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Studies of Antibiotics and Related Substances. XXXII. Syntheses of Neamine and Its Analogue¹⁾

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Neamine, an antibiotic glycoside, was synthesized from paromamine. The preferential tosylation of the primary hydroxyl group of tri-N-acetylparomamine was followed by the replacement of the tosyloxy group with an azido group. The azido derivative was catalytically hydrogenated and acetylated to give 4-O-(2, 6-diacetamido-2, 6-dideoxy-a-D-glucopyranosyl)-N, Ndiacetyldeoxystreptamine. De-N-acetylation of the product gave neamine. Furthermore, a structural isomer of neamine, namely $6 \cdot O \cdot (3, 6 \cdot \text{diamino} - 3, 6 \cdot \text{dideoxy} - \alpha - \text{D-glucopyranosyl})$ deoxystreptamine, was synthesized from 6-O-(3-amino-3-deoxy-a-D-glucopyranosyl)-deoxystrept amine which was obtained from kanamycin by partial hydrolysis.

Aminosugar glycosides of 2-deoxystreptamine have been found to have antibacterial activities, for example, neamine,²⁾ paromamine,³⁾ 4-O-(6amino -6- deoxy - α - D - glucopyranosyl) - deoxystrep-

tamine⁴) (6AD), and 5-O-(2-amino-2-deoxy- α -D-glucopyranosyl)-deoxystreptamine⁵) (5-O-2AD). Among them, paromamine⁶ and 5-O-2AD⁵ have recently been synthesized.

In connection to our investigation⁷) of the relationship between the structural and biochemical characteristics of these aminoglycosides, the present

¹⁾ Part XVI of "Studies of Aminosugars," by S. Umezawa. A brief communication has already been given of the synthesis of neamine: S. Umezawa, K. Tatsuta, T. Tsuchiya and E. Kitazawa, J. Antibiotics, **A20**, 53 (1967). A part of this paper was presented at the 20 th Annual Meeting of the Chemical Society of

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paper describes the synthesis of neamine (V) from paromamine and another synthesis of 6-O-(3, 6diamino-3, 6-dideoxy - α - D - glucopyranosyl) - deoxystreptamine (X), which is a structural isomer of the neamine, from 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-deoxystreptamine⁸⁾ (3AD).



Tri - N-acetylparomamine⁶ (I) contains one primary and four secondary hydroxyl groups. By the preferential tosylation of C-6 of I with p-toluenesulfonyl chloride, 4-O-(2-acetamido-2deoxy-6-O-tosyl- α -D-glucopyranosyl)-N, N'-diacetyldeoxystreptamine (II) was obtained in a 43%yield. In the next step, the replacement of the tosyloxy group of II with an azido group was carried out by heating II in dimethylformamide with sodium azide, thus affording 4-O-(2-acetamido-6 - azido - 2, 6 - dideoxy - α -D-glucopyranosyl)-N, N'diacetyldeoxystreptamine (III). The azido compound (III) was hydrogenated catalytically over platinum oxide in methanol and then N-acetylated with acetic anhydride to give 4-O-(2, 6-diacetamido - 2, 6 - dideoxy - α - D - glucopyranosyl) - N, N' diacetyldeoxystreptamine (IV) in a 85% yield.

The absolute structure of the synthetic product IV and its identity with the tetra-N-acetylneamine^{2b)} were confirmed by the copper complex method.⁹⁾ Tetra-N-acetylneamine includes two pairs of adjacent hydroxyl groups which make the projected angles of about $+60^{\circ}$ and -60° ; one in the glucose moiety and the other in the deoxystreptamine moiety. Accordingly, the $\mathcal{A}[M]_{CuAm}$ of tetra-N-acetylneamine may be expected to be nearly zero as a result of internal compensation.

The determination of the $\mathcal{A}[M]_{CuAm}$ values of the synthetic product and the corresponding tetra-Nacetyl derivative of natural neamine gave values of +34 and +59 respectively. Thus, the observed values for Δ [M]'s of IV and tetra-N-acetylneamine agreed within the range of experimental error, and the result indicated that the product IV is identical to tetra-N-acetylneamine, in which the four acetamido groups are located at C-2- and 6positions of glucose moiety and at C-1- and 3positions of 2-deoxystreptamine moiety. Thinlayer chromatography of the synthetic product IV illustrated that its R_f -value corresponds to that of tetra-N-acetylneamine.

