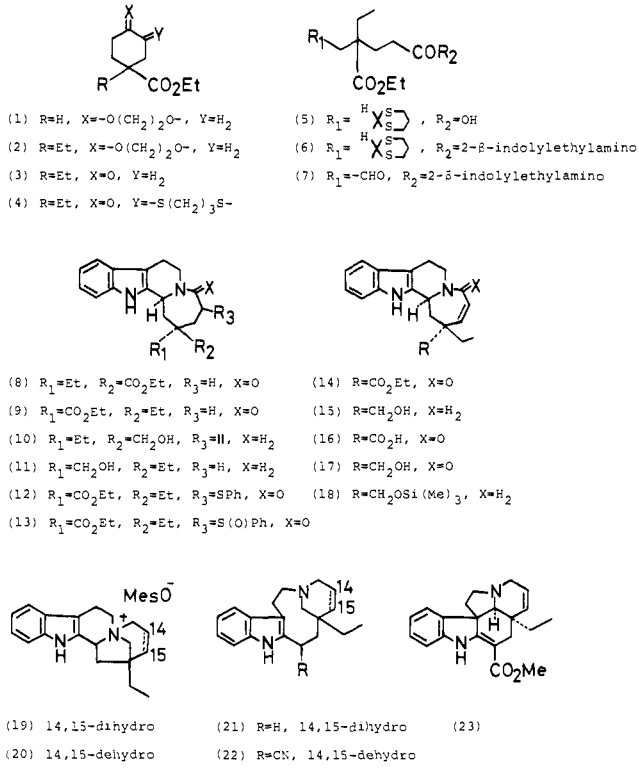


sponding amino alcohol **11** (mp 219–221°, ir (Nujol) 3320 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) (δ) 0.83 (3 H, t, J = 7.0 Hz)) in 98% yield. The α-ethyl amino alcohol **10** was converted (CH_3SO_2Cl–pyridine, 0°, 3 h) to the mesylate which was refluxed in chloroform for 3 h to give the amorphous quaternary salt **19**,[10] NMR (D_2O) (δ) 0.71 (3 H, t, J = 6.5 Hz), 2.93 (3 H, s), almost quantitatively. Similarly the β-ethyl isomer **11** afforded the isomeric quaternary salt **19**, mp 206–208° dec, NMR (D_2O) (δ) 1.04 (3 H, t, J = 6.8 Hz), 2.90 (3 H, s), quantitatively. Reduction of both isomeric salts with sodium in liquid ammonia[10] provided *dl*-quebrachamine (**21**) in about 80% yield, respectively (total yield 22.3% from **2**).

