
Carbon utilization	D-glucose, D-fructose, i-inositol, rhamnose and D-mannitol are utilized for growth. No growth or only trace of growth on L-arabinose, D-xylose, sucrose and raffinose.

1) Location: *Minato-ku, Tokyo, 108, Japan.*
2) S. Ōmura, H. Tanaka, J. Awaya, Y. Narimatsu, Y. Konda, and T. Hata, *Agr. Biol. Chem.*, **38**, 899 (1974).
3) M. Onda, Y. Konda, Y. Narimatsu, S. Ōmura, and T. Hata, *Chem. Pharm. Bull.* (Tokyo), **21**, 2048 (1973).
4) T.G. Pridham, C.W. Hesseltine, and R.G. Benedict, *Appl. Microbiol.*, **6**, 52 (1958).
5) E. Baldacci, *Giorn. Microbiol.*, **6**, 10 (1958).

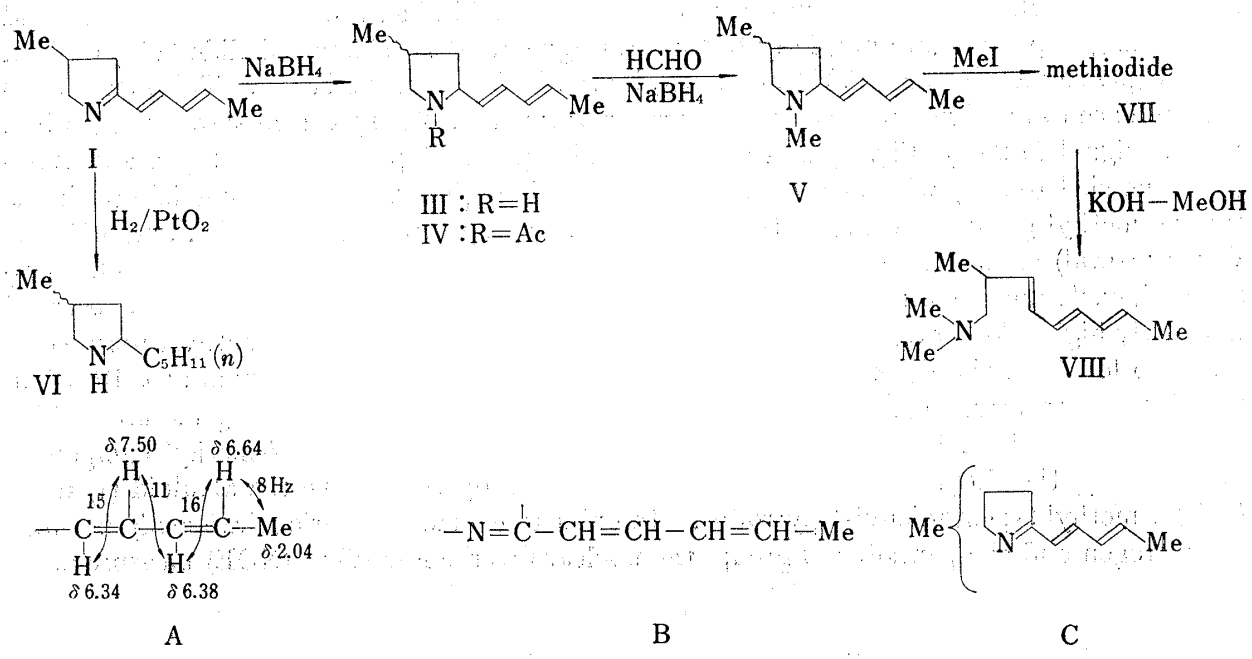

Chart 1

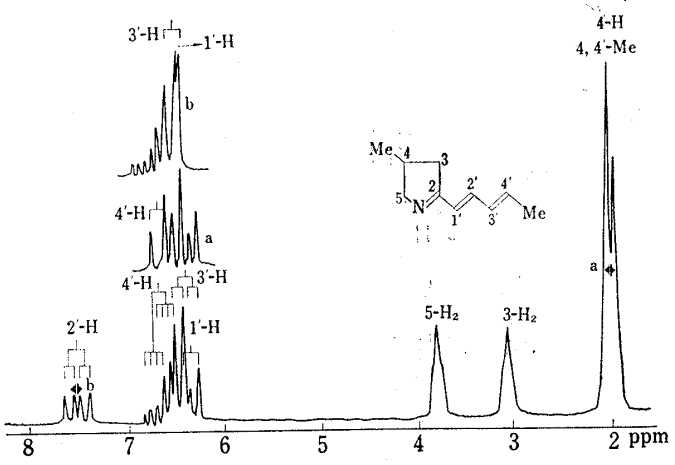

Fig. 1. NMR Spectrum of I Picrate

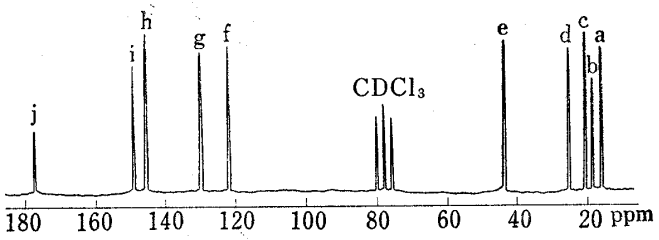

Fig. 2. ^{13}CNMR Spectrum of I Hydrochloride

The number of hydrogen atoms bonded to each carbon atom was determined by using off-resonance decoupling technique.

a: 17.357 4-Me	f: 121.382 C-4′
b: 19.185 C-4	g: 130.273 C-3′
c: 20.032 4′-Me	h: 145.292 C-1′
d: 25.328 C-3	i: 148.222 C-2′
e: 43.822 C-5	j: 176.886 C-2

The alkaloid (I) is a *tert*-amine, whose empirical formula, $C_{10}H_{15}N$, is determined by the microanalyses and the mass spectra of the I picrate and the I hydrochloride. The $NaBH_4$ reduction of I afforded a dihydro *sec*-amine (III), $C_{10}H_{17}N$, which was converted into an acetamide (IV) by acetylation and methylated to a *tert*-amine (V) by the Eschweiler-Clarke method, indicating the presence of a >C=N— group. On hydrogenation over Adams' platinum, I consumed three moles of hydrogen to give a hexahydro *sec*-amine (VI), $C_{10}H_{21}N$, showing the presence of two olefinic groups in addition to the >C=N— group. The nuclear magnetic resonance (NMR) spectrum (100 MHz, CF_3COOD) of the I picrate is recorded in Fig. 1. Double irradiation experiments display the presence of spin–spin interactions of the signals at δ 2.04 (d, Me) and 6.64 (dq, 1H) with J=8 Hz, 6.64 and 6.38 (q, 1H) with J=16 Hz, 6.38 and 7.50 (q, 1H) with J=11 Hz,[6] and 7.50 and 6.34 (d, 1H) with J=15 Hz, *i.e.*, the *trans,trans*-pentadienyl group (A) in I (Chart 1). Judging from the observed λ_{max}^{MeOH} at 267 (84700) and 307 mμ

