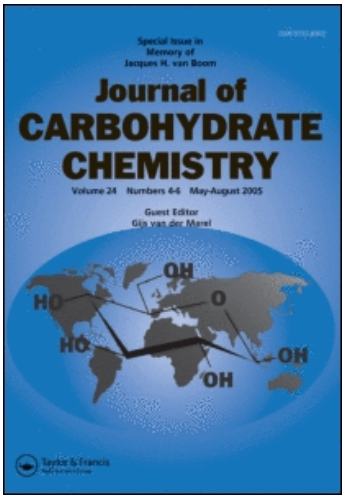

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SYNTHESIS OF SUCROSE 4'-(L-ARABINOSE-2-YL PHOSPHATE) (AGROCINOPINE A) USING AN ARABINOSE 2-H-PHOSPHONATE INTERMEDIATE

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ABSTRACT

The first completed chemical synthesis of Agrocinopine A (5) is presented. The key step in the synthesis was the pivaloyl chloride promoted condensation of a blocked arabinose 2-H-phosphonate derivative 3 with a protected sucrose derivative 1 having a free hydroxyl at 4'-OH. The resulting H-phosphonic acid diester was oxidised *in situ* with iodine to produce the corresponding phosphodiester 4, which was subsequently deblocked to give Agrocinopine A.

INTRODUCTION

Opines are compounds found specifically in plant crown gall tumors. These tumors are induced by *Agrobacterium tumefaciens* bacteria carrying a tumor-inducing (Ti) plasmid. The bacteria transfer a small segment of their Ti-plasmid DNA into the plant cells. This T-DNA is stably incorporated into the plant cell genome, and it codes for uncontrolled plant cell growth and production of opines. The inciting bacteria can utilize the opines as a nutrient source, because the non-transferrable plasmid DNA, which is exterior to the transferred T-DNA, also codes for the opine catabolism genes. Thus, the bacteria

