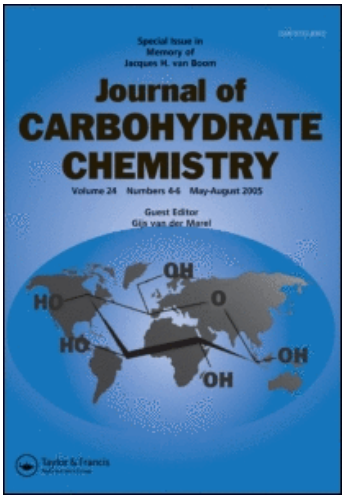

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Synthesis of D-Glucos-2-yl Sucros-2-yl Phosphate (Agrocinopin C) and *bis*(D-glucos-2-yl) Phosphate (Agrocinopin D)

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SYNTHESIS OF D-GLUCOS-2-YL SUCROS-2-YL PHOSPHATE (AGROCINOPIN C) AND BIS(D-GLUCOS-2-YL) PHOSPHATE (AGROCINOPIN D)

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ABSTRACT

The synthesis of a substance with the revised structure proposed for agrocinopin C, D-glucos-2-yl sucros-2-yl phosphate, *via* the hydrogenphosphonate approach to phosphodiesters is presented. Agrocinopin D, related to agrocinopin C by the lack of the fructofuranosyl part of the sucrose moiety, is synthesized *via* direct formation of hydrogenphosphonate diesters from appropriately protected monosaccharides and phosphonic acid.

INTRODUCTION

Agrocinopin C and D belong to a class of compounds occuring in plant crown gall tumors. These tumors are induced by transfer of a DNA segment (T-DNA) from a plasmid in *Agrobacterium tumefaciens* bacteria to the plant cell where it is incorporated in the genome. The T-DNA codes for the production of agrocinopins and also uncontrolled cell growth.

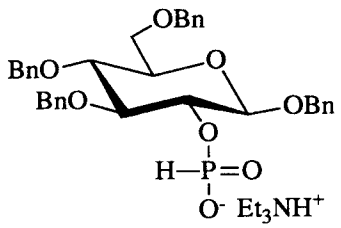
In a programme of synthesizing agrocinopins we have previously reported synthesis of agrocinopin A[1] and a phosphodiester corresponding to the first structural proposal of agrocinopin C[2], D-glucos-2-yl sucros-6-yl phosphate. The latter proved to be not identical to the native compound. A reinvestigation of the structure of the natural material indicated that the phosphate linkage to the sucrose part of the diester should be at O-2[3] instead of at O-6, as previously thought. Since agrocinopin D is related to agrocinopin C by the lack of the fructofuranosyl part of sucrose, this also means that the structure of agrocinopin D is revised. Thus, agrocinopin D is a symmetric phosphodiester in which O-2 of two D-glucose units are connected *via* a phosphate bridge.

The general strategy for the synthesis of agrocinopin C was to use the hydrogenphosphonate approach as in our previous syntheses of agrocinopin A and D-glucos-2-yl sucros-6-yl phosphate. A similar approach was chosen for the synthesis of agrocinopin D, but since this compound is a symmetrical phosphate the reaction conditions were modified to produce the hydrogenphosphonate diester directly by the condensation of phosphonic acid and appropriately protected monosaccharide derivatives.

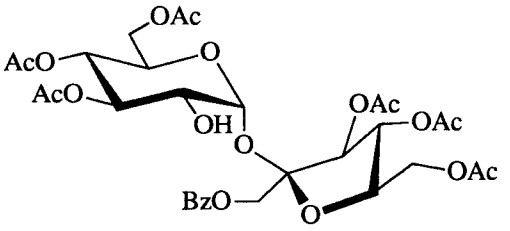
RESULTS AND DISCUSSION

For the synthesis of agrocinopin C the hydrogenphosphonate **1**[2] and the sucrose derivative **2**[4] were used. Condensation of these in pyridine, using pivaloyl chloride[5] as activating agent, gave the fully protected hydrogenphosphonate derivative, which was oxidized[6] *in situ* to give protected agrocinopin C (**3**) in 86 % yield after purification on a silica gel column. Deprotection was accomplished by Zemplén deacylation followed by hydrogenolysis at 400 kPa over palladium on charcoal to give almost pure deprotected phosphodiester. Purification on a Bio-Gel P-2 column gave 61% of **4**. The ^{13}C spectrum recorded for the synthetic product was in accordance with the one recorded for the natural product.

