Synthesis of Streptolidine (Roseonine, Geamine)[1]

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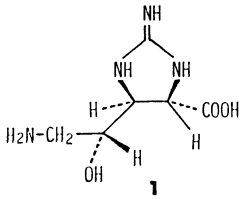
Streptolidine (roseonine, geamine) (**1**), a guanidino amino acid component of the streptothricin group antibiotics, was synthesized starting from D-ribose (**8**). The epimino compound (**11b**) was prepared from **8** in five synthetic steps. Azidolysis of **11b** gave the arabino-type compound (**12b**), which in turn was converted to the key intermediate tribenzyloxycarbamido lactone (**23b**) by oxidation of *C*-1. The lactone **23b** was rearranged to the free lactam (**4**) after deprotection, and then guanidinated with cyanogen bromide. The identity of the hydrolysis product of this guanidino lactam with natural streptolidine in all respects certified the proposed structure involving the steric configurations.

Streptolidine is a guanidino amino acid which widely distributes as a constituent of a number of *Streptomyces* antibiotics. It was first isolated from the hydrolyzate of streptothricin and streptolin.[2] The two amino acids thereafter reported, *i.e.*, roseonine from roseothricin[3] and geamine from geomycin,[4] were identified as the same substance. In 1961 Carter *et al.*[5] deduced the chemical structure of this amino acid by means of degradation studies. Its absolute configuration was recently established by X-ray crystallography as **1**.[6] As reported in our preliminary communication,[1] we synthesized streptolidine of the natural configuration from D-ribose.

Results and Discussion

Streptolidine has a unique structure in which a 2-amino-2-imidazoline ring and three asymmetric centers are involved. This peculiar feature in the structure seems to be the reason for delay in the chemical synthesis of the rather small molecule so far. The key intermediate of the synthesis is desired to be (2*S*,3*S*,4*R*)-2,3,5-triamino-4-hydroxypentanoic acid (**2**) or its equivalent, *e.g.*, its lactone (**3**) or lactam form (**4**). In this study, natural pentoses of the corresponding stereochemistry were chosen as the starting materials. Thus, attempts were made to convert three hydroxy groups on *C*-2, 3 and 5 of furanoside molecules stereoselectively into amino groups. In this way, the trouble in separation of stereoisomeric mixture otherwise synthesized can be avoided.



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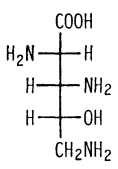

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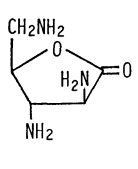

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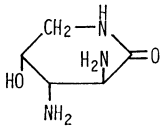

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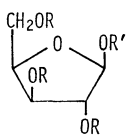
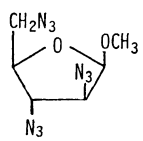


5 R=R′=H
6 R=CH_3SO_2, R′=CH_3



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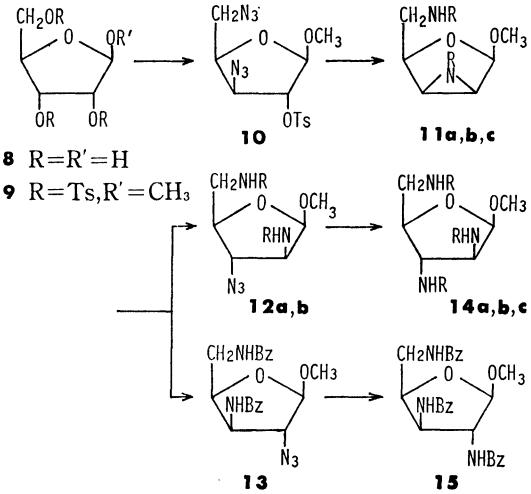
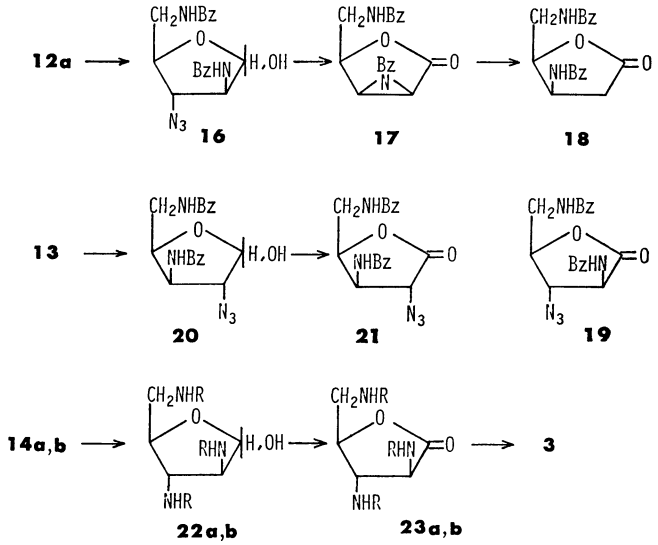
Many procedures have been presented to introduce an amino group into a sugar moiety. Among them the S_N2-type displacement of *O*-sulfonate with azide anion seems to be the most promissing one in our case. A synthetic pathway for preparation of **3** from D-xylose (**5**) was first investigated. When methyl 2,3,5-tri-*O*-mesyl-β-D-xylofuranoside (**6**) was treated with sodium azide under various conditions, however, 2,3,5-triazide (**7**) could not be obtained in an appreciable yield. Thus, the approach to **3** by this way was abandoned. The details on this experiment will be presented elsewhere.