In the next step, de-N-acetylation of IV with hydrazine, followed by chromatography on a column of Dowex 1×2 (OH form), gave a free base, which was then neutralized with hydrochloric acid to afford the tetrahydrochloride of 4-O-(2, 6-diamino - 2, 6 - dideoxy - α - D - glucopyranosyl)deoxystreptamine (V) in a 72% yield. On paper chromatography, the synthetic product V and the natural neamine showed identical mobilities. The antibiotic spectra and minimal inhibitory concentrations (MIC) of the synthetic product V against test organisms were in agreement with those of the natural neamine as shown in Table 1. Since the synthesis of paromamine⁶) has been established, the above synthesis may be regarded as a total synthesis of neamine.

6 - O - $(3, 6 - \text{Diamino} - 3, 6 - \text{dideoxy} - \alpha - D - \text{gluco}$ pyranosyl)-deoxystreptamine (X) was prepared likewise from 3AD, which was obtained from kanamycin by partial hydrolysis. Tri-N-acetyl derivative⁸) (VI) of 3AD was preferentially tosylated to give $6-O-(3-acetamido-3-deoxy-6-O-tosyl-\alpha-D$ glucopyranosyl) - N, N' - diacetyldeoxystreptamine (VII) in a 44% yield. The tosyloxy group of VII was then replaced by an azido group to afford 6-O-(3-acetamido-6-azido - 3, 6 - dideoxy - α - D - glucopyranosyl)-N, N'-diacetyldeoxystreptamine (VIII) in a 83% yield. The azido compound (VIII) was converted to 6-O-(3, 6-diamino-3, 6-dideoxy- α -Dglucopyranosyl)-deoxystreptamine (X) via tetra-N-acetylated derivative (IX), in the same way as described in the preparation of V.

Structural proof was obtained from the nuclear magnetic resonance spectrum of 6-O-(3-acetamido-3, 6-dideoxy - α -D - glucopyranosyl) - N, N' - diacetyldeoxystreptamine (XII), which was prepared from mono-O-tosylated derivative (VII) via deoxyiodo derivative (XI). The signal at τ 8.79 (doublet, J=7 cps) may be assigned to three methyl protons, the singlets at τ 8.04, 8.01 and 7.97, with the relative intensities of 3:3:3 may be ascribed to nine N-acetyl protons, and the doublet at τ 5.01 (I=3 cps), with a relative intensity of 1, may be assigned to the anomeric hydrogen. These results indicated that the primary alcoholic group in the 3-amino-3-deoxy-D-glucose moiety of 3AD is

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This Bulletin, 39, 1235 (1966).

preferentially tosylated and consequently aminated by replacing the tosyloxy group with an amino group.

As shown in Table 1, the synthetic product V (or natural neamine) and 6AD, in which aminoglucose moieties were linked to C-4-position of 2-deoxystreptamine, have antibacterial activities. On the other hand, compound X and 3AD did not show any antibacterial activity and their aminoglucose moieties were linked to C-6-position of 2-deoxystreptamine. The structural similarities of V and X did not reflect any biochemical similarities. It seems, therefore, that C-4-linked aminoglycosides of 2-deoxystreptamine (neamine or V, paromamine, and 6AD) have antibacterial activities but C-6-linked derivatives (X and 3AD) are hardly endowed with antibacterial activities.

Experimental

Thin-layer Chromatography, Silica-gel Column Chromatography, and Paper Chromatography. Thin-layer chromatography was conducted by the use of silica gel (Daiichi Pure Chemicals Co.); the prepared plate was activated at 110°C and then stored in a desiccator. The spray reagent used was concentrated sulfuric acid. Solvent systems used: ethanol-benzene (3:1) (Solvent A) and ethanol-benzene (5:1) (Solvent B). Silica-gel column chromatography was carried out by the use of silica gel (Kanto Chemical Co.) activated at 110°C before use. Paper chromatography was conducted by the descending technique on Toyo filter paper No. 50, and the substances were detected by the use of ninhydrin spray (0.25% in pyridine). Solvent system used: n-butanol - pyridine - water - acetic acid (6:4:3:1) (Solvent C).