Tabersonine precursor **22** was obtained from β-ethyllactam **9** via ten steps in 13.7% total yield (3.4% from 2). The β-ethyllactam **9** was converted (LDA–PhSSPh)[11] into the sulfide **12** in 91%: mp 166–167°; NMR ($CDCl_3$) (δ) 6.00 (1 H, d, J = 8.3 Hz). Oxidation and pyrolysis ((1) MCPBA; (2) toluene, reflux, 30 min)[12] transformed **12** into the α,β-unsaturated lactam **14** (mp 167–168°; ir (Nujol) 3260, 1720, 1648, 1598 cm^{-1}; NMR ($CDCl_3$) (δ) 6.08 (1 H, d, J = 12.0 Hz), 6.28 (1 H, dd, J = 12.0, 2.0 Hz)) in 95% yield from the sulfide **12** via the sulfoxide **13**. Since $LiAlH_4$ reduction failed to afford the desired unsaturated amino alcohol **15** as a major product (~5%), an alternative five-step method was developed. The unsaturated lactam **14** was hydrolyzed with potassium hydroxide to give the carboxylic acid **16** (mp 179.5–180°; ir (Nujol) 3400, 3050–2300, 1690, 1620, 1563 cm^{-1}; NMR ($CDCl_3$) (δ) 6.00 (1 H, d, J = 12.5 Hz), 6.48 (1 H, d, J = 12.5 Hz)) in 95% yield and the latter was converted to the unsaturated lactam alcohol **17** (mp 233–234°; ir (Nujol) 3350, 3240, 1630, 1579 cm^{-1}; NMR ($CDCl_3$+ Me_2SO-d_6) (δ) 5.88 (1 H, d, J = 10.5 Hz), 6.15 (1 H, d, J = 10.5 Hz)) by treating with ethyl chloroformate and triethylamine at room temperature, followed by $NaBH_4$ in aqueous tetrahydrofuran.[13] The unsaturated lactam alcohol **17** was silylated (Me_3SiCl–Et_3N, 20°) to give the silylether **18** which on reduction ($LiAlH_4$, THF, 0°) yielded the desired unsaturated amino alcohol **15** (mp 201–203.5°; ir (Nujol) 3400–3100 cm^{-1}; NMR ($CDCl_3$) (δ) 0.92 (3 H, t, J = 7.0 Hz), 2.00 (2 H, d, J = 9.0 Hz), 3.48 (1 H, d, J = 11.0 Hz), 3.74 (1 H, d, J = 11.0 Hz), 4.55 (1 H, t, J = 9.0 Hz), 5.50 (1 H, dd, J = 11.0, J = 2.8 Hz), 5.96 (1 H, ddd, J = 11.0, 6.0, J = 3.0 Hz)) in 58% yield from **14.**

Conversion of the unsaturated alcohol **15** to the known quaternary salt **20**[14] (mp 261° dec,[15] NMR (D_2O) (δ) 0.67 (3 H, t, J = 7.0 Hz), 1.41 (2 H, q, J = 7.0 Hz), 2.46 (3 H, s), 5.95 (1 H, d, J = 10.0 Hz)) was carried out as its saturated one **11** in quantitative yield. To confirm its formation, according to Ziegler and co-worker,[14] the salt **20** was transformed into 14,15,16,17-tetrahydroquebrachamine (58.2%) by $LiAlH_4$ and into 16-cyano-14,15-didehydroquebrachamine (**22**), (27.4%)[16] by potassium cyanide. Since the latter (**22**) has been converted to *dl*-tabersonine (**23**),[14] this constitutes an alternative synthesis of **23.**

The relatively simple sequence described here provides an efficient route to the non-tryptamine moiety of the Aspidosperma type and related indole alkaloids.

Acknowledgment. This research was supported by the grants from the Ministry of Education and by a Grant-in-Aid from Takeda Scientific Research Foundation.

References and Notes

(1) J. A. Marshall and D. E. Seits, *J. Org. Chem.*, **39**, 1814 (1974).
(2) M. Hesse, "Indolalkaloide in Tabellen", Springer Verlag, Berlin, 1964 and 1968.
(3) H. Musso, K. Naumann, and K. Grychtol, *Chem. Ber.*, **100**, 3614 (1967).
(4) D. S. Watt, *Tetrahedron Lett.*, 707 (1974).
(5) R. B. Woodward, I. J. Pachter, and M. L. Scheinbaum, *J. Org. Chem.*, **36**, 1137 (1971).
(6) J. C. A. Chivers and S. Smiles, *J. Chem. Soc.*, 697 (1928).
(7) Satisfactory mass spectroscopic data and analytical data were obtaine for all new compounds.
(8) M. Fetizon and M. Jurion, *J. Chem. Soc., Chem. Commun.*, 382 (1972).
(9) Stereochemcial assignment of the products will be described in the full paper.
(10) J. P. Kutney, N. Abdurahman, C. Gletsos, P. LeQuesne, E. Piers, and I. Vlattas, *J. Am. Chem. Soc.*, **92**, 1727 (1970).
(11) The same conversion was carried out on the α-ethyl isomer **8**, but a trace amount of desired sulfide was obtained with high recovery of the starting material.
(12) B. M. Trost and T. N. Salzmann, *J. Am. Chem. Soc.*, **95**, 6840 (1973).
(13) K. Ishizumi, K. Koga, and S. Yamada, *Chem. Pharm. Bull.*, **16**, 492 (1968).
(14) F. E. Ziegler and G. B. Bennett, *J. Am. Chem. Soc.*, **95**, 7458 (1973).
(15) Discrepancy from the reported value (mp 285° dec) might indicate a stereoisomeric relationship. However, this was not an important problem, because the isomeric center was lost in the following step.
(16) 10% of the corresponding carboxamide derivative was also obtained.