8 R=R′=H
9 R=Ts,R′=CH_3
10
11a,b,c
12a,b
13
14a,b,c
15
a: R=Bz=C_6H_5CO Ts=*p*-$CH_3C_6H_4SO_2$
b: R=$C_6H_5CH_2OCO$
c: R=H

Recently, Hildesheim *et al.*[7] prepared two isomers of monoazido dibenzamido trideoxypentose (**12a** and **13**) through azidolysis of an epimino compound (**11a**) derived from D-ribose (**8**). They assigned tentatively one isomer with a smaller $J_{1,2}$-value of 1 Hz to xylo-type (**13**) and the other with a larger value to arabino-type (**12a**) structure respectively. Each compound could be reduced and benzoylated to give methyl tribenzamido trideoxy xylofuranoside (**15**) and arabinofuranoside (**14a**). Since the compounds **12a** and **14a** have the same steric fashion as in **3**, oxidation of **12a** or **14a** at *C*-1 would be expected to lead to the desired intermediate **3** after several steps of further transformations. Thus, the methyl glycoside of **12a** was hydrolyzed with hydrochloric acid without ap-

preciable cleavage of benzoyl group. However, oxidation of the resulting compound **16** with chromium trioxide in pyridine afforded a mixture of two products. Although the main product showed two carbonyl bands due to amides (1680 and 1645 cm^{-1}) in its IR spectrum, the azide band disappeared, and no signal of an olefin proton in its NMR spectrum was observed. These results together with its molecular formula ($C_{19}H_{16}N_2O_4$) suggest an epimino structure (**17**) for this compound. This assignment was confirmed by the following transformation. Catalytic hydrogenation of **17** afforded a product of a molecular formula $C_{19}H_{18}N_2O_4$, whose NMR spectrum showed signals at δ 2.50 (1H, d, J=10 Hz) and 2.82[8] (1H, q, J=10, 5 Hz). These signals could be attributed to the two hydrogens on *C*-2 of the structure shown in **18**. The epimino ring in **17** obviously resulted from an elimination of the azido group with a participation of the neighboring benzamido group. As a minor product in the oxidation reaction of **16**, a small amount of **19** of the desired azido lactone structure was isolated. This structure was supported from its IR spectrum (2140, 1770 cm^{-1}). Further progress of the synthesis using this azido lactone had to be given up, because of a small amount available.

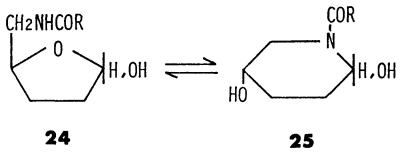


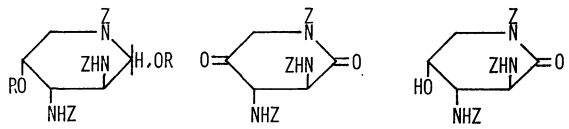
a: R=Bz=C_6H_5CO
b: R=$C_6H_5CH_2OCO$

In a parallel experiment, **13** was hydrolyzed to **20**, which was oxidized with chromium trioxide-pyridine to afford an azido lactone (**21**) (ν_{max} 2130, 1790 cm^{-1}) without elimination of the azido group successfully. Decoupling experiments on this lactone (**21**) showed that a doublet signal at δ 4.12 (1H, J=10 Hz) changed to a singlet when the signal at δ 5.25 (1H, quartet-like) coupling further with an NH proton at δ 9.07 (1H, d, J=9 Hz) was irradiated. This result indicates clearly that the signal at δ 4.12 should be due to the proton on *C*-2 and the structure of this lactone must be represented as α-azido structure of xylo-type as shown in **21**. Consequently, it was unequivocally established that both **13** and **20** have the xylo-type configuration in accordance with Hildesheim's assignment. Comparison of the behaviors of **16** and **20** in oxidation reaction indicates that the facile elimination of an azido group occurs particularly at β-position but not α-position to a carbonyl function.