General Procedure for Nuclear Magnetic Resonance Spectrometry. The NMR spectrum was determined at a frequency of 60 Mcps with a Varian A60 spectrometer in deuterium oxide. Sodium 2, 2dimethyl-2-silapentane-5-sulfonate was used as an internal reference in the sample. Peak positions are given in τ -values.

4-O-(2-Acetamido-2-deoxy-6-O-tosyl-a-D-glucopyranosyl)-N,N'-diacetyldeoxystreptamine (II). To a cold (-15°C) solution of tri-N-acetylparomamine⁶) (I, 1.22 g, 2.71 mmol) in dry pyridine (49 ml) was added p-toluenesulfonyl chloride (1.08 g, 5.67 mmol), and the mixture was stirred at the same temperature for 1 hr and allowed to stand overnight at 0°C and then 5°C for 2 days. After the addition of a small volume of water, the mixture was left for 30 min at 0°C. The mixture was evaporated and the residue was dried by coevaporation with toluene. The solid obtained was treated with methanol (3 ml) - acetone (20 ml) to separate the unchanged I (440 mg), which is insoluble in acetone. Concentration of the filtrate afforded a crude mono-tosyl derivative, which virtually showed a single spot $(R_f 0.42)$ on a thin-layer chromatogram with Solvent A. The crude product was dissolved in methanol and chromatographed on a silica-gel column $(48 \times 265 \text{ mm})$ with a solvent mixture of ethanolbenzene (3:1). Every fraction (15 g) was tested by thin-layer chromatography. The substance having an

 R_f 0.42 appeared in the fractions of tube Nos. 29—36, which were combined and evaporated under reduced pressure, and the residue was recrystallized from methanol-ether; yield 710 mg (43%); mp 169—172°C. (decomp.), $[\alpha]_2^{sb}$ +68° (c 0.5, methanol). IR spectrum (KBr disk): 3400 (ν OH), 3320 (ν NH), 1650 (amide I), 1600 (phenyl), 1550 (amide II), 1175 (ν_s SO₂) cm⁻¹. Found: C, 49.45; H, 5.86; S, 5.66%. Calcd for

 $C_{25}H_{37}O_{12}N_3S$: C, 49.74; H, 6.18; S, 5.31%.

4-O-(2-Acetamido-6-azido-2, 6-dideoxy-a-D-glucopyranosyl)-N, N'-diacetyldeoxystreptamine (III). To a solution of II (431 mg) in dry N, N-dimethylformamide (4.3 ml) was added sodium azide (434 mg), and the suspension was vigorously agitated at 100°C for 6.5 hr. The resulting dark solution was filtered, and the filtrate was evaporated and coevaporated with toluene to obtain a solid substance, which showed essentially a single spot $(R_f \ 0.39)$ on a thin-layer chromatogram with Solvent A. The dark solid substance was dissolved in methanol and chromatographed on a silica-gel column $(32 \times 221 \text{ mm})$ with a solvent mixture of ethanol-benzene (3:1). Fractions (15 g each) were tested by thin-layer chromatography. The The substance having an R_f 0.39 was eluted in the fractions of tube Nos. 34-41. The fractions were combined and evaporated to give a solid substance which was recrystallized from methanol-ether; yield 119 mg (35%); mp 241—245°C (decomp.), $[\alpha]_D^{25}$ +114° (c 0.5, water). IR spectrum (KBr disk): $3440 (\nu OH)$, 3300 (vNH), 2110 (azide), 1650 (amide I), 1550 (amide II) cm⁻¹.

Found: C, 45.26; H, 6.49; N, 17.53%. Calcd for $C_{18}H_{30}O_9N_6$: C, 45.56; H, 6.37; N, 17.71%.