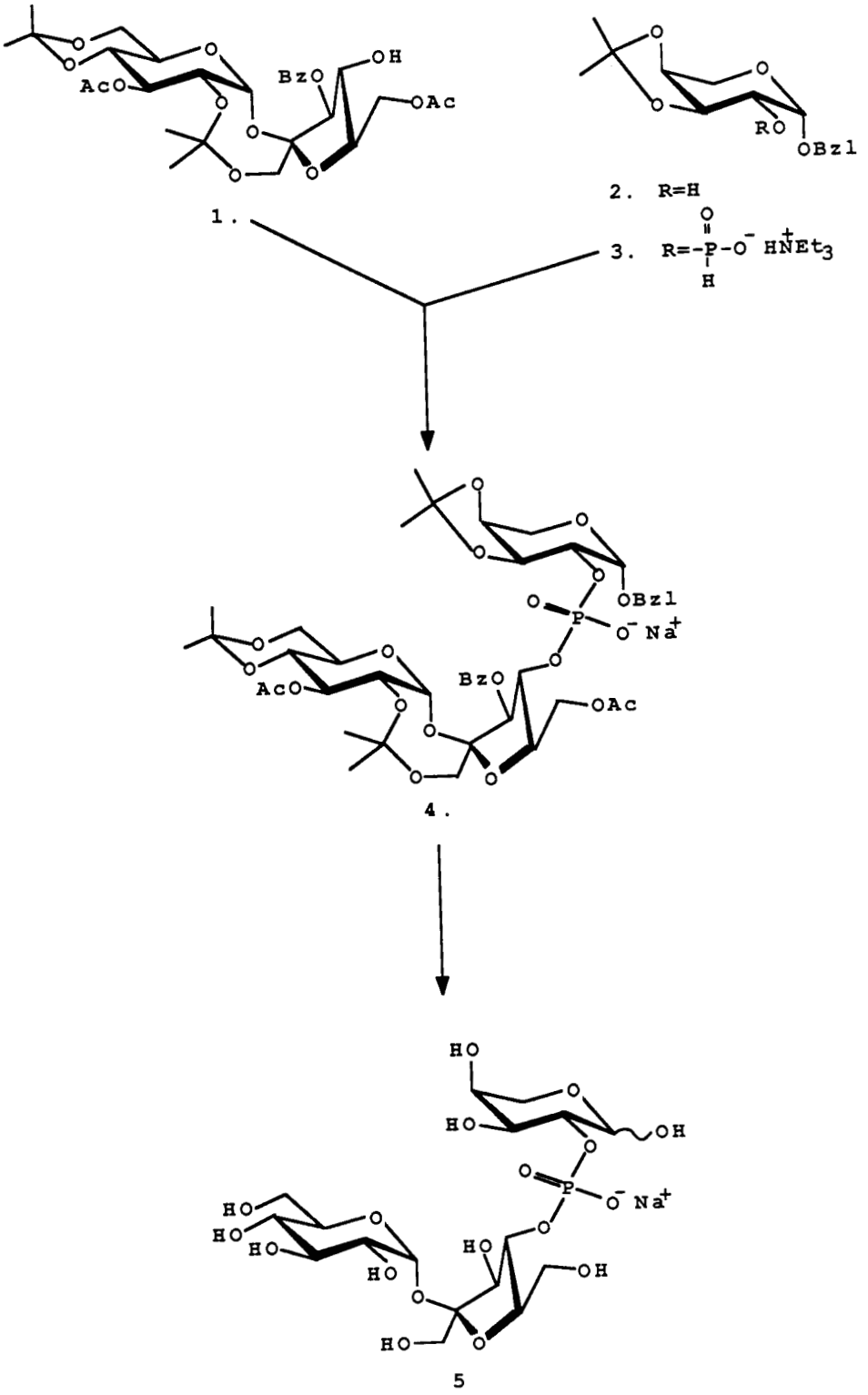
induce the plant biosynthetic machinery to produce nutrients they alone can use, thereby occupying a unique ecological niche.

Several different chemical classes of opines have been described[1,2]. Agrocinopine A is a sucrose arabinose phosphodiester with the structure[3] 5. We now report the first complete chemical synthesis of Agrocinopine A. The synthesis follows a route which differs from the recently reported[4] preparation of a protected Agrocinopine A derivative.

RESULTS AND DISCUSSION

The strategy adopted for synthesis of Agrocinopine A was to prepare protected arabinose and sucrose derivatives having free hydroxyls in the 2 and 4' positions, respectively, and then, using these derivatives and the phosphonate[5,6] coupling method, to create a phosphodiester which upon deprotection should give Agrocinopine A.

The sucrose synthon chosen was compound 1, prepared[7] in four steps from sucrose. Compound 2 was used as the arabinose synthon, and was prepared in two steps from L-arabinose using the sequence published[8] for the preparation of the corresponding D-compound. The H-phosphonic acid monoester 3 was prepared in 80 % yield from 2 by treatment[9] with a mixture of phosphorous trichloride, triethylamine, and imidazole in acetonitrile. The reason for preparing the monoester from the arabinose rather than from the sucrose synthon was that the latter was available in smaller quantities and it was therefore desireable to use it as late as possible in the synthetic sequence. Condensation[10] of 1 with 3 in pyridine using pivaloyl chloride as condensing agent gave a H-phosphonic acid diester, which was oxidised[11] in situ with iodine in pyridine-water to give the corresponding phosphodiester 4 in 80 % yield. Deprotection of 4 was accomplished by treatment with, successively, 60% aqueous acetic acid, methanolic sodium methoxide, and hydrogen/palladium on charcoal. The yield of the sodium salt of Agrocinopine A (5) after purification by ion exchange chromatography, gel filtration and C-18 Sep-Pak treatment was 70 %. The ^{13}C and ^{1}H NMR spectra of the synthetic product agreed well with that of material[3,12] isolated from natural sources. The synthetic material also showed the expected biological activity in the agrocin 84 assay[2].