6) This coupling constant value corresponds to an s-*trans* conformation. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1966, p. 285; C. Vos and P.E.J. Verwiel, *Tetrahedron Letters*, **1973**, 5173.

(55200) in the ultraviolet (UV) spectrum of the I hydrochloride, the two olefinic groups and the imino group conjugate mutually to form a fragment (B). The methiodide (VII) derived from V gave a methine base (VIII), $C_{12}H_{21}N$, by the Hofmann degradation. This fact reveals the presence of an N-heterocycle in I. Further, from the NMR spectrum showing a doublet methyl signal (δ 2.04) (Fig. 1) and the empirical formula, I possesses the 2-pentadienyl-1-pyrroline skeleton (C) with a methyl group in the ring. There are three possible positions for the methyl group to locate in the ring. The NMR spectra of III, IV, VI, and VII (see Experimental) show that both adjacent carbons to the nitrogen are unsymmetrically substituted, excluding the 5-methyl compound. Since the signal due to the MeCH< is observed in a fairly high field (δ *ca.* 2.00) in the NMR spectrum of the I picrate, the methyl group seems likely to locate at the C-4 position rather than the C-3 position. The signal at δ 19.185 in the ^{13}CNMR spectrum ($CDCl_3$) of the I hydrochloride corresponds to a ring methine which is not affected[7] by α-functional group (Fig. 2) and supports also the 4-methyl compound. Conclusively, the chemical reactions and the spectral properties prove the alkaloid (I) to be (E,E)-4-methyl-2-pentadienyl-1-pyrroline. In order to decide the steric relationship between the nitrogen and the pentadienyl group, the nuclear Overhauser effect (NOE) measurements