This finding leads to a strategy that the azido group on *C*-3 of **12a** should be reduced prior to the oxidation of *C*-1 in the synthetic approach towards **3**. Thus, the azido glycoside **12a** was first converted to tribenzamido derivative (**14a**) of arabino-type, which was then oxidized to tribenzamido lactone (**23a**) with chromium trioxide in pyridine through a hydrolysis product **22a**. However, the benzoyl groups of **23a** resisted against removal by acid hydrolysis. Thus, on refluxing in 6 M hydrochloric acid, **23a** gave a complex mixture of several products. Methanolysis with dry hydrogen chloride could not give an improved result.

Therefore, the benzoyl group was replaced with benzyloxycarbonyl group for the amino protection. In a simillar manner as in the benzoyl derivative (**11a**), the dibenzyloxycarbonyl epimino compound (**11b**) was prepared from D-ribose. Treatment of **11b** with sodium azide in dimethylformamide (DMF) gave a single product of the arabino-type azido derivative (**12b**). Catalytic hydrogenation of **12b** afforded triamino compound **14c** as trihydrochloride. Both compounds **12b** and **14c** certainly maintain the arabino-type configuration, since benzoylation of **14c** afforded **14a** of the determined structure. After the exhausted benzyloxycarbonylation of **14c** to **14b**, the glycoside linkage was hydrolyzed by refluxing with *p*-toluenesulfonic acid in dioxane to give **22b** in about 35% yield accompanied with 40% of unchanged **14b**. A longer refluxing as far as the starting material disappeared, or use of hydrochloric acid for the hydrolysis gave rather worse results presumably because of the acid-lability of the benzyloxycarbonyl group. The hydrolysis product was isolated through silica gel chromatography and recrystallized from chloroform. Although a compound with a 5-acylaminopentose structure is known to exist often as an equilibrium mixture of the corresponding furanose (**24**) and piperidine (**25**) form,[9] this product seemed to remain mainly in the furanose form (**22b**) during the isolation procedure since a γ-lactone was formed by oxidation of **22b** and no change was observed in the IR spectrum in repeated recrystallization. However, when **22b** was treated with acetic anhydride in pyridine, a diacetate (**26**) was obtained as a sole





26 R=CH_3CO
27 R=H
Z=$C_6H_5CH_2OCO$

product. This fact might be well elucidated by assuming that the piperidine form (**27**) is preferred in pyridine solution.[10] Similar preferences of piperidine form in *N*-benzyloxycarbonyl derivatives were also reported in some pentoses[11] and in idose series,[12] though it is not a case in the corresponding *N*-acetyl or benzoyl derivatives.[13]

Oxidation of **22b**[14] was effected with chromium trioxide in acetic acid, and the product was isolated by silica gel chromatography. This product could not be obtained in a crystalline state, although its IR spectrum showed, in accordance with the expected γ-lactone structure (**23b**), a carbonyl band at 1800 cm^{-1} together with a broad band at around 1710 cm^{-1} due to benzyloxycarbonyl groups. Removal of the benzyloxycarbonyl groups from this oxidation product with hydrogen bromide in acetic acid afforded an extremely hygroscopic hydrobromide as white powder, whose IR spectrum showed a weak absorption band at 1650 cm^{-1} besides an intensive one at 1800 cm^{-1} due to γ-lactone. The former band should be attributed to an amide carbonyl group of δ-lactam structure (**4**). The elemental analysis of this hydrobromide, in one experimental run, showed the composition of a 7: 3 mixture of lactone (**3**) trihydrobromide and lactam (**4**) dihydrobromide. Since it is improbable that the conversion of **3** into **4** occurred during the deprotection stage or thereafter when no basic condition was employed, the above observation suggests that the oxidation product **23b** might have already contained a small amount of lactam form **29** which arised from **27**. When an aqueous solution of a mixture of the hydrobromide of **3** and **4** was passed through a column of Amberlite IR-45 (OH^- form) and the eluate evaporated *in vacuo*, the residue showed only a carbonyl band due to lactam (**4**) and no other compound was eluted at all. This indicates the easy transformation of **3** trihydrobromide into **4** on neutralization.

