Pyrrolopyrimidine Nucleosides. Part XI.[1] Influence of Amino-groups at C-4 and C-6 or an Amino-group at C-6 on the Reactivity of a 5-Cyano-group in Pyrrolo[2,3-*d*]pyrimidine Nucleosides

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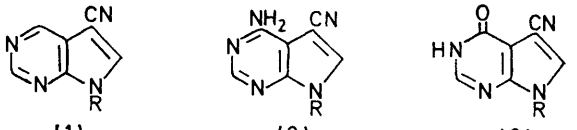
The syntheses of 6-amino-7-(β-D-ribofuranosyl)pyrrolo[2.3-*d*]pyrimidine-5-carbonitrile (7) and its 4,6-diamino-analogue (12) are described. Nucleophilic addition to the 5-cyano-group of both (7) and (12) was found to be more difficult under both acidic and basic conditions than addition to the nitrile group of toyocamycin (3) or 7-(β-D-ribofuranosyl)pyrrolo[2.3-*d*]pyrimidine-5-carbonitrile (1). The reaction of hydrazine with 6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, which furnished a tricyclic nucleoside, is discussed.

We have demonstrated[1-4] that a 4-amino- or 4-oxo-group in certain 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-carbonitriles [*e.g.* (1)—(3)] can have a deactivating effect towards nucleophilic addition to the cyano-group under both basic and acidic conditions. Although an amino-group in the pyrimidine ring influences the susceptibility of the 5-cyano-group in the pyrrole ring towards nucleophilic attack by increasing the electron density at the cyano-group, the electronic effects in this case are more pronounced in the pyrimidine ring since the rings are somewhat compartmentalized.[5] Therefore, an amino-group on the pyrrole ring would be



(1) (2) (3)

R = β-D-ribofuranosyl

expected to have a more significant deactivating effect

[1] Part X, B. C. Hinshaw, K. H. Schram, and L. B. Townsend, preceding paper.

[2] K. H. Schram and L. B. Townsend, *Tetrahedron Letters*, 1971, 4757.

[3] B. C. Hinshaw, J. F. Gerster, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, 1970, **35**, 236.

[4] L. B. Townsend and G. H. Milne, *Ann. New York Acad. Sci.*, in the press.

[5] A. Albert, 'Heterocyclic Chemistry,' Oxford University Press, New York, 1968.

on the cyano-group than an amino-group on the pyrimidine ring. This prompted us to synthesize analogues of toyocamycin (2) with the amino-group on the pyrrole rather than the pyrimidine moiety, and to study the effect of this transformation on the reactivity of the 5-cyano-group.

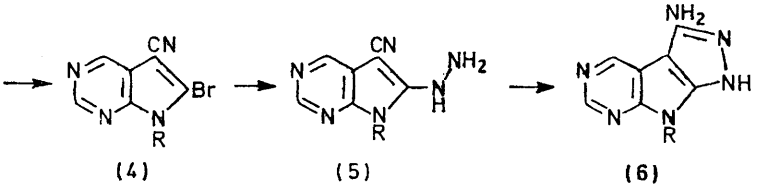
Although there have been reports of electrophilic halogenation reactions of certain 4-substituted pyrrolopyrimidine nucleosides [6] and of toyocamycin [7] (2), electrophilic substitution in a pyrrolo[2,3-*d*]pyrimidine nucleoside with only a strongly electron-attracting substituent (CN, NO_2, *etc.*) at C-5 has not been reported. Treatment of compound (1) with water saturated with bromine gave the 6 bromo-derivative (4), the ^{1}H n.m.r. spectrum of which showed only two peaks (δ 9·05 and 9·30) which could be assigned [1,6] to aromatic protons. The i.r. spectrum indicated retention of the cyano-group. The reaction of (4) with hydrazine could result in either addition to the nitrile function to produce the carboxamidrazone derivative or displacement of the bromo-group to form the 6-hydrazino-compound. A previous study [7] has shown that the second pathway is preferred with 6-bromotoyocamycin. However, the presence of an amino-group at C-4 could deactivate the nitrile function of 6-bromotoyocamycin towards nucleophilic addition in comparison to the cyano-group of (4). The possibility of competing reactions was therefore envisaged.