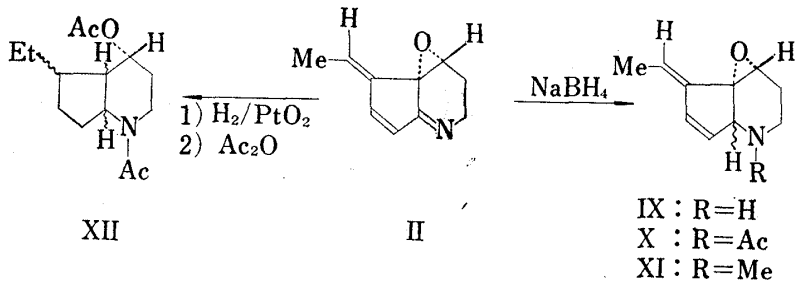

Chart 2

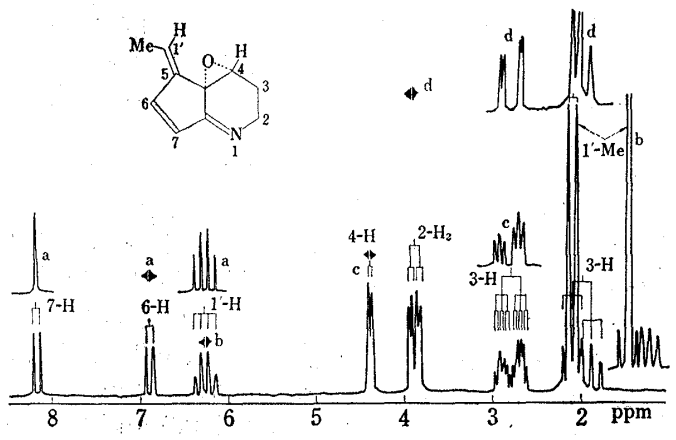

Fig. 3. NMR Spectrum of II Picrate

were carried out in the NMR spectrum of the I picrate (100 MHz, CF_3COOD). Since irradiation at δ 3.05 results in an increase in area of 11% for the signal at δ 7.50, the C-3 methylene group and the C-1′ hydrogen are assigned to be *trans*.

The alkaloid (II), $C_{10}H_{11}ON$, is a *tert*-amine. II was readily reduced by $NaBH_4$ to a dihydro *sec*-amine, $C_{10}H_{13}ON$ (IX), which gave an acetamide (X) and an N-methyl *tert*-amine (XI) by acetylation and the Esch-

7) J.B. Stother, "Carbon-13 NMR Spectroscopy," Academic Press, New York and London, 1972, pp. 80—85 and 269—272.

weiler-Clarke method, respectively. Hence, II must contain a >C=N— group. The NMR spectrum (100 MHz, CF_3COOD) of the II picrate indicates the presence of an ethylidene group (δ 2.18, d and 6.32, q with J=8 Hz) and a *cis* ethylene group (δ 6.94, d and 8.44, d with J=6 Hz) (Fig. 3). Double irradiation examinations show the presence of spin–spin interactions of the signals at δ 4.46 (d, 1H) and 2.70 (dsext, 1H) with J=2.7 Hz, 2.70 and 3.90 (dd, 1H) with J=3.3 Hz, and 3.90 and 2.10 (dt, 1H) with J= 10 Hz. From the above spectral data, II contains two fragments (D) and (E) (Chart 2). Since the infrared (IR) spectrum ($CHCl_3$) of XI shows no carbonyl and no hydroxyl group, II is considered to possess the oxygen in an ethereal form. Actually, hydrogenation over Adams' platinum and subsequent acetylation gave an acetoxy amide (XII),[8] $C_{14}H_{23}O_3N$. Taking account of the above results and the empirical formula, 4,4a-epoxy-5-ethylidene-3,4,4a,5-tetrahydro-2H-1-pyrindine can be assigned for the alkaloid (II) as a possible structure. This compound has been proposed for the structure of abikoviromycin[9] which was isolated from *Streptomyces abikoensis* and found to possess antiviral activity. The structure of II is defined by establishing the XI methiodide as 4,4a-epoxy-5-ethylidene-1-methyl-2,3,4,4a,5,7a-hexahydro-1H-1-pyrindine methiodide derived from abikoviromycin.

The alkaloid (I) was found to possess fat clearing activity, the details of which will be described eleswhere.

