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Synthesis of the Glycopeptide - O-(3,4-Di-O-Methyl-2-O-[3,4-di-O-methyl-α-L-rhamnopyranosyl]-α-L-rhamnopyranosyl)-L-alanilol: An Unusual Part Structure in the Glycopeptidolipid of Mycobacterium fortuitum

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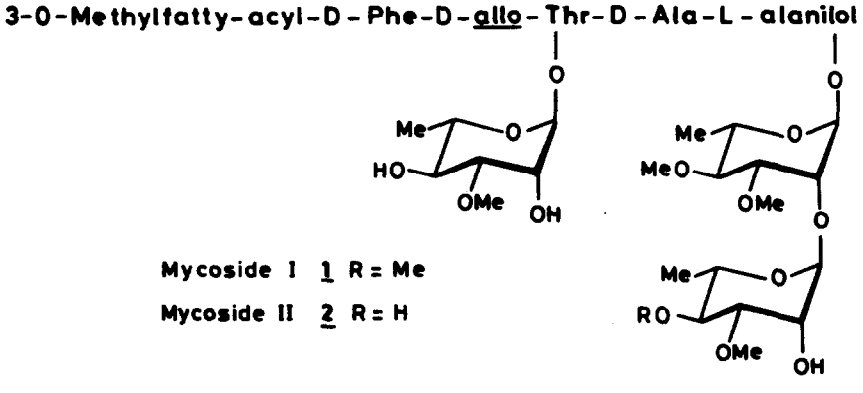
Summary : The stereoselective synthesis of the title compound, an unusual variant in the structure of glycopeptidolipid of M. fortuitum, by involving glycosyltrichloroacetimidate methodology has been described.

Inspite of several advances[1] made in glycopeptide synthesis, there is hardly any synthetic study[2] on the glycopeptide segments of species-specific C-mycoside glycopeptidolipids. Since C-mycosides are present on the surface of the bacteria, they are the defining factors in biological recognition and are involved in the colony morphology[3]. The basic structure of the glycotetrapeptide of C-mycosides has been proposed[4] as D-Phe-D-allo-thr-D-ala-L-alanilol-(3,4-di-O-methyl- α-L-rhamnopyranoside). However recently a few variants in the structure of C-mycosides such as Mycobacterium (M.) fortuitum[5] and M. xenopi[6] have been reported.

M. fortuitum is considered as an etiological agent of skin, soft tissue, postsurgical and some pulmonary human infections[7]. The bacteria does not respond to antituberculotic drugs and its response to antimicrobial agents depends upon species and subvariants which calls for precise identification of bacterial species. Two major C-mycosides (I and II) were isolated[5] from M. fortuitum biovar. Peregrinum and their structure determined by spectroscopic methods as

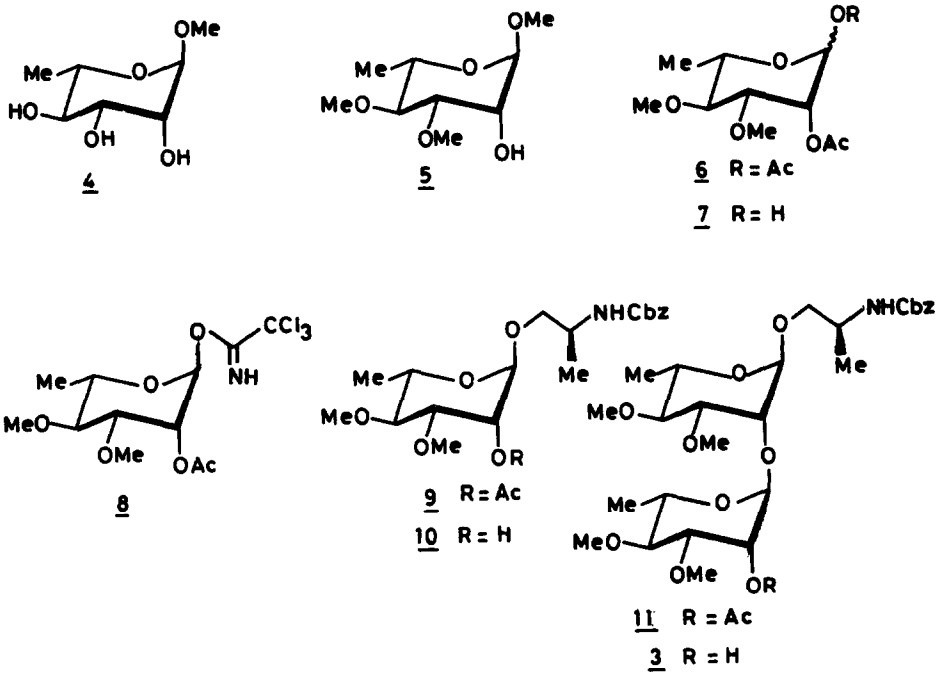


1 and 2 respectively. The presence of 3-O-methyl- α-L-rhamnopyranose instead of usual 6-deoxy-L-talose at the D-allo-threonine residue, and a oligosaccharide unit at the terminal L-alanilol instead of at D-allo-threonine are indeed fascinating and unknown[3] features for the glycopeptidolipids of C-mycosides. The structures of the oligosaccharides of mycoside I and II were established[5] as O-(3,4-di-O-methyl- α-L-rhamnopyranosyl)-(1 - 2)-3,4-di-O-methyl- α-L-rhamnopyranose and O-(3-O-methyl- α-L-rhamnopyranosyl)-(1 - 2)-3,4-di-O-methyl-α-L-rhamnopyranose respectively. Herein we report a stereoselective synthesis of the title glycopeptide (3) by employing O-glycosylated trichloroacetimidate methodology[8].