The reaction of hydrazine with (4) in fact afforded only (t.l.c.) the 6-hydrazino-compound (5) [ν_{CN} 2 200 cm^{-1}; δ 8·60 (s) and 8·57 (s) (H-2 and H-4), 6·35 (d, H-1′), 3·4—5·6 (carbohydrate protons [8]), and 5·68br (3H, exchangeable, NH·NH_2)].

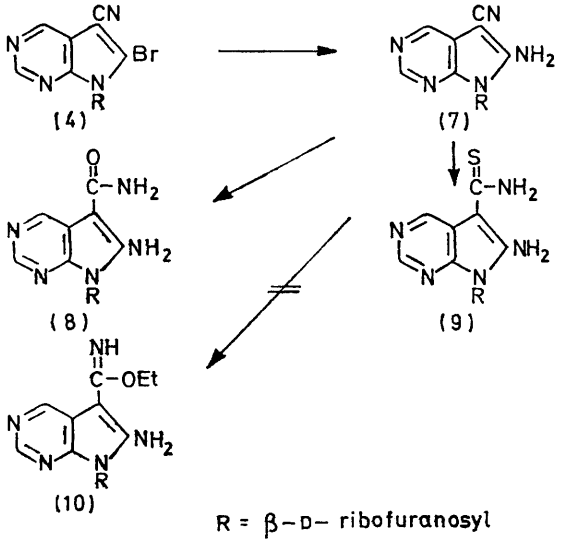
Ring closure of (5) to afford 3-amino-8-(β-D-ribofuranosyl)pyrazolo[4′,3′:4,5]pyrrolo[2,3-*d*]pyrimidine (6), showing no ν_{CN}, was effected by boiling water. The ^{1}H n.m.r. spectrum of (6) showed peaks for the aromatic (δ 9·45 and 9·25) and carbohydrate protons, lacked the hydrazino-signal δ 5·7, and contained a broad peak in the δ 7—8·7 region assigned to the 3-amino-group and the pyrazole proton. This ring closure reaction corroborated the site of bromination of (4).

Treatment of (4) with liquid ammonia in a sealed vessel at 110—120° for 12 h resulted in displacement* of the 6-bromo-group to afford the 6-amino-compound (7) in 74% yield [ν_{CN} 2 200 cm^{-1}; δ 7·9br (2H, exchangeable, 6-NH_2), 8·6 (H-4), and 9·0 (H-2)]. Additional evidence that nucleophilic displacement had occurred was provided by a large bathochromic shift [9] in the u.v. spectrum (Table). The reactivity of the cyano-group of (7) towards various nucleophiles was then studied.

Treatment of (7) with hydrogen peroxide in base gave an isomer of sangivamycin, 6-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8) [no ν_{CN}; δ 6·9 (6-NH_2) and 7·95 ($CONH_2$)]. T.l.c. showed the presence of at least two highly fluorescent minor products. The low yield of (8) (38%) in comparison with the conversion of toyocamycin into sangivamycin (65%) [10] under similar conditions indicated that side reactions were occurring. Since an amino-group in the 6-position of a pyrrolo[2,3-*d*]pyrimidine resides on an electron-rich ring system,[5] it is possible that *N*-oxidation was occurring



SCHEME 1



SCHEME 2

* Displacement of a 6-bromo-group in a pyrrolopyrimidine nucleoside has been accomplished by using methanolic ammonia in a sealed vessel.[9]

[6] J. F. Gerster, B. Carpenter, R. K. Robins, and L. B. Townsend, *J. Medicin. Chem.*, 1967, **10**, 326.

[7] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 1971, **8**, 703.

[8] L. B. Townsend, in 'Synthetic Procedures in Nucleic Acid Chemistry,' vol. II, eds. W. W. Zorbach and R. S. Tipson, Wiley, New York, 1973, pp. 267—398.

[9] M. Bobek, R. L. Whistler and A. Bloch, *J. Medicin. Chem.*, 1972, **15**, 168.