Experimental

Melting points were determined on a micro hot-stage and are uncorrected. UV spectra were measured with a Hitachi EPS-2U. IR spectra were recorded on a JASCO IR-G. NMR spectra and NOE were measured with a Varian T-60 and a JEOL's JNM-4H-100. ^{13}CNMR spectra were taken on a Varian XL-100 at 25.1 MHz using TMS as reference. Mass spectra were recorded on a JEOL's JMS-01S.

Production and Isolation——Fermentation was carried out in a 400 liter jar fermentor at 27° for 48 hr in a medium (200 liter) containing 2% glucose, 0.5% peptone, 0.3% dry yeast, 0.5% meat extract, 0.5% NaCl, and 0.3% $CaCO_3$ (pH 7.0 before sterilization). After the broth filtrate was made alkaline (pH 10) with conc. NH_4OH, the alkaloids were extracted with butyl acetate (40 liter) and then transferred into 0.1N HCl (8 liter). The aqueous layer was made alkaline (pH 10) with conc. NH_4OH and extracted with ether (1.6 liter). The ethereal layer was dried over Na_2SO_4 and evaporated *in vacuo* to one-third volume, to which a saturated solution of picric acid in ether was added until no more precipitation occurred. Crude picrate (15 g) was obtained by filtration. Crude picrate (1.2 g) was dissolved in chloroform (50 ml) and then filtered to remove insoluble tar (500 mg). To the chloroform solution was added petr. ether (5 ml) until precipitation occurred. Alkaloid (II) picrate (287 mg) was collected and recrystallized from ethyl acetate–methanol to give yellow needles (250 mg), mp 136—137°. *Anal.* Calcd. for $C_{16}H_{14}O_8N_4$: C, 49.24; H, 3.62; N, 14.35. Found: C, 49.11; H, 3.56; N, 14.41. Mass Spectrum Calcd. for $C_{16}H_{14}O_8N_4$–$C_6H_3O_7N_3$: M, 161.084. Found: $M^+-C_6H_3O_7N_3$, 161.083. Acid sulfate: needles, mp 140—141° (decomp.). $[\alpha]_D^{20}=+14°$ (c=0.33, H_2O). To the above filtrate was petr. ether (10 ml) to give crude alkaloid (I) picrate (110 mg) which was made alkaline with 20% aq. NaOH (1 ml) and extracted with chloroform. The chloroform residue was chromatographed on silica gel (5 g) by using chloroform as eluent to give alkaloid (I) (50 mg) as picrate, yellow granules, mp 168—169° (from ethyl acetate–methanol). *Anal.* Calcd. for $C_{16}H_{18}O_7N_4$: C, 50.73; H, 4.73; N, 14.58. Found: C, 50.79; H, 4.80; N, 14.81. I hydrochloride: plates, mp 150° (decomp.) (from chloroform–ether). $[\alpha]_D^{20}=+21.5°$ (c=0.32, H_2O). *Anal.* Calcd. for $C_{10}H_{16}NCl$: C, 64.68; H, 8.68; N, 7.54. Found: C, 64.31; H, 8.71; N, 7.10. Mass Spectrum Calcd. for $C_{10}H_{16}NCl$–HCl: M, 149.120. Found: M^+—HCl, 149.122.

8) Since, because of lack of the sample, the position of the acetoxy group could not be decided, the structure of XII was depicted in Chart 2 according to Gurevich, *et al.*

9) Acid sulfate: mp 140—141° (decomp.), $[\alpha]_D=+24°$ (c=1, H_2O). Picrate: mp 137—140° (decomp.). A.I. Gurevich, M.N. Kolosov, V.G. Korobko, and V.V. Onoprienko, *Tetrahedron Letters*, **1968**, 2209; *idem*, *Chem. Natur. Comp.*, **7**, 93 (1973); H. Umezawa, T. Tazaki, and S. Fukuyama, *Jap. Med. J.*, **4**, 331 (1951); Y. Sakagami, R. Utahara, K. Yagishita, and H. Umezawa, *J. Antibiotics* (*Tokyo*), *Ser. A*, **11**, 231 (1958); Y. Kōno, S. Takeuchi, H. Yonehara, F. Marumo, and Y. Saito, *J. Appl. Crystallography*, **B 27**, 2341 (1971). The stereostructure of abikoviromycin was firstly proposed by Gurevich, *et al.* Subsequently, Kōno, *et al.* revised the configurations at C-4 and C-4a, and also decided the E configuration for the *exo* double bond by the X-ray analysis of latumcidin which was identified as abikoviromycin by the other workers.