4-O-(2, 6 - Diacetamido -2, 6 - dideoxy - α -D-glucopyranosyl)-N, N'-diacetyldeoxystreptamine (IV), (Tetra-N-acetylneamine). A sample (200 mg) of III was dissolved in methanol (20 ml) and hydrogenated over platinum oxide (100 mg) under 3.5 atm of hydrogenpressure at 40°C for 4 hr in a Paar shaker-type apparatus. After removal of the catalyst, the ninhydrin-positive solution was N-acetylated with acetic anhydride (3.5 ml) for 30 min. The resulting mixture was evaporated *in* vacuo to give a solid substance which was recrystallized from methanol; yield 176 mg (85%); mp 326°C (decomp.), $[\alpha]_{156}^{15}$ +95° (c 0.50, water), $[\alpha]_{156}^{15}$ +178° (c 0.50, water), $[\alpha]_{156}^{15}$ +185° (c 0.50, CuAm),¹⁰⁰ Δ [M]_{CuAm} +34. IR spectrum (KBr disk): 3450 (ν OH), 3330 (ν NH), 1665 (amide I), 1555 (amide II) cm⁻¹. Found: C, 48.50; H, 7.25; N, 11.02%. Calcd for

 $C_{20}H_{34}O_{10}N_4$: C, 48.97; H, 6.99; N, 11.42%. The tetra-*N*-acetylneamine^{2b} obtained from natural

neamine by N-acetylation showed $[\alpha]_{559}^{15} + 94^{\circ}$ (c 0.49, water), $[\alpha]_{436}^{15} + 172^{\circ}$ (c 0.49, water), $[\alpha]_{436}^{15} + 184^{\circ}$ (c 0.49, CuAm), $\Delta[M]_{CuAm} + 59$.

On a thin-layer chromatogram with Solvent B, the product IV showed a single spot $(R_f \ 0.30)$ and the R_f -value agreed with that of tetra-N-acetylneamine.

4-O-(2, 6-Diamino-2, 6-dideoxy- α -D-glucopyranosyl)-deoxystreptamine (V), (Neamine). A mixture of IV (50 mg) and 80% hydrazine hydrate (1 ml) in

 $\Delta[M]_{CuAm} =$

$$([\alpha]_{436}$$
CuAm $- [\alpha]_{436}$ water) $\times \frac{Mol wt}{100}$; see Ref. 9.

¹⁰⁾ CuAm: Cuprous chloride in aqueous ammonia; cuprous chloride (1.60 g) was dissolved in 15 N ammonia (100 ml) at 15° C.

a sealed tube was heated in a boiling water bath for 20 hr. After the hydrazine had been removed in vacuo, the residue was dissolved in water and chromatographed on a column (11×42 mm) of Dowex 1X2 (OH form) with water. A ninhydrin-positive eluate between 3—6 ml was neutralized with hydrochloric acid to pH 2 and evaporated to dryness. The residue was recrystallized from aqueous methanol-acetone affording the tetrahydrochloride of V; yield 34 mg (72%); mp 233°C (decomp.), $[\alpha]_{1}^{s_{1}} + 86.8^{\circ}$ (c 0.50, water). IR spectrum (KBr disk): 3400—3300 (ν OH, NH), 2900 (ν CH), 1600 (δ_{as} NH₃⁺), 1500 (δ_{s} NH₃⁺) cm⁻¹. Found: C, 30.64; H, 6.94; N, 11.64%. Calcd for

Found: C, 30.64; H, 6.94; N, 11.64%. Calcd for $C_{12}H_{26}O_6N_4$ ·4HCl: C, 30.78; H, 6.46; N, 11.97%.

On a paper chromatogram with Solvent C, the product V showed a single spot ($R_{f \text{ paromamine}} 0.47$) and the R_{f} -value agreed with that of natural neamine. Infrared spectra of the product V and the natural neamine were completely superimposed.