Methyl 3,4-di-O-methyl- α-L-rhamnopyranoside (5) was prepared from methyl α-L-rhamnopyranoside (4) in four steps[9]: i) formation of 2,3-O-isopropylidene derivative, ii) methylation at 0-4, iii) hydrolysis of 2,3-O-isopropylidene groups and iv) selective methylation at 0-3 via the corresponding 2,3-dibutyltin acetal. Subsequent hydrolysis of anomeric methoxyl group in 5 with 3N sulfuric acid in dioxane in a boiling water bath for 6h gave the hemiacetal which was conventionally acetylated with acetic anhydride and pyridine to provide the 1,2-diacetate (6). From the ^{1}H NMR spectrum, 5 appeared to be a mixture of α- and β-pyranoses in which α-isomer was predominating. In order to selectively[10] deblock the acetyl group present at the anomeric position, 6 was heated under reflux with freshly prepared tri-n-butyltin methoxide in dichloroethane for 3h to give 7 (80%). Treatment[11] of 7 with trichloroacetonitrile in the presence of DBU in dichloromethane furnished the trichloroacetimidate derivative (8). The imidate (8) was used as a common glycosylating agent in both the O-glycosylation reactions. The presence of an acetyl group at O-2 in 8 was expected to favour α selectivity during the O-glycosylation reaction.

Condensation of 8 with N-carbobenzyloxy-L-alanilol in the presence of 4°A molecular sieves and borontrifluoride-etherate (BF_3:OEt_2) in dichloromethane at -20° for 30 minutes resulted in the formation of 9 (70%). The ^{1}H NMR spectrum of 9 indicated H-1 as a doublet (J = 1.3 Hz) at 4.68 ppm while ^{13}C NMR should anomeric carbon C-1 at 97.78 ppm. However, the precise assignment of α-configuration at the anomeric center was confirmed at the later stage of the synthesis. The acetyl group in 9 was cleaved with methanolic sodium methoxide under Zemplen condition to furnish 10. Subsequently, 10 was further O-glycosylated with 8 under same conditions as described above to give 11 (60%). The anomeric protons in the ^{1}H-NMR spectrum of 10

appeared at 4.69 ppm (J = 1.5 Hz) and 4.95 ppm (J = 1.5 Hz). Finally **11** was deacetylated under Zemplen condition to give the title glycopeptide (**3**). The ^{1}H-NMR spectrum of **3** was in full agreement with its structure. The high coupling constant valves (J=169.3Hz) for both the anomeric carbons in a partially decoupled ^{13}C-NMR spectrum of **3** confirmed the α-configuration at both these centers.



2-O-Acetyl-3,4-di-O-methyl-α,β-L-rhamnopyranose (7):

Methyl 3,4-di-O-methyl-α-L-rhamnopyranoside (**5**) (3.09 g, 15.0 mmol), 3N H_2SO_4 (6 mL), and dioxan (10 mL) were heated under reflux for 6h, diluted with dioxane and neutralised with solid $BaCO_3$. The solid was filtered and washed with dioxan. The filtrate was concentrated, and codistilled with toluene. The residue (2.04 g) was treated with acetic anhydride (3 mL) and pyridine (10 mL) for 18 h and worked-up. The residue was purified by column chromatography on silica gel with ethyl acetate-light petroleum (1:9) as eluent to give **6** (3.55 g, 86%) as a syrup; ^{1}H NMR ($CDCl_3$) (α-anomer) δ 1.34 (d, 3H, J = 6.5 Hz, CH_3), 2.13, 2.18 (6H, 2s, 2Ac), 3.14 (t, 1H, J = 10.0 Hz, H-4), 3.48, 3.62 (2s, 6H, 2 OMe), 5.32 (m, 1H, H-2), 6.08 (d, 1H, J = 1.5 Hz, H-1).

6 (1.38 g, 5.0 mmol) and freshly prepared tri-n-butyltin methoxide (1.5 mL) in $ClCH_2CH_2Cl$ (15 mL) were heated under reflux for 3

h and concentrated. The residue was chromatographed on silica gel by using ethyl acetate-light petroleum (gradient 0:1 - 3:7) to give **7** (0.94 g, 80%), as a syrup, ^{1}H-NMR ($CDCl_3$) (α -anomer) δ 1.28 (d, 3H, J = 6.4 Hz, CH_3), 2.14 (s, 3H, Ac), 3.40, 3.55 (2s, 6H, 2 OMe), 5.1 (bs, 1H, H-1), 5.25 (m, 1H, H-2).