(E,E)-4-Methyl-2-pentadienylpyrrolidine (III)——I picrate (180 mg) was added to 20% aq. NaOH (2 ml) and extracted with benzene (100 ml). To the benzene solution was added ethanol (20 ml) and then $NaBH_4$ (50 mg). The reaction mixture was stirred for 15 min at room temperature. The usual work-up gave an oil (65 mg), whose chromatography on silica gel (3 g) using chloroform–methanol (10: 1 v/v) as eluent gave III (20 mg) as oil. Hydrochloride: needles, mp 186—188° (from ethyl acetate). UV λ_{max}^{EtOH} mμ: 224 (23600) and 229 (24300). NMR (60 MHz, $CDCl_3$): δ 6.4—5.5 (4×vinyl-H), *ca.* 3.43 (2-H and 5-H), 2.93 (q, J=6 and 14 Hz, 5-H), and *ca.* 1.77 (3-H_2, 4-H, and 2-Me). Mass Spectrum Calcd. for $C_{10}H_{18}NCl$–HCl: M, 151.136. Found: M^+−HCl, 151.134. Acetamide (IV): IV was prepared as needles, mp 73° (from *n*-hexane), by acetylation of III with acetic anhydride in the presence of pyridine. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1640 (MeCON). NMR (60 MHz, CCl_4): δ 6.0—5.35 (4×vinyl-H), 4.90 (m, 2-H), 4.00 (bs, 5-H), 2.90 (bs, 5-H), 1.90 (MeCO), and *ca.* 1.65 (3-H_2, 4-H, and 2×Me). Mass Spectrum Calcd. for $C_{12}H_{19}ON$: M, 193.146. Found: M^+, 193.144.

(E,E)-1,4-Dimethyl-2-pentadienylpyrrolidine (V)——To a solution of III (135 mg) in ethanol (5 ml) was added formalin (0.1 ml) and then $NaBH_4$ (40 mg). The reaction mixture was refluxed for 30 min. After work-up, V (65 mg) was obtained as oil. Mass Spectrum Calcd. for $C_{11}H_{19}N$: M, 165.151. Found: M^+, 165.152. Methiodide (VII): scales, mp 164—167° (from ethyl acetate–methanol). UV λ_{max}^{MeOH} mμ: 229 (32500). NMR (60 MHz, $CDCl_3$): δ 6.66 (q, J=10 and 15 Hz, 2′-H), *ca.* 6.0 (1′-H and 4′-H), 5.47 (q, J=10 and 15 MHz, 3′-H), 4.60 (m, 2-H), 3.90 (bs, 5-H_2), 3.38 and 3.10 (2×N–Me), and 1.93—1.73 (3-H_2, 4-H, and 2×Me). *Anal.* Calcd. for $C_{12}H_{22}NI$: C, 46.91; H, 7.22; N, 4.56. Found: C, 46.65; H, 7.48; N, 4.47. Mass Spectrum Calcd. for $C_{12}H_{22}NI$–HI: M, 179.167. Found: M^+−HI, 179.163.

4-Methyl-2-*n*-pentylpyrrolidine (VI)——A solution of I hydrochloride (17 mg) in ethanol (10 ml) was hydrogenated over platinum black obtained from PtO_2 (4 mg) at room temperature for 1 hr. The usual work-up gave VI hydrochloride (13 mg) as needles, mp 139—141° (from ethyl acetate). NMR (100 MHz, $CDCl_3$): δ 2.90 (m, 2-H), 3.40 (bd, J=13 Hz, 5-H), 2.77 (bd, J=13 Hz, 5-H), 1.90—1.20 (11×H), 1.30 (4-Me), and 0.87 (t, J=7.5 Hz, 4′-Me). Mass Spectrum Calcd. for $C_{10}H_{21}N$: M, 155.167. Found: M^+, 155.163.

Methine Base (VIII)——A solution of VII (100 mg) in 30% KOH–MeOH (2 ml) was refluxed for 2.5 hr. The usual work-up afforded an oil (57 mg), whose chromatography on silica gel (3 g) using chloroform–methanol (20: 1 v/v) as eluent gave VIII (45 mg) as oil. UV λ_{max}^{MeOH} mμ: 255 (29500), 263 (38340), and 274 (30500). NMR (100 MHz, $CDCl_3$): δ 6.40—5.00 (6×vinyl-H), *ca.* 2.20 (N–CH_2), 2.20 (3×Me), 1.50 (m, CHMe), and 1.46 (q, J=4 Hz, Me). Mass Spectrum Calcd. for $C_{12}H_{21}N$: M, 179.167. Found: M^+, 179.167.