6-O-(3-Acetamido-3-deoxy-6-O-tosyl-α-D-glucopyranosyl)-N, N'-diacetyldeoxystreptamine (VII). To a cold (-15°C) solution of 6-O-(3-acetamido-3deoxy- α -D-glucopyranosyl) - N, N' - diacetyldeoxystreptamine⁸) (VI, 2.00 g, 4.46 mmol) in dry pyridine (80 ml) was added *p*-toluenesulfonyl chloride (1.28 g, 6.72 mmol), and the mixture was stirred at the same temperature for 1 hr and kept at 5°C for 5 days. After a small volume of water had been added and the mixture had been set aside for 30 min, the mixture was evaporated and coevaporated with toluene to give a solid substance, which showed three spots having R_f values of 0.18 (unchanged VI), 0.32 (main) and 0.78 (minor) on a thin-layer chromatogram with Solvent A. The solid substance was treated with ethanol (42 ml) - acetone (170 ml) to separate the unchanged VI (0.69 g), which was insoluble in acetone. After concentration of the filtrate, the residue was dissolved in a solvent mixture of ethanol-benzene (3:1) and chromatographed on a silica-gel column $(25 \times 380 \text{ mm})$ with the same solvent. After about 230 ml of eluate, fractions (15 g each)were tested by thin-layer chromatography. The substance having an R_f value of 0.32 appeared in the fractions of tube Nos. 22-35, which were combined and evaporated under reduced pressure. The residue was recrystallized from ethanol-acetone affording VII; yield 1.18 g (44%); mp 184—189°C (decomp.), $[\alpha]_{D}^{20}$ $+59^{\circ}$ (c 0.68, methanol). IR spectrum (KBr disk): 3400 (vOH), 3320 (vNH), 1650 (amide I), 1600 (phenyl), 1560 (amide II), 1180 ($\nu_s SO_2$) cm⁻¹.

Found: C, 49.48; H, 6.09; S, 5.36%. Calcd for $C_{25}H_{37}O_{12}N_3S$: C, 49.74; H, 6.18; S, 5.31%.

6-O-(3-Acetamido-6-azido-3, 6-dideoxy-a-D-glucopyranosyl)-N,N'-diacetyldeoxystreptamine (VIII). To a solution of VII (80 mg) in dry N, N-dimethylformamide (0.8 ml) was added sodium azide (80 mg), and the suspension was treated in a way described in the synthesis of III. The product showed essentially a single spot (R_f 0.25) on a thin-layer chromatogram with Solvent A, and was dissolved in methanol and chromatographed on a silica-gel column (9×150 mm) with a solvent mixture of ethanol-benzene (3 : 1). After elution of about 6 ml of the solution, fractions (3g each) were tested by thin-layer chromatography. The substance having an R_f value of 0.25 was eluted in the fractions of tube Nos. 10—19. These fractions were combined and evaporated to give a solid, which was recrystallized from methanol-ether; yield 52 mg (83%); mp 239—241°C (decomp.), $[\alpha]_D^{20}$ +88°C (c 0.54, water). IR spectrum (KBr disk): 3400—3340 (ν OH, NH), 2110 (azide), 1650 (amide I), 1555 (amide II) cm⁻¹.

Found: C, 45.29; H, 6.59; N, 17.62%. Calcd for $C_{18}H_{30}O_9N_6$: C, 45.56; H, 6.37; N, 17.71%.

6-0-(3, 6-Diacetamido - 3, 6 - dideoxy - a - D - glucopyranosyl)-N, N'-diacetyldeoxystreptamine (IX). A sample (100 mg) of VIII was dissolved in methanol (10 ml) and hydrogenated over platinum oxide (40 mg), followed by N-acetylation with acetic anhydride (1 ml) as has been described in the synthesis of IV. The product obtained showed essentially a single spot $(R_f \ 0.23)$ on a thin-layer chromatogram with Solvent B, and was dissolved in a solvent mixture of ethanol-benzene (5:1) and chromatographed on a silica-gel column $(10 \times 240 \text{ mm})$ with the same solvent. An eluate between 102-146 ml was evaporated to dryness and the residue was recrystallized from methanol affording needles of IX; yield 62 mg (60%); mp 293-296°C (decomp.), $[\alpha]_{\rm D}^{20}$ +63° (c 0.67, water). IR spectrum (KBr disk): 3400-3320 (vOH, NH), 1650 (amide I), 1555 (amide II) cm.⁻¹

Found: C, 48.76; H, 7.21; N, 11.12%. Calcd for $C_{20}H_{34}O_{10}N_4$: C, 48.97; H, 6.99; N, 11.42%.

