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THE SYNTHESIS OF NETILMICIN *via* COMPLEXING OF VICINAL AND *NON*-VICINAL AMINO-HYDROXYL GROUP PAIRS WITH DIVALENT TRANSITION-METAL CATIONS*

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ABSTRACT

Sisomicin was converted into 3,2′,6′-tri-*N*-acetylsisomicin by acetylation of its 3″,4″-vicinal-*cis* and 1,2″-*non*-vicinal amino-hydroxyl group pair complex of copper(II) or cobalt(II) acetate. The tri-*N*-blocked derivative was reductively monoalkylated at the 1-position with acetaldehyde and sodium cyanoborohydride and then *N*-deprotected to give netilmicin.

INTRODUCTION

Netilmicin (**6**) is the latest semisynthetic aminoglycoside-aminocyclitol antibiotic to enter the market. It is the 1-*N*-ethyl derivative of sisomicin (**1**), an antibiotic produced by *Micromonospora purpurea*. Its first synthesis[1,2], proof of structure[2], microbiological spectrum of activity[3], and report of clinical utility[4] have appeared in the literature[5]. This paper describes a much improved and practical synthesis of the antibiotic from sisomicin employing the novel vicinal and *non*-vicinal transition-metal complexing of aminoglycoside-aminocyclitol antibiotics developed in our laboratory[6,7].

RESULTS AND DISCUSSION

As pointed out earlier[6], divalent transition-metal cations form five types of reversible complexes with aminoglycoside-aminocyclitol antibiotics in solution owing to the presence of five types of amino and hydroxyl group pairs. The five types are: primary amino-hydroxy *cis*-vicinal, primary amino-hydroxy *trans*-vicinal, secondary amino-hydroxy *cis*-vicinal, secondary amino-hydroxy *trans*-vicinal, and primary amino-hydroxy *non*-vicinal. A *non*-vicinal amino and hydroxy group pair is one in which the two groups are located several bonds away on different rings, yet are in proximity owing to a unique stereochemical consequence as a result of

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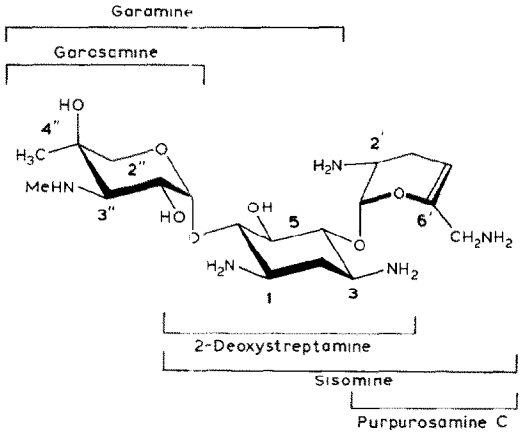
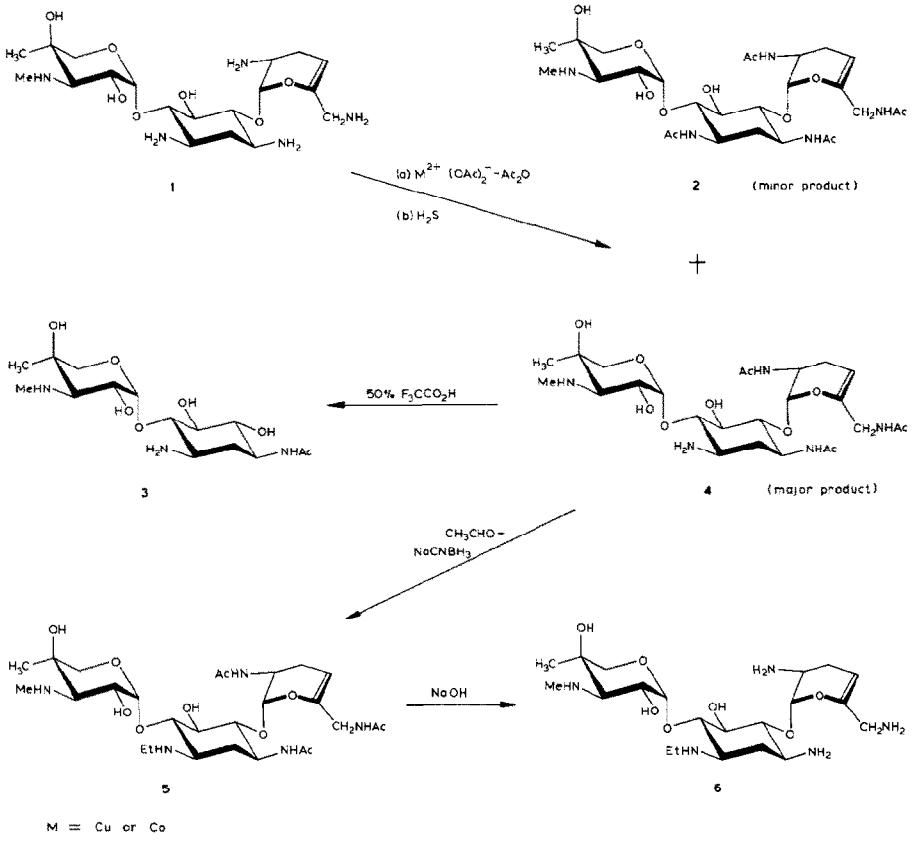