4,4a-Epoxy-5-ethylidene-2,3,4,4a,5,7a-hexahydro-1H-1-pyrindine (IX)——II picrate (100 mg) was added to 20% aq. KOH (1 ml) and extracted with chloroform (50 ml). To the chloroform solution was added ethanol (20 ml) and then $NaBH_4$ (40 mg). The reaction mixture was stirred for 1 hr at room temperature. The usual work-up gave an oil (35 mg). After preparative thin-layer chromatography (TLC),[10] the zone with *Rf* 0.45 gave IX (20 mg) as oil. UV λ_{max}^{EtOH} mμ: 245 (6090). Mass Spectrum Calcd. for $C_{10}H_{13}ON$: M, 163.099. Found: M^+, 163.100. Acetate (X): X was prepared as oil by acetylation of IX with acetic anhydride in the presence of pyridine. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1650 (MeCON). Mass Spectrum Calcd. for $C_{12}H_{15}O_2N$: M, 205.110. Found: M^+, 205.112.

4,4a-Epoxy-5-ethylidene-1-methyl-2,3,4,4a,5,7a-hexahydro-1H-1-pyrindine (XI)——To a solution of IX (90 mg) in methanol (30 ml) was added formalin (0.12 ml) and then $NaBH_4$ (60 mg). The reaction mixture was refluxed for 1 hr. The usual work-up afforded an oil (55 mg), whose chromatography on silica gel (3 g) using chloroform as eluent gave XI (40 mg) as oil. NMR (60 MHz, CCl_4): δ 6.60 (dd, J=2 and 6 Hz, 6-H), 6.12 (dd, J=2 and 6 Hz, 7-H), 5.03 (dq, J=2 and 8 Hz, 1′-H), 3.07 (d, J=2 Hz, 7a-H), 2.97 (d, J=2 Hz, 4-H), 2.33 (m, 2-H_2), 2.27 (s, 1-Me), 2.03 (m, 3-H_2), and 1.77 (d, J=8 Hz, 1′-Me). Mass Spectrum Calcd. for $C_{11}H_{15}ON$: M, 177.115. Found: M^+, 177.116. Methiodide: plates, mp 231—232° (decomp.) (from ethyl acetate–methanol). NMR (60 MHz, $CD_3OD/CDCl_3$ 1: 5 v/v): δ 7.00 (dd, J=2 and 6 Hz, 6-H), 6.28 (dd, J=2 and 6 Hz, 7-H), 5.28 (dq, J=2 and 8 Hz, 1′-H), 4.60 (d, J=2 Hz, 7a-H), 3.84 (dt, J=3 and 14 Hz, 2-H), 3.64 (m, 2-H), 3.42 (s, N–Me), 3.30 (d, J=3 Hz, 4-H), 2.93 (N–Me), 2.41 (m, 3-H_2), and 1.82 (d, J=8 Hz, 1′-Me). *Anal.* Calcd. for $C_{12}H_{18}ONI$: C, 45.15; H, 5.64; N, 4.38. Found: C, 45.18; H, 5.77; N, 4.38. Methiodide was identified as 4,4a-epoxy-5-ethylidene-1-methyl-2,3,4,4a,5,7a-hexahydro-1H-1-pyrindine methiodide derived from abikoviromycin by mixed mp and NMR spectroscopy.

4-Acetoxy-1-acetyl-5-ethyl-octahydro-1H-1-pyrindine (XII)——I, which was obtained from I picrate (100 mg) by the usual way, was dissolved in 0.1N HCl (15 ml) and hydrogenated over platinum black derived from PtO_2 (10 mg) at room temperature for 2 hr. The usual work-up afforded an oil (35 mg) which gave oily compounds by acetylation. After preparative TLC,[11] the zone with *Rf* 0.67 gave XII (6 mg) as oil. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1700 (AcO) and 1625 (AcN). Mass Spectrum Calcd. for $C_{14}H_{23}O_3N$: M, 253.167. Found: M^+, 253.166.

Acknowledgement We thank Meiji Seika Kaisha, Ltd. for the gift of 1,7a-dihydroabikoviromycin.

10) Silica gel plate (0.5 mm), chloroform–methanol (5: 1 v/v).
11) Silica gel plate (0.5 mm), chloroform–methanol (50: 1 v/v).