EXPERIMENTAL

General procedures. Concentrations were performed at 1-2 kPa at <40°C (bath). Optical rotations were recorded at 22-24°C for 0.5-1 % solutions in chloroform, unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si, δ = 0.00) unless otherwise stated, using JEOL JNM-GSX 270 or Bruker AM 500 instruments. NMR spectra were invariably in agreement with the postulated structures, and only selected data are given. TLC was performed on Silica Gel F_{254} (Merck) with detection by U.V. light when applicable or by spraying with 8 % aqueous sulfuric acid and charring. Column chromatography was performed on Matrex™ Silica gel 60 (0.035-0-070 mm, Amicon Corp.) with loadings in the range 1/25 - 1/100 and elution with suitable solvent mixtures. Organic solutions were dried over Na_2SO_4 prior to concentrations.

Benzyl 3,4-O-isopropylidene-β-L-arabinopyranoside 2-hydrogenphosphonate, triethylammonium salt (3). Imidazole (0.485 g) was dissolved in acetonitrile (12.5 mL) and the solution was kept on an ice-water bath. Phosphorous trichloride (0.187 ml) was added, followed by triethylamine (1.05 mL). The mixture was stirred for 15 min, then benzyl 3,4-O-isopropylidene-β-L-arabinopyranoside[8] (2, 0.140 g) in acetonitrile (12.5 mL) was added during 15 min, and the mixture was stirred for two hours at room temperature. Water (3 mL) was then added, and the clear solution was stirred for another 30 min. The solution was concentrated and then partitioned between chloroform and water. The aqueous phase was washed several times with chloroform, the combined organic phases were washed once with water, then dried and concentrated. Purification of the residue by column chromatography (90:10:1 chloroform - methanol - triethylamine as eluant) gave pure 3 (0.183 g, 82 %), $[\alpha]_D$ +135°; ^1H NMR δ 3.97 (d, 2H, H-5a, H-5b), 4.23 (m, 1H, H-4), 4.33 (m, 2H, H-2, H-3), 4.53, 4.75 (2 doublets, 2H, J 11.9 Hz, $PhC\underline{H}_2$), 5.10 (d, 1H, J 2.8 Hz, H-1), 6.98 (d, 1H, J 639.3 Hz, H-P); ^{13}C NMR δ 8.6 ($\underline{C}H_3$-CH_2-N), 26.4, 28.1 ($\underline{C}H_3$-C), 45.6 (CH_3-$\underline{C}H_2$-N), 59.1 (C-5), 69.7 ($Ph\underline{C}H_2$), 72.8 (d, J 5.5 Hz, C-2), 73.7 (C-4), 74.8 (d, J 4.6 Hz, C-3), 97.7 (d, J 1.8 Hz, C-1), 109.0 (CH_3-$\underline{C}$).