Seiichi Takano,* Susumi Hatakeyama, Kunio Ogasawara
Pharmaceutical Institute, Tohoku University
Aobayama, Sendai, Japan 980
Received December 31, 1975

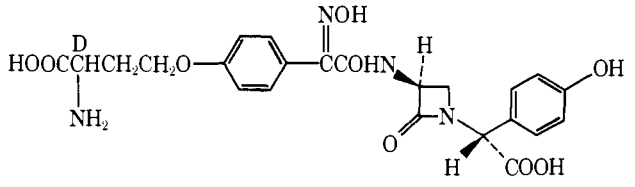
Nocardicin A and B, Novel Monocyclic β-Lactam Antibiotics from a *Nocardia* Species

Sir:

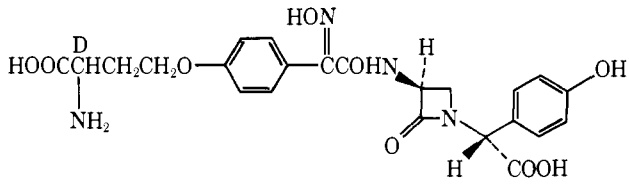
In view of their outstanding antimicrobial activity, the β-lactam antibiotics have attracted great interest in recent years.[1] In the present communication we report the structure of two unique monocyclic β-lactam antibiotics, nocardicin A (**1**) and B (**2**), which are structurally and biologically related to the penicillins and cephalosporins.

Nocardicin A (**1**)[2] ($C_{23}H_{24}O_9N_4$, mp 214–216° dec, $[\alpha]_D$ −135.0° (H_2O),[3] pK_a 3.2, 4.5, 10.0, 11.6, and 12.7 (potentiometry), positive ninhydrin test) was isolated as a major component from a strain of *Nocardia*.[4] Acetylation of **1** with Ac_2O in MeOH (0°) and subsequent methylation with CH_2N_2 gave the monoacetyl-tetramethyl derivative **3**, while acetylation of **1** with Ac_2O in H_2O (pH ca. 9, room temperature), followed by methylation with CH_2N_2 gave the triacetyl-dimethyl derivative **4.** Hence one amino, two carboxyl, and two weakly acidic hydroxyl groups are present in **1.**

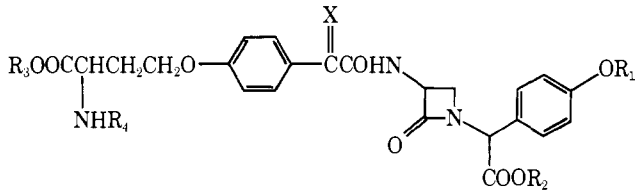
The ^1H NMR analysis of **1** (Na salt in D_2O) revealed all



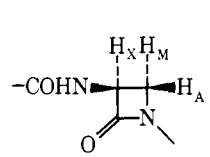
1



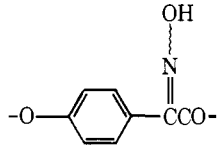
2



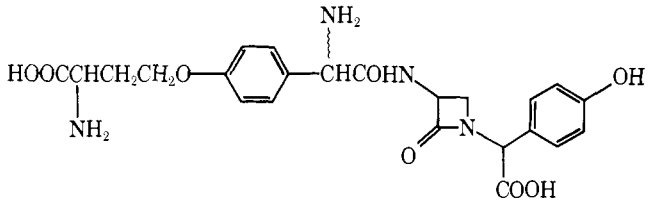
3, $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = CH_3$; $R_4 = COCH_3$; X = $NOCH_3$
4, $R_1 = COCH_3$; $R_2 = CH_3$; $R_3 = CH_3$; $R_4 = COCH_3$; X = $NOCOCH_3$
6, $R_1 = R_2 = R_3 = R_4 = H$; X = O