For the synthesis of agrocinopin D, an obvious alternative was the condensation of H-phosphonate **1** and its precursor benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside. However, since agrocinopin D is a symmetrical phosphodiester we looked for a way to create the phosphodiester linkage in one step from the alcohol. Hata *et al.*[7] has



1



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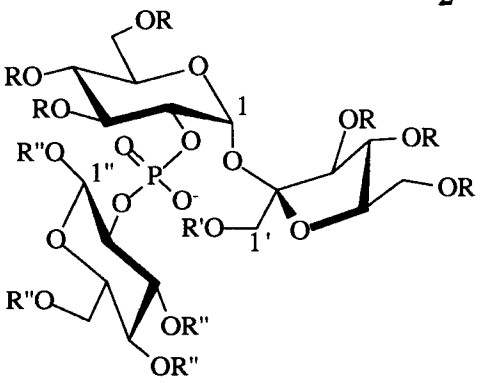
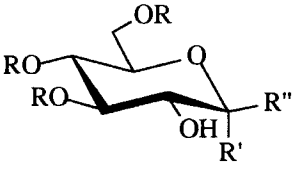


3 R=Ac, R'=Bz, R"=Bn
4 R=R'=R"=H

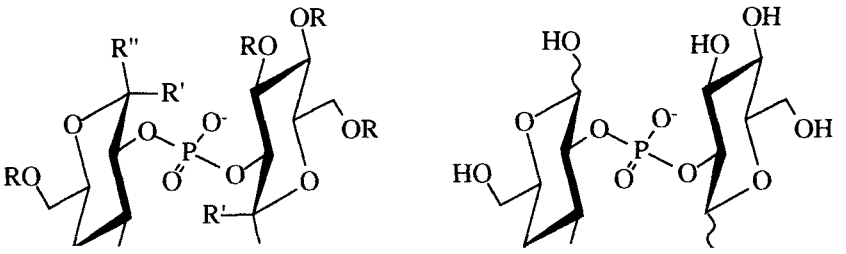
reported, in the search for a convenient preparation of nucleoside H-phosphonate monoesters, that reaction between a nucleoside and 3 equivalents of phosphonic acid in pyridine with pivaloyl chloride (6 eq.) produced a 3:2 mixture of H-phosphonate mono- and diesters. Furthermore, they found that treatment of a nucleoside with tribenzoyl phosphite in pyridine gave, as main product, the diester. Later, Stawinski and Thelin made a study on the acyl chloride activation of phosphonic acid.[8] They noted that on addition of 3 equivalents of pivaloyl chloride to an equimolar mixture of phosphonic acid and a partially protected nucleoside, the main product formed was the hydrogenphosphonate diester.



5 R=Bn, R'=H, R"=OBn
6 R=Ac, R'=OAc, R"=H

Based on these reports we reasoned that reaction of phosphonic acid with two equivalents of an alcohol and, at least, two equivalents of pivaloyl chloride in pyridine should constitute a useful preparative route to hydrogenphosphonate diesters. Indeed, this proved to be the case. Agrocinopin D was synthesised in two ways, using the tetrabenzyl[2] (**5**) and the tetraacetyl[9] (**6**) derivatives of D-glucose respectively. Treatment of these with 0.5 molar equivalents of phosphonic acid and 1.5 molar equivalents of pivaloyl chloride in dry pyridine followed by *in situ* oxidation by iodine in pyridine-water 98:2 yielded bis(benzyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranosid-2-yl) phosphate (**7**) and bis(1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranos-2-yl) phosphate (**8**) in 82% and 63%, respectively. We found two equivalents of the coupling agent to be insufficient to make the reaction go to completion. In our hands at least 2.5 eq. was needed. Since the coupling proceeded in less than 10 minutes we reasoned that the risk of side reactions were negligible, and thus a slight excess of reagent could be allowed.