The final stage of streptolidine synthesis was carried out as follows. An aqueous solution of a mixture of the hydrobromide of **3** and **4** was brought to pH 11 with 1 M sodium hydroxide. The preferred formation of **4** in this solution was proved by isolation of its bis-4-hydroxyazobenzene-4'-sulfonic acid (HABS) salt. The solution was then treated directly with cyanogen bromide. In this reaction, the amino group on *C*-5 in the lactam form was protected against an action of cyanogen bromide and a selective formation of the 2-amino-2-imidazoline ring between *C*-2 and *C*-3 was expected. The reaction mixture was refluxed with hydrochloric acid to hydrolyze the lactam ring and then subjected to a column of Dowex 50×8 (NH_4^+ form). Elution with 0.2 M ammonia afforded a fraction which showed a single ninhydrin positive spot identical with natural streptolidine on both paper chromatography (PPC) and paper electrophoresis (PEP).

Table 1. Comparison of synthetic and natural streptolidine

	Synthetic	Natural
R_f value on PPC[a]	0.29	0.29
Mobility on PEP[b]	11.0 cm	11.0 cm
Elution time on amino acid analysis	47.0 min	47.0 min
Bis-HABS salt		
mp	250—254°C (dec.)	250—254°C (dec.)
$[\alpha]_D$ in MeOH	+20.5° (*c* 0.29)	+22.1° (*c* 0.29)
Dihydrochloride		
mp	239 °C (dec.)	241 °C (dec.)
$[\alpha]_D^{20}$ in H_2O	+52.2° (*c* 0.16)	+54.2° (*c* 0.15)

a) Isopropanol–HCOOH–H_2O (4:1:1) b) Pyridine–AcOH–H_2O (30:4:966)

This product was isolated as crystalline bis-HABS salt, mp 250—254 °C (decomp.), $[\alpha]_D^{21}$ +20.5° (methanol), which was identical with the same salt of the natural amino acid, mp 250—254 °C (decomp.), $[\alpha]_D^{19}$ +22.1° (methanol). Identity was further confirmed by comparison of mp and IR spectrum of dihydrochloride, mobility on PEP, R_f value on PPC as well as the elution time on amino acid analysis with those of the natural specimen. Consequently, the structure and the stereochemistry of streptolidine were unequivocally established as **1** by the present chemical synthesis.

Experimental

All melting points are uncorrected. The NMR spectra were taken on a Varian T-60 spectrometer in deuteriochloroform unless otherwise stated, using tetramethylsilane as an internal standard. Chemical shifts and coupling constants were recorded in δ values and Hz, and frequencies in cm^{-1}. Optical rotations were measured with Perkin-Elmer polarimeter model 141. Paper electrophoresis (PEP) was carried out in a pyridine–acetic acid–water (30: 4: 966) buffer solution.

3-Azido-2,5-dibenzamido-2,3,5-trideoxy-D-arabinofuranose (16). To a solution of methyl 3-azido-2,5-dibenzamido-2,3,5-trideoxy-β-D-arabinofuranoside (**12a**)[7] (200 mg) in dioxane (60 ml) was added 2 M HCl (40 ml), and the mixture was heated under reflux for 2.5 hr. After cooling, the solution was extracted with CH_2Cl_2. The extract was washed with water, dried over $MgSO_4$ and evaporated to give **16** (155 mg, 80%). Recrystallization was effected from ethyl acetate; mp 187—188 °C.

Found: C, 59.65; H, 5.05; N, 18.53%. Calcd for $C_{19}H_{19}N_5O_4$: C, 59.83; H, 5.02; N, 18.36%.

Oxidation of 16 with Chromium Trioxide in Pyridine. To an ice-cooled solution of CrO_3 (240 mg, 2.4 mmol) in dry pyridine (15 ml) was added a solution of **16** (155 mg, 0.41 mmol) in dry pyridine (3 ml). The mixture was stirred at 0 °C for 2 hr, then at room temperature overnight, and extracted with CH_2Cl_2. The extract was treated as usual and evaporated *in vacuo* to leave a brown residue, which was chromatographed on silica gel column. Ethyl acetate–*n*-hexane (2: 1) eluted **17** (100 mg, 73%), which was recrystallized from ethyl acetate–*n*-hexane; mp 196—197 °C; ν_{max} (Nujol) 1680, 1645; $[\alpha]_D^{28}$ +55.6° (*c* 1.87, ethyl acetate).

Found: C, 67.76; H, 4.82; N, 8.48%. Calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.80; N, 8.33%.

Further elution with the same solvent afforded a small amount of **19**; mp 167—169 °C; ν_{max} (Nujol) 2140, 1770.