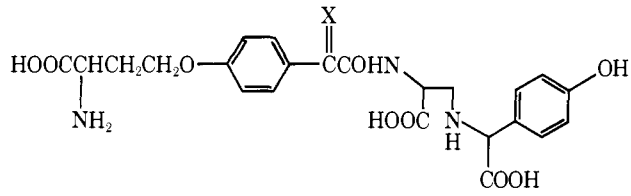
a



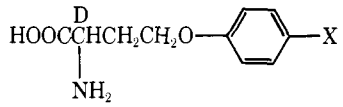
b



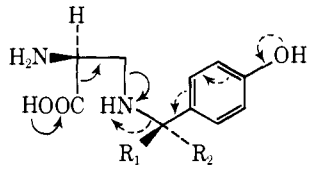
5



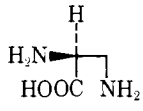
7, X = O
8, X = NOH



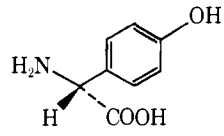
9, X = C(=NOH)COOH
10, X = COCOOH
15, X = COOH



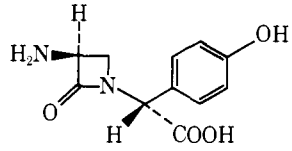
11, R_1 = H; R_2 = COOH
12, R_1 = COOH; R_2 = H



13



14



16

partial structures. Moiety a is derived on the following grounds: an AMX[5] system is present at 3.14, 3.97, and 5.01 ppm (J_{AM} = 6 Hz, J_{AX} = 2 Hz, J_{MX} = 5 Hz); proton X is further coupled to the amide proton when measured in Me_2SO-d_6 (N-H, 9.12 ppm, d, J = 8 Hz); and a β-lactam ir band (KBr) is present at 1730 cm^{-1} in **1**-Na salt. A 2 H quintet (J = 6 Hz) at 2.39 ppm coupled to a 2 H triplet (J = 6 Hz) at 4.22 ppm and a 1 H triplet (J = 6 Hz) at 3.81 ppm[6] suggests the presence of the homoserine unit. Two sets of AB systems centered at 7.07 (4 H, J = 9 Hz) and 7.22 ppm (4 H, J = 9 Hz), respectively, indicate the presence of two para substituted aromatic groups, which are further characterized as being a para alkylated phenol(p-hydroxyphenylglycine) and a conjugated alkoxyphenyl derivative (e.g., partial structure b) from uv data: $\lambda_{max}^{EtOH-H_2O}$ 220 nm (ϵ, 21 000) and 272 (16 000), $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 245 nm (ϵ, 23 500) and 285 (11 300). A 1 H singlet at 5.33 ppm is assigned to the benzylic proton.

These partial structures were further corroborated by the ^{13}C NMR spectrum[7] which indicates 3 methylene (30.63 (t), 47.02 (t), and 66.01 ppm (t)), 3 methine (54.17 (d), 54.90 (d), and 61.58 ppm (d)), and 12 aromatic carbon signals (115.88 (d, two C), 116.54 (d, two C), 123.95 (s), 127.46 (s), 128.68 (d, two C), 131.04 (d, two C), 156.41 (s), 160.53 (s)). The spectrum shows five additional signals in the downfield region: four of them, 166.84 (s), 168.54 (s), 174.73 (s), and 176.61 ppm (s), are assigned to carboxyl (two), amide, and β-lactam carbonyl groups. The remaining signal at 153.74 ppm (s) is assignable to the oxime group (partial structure b), which constitutes one of two weakly acidic hydroxyl functions in **1** (the other being the phenolic hydroxyl).