Preparation of **9** was accomplished either from **7** by catalytic hydrogenolysis or by deacetylation of **8** with saturated methanolic ammonia. The former procedure gave virtually no byproducts according to TLC (isopropanol-pyridine-water 7:2:1) and the isolated yield after ion-exchange chromatography on a DEAE-Sephadex column, eluted with 0.08M NaCl, followed by desalination on a Bio-Rad P2 column, was 89%. Deacylation of **8** gave a less clean reaction and afforded **9** together with some byproducts with a higher mobility on TLC. This may be due to phosphate cleavage *via* a cyclic intermediate formed by attack from 1-OH when in an α-configuration. Isolated yield following the same purification procedure as above was 71%. Both routes gave material with identical ^{1}H and ^{13}C NMR spectra.



The mixture of isomers in solution due to the free anomeric centers of the glucose moieties in both agrocinopin C and D makes the NMR spectra of these compounds complex. Partial assignment of the signals has been made using 1D and 2D (H,H- and C,H-COSY) NMR techniques and by comparing shift values with those obtained for glucose, sucrose and monophosphorylated methyl α-D-mannopyranosides.[10]

EXPERIMENTAL

General methods. Concentrations were performed at 1-2-kPa at <40 °C (bath). Melting points are corrected. Optical rotations were recorded at room temperature for solutions in chloroform, unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal acetone δ_H 2.23 ppm and δ_C 31.0 ppm) using a JEOL JNM-GSX 270 instrument. For ^{31}P NMR 2% H_2PO_4 (δ 0.0 ppm) was used as the external reference. FAB-MS were recorded in the negative mode on a JEOL JMS-SX 102 mass spectrometer. TLC was performed on Silica Gel F_{254} (Merck) with detection by UV light when applicable and by charring with sulfuric acid. Column chromatography was performed on Matrex™ Silica Gel 0.035-0.070 mm (Amicon Corp).

Sodium (Benzyl 3,4,6-Tri-*O*-benzyl-β-D-glucopyranosid-2-yl) (3,4,6,3',4',6'-Hexa-*O*-acetyl-1'-*O*-benzoylsucros-2-yl) Phosphate (3). Compounds **1** (230 mg, 0.32 mmol) and **2** (228 mg, 0.32 mmol) were co-concentrated twice from dry pyridine (4 mL). The residue was dissolved in dry pyridine (4 mL) and pivaloyl chloride (98 μL, 0.8 mmol) was added. When the reaction was complete according to TLC (chloroform-methanol 3:1 and toluene-ethyl acetate 6:1), iodine (165 mg, 0.66 mmol) in pyridine-water (96:4, 4 ml) was added and the stirring continued for 15 min. The reaction mixture was then partitioned between ethyl acetate and saturated aqueous sodium bisulfite. The organic phase was washed with 2 M aqueous sulfuric acid, saturated aqueous sodium hydrogencarbonate, and water, dried over sodium sulfate, filtered, and concentrated. The residue was purified on a silica gel column eluted with chloroform-methanol 12:1. Fractions containing the phosphodiester were pooled, concentrated, dissolved in chloroform-methanol 1:1 and passed

through a Dowex-50(H^+) column followed by a Amberlyst IRC-50(Na^+) column. Concentration of the eluate gave **3** (378 mg, 86 %), $[\alpha]_D$ +6° (*c* 0.8).

Anal. Calcd for $C_{65}H_{72}NaO_{26}P$: C 59.0, H 5.5, P 2.3. Found: C 59.0, H 5.6, P, 2.2.