3,5-Dibenzamido-2,3,5-trideoxy-D-xylono-1,4-lactone (18). Hydrogen was passed through a stirred solution of **17** (400 mg, 1.06 mmol) in methanol (20 ml) and concentrated hydrochloric acid (0.13 ml) in the presence of palladium black. After 20 hr, the catalyst was removed by filtration and the solvent evaporated *in vacuo*. The residue was dissolved in

CH_2Cl_2, washed with water, dried over $MgSO_4$ and evaporated to give **18** (216 mg, 52%), which was recrystallized from ethyl acetate–*n*-hexane; mp 212—213 °C; $[\alpha]_D^{28}$ +32.0° (*c* 1.78, ethyl acetate–DMF (3:1)). NMR (in pyridine): 2.50 (1H, d, $J=10$), 2.82 (1H, q, $J=5$, 10).

Found: C, 67.33; H, 5.36; N, 8.28%. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28%.

*2-Azido-3,5-dibenzamido-2,3,5-trideoxy-*D*-xylofuranose (20).* To a solution of methyl 2-azido-3,5-dibenzamido-2,3,5-trideoxy-β-D-xylofuranoside (**13**)[7] (2.75 g, 6.96 mmol) in dioxane (200 ml) was added 1 M HCl (40 ml), and the mixture was heated under reflux for 1.5 hr. After cooling, a reaction mixture was extracted with CH_2Cl_2 and the extract was treated as usual. The residue obtained by evaporation was chromatographed on silica gel column. Elution with CH_2Cl_2–ethyl acetate (4:1) gave unchanged **13** (1.20 g, 44%) and then **20** (0.315 g, 12%). The latter was recrystallized from $CHCl_3$–*n*-hexane; mp 177—179 °C (decomp.).

Found: C, 59.68; H, 5.07; N, 18.48%. Calcd for $C_{19}H_{19}N_5O_4$: C, 59.83; H, 5.02; N, 18.36%.

*2-Azido-3,5-dibenzamido-2,3,5-trideoxy-*D*-xylono-1,4-lactone (21).* To an ice-cooled solution of CrO_3 (420 mg, 4.2 mmol) in dry pyridine (15 ml) was added a solution of **20** (240 mg, 0.63 mmol) in dry pyridine (5 ml). The mixture was stirred at 0° for 20 hr, and extracted with CH_2Cl_2. Working up in the usual manner and evaporation of the solvent *in vacuo* afforded a brown residue, which was chromatographed on silica gel column. Elution with ethyl acetate–*n*-hexane gave **21** (160 mg, 67%), which was recrystallized from ethyl acetate–*n*-hexane; mp 164—166 °C (decomp.); ν_{max} ($CHCl_3$) 2130, 1790. NMR ($CDCl_3$, 100 MHz): around 3.6 and 4.2 (each 1H, m; 2H-5), 4.12 (1H, d, $J=10$; H-2), 4.85 (1H, broad; H-4), 5.25 (1H, q; H-3), 7.3—8.2 (11H; aromatic H and NH-5), 9.07 (1H, d, $J=9$; NH-3).

Found: C, 59.74; H, 4.59; N, 18.03%. Calcd for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46%.

*2,3,5-Tribenzamido-2,3,5-trideoxy-*D*-arabinofuranose (22a).* A mixture of methyl 2,3,5-tribenzamido-2,3,5-trideoxy-β-D-arabinofuranoside (**14a**)[7] (1.80 g, 3.80 mmol), $CuCl_2 \cdot 2H_2O$ (2.1 g), dioxane (20 ml) and 0.5 M HCl (40 ml) was heated under reflux for 2 hr. After cooling, the mixture was evaporated *in vacuo* to dryness. To the residue was added water (50 ml) and $CHCl_3$ (150 ml), and the mixture was heated under reflux for 30 min. The organic layer was separated, treated as usual and evaporated *in vacuo* to leave a residue, which was recrystallized from $CHCl_3$ to afford **22a** (0.46 g, 26%); ν_{max} (Nujol) 3450, 3340, 1700—1670 (broad).

From the mother liquor was recovered the unchanged **14a** (1.20 g, 67%).

*2,3,5-Tribenzamido-2,3,5-trideoxy-*D*-arabono-1,4-lactone (23a).* To an ice-cooled solution of CrO_3 (0.70 g, 7 mmol) in dry pyridine (30 ml) was added **22a** (600 mg, 1.31 mmol) in dry pyridine (20 ml). The mixture was stirred at 0°C for 4 hr, then at room temperature for 20 hr, and worked up as usual. After evaporation of the solvent *in vacuo*, the residue was chromatographed on silica gel column. Elution with $CHCl_3$–ethyl acetate (1:2) afforded **23a** (470 mg, 78%), which was recrystallized from dioxane–water; mp 217—218 °C; $[\alpha]_D^{28}$ −102.4° (*c* 1.45, DMF).