Scheme 1. Vicinal and *non*-vicinal aminohydroxy group pairs in sisomicin (**1**): 1,2″-*non*-vicinal, 2′,5-*non*-vicinal, and 3″,4″-vicinal.

conformational preference about the glycosidic linkage and possible further stabilization of the conformation by intramolecular hydrogen-bonding between the two groups[7]. In sisomicin (**1**) are a vicinal *cis* amino and hydroxy group pair at 3″,4″-position, and two *non*-vicinal amino and hydroxyl group pairs at 1, 2″ and 2′,5-positions (see Scheme 1). Under suitable conditions, the amino-hydroxyl group pairs form reversible complexes with divalent transition-metal cations, the extent to which these cation complexes are formed in solution being dependent on the type of transition-metal salt, availability of the amino hydroxyl group pair for complexing, stability of the complex, and nature of the solvent[7]. The noncomplexed amino groups are then allowed to react with suitable *N*-blocking reagents to give, after removal of the transition metal, selectively *N*-protected derivatives[7]. By making use of the reactivity differences among the noncomplexed amino groups, it is possible to achieve further selectivity. In the case of sisomicin (**1**), as we have shown previously, complexing occurs at the 3″,4″ and 1,2″-positions[7]. No complexing takes place between the amino and hydroxyl group pair at the 2′,5-position owing to inaccessability of OH-5 which is buried between two sugar residues[7]. *N*-Protection at the 3,2′ and 6′-positions of sisomicin (**1**) could therefore be readily achieved[7].

Treatment of sisomicin (**1**) with 14 equivalents of cupric acetate dihydrate in aqueous *N,N*-dimethylformamide, followed by 3 equivalents of acetic anhydride afforded 3,2′,6′-tri-*N*-acetylsisomicin (**4**) as the major product. It was isolated in 75% yield by column chromatography on silica gel after removal of cupric ions with hydrogen sulfide. 1,3,2′,6′-Tetra-*N*-acetylsisomicin (**2**) was formed as a by-product which was isolated in 11% yield.

The structure of **2** was readily proven by a direct comparison of its physicochemical properties with those of an authentic sample prepared by an alternate



method[8]. The structure of **4** was proven as follows: The ^{1}H-n.m.r. spectrum showed the presence of three *N*-acetyl groups. The absence of an acetyl group at NH-3″ was inferred from the chemical shift (δ 2.51) and the sharp singlet nature of the *N*-methyl signal[9]. The electron impact (e.i.) mass spectrum showed a molecular ion at *m/z* 573 consistent with the structure. The absence of an acetyl group in the garosamine moiety of the molecule was further confirmed by the ion at *m/z* 160 which is characteristic of the unsubstituted garosaminyl cation[10]. The presence of a single acetyl group in the 2-deoxystreptamine portion of the molecule was clearly evidenced by ions at *m/z* 233, 215, 205, and 187. The ions characteristic of a fragmentation pattern of the protonated formyl 2-deoxystreptamine are 191→173, 191→163, 145. The presence of two acetyl groups in the purpurosamine C portion was clear from the ions at *m/z* 211 (expected for purpurosamine C glycosyl cation, 127), and 443, 425, 415, and 397 (the characteristic fragmentation pattern[10] of protonated formyl sisomine is 317→299, 317→289→271). From the aforementioned studies the only question that remained unanswered was the position of the acetyl group in the 2-deoxystreptamine part. To answer this question, compound **4** was

selectively hydrolyzed in aqueous acid, utilizing the higher reactivity of the vinyl ether, to give 3-*N*-acetylgaramine (**3**).