^1H NMR δ 3.50 (H-5, ddd, $^3J_{H-5,H-6}$ 2.7/3.5 Hz), 3.58 (H-4, dd, $^3J_{H-4,H-5}$ 9.5 Hz), 3.64 (H-6, m), 3.68 (H-3, dd, $^3J_{H-3,H-4}$ 8.3 Hz), 4.10 (H-6', m), 4.23 (H-5", m), 4.29 (H-2, dd, $^3J_{H-2,H-3}$ 8.4 Hz), 4.31 (H-5', m), 4.34 (H-6", m), 4.52 (H-2', dd, $^3J_{H-2',H-3'}$ 9.8 Hz), 4.58 (H-1, d, $^3J_{H-1,\ H-2}$ 7.1 Hz), 4.79 (H-1") 4.90 (H-4', dd, $^3J_{H-4',H-5'}$ 9.7 Hz), 5.40 (H-3', dd, $^3J_{H-3',H-4'}$ 9.9 Hz), 5.47 (H-4", dd, $^3J_{H-4",H-5"}$ 7.3 Hz), 5.66 (H-3", d, $^3J_{H-3",H-4"}$ 7.3 Hz), 5.78 (H-1', d, $^3J_{H-1',H-2'}$ 3.5 Hz)

^{13}C NMR δ 20.1, 20.2, 20.3, 20.3, 20.4, 20.6 (6x*C*H_3CO), 62.6 (C-6"), 64.2 (C-1', C-6'), 68.4 (C-5"), 68.6 (C-4"), 68.7 (C-6), 70.3 (Ph*C*H_2), 70.8 (C-3", $J_{C,P}$ 5.4 Hz), 71.8 (C-2", $J_{C,P}$ 4.8 Hz), 73.1 (Ph*C*H_2), 74.4 (C-5), 74.5, 74.7 (2xPh*C*H_2), 74.7 (C-4'), 75.4 (C-3'), 77.0 (C-2, $J_{C,P}$ 6.1 Hz), 77.4 (C-4), 78.5 (C-5'), 84.2 (C-3, $J_{C,P}$ 3.4 Hz), 91.0 (C-1", $J_{C,P}$ <1 Hz), 100.1 (C-1, $J_{C,P}$ 3.4 Hz), 103.0 (C-2'), 127.5-138 (Ar-*C*), 166.3 (Bz*C*O), 169.8, 170.2, 170.3, 170.7, 170.8, 171.1 (6xCH$_3$*C*O)

^{31}P NMR δ 0.48 ppm (t, J=9.2 Hz)

Sodium D-Glucos-2-yl Sucros-2-yl Phosphate (4). Compound **3** (50 mg) was dissolved in methanolic sodium methoxide (5 mL, 10 mM). After 5 h TLC (ethyl acetate-acetic acid-methanol-water 9:3:3:2) showed complete reaction and the solution was neutralized with Dowex 50(H^+). The resin was filtered off and the filtrate was concentrated to dryness. The residue was taken up in methanol-water (1:1, 5 mL) and Pd/C (10 mg, 10%) was added. The mixture was hydrogenolyzed at 400 kPa for 15 h. The catalyst was filtered off and the filtrate was concentrated. The residue was taken up in water and applied on a DEAE-Sephadex column (25x300 mm). The column was washed with 5 bed volumes of water, then eluted isocratically with 0.08 M NaCl. The fractions containing **4** were pooled and lyophilized, dissolved in 0.5 mL water and desalinated on a Bio-Rad P2 column (30x500 mm) to give **4** (14 mg, 61%), $[\alpha]_D$ +56.5° (*c* 1.0, water, equil.)

Anal. Calcd for $C_{18}H_{32}NaO_{19}P{\cdot}H_2O$: C 34.6, H 5.5. Found: C 34.2, H 5.1.