[10] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1968, **90**, 524.

since aniline is oxidized to azobenzene and pyridine is oxidized to pyridine *N*-oxide by peroxide in base.[11]

U.v. spectra of some pyrrolopyrimidine nucleosides

Compound	pH 1		MeOH		pH 11	
	$\lambda_{max.}$/nm	$\varepsilon_{max.}$ [a]	$\lambda_{max.}$/nm	$\varepsilon_{max.}$	$\lambda_{max.}$/nm	$\varepsilon_{max.}$
(4)	274	9·0	274	11·0	282	10·1
	226	17·4				
(5)	303	8·0	314	5·5	312	8·0
	245	21·1	240	11·3	232	15·0
(6)	320	7·3	(320) [b]		(314)	
	261	6·9	248	9·0	246	17·3
(7)	299	9·6	311	10·3	304	9·3
	(252)		279	10·7	(274)	
	239	29·2	(252)		250	10·0
			236	26·1	232	23·0
(8)	302	11·8	(312)		(310)	
	260	16·8	282	14·9	281	17·1
	242	31·0	267	14·3	234	25·1
			236	21·4		
(9)	330	18·4	338	21·5	338	20·6
	277	15·4	270	12·0	268	15·4
	246	28·0	238	24·6	238	45·0
(12)	293	15·0	292	24·2	290	19·9
	(230)					
	368	14·6	363	16·0	362	13·6
	304	13·0	(298)		(297)	
	278	18·1	273	22·8	271	21·2
	(249)		(238)		235	16·4
(15)	294	15·3	293	17·9	293	18·2

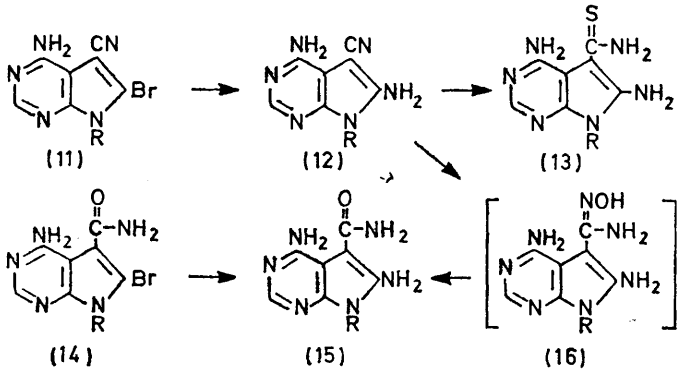
[a] $\varepsilon \times 10^{-3}$. [b] Shoulders in parentheses.

The reduced reactivity of the cyano-group of (7) in comparison with that of (1) or (2) is clearly shown in the preparation of the thioamide (9). Treatment of (7) with pyridine, triethylamine, and hydrogen sulphide under conditions essentially identical with those reported[3] for the conversion of (2) into a 5-thioamide produced no reaction. A recent report[12] states that nitriles unreactive under the above conditions can be converted into thioamides by dimethylformamide, dimethylamine, and hydrogen sulphide. Treatment of (7) in this way gave the thioamide (9), but in very low yield (<10%) [no ν_{CN}; δ 9·35 ($CSNH_2$) and 8·35 (6-NH_2) in $(CD_3)_2SO$]. A higher yield of (9) (21%) was obtained when (7) was treated with sodium hydrosulphide in methanol saturated with hydrogen sulphide in a sealed vessel for 12 h at 120—130°.

Since the nitrile group of (5) showed a reduced tendency towards nucleophilic attack under basic conditions it was expected that reactions performed under acidic conditions would be extremely slow. The initial reaction of a nitrile group under acidic conditions is generally thought to be protonation of the lone pair of electrons on the nitrogen of the nitrile group.[13] If protonation of the 6-amino-group occurred, this would be expected to retard or prevent protonation at the nitrile. This may explain why the attempted conversion of (7) into the imidate (10) by hydrogen chloride in ethanol, conditions identical with those used to convert toyocamycin (2) into an imidate, was unsuccessful, even with a prolonged reaction time. Thus transposition of the 4-amino-group of toyocamycin (2) to the 6-position produces a definite deactivating effect on the cyano-group towards nucleophilic attack under both acidic and basic conditions.