In tetraamine copper solution (TaCu), the c.d. spectrum of **3** gave $[\theta]^{290}_{TaCu}$ −6260, which meant that the acetyl group was present at NH-3 (ref. 11). If the acetyl group were at NH-1, the value expected would be twice that observed[11]. Therefore, the structure of **4** was proven to be 3,2′,6′-tri-*N*-acetylsisomicin.

The yield of **4** was further improved by enhancing the selectivity. Thus, it was increased to 87% by conducting the reaction in the presence of 2 equivalents of cobalt(II) acetate tetrahydrate in *N,N*-dimethylformamide. This could be further improved to 95% by increasing the amount of cobalt(II) acetate tetrahydrate to 3 equivalents and changing the solvent to dimethyl sulfoxide.

Reductive alkylation of **4** with acetaldehyde and sodium cyanoborohydride in aqueous acid gave 3,2′,6′-tri-*N*-acetyl-1-*N*-ethylsisomicin (**5**) in 70% yield after chromatography on silica gel. The data presented in the experimental section are consistent with the structure. *N*-Deacetylation of **5** with aqueous sodium hydroxide gave, after purification on silica gel, 1-*N*-ethylsisomicin (**6**, netilmicin) in 90% yield. The product was identical in its physicochemical and microbiological properties with an authentic sample.

EXPERIMENTAL

General. — Details of experimental techniques have been given in earlier publications[12,13].

*3,2′,6′-Tri-*N*-acetylsisomicin* (**4**) *by use of cupric acetate dihydrate.* — Lyophilized sisomicin (**1**, 1.3 g, 3 mmol) was dissolved in water (16 mL) and *N,N*-dimethylformamide (54 mL) was added with stirring. To the stirred solution was added cupric acetate dihydrate (9 g, 41.5 mmol) and the mixture kept with stirring at room temperature for 35 min. A freshly prepared M solution of acetic anhydride in *N,N*-dimethylformamide (9.9 mL) was added dropwise at the rate of 25 drops/min. After the addition was complete, the mixture was stirred for a further period of 30 min and diluted with water (20 mL). Hydrogen sulfide was bubbled through the solution for 10 min to precipitate cupric sulfide, and to ensure complete precipitation the mixture was stirred for additional 30 min. The suspension was filtered through a pad of Celite, the residue thoroughly washed with water (3 × 10 mL), and the filtrate evaporated to dryness *in vacuo*. The residue was chromatographed on a column (2.5 × 96 cm) of silica gel (150 g) with 30:10:1 chloroform–methanol–ammonium hydroxide as the eluant. The fractions containing **4** were pooled, evaporated to dryness, and lyophilized to give 1.29 g (75%). Fractions containing **2** were processed in a similar manner to give 0.2 g (11%). Compound **4** showed $[\alpha]^{26}_{D}$ +186.7° (*c* 0.45, water); ^{1}H-n.m.r. (D_2O); δ 1.94, 1.98, 2.0 (9 H, 3 NCOCH_3), 1.22 (s, 3 H, C-CH_3), 2.51 (s, 3 H, N-CH_3), 2.59 (d, 1 H, *J* 9.5 Hz, H-3″), 5.10 (d, 1 H, *J* 4.2 Hz, H-1″), and 5.51 (d, 1 H, *J* 2.5 Hz, H-1′); m.s.: *m/z* 573 (M^+), 443, 425, 415, 397 (protonated formyl tri-*N*-acetylsisomine series), 397,

374, 364, 346 (protonated formyl *N*-acetylgaramine series), 233, 215, 205, 187 (protonated formyl *N*-acetyl-2-deoxystreptamine series), 211 (di-*N*-acetylpurpurosamine C cation), and 160 (garosaminyl cation).

Anal. Calc. for $C_{25}H_{43}O_{10}N_5 \cdot H_2CO_3$: C, 49.13; H, 7.14; N, 11.02. Found: C, 49.10; H, 7.02; N, 11.38.

*3,2',6'-Tri-*N*-acetylsisomicin* (**4**) *by use of cobalt(II) acetate tetrahydrate.* — Lyophilized sisomicin (**1**, 0.447 g, 1 mmol) was dissolved in *N,N*-dimethylformamide (20 mL) and cobalt(II) acetate tetrahydrate (0.516 g, 2.07 mM) was added with stirring. After 20 min at room temperature, a freshly prepared M solution of acetic anhydride in oxolane (3 mL, 3 mmol) was added dropwise. After 1 h at room temperature, water (10 mL) was added and hydrogen sulfide bubbled through the solution as described earlier until all the cobalt had completely precipitated. The solids were removed by filtration through Celite and washed with water. The combined filtrates were evaporated *in vacuo* and the residue chromatographed on silica gel (50 g) with 40:20:7 chloroform–methanol–ammonium hydroxide as the eluant. The homogeneous fractions containing the product were pooled, concentrated, and lyophilized to give pure **4** (0.5 g, 87%).