Hydrogenation of **1** over 10% Pd-C gave an isomeric mixture of amines **5**, which supports the presence of an oxime group in **1**. This was further clarified as follows. Treatment of **1** with $NaHSO_3$ (80°, 3 h) afforded the keto derivative **6**: λ_{max}^{EtOH} 300 nm (ϵ, 15 400). On treatment with 1 N HCl (room temperature), **6** was converted to **7** which was also derived by direct hydrolysis of **1** with 3 N HCl (room temperature). Reaction of **7** with NH_2OH (H_2O, 80°) yielded **8**, which was also obtained from **1** by treatment with 1 N NaOH. This sequence of reactions establishes the presence of the oxime group in **1**.

The ease of hydrolysis of **1** and **6** described above also shows the presence of the β-lactam moiety in **1**.

Acid degradation of **1** with 6 N HCl (reflux, 1 h) resulted in the formation of **9**, **10**, and **11**, which were fully characterized by further degradation reactions. Oxime acid **9** was again hydrolyzed with 6 N HCl (reflux, 1 h) to give **10**, which, on treatment with NH_2OH, reverted to **9**. Further acid hydrolysis (6 N HCl, reflux 3 h) of fragment **11** gave, in addition to recovered **11**, the epimer **12**, $\alpha\beta$-diaminopropionic acid (HCl salt) **13**, and p-hydroxyphenylglycine (HCl salt) **14**. The geneses of **13** and **14** can be rationalized by the arrows shown

in structure **11.** These chemical data are in full agreement with structure **1** for nocardicin A.

The absolute configuration of the acylamino group on the β-lactam ring and the carboxyl group of the *p*-hydroxyphenylglycine moiety were established to be L and D, respectively, from optical data of **13,** $[\alpha]_D$ +20.3° (1 N HCl),[8] and **14,** $[\alpha]_D$ −80.0° (0.1 N HCl) (54% optical purity).[9] With regard to the stereochemistry of the remaining homoserine unit, the benzoic acid derivative **15,** obtained by treatment of **10** with H_2O_2, was hydrogenated over Pt in 3 N HCl to generate D-α-aminobutyrolactone (HCl salt), $[\alpha]_D$ +29.0 (0.1 N HCl);[10] the absolute configuration of the homoserine part is thus D.

The oxime configuration was established to be syn to the acylamino group on the following grounds. Nocardicin B (**2**), $C_{23}H_{24}O_9N_4$, mp 262–264° dec, $[\alpha]_D$ −162.0°(H_2O),[3] isolated as a minor product from the same culture, was shown to be a stereoisomer of **1** at the oxime function; on treatment with $NaHSO_3$, **2** was also converted to the keto derivative **6.** The ^1H NMR spectrum of **1** (Me_2SO-d_6) shows the amide proton at 9.12 ppm (as described above), while in **2** it is at 8.81 ppm (d, J = 8 Hz). This difference in the chemical shift of the amide protons suggests the presence of an internal hydrogen bonding between the oxime O and amide H in **1.** This is possible only when the oxime OH is syn to the amide group.[11,12]

The structures of nocardicin A and B are hence established as being **1** and **2,** respectively. Nocardicin A is active against a variety of gram-negative bacteria and shows an especially high antimicrobial activity against *Pseudomonas,* while the activity of nocardicin B is weaker.[13] These antibiotics are unique in several respects: (1) they are the first examples of monocyclic β-lactam antibiotics[14] possessing relatively high potency; (2) they have an oxime function[15] whose syn relation to the acylamino group is favored for antimicrobial activity; (3) they contain *p*-hydroxyphenylglycine (two such units) which is found rarely in nature;[16] (4) their structures are stereochemically related to the penicillin molecule (carboxyl, α; acylamino, β); and (5) similarly to penicillins and cephalosporins, they are enzyme inhibitors in the cell wall biosynthesis of bacteria.[17]

Chemical modification of nocardicins and preparation of new 3-acyl derivatives of 3-aminonocardicinic acid (3-ANA) **16**[18] are in progress.