^{1}H NMR δ 3.40-3.56 (H-4α, H-4β, H-4"α, H-4"β, H-5"β), 3.60-3.78 (H-3"β, H-6'), 3.78-3.94 (H-1',H-2"β, H-3α, H-3"α, H-5', H-6, H-6"), 3.97-4.12 (H-2α, H-2"α, H-4'), 4.23 (H-3'), 4.76 (H-1"β), 5.42 (H-1"α), 5.57-5.58 (H-1).

^{13}C NMR δ 60.85, 61.27, 61.43, 61.72, 63.11 (primary carbons), 69.85, 70.01, 70.17, 70.36, 72.07, 72.22 ($^3J_{C,P}$ 5.0 Hz), 72.42 ($^3J_{C,P}$ 6.4 Hz), 72.98, 73.04, 74.63 (C-4'), 74.67, 75.37 ($J_{C,P}$ 6.0 Hz), 75.48, 75.91 (C-3"β, $^3J_{C,P}$ 3.2 Hz), 76.18 ($J_{C,P}$ 6.0 Hz), 76.46 (C-3'), 76.64 (C-5"β), 79.71 (C-2"β, $^2J_{C,P}$ 6.4 Hz), 82.20 (C-5'), 91.65 (C-1, $^3J_{C,P}$ 6.0 Hz), 91.68 (C-1"α, $^3J_{C,P}$ 5.0 Hz), 95.85 (C-1"β, $^3J_{C,P}$ 4.1 Hz), 104.67(C-2').

^{31}P NMR δ -0.59 (t, $J_{P,H}$ 8.8 Hz), -1.01 (t, $J_{P,H}$ 8.4 Hz) ratio 1:2.2

FAB-MS 583.08 $(M-Na)^-$ and 605.05 $(M-H)^-$.

Sodium Bis(benzyl 3,4,6-Tri-*O*-benzyl-β-D-glucopyranosid-2-yl) Phosphate (7). Benzyl 3,4,6-tetra-*O*-benzyl-β-D-gluco-pyranoside (**5**) (0.54 g, 1 mmol) and pyridinium phosphite (0.08 g, 0.5 mmol) were co-concentrated twice from dry pyridine. The residue was dissolved in dry pyridine (5 mL) and pivaloyl chloride (0.18 mL, 1.5 mmol) was added at room temperature. After one hour, iodine (0.25 g, 1 mmol) in pyridine-water (96:4, 5 mL) was added. After 15 min, the reaction mixture was partitioned between dichloromethane and saturated aqueous sodium bisulphite. The organic phase was washed with water, dried with sodium sulphate and concentrated. Purification on a silica gel column (chloroform/methanol 18:1) and consecutive ion exchanges on Dowex-50(H^+) and Amberlyst IRC-50(Na^+) columns in chloroform/methanol 3:1 gave pure **7** (0.48 g, 82 %), $[\alpha]_D$ -20° (*c* 1.1).

Anal. Calcd for $C_{68}H_{70}NaO_{14}P{\cdot}H_2O$: C 69.0, H 6.1, P 2.6. Found: C 68.9, H 6.0, P 2.6.

^{1}H NMR δ 3.35 (H-5), 3.45-3.58 (H-3, H-4, unresolved signals), 3.59-3.74 (H-6a, H-6b), 4.27-4.40 (H-1, H-2, unresolved signals) 4.48-5.13 (8H, 4xPhCH_2), 7.12-7.53 (20H, Ar-H).

^{13}C NMR δ 68.7 (C-6), 70.3, 73.1 (2xPhCH_2), 74.4 (C-5), 74.4, 74.6 (2xPhCH_2), 77.3 (C-4), 77.6 (C-2, $^2J_{C,P}$ 5,8 Hz), 83.8 (C-3, $^3J_{P,C}$ 3.3 Hz), 100.1 (C-1, $^3J_{P,C}$ 2.4 Hz), 127.3-128.2, 137.2-138.5 (Ar-C).