6-O-(3, 6-Diamino-3, 6 - dideoxy - α - D - glucopyranosyl)-deoxystreptamine (X). A mixture of IX (200 mg) and 80% hydrazine hydrate (4 ml) in a sealed tube was heated at 130°C for 40 hr. The resulting mixture was treated in a way described in the synthesis of V. A ninhydrin-positive eluate was neutralized with hydrochloric acid to pH 2 and evaporated to dryness. The residue was recrystallized from aqueous methanol-ethanol affording the tetrahydrochloride of X; yield 141 mg (74%); mp 170–173°C (decomp.), [α] $_{30}^{\circ}$ +69° (α 0.66, water). IR spectrum (KBr disk): 3400–3300 (ν OH, NH), 1600 (δ_{as} NH $_{3}^{+}$), 1500 (δ_{s} NH $_{3}^{+}$) cm⁻¹.

Found: C, 30.67; H, 6.77; N, 11.75%. Calcd for $C_{12}H_{26}O_6N_4$.4HCl: C, 30.78; H, 6.46; N, 11.97%.

On a paper chromatogram with Solvent C, the product X showed a single spot $(R_{f \text{ sAD}} 0.62)$.

6-O-(3-Acetamido-3, 6 - dideoxy - a - D - glucopyranosyl) - N, N' - diacetyldeoxystreptamine (XII). A solution of the tosylate (VII, 500 mg) in dry N, Ndimethylformamide (5 ml) containing sodium iodide (380 mg) was stirred at 130°C for 6 hr. The resulting mixture was filtered, and the filtrate was evaporated to give a syrup, which was dried by coevaporation with toluene. The dark solid obtained showed essentially a single spot $(R_f \ 0.34)$ on a thin-layer chromatogram with Solvent A. The solid was dissolved in a solvent mixture of ethanol-benzene (3:1) and chromatographed on a silica-gel column $(25 \times 230 \text{ mm})$ with the same solvent. An eluate between 416-500 ml was evaporated to give a deoxyiodo derivative (XI, 350 mg), which gave a strong positive Beilstein test for halogen. The infrared spectrum of the product showed the absence of the absorption at 1180 cm^{-1} due to a tosyl group.

The product XI (350 mg) was dissolved in 99% ethanol (25 ml) and hydrogenated over Raney nickel (W-4) (0.5 ml) under 3.5 atm of hydrogen-pressure at 40°C for 2 hr. The mixture was filtered, and the filtrate was again treated with Raney nickel and hydrogen for 4 hr. After removal of the catalyst, the filtrate was

Test organisms	v	Х	Neamine	Paromamine	6AD	3AD
Bacillus subtilis PCI 219	4	>1000	4	125	50	>1000
Mycobacterium tuberculosis 607	30	>1000	30	>1000	200	>1000
M. pyogenes var. aureus 209p	30	>1000	30	>1000	50	>1000
E. coli	125	>1000	62.5	500	1000	>1000

Table 1. The MIC's of compounds V, X, neamine, paromamine, 6AD and 3AD as determined by the dilution method in souillon, mcg/ml

evaporated to give a solid substance, which showed essentially a single spot (R_f 0.18) on a thin-layer chromatogram with Solvent A. The solid was dissolved in a solvent mixture of ethanol-benzene (3:1), and chromatographed on a silica-gel column ($16 \times 200 \text{ mm}$) with the same solvent. An eluate between 275—480 ml was evaporated to dryness and the residue was recrystallized from methanol affording XII; total yield 180 mg (50%); mp 285°C (decomp.), [α]²⁰ +67° (c 0.65, water). Beilstein test negative. NMR spectrum data: τ 8.79 (doublet, J=7 cps, 3 protons, 6-CH₃) τ 8.9 and 8.4 (multiplets, 1 proton each, 2-CH₂ of decoxystreptamine), τ 8.04, 8.01 and 7.97 (singlets, 3 protons each, NAc), τ 5.01 (doublet, J=3 cps, 1 proton, anomeric hydrogen); IR spectrum (KBr disk): 3430 (ν OH), 3300 (ν NH), 2910 (ν CH), 1650 (amide I), 1555 (amide II) cm⁻¹.

Found: C, 49.64; H, 7.42; N, 9.43%. Calcd for $C_{18}H_{31}O_9N_8$: C, 49.87; H, 7.21; N, 9.69%.

Preliminary Bioassay. The antibiotic spectra and minimal inhibitory concentrations (MIC) of compounds V and X were shown in Table 1 and compared with natural neamine, paromamine, 6AD and 3AD.

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