References and Notes

(1) For recent reviews on the chemistry and biology of the β-lactam antibiotics, see, e.g., J. H. C. Nayler, *Adv. Drug Res.*, **7,** 1 (1973); D. N. McGregor, *Fortschr. Chem. Org. Naturst.*, **31,** 1 (1974); A. K. Mukerjee and A. K. Singh, *Synthesis,* 547 (1975); R. J. Stoodley, *Tetrahedron,* **31,** 2321 (1975); J. C. Jaszberenyi and T. E. Gunda, *Prog. Med. Chem.*, **12,** 395 (1975).

(2) The fermentation, isolation, and characterization of nocardicins have been carried out by H. Aoki, H. Sakai, M. Kohsaka, T. Konomi, J. Hosoda, T. Kubochi, E. Iguchi, and H. Imanaka, *J. Antibiot.*, in press.

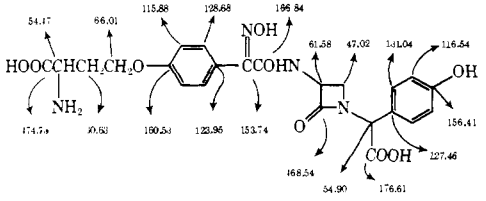
(3) The $[\alpha]_D$ measurements were performed on the sodium salts: **1,** $C_{23}H_{23}O_9N_4Na$, mp 234–235° dec; **2,** $C_{23}H_{23}O_9N_4Na$, mp 257–260° dec.

(4) *Nocardia uniformis* var. *tsuyamanensis* ATCC 21806.

(5) For data of geminal and vicinal coupling constants of substituted β-lactams, see K. D. Barrow and T. M. Spotswood, *Tetrahedron Lett.*, 3325 (1965); P. V. Demarco and R. Nagarajan, "Cephalosporins and Penicillins, Chemistry and Biology". H. Flynn, Ed., Academic Press, New York, N.Y., 1972, pp 330–340.

(6) Upon acetylation, this 1 H triplet underwent a downfield shift to 4.70 ppm (**3** in $CDCl_3$).

(7) Each signal in the ^{13}C NMR spectrum was assigned as follows by comparison with the spectra of degradation products. Details will be discussed in a forthcoming full paper.

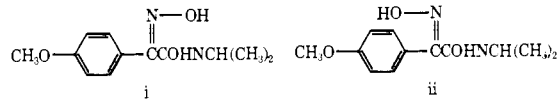


(8) Lit. $[\alpha]_D$ +25.2° (**1** N HCl): S. M. Birnbaum, R. J. Koegel, S.-C. J. Fu, and J. P. Greenstein, *J. Biol. Chem.*, **198,** 335 (1952).

(9) During the reaction, partial racemization took place in **14;** prolonged heating led to complete racemization of **14.** An optically pure sample (HCl salt) showed $[\alpha]_D$ −149° (0.1 N HCl) (lit. $[\alpha]_D$ −108° (H_2O)): A. A. W. Long, J. H. C. Naylor, H. Smith, T. Taylor, and N. Ward, *J. Chem. Soc. C,* 1920 (1971).

(10) L-α-Aminobutyrolactone (HCl salt) prepared from L-homoserine for comparison showed $[\alpha]_D$ −29.0° (0.1 N HCl) (lit. $[\alpha]_D$ +26.7°(H_2O) for the D isomer): S. M. Birnbaum and J. P. Greenstein, *Arch. Biochem. Biophys.*, **42,** 212 (1953).