This prompted us to study the effect of two amino-groups, one in the pyrimidine ring at C-4 and another in the pyrrole ring at C-6. It was considered that this would further decrease the reactivity of the cyano-group towards nucleophilic addition.

The reaction of 6-bromotoyocamycin[10] (11) with liquid ammonia under conditions similar to those used for the preparation of (7) gave 6-aminotoyocamycin (12) in 74% yield [δ 6·2 (2H, 6-NH_2); ν_{CN} 2 210 cm^{-1}; significant bathochromic u.v. shift (Table)].



SCHEME 3

Synthesis of the 6-amino-analogue of 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (thiosangivamycin) was initially attempted under conditions (pyridine, triethylamine, and hydrogen sulphide) identical with those used in the conversion of toyocamycin (2) into thiosangivamycin, but only starting material was recovered. Dimethylformamide, dimethylamine, and hydrogen sulphide were also ineffective. However, when (12) was heated with methanolic sodium hydrosulphide in a sealed vessel, 6-aminothiosangivamycin (13) was obtained [no ν_{CN}; large

[11] G. B. Payne, P. H. Deming, and P. H. Williams, *J. Org. Chem.*, 1961, **26**, 659.

[12] E. E. Gilbert, E. J. Rumanowski, and P. E. Newallis, *J. Chem. Eng. Data*, 1968, **13**, 130.

[13] F. C. Schaefer, 'The Chemistry of the Cyano Group,' ed. Z. Rappoport, Interscience, New York, 1970.

bathochromic u.v. shift (Table); δ 8·3 (4H, 4-NH_2 and $CSNH_2$) and 6·4 (6-NH_2) in $(CD_3)_2SO$].

Hydration of the nitrile group of (12) to produce 4,6-diamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (15)(6-aminosangivamycin) with hydrogen peroxide and base produced a mixture of several compounds (t.l.c.). The incidence of side reactions was greater than observed in the similar oxidation of (7), presumably owing to the increased electron density by the 6-amino-group of (12). Therefore, alternative methods of converting the nitrile into a carboxamide group were investigated. Polyphosphoric acid has been reported [14] to convert unhindered nitriles into amides; *e.g.* when benzonitrile is heated at 110° for 1 h in polyphosphoric acid, a 96% yield of benzamide is obtained. Although the reaction conditions may be considered extreme for nucleosides, it was assumed that the more stable glycosidic bond of the pyrrolopyrimidine nucleosides [15] (in comparison with the purine nucleosides) would not be hydrolysed by this treatment. However, treatment of (12) under the above conditions resulted in the formation of resinous material containing several components (t.l.c.). The use of ion-exchange resins has also been reported to form carboxamides from nitriles under relatively mild conditions with no hydrolysis to the corresponding carboxylic acids.[16-18] However when (12) was heated with IRA-400 (OH) resin in water at reflux temperature for 20 h, only starting material was isolated.

An alternative route to (15) was then studied which involved nucleophilic displacement of the 6-bromo-group of 6-bromosangivamycin [7] (14) with liquid ammonia in a sealed vessel. Use of the conditions described for the synthesis of 6-aminotoyocamycin (12) gave the amide (15), which showed a bathochromic u.v. shift (Table) and ^1H n.m.r. signals for the carbohydrate protons and the aromatic proton (δ 9·3), and three peaks (each 2H) corresponding to the 4-amino and 5-carboxamide groups (δ 7·4 and 7·03; specific assignment not made) and the 6-amino-group (δ 6·47).

The reaction of (12) with hydroxylamine in ethanol was expected to yield 4,6-diamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide oxime (16). The i.r. spectrum confirmed that a modification of the cyano-function had occurred as the strong band for the nitrile group in the 2 200 cm^{-1} region had disappeared. Elemental analysis and the lack of an NOH signal at δ 9—10 in the ^1H n.m.r. spectrum [3] indicated that hydrolysis of the amidoxime group had also occurred, to produce (15). The product was identical (spectral data and m.p.) with that obtained from (14).

The above findings indicate that an amino-group on the pyrrole unit of the pyrrolopyrimidine system substantially deactivates the 5-cyano-group towards addition by nucleophilic reagents in comparison with the 5-cyano-group of toyocamycin (2).