Found: C, 67.85; H, 5.32; N, 8.87%. Calcd for $C_{26}H_{23}N_3O_5 \cdot 1/5C_4H_8O_2$: C, 67.75; H, 5.22; N, 8.85%.

Attempts for Removal of Benzoyl Groups of **23a**. *1) Hydrolysis with HCl*: Compound **23a** (20 mg) was heated under reflux in 6 M HCl (9 ml) and the progress of the reaction was followed by means of PEP. Even after 5 hr, the reaction was not finished, though there appeared three main spots and the reaction mixture turned into deep brown. Heating for a longer time caused considerable decomposition of the products. Use of acetic acid or dioxane as co-solvent or use of more dilute HCl did not improve the result.

2) Methanolysis: A 15% solution of dry HCl in absolute methanol (10 ml) was added to **23a** (400 mg) and the mixture was heated under reflux for 17 hr. PEP showed the presence of several ninhydrin-positive spots in the mixture, from which **3** could not be isolated.

*Methyl 5-Benzyloxycarbamido-2,3-benzyloxycarbonylepimino-2,3,5-trideoxy-β-*D*-lyxofuranoside (11b).* Benzyloxycarbonyl chloride (18.1 g, 105 mmol) was added portionwise to an ice-cooled solution of methyl 5-amino-2,3-epimino-2,3,5-trideoxy-β-D-lyxofuranoside (**11c**)[7] (5.6 g, 44 mmol) and triethylamine (14.6 ml, 105 mmol) in ethanol (250 ml) during 20 min with stirring. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 2.5 hr. Then water (400 ml) was added and crystals of **11b** were filtered off (13.5 g, 75%). Recrystallization was effected from ethyl acetate–*n*-hexane; mp 115.5—116.5 °C, $[\alpha]_D^{24}$ −35.7° (*c* 2.02, ethyl acetate).

Found: C, 64.18; H, 5.85; N, 6.79%. Calcd for $C_{22}H_{24}N_2O_6$: C, 64.06; H, 5.87; N, 6.79%.

*Methyl 3-Azido-2,5-dibenzyloxycarbamido-2,3,5-trideoxy-β-*D*-arabinofuranoside (12b).* Sodium azide (10.0 g, 155 mmol) was added to a solution of **11b** (12.8 g, 31.0 mmol) in dry DMF (200 ml), and the mixture was heated at 90 °C for 1 hr with stirring. After cooling, the mixture was filtered through Celite and evaporated to dryness *in vacuo*. Water and CH_2Cl_2 were added to the residue. The organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The remaining syrup was passed through a column of silica gel. Elution with benzene–ethyl acetate (4:1) afforded **12b** (6.5 g, 46%), which was recrystallized from ethyl acetate–*n*-hexane; mp 103.5—105 °C; $[\alpha]_D^{24}$ −27.3° (*c* 2.20, ethyl acetate). NMR: 3.39 (3H, s; CH_3O), 4.77 (1H, d, $J=5$; H-1), 5.16 and 5.19 (each 2H, s; $2C_6H_5C\underline{H}_2O$) and near 7.3 (10H; aromatic).

Found: C, 58.15; H, 5.52; N, 15.30%. Calcd for $C_{22}H_{25}N_5O_6$: C, 58.01; H, 5.53; N, 15.38%.

*Methyl 2,3,5-Triamino-2,3,5-trideoxy-β-*D*-arabinofuranoside (14c) Trihydrochloride.* A solution of **12b** (3.8 g, 8.35 mmol) and concentrated hydrochloric acid (2.5 ml) in methanol (60 ml) was added with 5% palladium on carbon (0.4 g). A slow stream of hydrogen was passed through this mixture with stirring at room temperature overnight. The catalyst was filtered off and ether was added to the filtrate. Trihydrochloride of **14c** was crystallized out as needles; 1.85 g (82%). It was recrystallized from water–methanol–ether; mp 240 °C (decomp.); $[\alpha]_D^{19}$ −57.1° (*c* 1.81, water).

Found: C, 26.71; H, 6.77; N, 15.37; Cl, 39.14%. Calcd for $C_6H_{18}N_3O_2Cl_3$: C, 26.63; H, 6.71; N, 15.53; Cl, 39.31%.