3-N-*Acetylgaramine* (**3**). — Compound **4** (0.275 g) was dissolved in 50% trifluoroacetic acid (4 mL) and set aside at room temperature for 18 h. After removal of the solvents *in vacuo*, the residue was chromatographed on a column of silica gel (25 g) with 100:50:17 chloroform–methanol–ammonium hydroxide as the eluant. Fractions (2 mL) were collected, and the homogeneous fractions pooled, concentrated, and lyophilized to give pure **3** (95 mg), $[\theta]^{290}_{TaCu}$ −6260; ^{1}H-n.m.r. (D_2O): δ 1.98 (s, 3 H, NCOCH_3), 1.28 (s, 3 H, C-CH_3), 2.8 (s, 3 H, N-CH_3), and 5.15 (1 H, *J* 3.5 Hz, H-1').

*2',3,6'-Tri-*N*-acetyl-1-*N*-ethylsisomicin* (**5**). — The pH of a stirred and cooled (3°) solution of **4** (1.136 g) in water (20 mL) was brought to 2.7 by the addition of 0.1M HCl. A freshly prepared M solution of acetaldehyde in oxolane (2.2 mL) was added, followed by a solution of sodium cyanoborohydride (160 mg) in water which was added dropwise while maintaining the pH at 2.7 by the addition of 0.1M HCl. The mixture was stirred for 1 h at ~3°. A 0.44-mL portion of the above stock solution of acetaldehyde was added, followed by sodium cyanoborohydride (35 mg) in a little water while maintaining the pH at 2.7 by the addition of 0.1M HCl. After 1 h, the above process was repeated with 0.22 mL of the acetaldehyde solution and 10 mg of sodium cyanoborohydride. After an additional hour, the last step was again repeated. The mixture was stirred for 18 h at room temperature, and then stirred for 1 h with Amberlite IRA-401S (OH^-) ion-exchange resin to bring the pH to 9. The resin was removed by filtration and washed thoroughly with water. The solution was evaporated to dryness *in vacuo* and the residue chromatographed on silica gel (100 g) with 120:40:1 chloroform–methanol–ammonium hydroxide as the eluant. The homogeneous fractions (15-mL fractions) were pooled, concentrated, and lyophilized to give a 70% yield (0.84 g) of **5**, $[\alpha]^{26}_D$ +165° (*c* 0.8, in water); ^{1}H-n.m.r. (D_2O): δ 1.05 (t, 3 H, *J* 7.0 Hz, CH_3CH$_2$), 1.17 (s, C-

CH_3), 1.84, 1.9, 1.91 (N-COCH_3's), 2.52 (s, 3 H, N-CH_3), 4.80 (m, H-4'), 4.92 (d, $J_{1'',2''}$ 4.0 Hz, H-1''),5.4 (d, $J_{1',2'}$ 2.5 Hz, H-1'); m.s.: *m/z* 601 (M^+), 402, 392, 374 (protonated formyl 3-*N*-acetyl-1-*N*-ethylgaramine series), 160 (garosaminyl cation), 471, 453, 443, 425 (protonated formyl tri-*N*-acetyl-1-*N*-ethylsisomine series), 211 (di-*N*-acetylpurpurosamine C cation), and 261 (protonated formyl 3-*N*-acetyl-2-deoxy-1-*N*-ethylstreptamine).

Anal. Calc. for $C_{27}H_{47}N_5O_{10} \cdot 1.5\ H_2O$: C, 51.58; H, 8.02; N, 11.14. Found: C, 51.47; H, 8.89; N, 10.91.