[14] H. R. Snyder and C. T. Elston, *J. Amer. Chem. Soc.*, 1954, **76**, 3039.
[15] G. Acs, E. Reich, and M. Mori, *Proc. Nat. Acad. Sci. U.S.A.*, 1964, **52**, 493.

EXPERIMENTAL

For general procedures, see the preceding paper. The solvent system for t.l.c. was Pr^nOH–EtOAc–H_2O (1 : 4 : 2 v/v/v; upper phase).

6-Bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (4).—7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile [1] (3) (200 mg) was added to water (10 ml) and stirred at room temperature. Water saturated with bromine was added dropwise until a precipitate began to form (*ca.* 10 min). Bromine water (3 × 10 ml) was then added at 10 min intervals and the mixture was finally stirred for an additional 30 min. The yellow precipitate was filtered off under aspirator vacuum, washed with acetone (50 ml), and air-dried to give *compound* (4) (200 mg, 56%), m.p. 243° (Found: C, 40·5; H, 3·25; N, 15·8. $C_{12}H_{11}BrN_4O_4$ requires C, 40·55; H, 3·1; N, 15·75%).

6-Hydrazino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine 5-carbonitrile (5).—Hydrazine (97%; 2·4 ml) was added to a mixture of the 6-bromo-compound (4) (800 m.g.) in ethanol (40 ml). The mixture was heated at reflux temperature for 0·5 h and the white precipitate was filtered off and air-dried to give *compound* (5) (410 mg, 60%), m.p. 264—265° (Found: C, 47·1; H, 4·5; N, 27·4. $C_{12}H_{14}N_6O_4$ requires C, 47·05; H, 4·5; N, 27·45%).

3-Amino-8-(β-D-ribofuranosyl)pyrazolo[4′,3′:4,5]pyrrolo-[2,3-d]pyrimidine (6).—The 6-hydrazino-derivative (5) (300 mg) was mixed with water (30 ml) and heated at reflux temperature for 6 h. The mixture was cooled to room temperature and acidified to pH 2 (pH paper) with 3N-hydrochloric acid. The solvent was removed under reduced pressure on a steam-bath, and the residue was triturated with boiling ethanol (30 ml). The ethanol-insoluble white powder was filtered off and air-dried to give *compound* (6) (210 mg, 57%), m.p. >300° (Found: C, 41·15; H, 4·5; N, 24·2. $C_{12}H_{15}ClN_6O_4$ requires C, 41·0; H, 4·5; N, 23·95%).

6-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (7).—*Method A.* The 6-bromo-compound (4) (1·0 g) and methanolic ammonia (saturated previously at −5°) (75 ml) were mixed and heated at 110—115° in a sealed vessel for 16 h. T.l.c. showed a mixture of two major products, R_F 0·8 and 0·5, which were bright blue under u.v. light (254 nm). The mixture was dissolved in the minimum of boiling methanol and kept at 5° for 18 h. The tan crystals were filtered off and air-dried to yield *compound* (7) (250 mg, 30%), m.p. 260—261° (Found: C, 49·2; H, 4·55; N, 24·1. $C_{12}H_{13}N_5O_4$ requires C, 49·15; H, 4·45; N, 24·05%).

Method B. Liquid ammonia (*ca.* 3 ml) was added to the 6-bromo-compound (4) (680 mg) in a steel vessel. The vessel was sealed, placed in an oil-bath preheated to 100—110°, and then heated at that temperature for 12 h. The residue left after evaporation of the excess ammonia was triturated with methanol (20 ml) at room temperature. The insoluble yellow solid was filtered off and air-dried to give compound (7) (70%), identical [mixed m.p., i.r. and u.v. spectra data, R_F (0·8)] with that prepared by method A.