*Methyl 2,3,5-Tribenzyloxycarbamido-2,3,5-trideoxy-β-*D*-arabinofuranoside (14b).* To an ice-cooled solution of **14c** (3.7 g, 14 mmol) and triethylamine (7.5 ml, 54 mmol) in ethanol (120 ml) was added benzyloxycarbonyl chloride (9.1 g, 54 mmol) portionwise during 20 min with stirring. After stirring in an ice-bath for 30 min, triethylamine (5.5 ml, 39 mmol) and benzyloxycarbonyl chloride (3.0 g, 18 mmol) were added and stirring was continued at room temperature for another 1 hr. The product (**14b**) crystallized out was filtered off and recrystallized from $CHCl_3$–*n*-hexane (6.9 g, 90%). An analytical sample was obtained by recrystallization from dioxane–water; mp 202—203 °C, $[\alpha]_D^{19}$ −17.0° (*c* 1.98, $CHCl_3$).

Found: C, 63.43; H, 6.12; N, 7.02%. Calcd for $C_{30}H_{33}N_3O_8 \cdot 1/2C_4H_8O_2$: C, 63.25; H, 6.14; N, 6.92%.

*2,3,5-Tribenzyloxycarbamido-2,3,5-trideoxy-*D*-arabinofuranose*

(22b). To a solution of **14b** (4.28 g) in dioxane (200 ml) was added 2 M aqueous solution of *p*-toluenesulfonic acid (200 ml). The mixture was heated under reflux for 2 hr, then concentrated to half of its volume *in vacuo*, and extracted with $CHCl_3$. The extract was washed with aqueous $NaHCO_3$ and water, dried over $MgSO_4$ and evaporated *in vacuo*. The resulting residue was chromatographed on silica gel column. Elution with $CHCl_3$–ethyl acetate (2: 1) first afforded the unchanged **14b** (1.80 g, 42%) and then **22b** (1.45 g, 35%). The latter was recrystallized from $CHCl_3$; mp 211—212 °C.

Found: C, 63.00; H, 5.71; N, 7.67%. Calcd for $C_{29}H_{31}N_3O_8$: C, 63.38; H, 5.69; N, 7.65%.

2,3,5-Tribenzyloxycarbamido-2,3,5-trideoxy-D-arabono-1,4-lactone (23b). To a solution of **22b** (2.0 g, 3.6 mmol) in acetic acid (240 ml) was added CrO_3 (0.36 g, 3.6 mmol) in acetic acid–water (4: 1, 3.6 ml). The mixture was stirred at room temperature for 1.5 hr, then added with water, and extracted with $CHCl_3$. The residue obtained by the usual working up was chromatographed on silica gel column. Elution with $CHCl_3$–ethyl acetate (2: 1) afforded unchanged **22b** (0.62 g, 31%) and further elution with the same solvent gave **23b** (0.52 g, 26%) as sticky syrup; ν_{max} ($CHCl_3$) 1800, 1730—1690 (broad).

1,4-Di-O-acetyl-2,3,5-tribenzyloxycarbamido-2,3,5-trideoxy-D-arabinopyranose (26). Acetic anhydride (0.05 ml) was added to a stirred solution of **22b** (29.0 mg) in pyridine (0.3 ml) under ice-cooling. The mixture was set aside at room temperature for 48 hr and then evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ and added with *n*-hexane to afford **26** (23.9 mg, 71%), which was recrystallized from $CHCl_3$–*n*-hexane; mp 186—186.5 °C. NMR: 1.89 and 2.09 (each 3H, s; $2CH_3CO$).

Found: C, 62.40; H, 5.53; N, 6.58%. Calcd for $C_{33}H_{35}N_3O_{10}$: C, 62.55; H, 5.57; N, 6.63%.

2,3,5-Triamino-2,3,5-trideoxy-D-arabono-1,4-lactone (3) Trihydrobromide and 2,3,5-Triamino-2,3,5-trideoxy-D-arabono-1,5-lactam (4) Dihydrobromide. Anisole (0.3 ml) and **23b** (455 mg), containing a small amount of **29** (see text), was dissolved in acetic acid (10 ml) which was previously saturated with dry hydrogen bromide. The mixture was allowed to stand at room temperature for 1.5 hr. Addition of anhydrous ether afforded very hygroscopic white powder (300 mg); ν_{max} (Nujol): 1800, 1650 (weak).

Found: C, 16.62; H, 3.92; N, 11.20; Br, 59.69%. Calcd for a 7: 3-mixture of **3** trihydrobromide ($C_5H_{14}N_3O_2Br_3$) and **4** dihydrobromide ($C_5H_{13}N_3O_2Br_2$): C, 16.51; H, 3.80; N, 11.56; Br, 59.33%.