3,6'-Di-O-acetyl-3'-O-benzoyl-1,2':4,6-di-O-isopropylidene-sucrose 4'-(1-O-benzyl-3,4-O-isopropylidene-β-L-arabinopyranos-2-yl phosphate), sodium salt (4). A mixture of compounds 1 (0.730 g)[7] and 3 (0.477 g) was concentrated twice from pyridine (30 mL) solution to remove moisture, then dissolved in pyridine (20 mL). Pivaloyl chloride (0.33 mL) was added to the stirred solution. When TLC indicated that all 3 had been consumed (10 min), iodine (0.41 g) in 95:5 pyridine-water (20 mL) was added. After stirring for another 10 min, the mixture was partitioned between ethyl acetate and saturated aqueous sodium bisulphite. The organic phase was washed with 2 M aqueous sulfuric acid, saturated aqueous sodium bicarbonate solution, and water. Drying and concentration left a syrup, which was purified by column chromatography (4:1 ethyl acetate-methanol as eluant) to give pure 4 (0.814 g, 78 %), $[\alpha]_D$ +68°; NMR data ($(CD_3)_2SO$: D_2O : $CDCl_3$, 4:1:4, $(CD_3)_2SO$ = δ_H 2.525 and δ_C 38.96): ^1H, δ 1.06, 1.17, 1.19, 1.27, 1.35, 1.44 (CH_3-C), 1.89, 1.94 (CH_3CO), 3.48 (d, J 12.3 Hz, F1), 3.67 (dd, $J_{1,2}$ 3.6, $J_{2,3}$ 9.2 Hz, G2), 4.06 (d, F1'), 4.46 (d, J 12.0 Hz, $PhCH_2$), 4.55 (ddd, $J_{4,5}$ 2.2, $J_{5,6}$ 4.0, $J_{5,6'}$ 9.8 Hz, F5), 4.57 (d, $PhCH_2$), 4.75 (dd, $J_{2,3}$ 9.2, $J_{3,4}$ 9.4 Hz, G3), 4.78 (ddd, $J_{3,4}$ 2.7, $J_{4,5}$ 2.2, $J_{4,P}$ 6.8 Hz, F4), 5.08 (d, $J_{1,2}$ 2.1 Hz, A1), 5.20 (d, $J_{3,4}$ 2.7 Hz, F3), 5.90 (d, $J_{1,2}$ 3.6 Hz, G1); ^{13}C, δ 18.5, 20.3 ($\underline{C}H_3CO$), 23.4, 25.0, 25.8, 27.6, 28.4 ($\underline{C}H_3C$), 58.1, 60.8, 63.2, 64.0, 66.1, 69.1, 70.1, 70.4, 71.0, 72.8, 73.4 (d, J 5.1 Hz), 74.1 (d, J 6.8 Hz), 78.3, 79.5 (d, 5.3 Hz), 81.7 (d, 3.3 Hz) (F1, F3-F6, A2-A5, G2-G6), 91.2 (G1), 97.0 (A1), 98.5, 100.5, 104.7, 108.0 (F2, CH_3-$\underline{C}$), 164.9 (PhCO), 169.0, 170.4 ($CH_3\underline{C}O$).

Anal. Calcd for $C_{44}H_{56}O_{21}PNa \times H_2O$: C, 53.2, H, 5.9. Found: C, 53.1, H, 5.9.

Sucrose 4'-(L-arabinose-2-yl phosphate) (Agrocinopine A), sodium salt (5). A solution of 4 (0.165 g) in 60 % aqueous acetic acid (17 mL) was heated to 50°C for 15 min, then concentrated. The residue was dissolved in methanol (17 mL) and 1 M methanolic sodium methoxide (0.16 mL) was added. When TLC indicated complete reaction, the mixture was neutralized with dry ice and then concentrated. The residue was dissolved in water (20 mL) and Pd/C (10 %, 70 mg) was added. The mixture was hydrogenated at room temperature and athmospheric pressure

for 24 hours, then filtered and concentrated. The residue was dissolved in water (5 mL) and applied to a column of DEAE-Sepharose (2.5 x 35 cm). The column was eluted with a linear gradient of 0-0.5 M sodium chloride (1000 mL total). Fractions containing 5 were concentrated and the residue was applied to a Bio-Gel P2 column (2.5 x 35 cm). Elution with water gave fractions containing 5, which were pooled and concentrated. The residue was taken up in water (1 mL) and the solution was passed through a Sep-Pak™ (1 x 1 cm, Waters Associates) column to remove hydrophobic impurities, the eluate was concentrated to give pure 5 (66 mg, 67 %), $[\alpha]_D$ +50° (water). Ryder et. al.[3] reports $[\alpha]_D$ +47.9° for natural Agrocinopine A. The ^{13}C and ^{1}H NMR spectra of the synthetic material were identical to those reported[3,12].

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