^{31}P NMR δ -2.1.

Sodium Bis(1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranos-2-yl) Phosphate (8). 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (**6**) (0.35 g, 1

mmol) was treated in the same way as **5**. Purification on a silica gel column (chloroform/methanol 9:1) and consecutive ion exchanges on Dowex-50(H^+) and Amberlyst IRC-50(Na^+) columns in chloroform/methanol 1:1 gave **8** (0.27 g, 63 %), $[\alpha]_D$ +85° (c 1.3).

Anal. Calcd for $C_{28}H_{38}NaO_{22}P{\cdot}H_2O$: C 42.1, H 5.0, P 3.9. Found: C 42.0, H 4.6, P 3.5.

^1H NMR δ 2.02, 2.06, 2.09, 2.20 (12H, 4xCH_3CO), 4.03-4.12 (H-5, H-6a), 4.18-4.28 (H-2, H-6b), 5.06 (H-4, $^3J_{H\text{-}4,H\text{-}5}$ 9.7 Hz), 5.36 (H-3, $^3J_{H\text{-}3,H\text{-}4}$ 9.6 Hz), 6.44 (H-1, $^3J_{H\text{-}1,H\text{-}2}$ 2.6 Hz).

^{13}C NMR δ 20.3, 20.3, 20.5, 20.7 (4xCH_3CO-), 61.7 (C-6), 67.9 (C-4), 69.6 (C-5), 70.7 (C-3, $^3J_{C,P}$ 6.4 Hz), 71.3 (C-2, $^2J_{C,P}$ 4.6 Hz), 90.3 (C-1), 169.7, 170.0, 170.8, 171.0 (4xCH_3CO-).

^{31}P NMR δ -0.2.

Sodium Bis(D-glucos-2-yl) Phosphate (9).

A. Compound **7** (100 mg) was dissolved in 10 mL of ethyl acetate-ethanol-water 1:3:1 and hydrogenolysed at 400 kPa over 10 mg Pd/C (10%). After 12 h the catalyst was filtered off and the filtrate concentrated to dryness. The residue was purified as described for **4** to give 34 mg (89%) of **9**.

B. Compound **8** (42 mg) was dissolved in 10 mL of saturated methanolic ammonia and left at 5 °C for 24 h. TLC showed complete reaction accompanied with some faster moving byproducts. The product was concentrated and purified as described above to give 17 mg (71%) **9**, $[\alpha]_D$ +51.4° (*c* 1.1, water, equil.).

Anal. Calcd for $C_{12}H_{22}NaO_{14}P{\cdot}H_2O$: C 31.2, H 5.2. Found: C 30.9, H 4.8.

^1H NMR δ 3.42-3.50 (H-4α, H-4β, H-5β), 3.60-3.90 (H-2β, H-3α, H-3β, H-5α, H-6α, H-6β), 4.00-4.09 (H-2α), 4.73 (H-1β, $^3J_{H,H}$ 7.9 Hz), 5.40 (H-1α, $^3J_{H,H}$ 3.7 Hz)

^{13}C NMR δ 61.46, 61.58 (C-6α, C-6β) 70.26, 70.32, 70.40, 70,48 (C-4α, C-4β), 72,15 (C-5α), 72.48, 72.56 (C-3α), 75.96, 76.00, 76.04 (C-3β), 76.29, 76.38, 76.41, 76.50 (C-2α), 76.69 (C-5β), 79.58, 79.66, 79.75 (C-2β), 91.69, 91.73, 91.77 (C-1α), 95.84, 95.90 (C-1β)

^{31}P NMR δ -0.03 (t, $^3J_{P,H}$ 8.8 Hz), -0.50 (t, $^3J_{P,H}$ 8.7 Hz), -0.80 (t, $^3J_{P,H}$ 8.4 Hz), ratio 1:5.4:6.6

FAB-MS 421.04 $(M\text{-}Na)^-$ and 443.01 $(M\text{-}H)^-$.

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