O-(2-O-Acetyl-3,4-di-O-methyl- α -L-rhamnopyranosyl)-N-carbobenzyloxy-L-alanilol (9):

A solution of **7** (0.30 g, 1.29 mmol), trichloroacetonitrile (1.2 mL), DBU (9 μL) in CH_2Cl_2 (5 mL) was stirred for 10 min. and poured over a small column of silica gel. Elution with CH_2Cl_2 gave **8** (0.41 g, 85%).

To the solution of **8** (0.38 g, 1.0 mmol), N-benzyloxycarbonyl-L-alanilol (0.21 g, 1.0 mmol), 4A° molecular sieves (0.5 g) in $CHCl_3$ (15 mL) at -20° under nitrogen was added BF_3:OEt_2 (9 μL) in $CHCl_3$. After 30 min. a few drops of pyridine were introduced to decompose BF_3:OEt_2, filtered and concentrated. The residue was chromatographed on silica gel by using ethyl acetate-light petroleum (1:6) to give **9** (0.27 g, 64%) as a syrup, $[\alpha]_D$ -41° (c 1.0, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ 1.18 (d, 3H, J = 6.2 Hz, CH_3), 1.29 (d, 3H, J = 6.0 Hz, CH_3), 2.12 (s, 3H, Ac), 3.06 (t, 1H, J = 10.2 Hz, H-4), 3.41, 3.56 (2s, 6H, 2 OMe), 3.91 (m, 1H), 4.68 (d, 1H, J = 1.3 Hz, H-1), 5.10 (s, 2H, $PhCH_2$), 5.25 (m, 1H), ^{13}C-NMR 97.78 (c-1'). Anal. Calcd. for $C_{21}H_{31}O_8N$: C, 59.3; H, 7.3. Found: C, 56.7; H, 7.3.

O-(3,4-Di-O-methyl-2-O-[3,4-di-O-methyl- α -L-rhamnopyranosyl]- α -L-rhamnopyranosyl)-L-alanilol (11):

To a solution of **9** (0.23 g, 0.54 mmol) in methanol, sodium (25 mg) was added. After 3h, the solution was deionised with Amberlite IR 120 (H) resin, filtered and concentrated. The residue was codistilled with toluene to remove traces of moisture and then the resulting product **10** (0.19 g) dissolved in dry $CHCl_3$ (7 mL). Molecular sieves 4A° (0.5g), and **8** (0.19 g, 0.5 mmol) were introduced into the solution and cooled to -20°. BF_3:OEt_2 (5 μL) in $CHCl_3$ (2 mL) was added and stirring continued for 30 min. After usual workup as described for **9**, the residue obtained was purified by column chromatography on silica gel with ethyl acetate-light petroleum (1:4) as eluent to give **11** (0.18 g, 60%), as a syrup, $[\alpha]_D$ -40° (c 2.1, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ 1.16 (d, 3H, J = 6.1 Hz, CH_3), 1.24 (d, 3H, J = 6.0 Hz, CH_3), 1.28 (d, 3H, J = 6.0 Hz, CH_3), 2.12 (s, 3H, Ac), 3.02 (t, 1H, J = 10.2 Hz, H-4), 3.04 (t, 1H, J = 0.0 Hz, H-4"), 3.38 (s, 6H, 2 OMe), 3.48, 3.49 (2s, 6H, 2 OMe), 4.69 (d, 1H, J = 1.5 Hz, H-1'), 4.95 (d, 1H, J = 1.5 Hz, H-1"), 5.10 (s, 2H, $PhCH_2$), 5.34 (m, 1H,

H-2"), 7.35 (s, 5H, Ph).

11 (0.18 g) was deacetylated with methanolic sodium methoxide as described for **10** to give a crude residue which was purified on silica gel column with ethyl acetate-light petroleum (1:1) as eluent. The pure product **3** (0.15 g, 88%) was isolated as a syrup, $[\alpha]_D$ -58° (c 1.6, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ 1.16 (d, 3H, J = 6.3 Hz, CH_3), 1.27 (d, 3H, J = 6.5 Hz, CH_3), 1.31 (d, 3H, J = 6.2 Hz, CH_3), 3.06 (t, 1H, J = 10.4 Hz, H-4'), 3.08 (t, 1H, J = 10.2 Hz, H-4"), 3.45, 3.52 (2s, 6H, 2 OMe), 3.54 (s, 6H, 2 OMe), 4.0 (m, 1H), 4.12 (m, 1H), 4.70 (d, 1H, J = 1.0 Hz, H-1'), 4.89 (bd, 1H), 5.04 (d, 1H, J = 1.0 Hz, H-1"), 5.10 (s, 2H, $PhCH_2$), 7.35 (s, 5H, Ph), ^{13}C-NMR ($CDCl_3$) δ 97.77 (C-1', J = 169.3 Hz), 98.88 (C-1", J = 169.3 Hz). Anal. Calcd. for $C_{27}H_{43}O_{11}N$: C, 58.2; H, 7.7. Found: C, 58.0; H, 7.8.

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