1-N-*Ethylsisomicin (Netilmicin)* (**6**). — Pure **5** (100 mg) was dissolved in M sodium hydroxide (10 mL) and the solution heated under reflux and nitrogen until only the product spot was seen on t.l.c. in 40:20:7 chloroform–methanol–ammonium hydroxide (required ~48 h). The volume of the cooled solution was brought to 50 mL with water and the solution stirred with water-washed Amberlite IRC-50 ion-exchange resin (H^+; 40 mL) until the pH of the solution was 5.5. The resin was removed by filtration and washed with water (t.l.c. of the water wash should indicate the absence of **6**). The resin was stirred with 7% ammonium hydroxide solution (100 mL) for 30 min. The supernatant solution was decanted and the resin treated with 7% ammonium hydroxide solution twice more (50 mL each time). The ammonium hydroxide extract was evaporated to dryness and the residue extracted with methanol. The methanol extract was evaporated to dryness and the residue chromatographed on a column (0.8 cm × 30 cm) of silica gel with 40:20:7 chloroform–methanol–ammonium hydroxide as the eluant. The homogeneous fractions containing the product were pooled, evaporated to dryness, and lyphilized to give pure netilmicin[1,2] (71 mg, 90%); ^{1}H-n.m.r. of the sulfate salt (D_2O): δ 1.33 (t, 3 H, *J* 7.0 Hz, CH_3CH_2), 1.35 (s, 3 H, C-CH_3), 2.93 (s, 3 H, N-CH_3), 3.18 (q, 2 H, *J* 7.0 Hz, CH_2CH_3), 3.45 (d, 1 H, *J* 11.0 Hz, H-3''), 5.20 (d, 1 H, *J* 4.0 Hz, H-1''), and 5.67 (d, 1 H, *J* 1.5 Hz, H-1'); m.s.: *m/z* 476 (MH^+), 475 (M^+), 458 ($M^+ - NH_3$), 332 (1-*N*-ethylgaramine cation −OH), 299 (1-*N*-ethylsisomine cation −OH), 219 (protonated formyl 2-deoxy-1-*N*-ethylstreptamine), 160 (garosaminyl cation), and 127 (purpurosamine C cation).

Anal. Calc. for $C_{21}H_{41}N_5O_7 \cdot 2.5\ H_2SO_4$: C, 34.99; H, 6.43; N, 9.72; SO_4, 33.32. Found: C, 34.43; H, 6.46; N, 9.81; SO_4, 32.41.

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REFERENCES

1 J. J. WRIGHT, P. J. L. DANIELS, A. K. MALLAMS, AND T. L. NAGABHUSHAN, U.S. Pat. 4 002 742 (1977); *Chem. Abstr.*, 87 (1977) 6298w.
2 J. J. WRIGHT, *Chem. Commun.*, (1976) 206–208.
3 G. H. MILLER, R. S. HARE, E. L. MOSS, JR., F. J. SABATELLI, AND J. A. WAITZ, *Clin. Trials J. (London)*, 17, 6, (1980) 242–267.

4 A. M. LERNER, L. A. CONE, W. JANSEN, M. P. REYER, D. C. BLAIR, AND G. E. WRIGHT, *Lancet*, (1983) 1123–1126.
5 T. L. NAGABHUSHAN, G. H. MILLER, AND M. J. WEINSTEIN, in A. WHELTON AND H. C. NEU (Eds.), *The Aminoglycosides*, Marcel Dekker, New York, 1982 pp. 3–27.
6 T. L. NAGABHUSHAN, *Abstr. Int. Symp. Carbohydr. Chem.*, 9 (1978) B.36, B.37.
7 T. L. NAGABHUSHAN, A. B. COOPER, W. N. TURNER, H. TSAI, S. MCCOMBIE, A. K. MALLAMS, D. RANE, J. J. WRIGHT, P. REICHERT, D. L. BOXLER, AND J. WEINSTEIN, *J. Am. Chem. Soc.*, 100 (1978) 5253–5254.
8 T. L. NAGABHUSHAN, J. J. WRIGHT, A. B. COOPER, W. N. TURNER, AND G. H. MILLER, *J. Antibiot.*, 31 (1978) 43–54.
9 H. REIMANN, D. J. COOPER, A. K. MALLAMS, R. S. JARET, A. YEHASKEL, M. KUGELMAN, H. F. VERNAY, AND D. SCHUMACHER, *J. Org. Chem.*, 39 (1974) 1451–1457.
10 P. J. L. DANIELS, A. K. MALLAMS, J. WEINSTEIN, J. J. WRIGHT, AND G. W. A. MILNE, *J. Chem. Soc., Perkin Trans. 1*, (1976) 1078–1088.
11 M. KUGELMAN, A. K. MALLAMS, AND H. F. VERNAY, *J. Antibiot.*, 26 (1973) 394–395.
12 T. L. NAGABHUSHAN, W. N. TURNER, P. J. L. DANIELS, AND J. B. MORTON, *J. Org. Chem.*, 40 (1975) 2830–2834.
13 J. J. WRIGHT, A. B. COOPER, P. J. L. DANIELS, T. L. NAGABHUSHAN, D. RANE, W. N. TURNER, AND J. WEINSTEIN, *J. Antibiot.* 29 (1976) 712–714.