Transformation of Trihydrobromide of 3 into 4. 1) On Ion Exchange Resin: A mixture of hydrobromide of **3** and **4**, obtained from **23b** (290 mg) was dissolved in water, subjected to a column of Amberlite IR-45 (OH^- form) and eluted with water. The eluate was evaporated *in vacuo* to afford **4** solely as hygroscopic amorphous solid: ν_{max} (Nujol) 1640.

2) With NaOH: The hydrobromide (40 mg) mentioned above was dissolved in water (8 ml). This solution was brought to pH 11 by addition of 1 M NaOH and left stand at room temperature for 2 hr. To this solution was added 4-hydroxyazobenzene-4′-sulfonic acid (HABS) to afford bis-HABS salt of **4**, which was recrystallized from water; mp 190 °C (decomp.); ν_{max} (Nujol) 1640.

Found: C, 46.34; H, 4.92%. Calcd for $C_5H_{11}N_3O_2 \cdot 2(C_{12}H_{10}N_2O_4S) \cdot 3H_2O$: C, 46.08; H, 4.94%.

Streptolidine (1). To an aqueous solution (90 ml) of **4**, prepared from **23b** (0.74 g, 1.35 mmol) using NaOH as mentioned above, was added a solution of BrCN (0.32 g, 3.0 mmol) in water (6.5 ml) portionwise over a period of 2.5 hr with stirring. During this time the pH of the reaction mixture fell to 6.9. The mixture was stirred at room temperature for additional 30 min and then evaporated *in vacuo* to dryness. The residue was heated in 6 M HCl (150 ml) under reflux for 2 hr and then evaporated *in vacuo*. The residue was dissolved in a little water and subjected to a column of Dowex 50×8 (NH_4^+ form, 1.5×36 cm). The column was first washed with water (500 ml) and eluted with 0.2 M NH_4OH. Eluate of each 2 ml was collected and each fraction was tested by means of PEP and PPC (2-propanol–formic acid–water 4:1:1). Fractions 21—31 showed a single ninhydrin-positive spot, which was identical with that of natural streptolidine. Fractions 18—20 and 32—41, containing the same substance overlapped with some other impurities, were combined and again chromatographed as above, affording fractions which showed also a single spot identical with streptolidine. These and the fractions 21—31 in the first chromatography were combined, concentrated *in vacuo* to a small volume, added with 4-hydroxyazobenzene-4′-sulfonic acid (HABS, 150 mg) and heated on a boiling water bath to obtain a clear solution. On cooling, crystals (95 mg) of bis-HABS salt of streptolidine were obtained. From the mother liquor was obtained more amount (37 mg) of them; total 132 mg (12.5% from **23b**). Recrystallization was effected from hot water; mp 250—254 °C (decomp.); $[\alpha]_D^{21}$ +20.5° (*c* 0.29, methanol).

Found: C, 46.23; H, 4.63; N, 14.22; S, 8.08%. Calcd for $C_6H_{12}N_4O_3 \cdot 2(C_{12}H_{10}N_2O_4S) \cdot 2H_2O$; C, 46.15; H, 4.65; N, 14.35; S, 8.21%.

Bis-HABS salt (81 mg) was subjected to PEP using TOYO No. 50 paper (4 pieces of 40 cm length×16 cm width; 1.3 mA/cm, 1.5 hr). The ninhydrin-positive substance was extracted from the paper with 2 M HCl and the extract concentrated *in vacuo* to afford streptolidine dihydrochloride (25.5 mg) as crystalline residue, which was recrystallized from water–ethanol; mp 239 °C (decomp.); $[\alpha]_D^{20}$ +52.2° (*c* 0.115, H_2O).

The IR spectra of bis-HABS salt and dihydrochloride of synthetic streptolidine were fully identical with those of natural specimen. R_f-values on PPC, mobilities on PEP and elution times on amino acid analysis of the synthetic and natural product were all identical (see Table 1).

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10) In accordance with this assumption, oxidation of **22b** with Collins' reagent in methylene chloride afforded a product, whose IR spectrum suggested the structure **28**. Oxidation of **22b** with chromium trioxide in pyridine gave no γ-lactone (**23b**) as well.

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13) Oxidation of **22a** with chromium trioxide in pyridine afforded exclusively the γ-lactone (**23a**), indicating the preference of furanose form even in pyridine in the case of benzoyl derivative.

14) This substance contained a small amount of its piperidine form (**27**), as judged from the formation of lactam derivative (**29**, see text) on oxidation.