6-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxamide (8).—Compound (7) (200 mg), concentrated ammonium hydroxide (10 ml), and 30% hydrogen peroxide (2 ml) were mixed and stirred at room temperature for 2 h and then kept at 5° for 12 h. The white precipitate was filtered off and recrystallized from the minimum of water to

[16] J. M. Bobbitt and D. A. Scola, *J. Org. Chem.*, 1960, **25**, 561.
[17] J. M. Bobbitt and R. E. Doolittle, *J. Org. Chem.*, 1964, **29**, 2298.
[18] A. Galat, *J. Amer. Chem. Soc.*, 1948, **70**, 3945.

give *compound* (8) (120 mg, 38%), m.p. 257—258° (decomp.) (Found: C, 46·4; H, 4·85; N, 22·6. $C_{12}H_{15}N_5O_5$ requires C, 46·6; H, 4·85; N. 22·65%).

6-*Amino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine*-5-*thiocarboxamide* (9).—Compound (7) (500 mg) was added to a solution containing sodium hydrosulphide (200 mg) in methanol (20 ml) which had been saturated at room temperature with hydrogen sulphide for 40 min. The mixture was placed in a steel vessel, and the vessel was sealed, heated at 110—120° for 12 h, then cooled to room temperature. The methanol was removed under reduced pressure and the residue was dissolved in the minimum of water and kept at 5° for 12 h. The pale orange precipitate was filtered off and air-dried to give *compound* (9) (120 mg, 21%), m.p. 237° (Found: C, 43·0; H, 4·8; N, 20·85. $C_{12}H_{15}N_5O_4S$, 0·5 H_2O requires C, 43·1; H, 4·8; N, 20·95%).

4,6-*Diamino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine*-5-*carbonitrile* (6-*Aminotoyocamycin*) (12).—6-Bromotoyocamycin [7] (11) (200 mg) was added to liquid ammonia (*ca.* 5 ml) in a steel vessel. The sealed vessel was placed in a preheated (100—110°) oil-bath and heated for 22 h at 100—110°. The vessel was cooled in ice–water and the ammonia was allowed to evaporate. The residue was triturated with methanol (20 ml) at room temperature. The white, methanol-insoluble powder was filtered off, recrystallized from the minimum of water, and dried *in vacuo* at 80° for 12 h to give *compound* (12) (120 mg, 72%), m.p. 258—260° (decomp.) (Found: C, 44·4; H, 4·95; N, 25·85. $C_{12}H_{14}N_6O_4,H_2O$ requires C, 44·45; H, 4·95; N, 25·95%).

4,6-*Diamino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine*-5-*thiocarboxamide* (6-*Aminothiosangivamycin*) (13).—A mixture of 6-aminotoyocamycin (12) (300 mg), sodium hydrosulphide (100 mg), and methanol (20 ml) (saturated at room temperature for 45 min with hydrogen sulphide) was heated at 120—130° for 16 h in a sealed vessel and then allowed to cool to room temperature. The crystals were filtered off, recrystallized from the minimum of water, and dried under reduced pressure at 80° for 12 h to give *compound* (13) (240 mg, 73%), m.p. 246—247° (Found: C, 42·25; H, 5·0; N, 24·5. $C_{12}H_{16}N_6O_4S$ requires C, 42·35; H, 4·7; N, 24·7%).

4,6-*Diamino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine*-5-*carboxamide* (6-*Aminosangivamycin*) (15).—*Method A*. A mixture of 6-aminotoyocamycin (12) (1·1 g) and hydroxylamine [19] (1·1 g) in ethanol (80 ml) was heated at reflux temperature for 16 h. The solid was filtered off, recrystallized from the minimum amount of water, and dried under reduced pressure at 80° for 5 h to give *compound* (15) (880 mg, 75%), m.p. 268—269° (Found: C, 44·2; H, 4·75; N, 25·8. $C_{12}H_{15}N_6O$ requires C, 44·45; H, 4·95; N, 25·9%).

Method B. 6-Bromosangivamycin [7] (14) (1·0 g) was added to liquid ammonia (*ca.* 5 ml) and the mixture was heated at 110—120° in a sealed vessel for 16 h. After removal of the excess ammonia by evaporation, the residue was triturated with ethanol (20 ml) and filtered. Recrystallization of the ethanol-insoluble solid from the minimum of water gave compound (15) (59%), identical (m.p., u.v. and i.r. spectra) with that obtained by method A.

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