(11) This behavior was also observed in model compounds i and ii: i (syn to the amide), 8.30 ppm (d, J = 8 Hz); ii (anti to the amide), 8.00 ppm; difference, 0.30 ppm.



i ii

(12) The uv absorptions due to partial structure b of nocardicin A and B were calculated by substraction of the absorbance of *p*-hydroxyphenylglycine from those of nocardicins: 1, $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ε, 14 900) and $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 283 nm (ε, 9500); **2,** $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ε, 8900) and $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 275 nm (ε, 9400). In comparison to **2,** the uv absorption band of **1** was longer and stronger in both neutral and basic media. This is in agreement with the data of models i and ii: i, $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ε, 15 800) and $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 283 nm (ε, 11 400); ii, $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ε, 9800) and $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 275 nm (ε, 9500). These data also confirmed that the oxime function is syn to the amide group in **1** and anti in **2.**

(13) Y. Mine, S. Nonoyama, H. Kojo, S. Fukada, M. Nishida, S. Goto, and S. Kuwahara, to be submitted for publication.

(14) Several compounds containing monocyclic β-lactam rings have been isolated from microorganisms: see, e.g., W. W. Stewart, *Nature (London),* **229,** 174 (1971); H. K. Schnoes and R. D. Durbin, *Biochem. Biophys. Acta,* **286,** 107 (1972); T. Takita, Y. Muraoka, T. Yoshioka, A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiot.*, **25,** 755 (1972); J. P. Scannell, D. L. Pruess, J. F. Blount, H. A. Ax, M. Kellett, F. Weiss, T. C. Demny, T. H. Williams, and A. Stempel, *J. Antibiot.*, **28,** 1 (1975).

(15) Aside from semisynthetic compounds, only few microbial products bearing an oxime function are known; see P. H. Wiley, R. R. Herr, F. A. Mackellar, and A. O. Argondelis, *J. Org. Chem.*, **30,** 2330 (1965); H. Sakakibara, H. Naganawa, M. Ohno, K. Maeda, and H. Umezawa, *J. Antibiot.*, **27,** 897 (1974); B. W. Bycroft and R. Pinchin, *J. Chem. Soc., Chem. Commun.*, 121 (1975).

(16) See, e.g., K. A. Smith, D. H. Williams, and G. A. Smith, *J. Chem. Soc., Perkin Trans. 1,* 2369 (1974).

(17) A study on the inhibitory mechanism of nocardicins will be reported by H. Aoki et al.; cf. ref 2.

(18) Preparation of **16** from nocardicin A will be reported elsewhere.

Masashi Hashimoto, Tada-aki Komori, Takashi Kamiya*

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532, Japan

Received January 16, 1976

Spectinomycin Biosynthesis Studied by Carbon Magnetic Resonance Spectroscopy[1]

Sir:

We have recently reported[2] that the biosynthesis of deoxystreptamine, the aminocyclitol moiety of neomycin, proceeds from glucose by a pathway in which [6-^{13}C]D-glucose labels C-2 of deoxystreptamine and [1-^{13}C]D-glucosamine[3] labels C-1 of deoxystreptamine (Figure 1, path b).[4] More recently we showed that [6-^{13}C]D-glucose labels C-6 of streptidine, the substituted aminocyclitol moiety of streptomycin,[1b] which would agree with earlier reports that [1-^{14}C]D-glucose labels C-5 of streptidine.[5]

Thus, the two aminocyclitols deoxystreptamine and streptidine are biosynthesized by different pathways. A third aminocyclitol antibiotic,[6] spectinomycin (Figure 2),[7,8] which is used clinically in the treatment of gonorrhea, contains a different aminocyclitol unit, actinamine, with similarities to both deoxystreptamine and streptidine. Actinamine does not contain the highly basic guanido groups of streptidine, but, unlike deoxystreptamine, it contains a hydroxyl group at C-2. A priori, then, either (or neither) of the two biosynthetic pathways might be followed.

No report exists of the precise location of label in the am-