Two novel acyl sucroses from *Petunia nyctaginiflora*

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Two acyl sucroses, isolated from *Petunia nyctaginiflora* Jussieu (Solanaceae) have been characterized chemically as 2,3,4-tri(5-methylhexanoyl)-α-D-glucopyranosyl-β-D-6-acetyl fructofuranoside 4 and 2,3,4-tri(6-methylheptanoyl)-α-D-glucopyranosyl-β-D-6-acetyl fructofuranoside 6, on the basis of chemical and spectroscopic evidence.

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*Petunia nyctaginiflora* (Solanaceae) is one of the few sources of acyl sucroses 1. Our interest in these compounds is due to the significant biological activities attributed to them 2-5. Previous investigation on *P. nyctaginiflora* resulted in the isolation and characterization of cyclolanosterol 6 and acyl sucroses 7.

Chromatography of the concentrated aqueous methanol soluble portion obtained from the fractionation of *n*-hexane extract of the aerial parts of *P. nyctaginiflora*, afforded a TLC homogenous viscous liquid, designated as PNA. The IR spectrum of PNA exhibited broad absorption bands at 3600 and 3500 and a strong absorption band at 1745 cm\(^{-1}\) inferring the presence of hydroxyl and ester carbonyl groups in the molecule. Examination of 1H NMR spectrum led to the identification of oxymethylene and oxymethine hydrogens showing signals spreaded over δ 3.50 and 5.60 along with one proton doublet at δ5.65 (J = 3.5 Hz) presumably due to an equatorial anomeric hydrogen of a sugar molecule. A singlet at δ 2.12 (3H), for low field methyl group confirmed the presence of an acetoxy group in the molecule. Also, the presence of a broad envelope of signal between δ 2.2 and δ 2.5 assignable to methylene and ketomethylene groups made us to assume that the signals were associated with long chain acyl function.

The IR and 1H NMR spectral data of PNA showed significant resemblance with those of two recently reported acyl sucrose derivatives viz., 2, 3, 4-tri (5-methylhexanoyl) sucrose 1 and 2,3,4-tri (6-methylheptanoyl) sucrose 2, designated as PNB I and PNB II respectively 7. The characterization of PNA was arrived on the basis of comparison of spectral data of PNB.

The 1H NMR spectrum of PNA acetate, prepared by acetylation of PNA with acetic anhydride-pyridine, was found to be virtually identical with that of PNB acetate. As a signal for an acetoxy methyl was originally present in the 1H NMR spectrum of PNA, it was concluded that, PNA is a monoacetyl derivative of PNB.

The position of the ester group was settled by studying 500 MHz 1H NMR spectrum of PNA, which showed the anomeric hydrogen signal at δ 5.65 (1H, d, J = 3.5 Hz) and three oxymethine hydrogen signal at a relatively lowfield at δ 4.94, 5.14, 5.54 (1H each, -CHO-CO-,), giving the proof for the presence of the fourth ester group on any of the primary hydroxyl groups, which is obviously the acetate function. Out of the three primary alcohols in the sucrose molecule (C-1’-H2 and C-6’-H2 of fructofuranoside and C-6-H2 of glucopyranoside), C-1’-H2 was excluded as the spectrum showed signals for geminally coupled AB quartet at δ 3.62, 2H.

Finally, the exact position of acetate group was assigned by comparing the H-H COSY spectrum of PNA and PNB. The signals for C-6’-H2 and C-6-H2 in PNA appeared at δ 4.34 and 3.78 respectively, but those were discerned around δ 3.86 (brm, 4H) along with signals for other carbinyl hydrogens in the spectrum of PNB. A downfield shift of 0.5 ppm was observed for C-6’-H2 which undoubtedly made us to assign the acetate group at C-6’-H2 in the molecule.

Once the gross structure of PNA was settled, its purity was verified by HPLC analysis of its benzoate derivative and it was found to be a mixture of several compounds, like its congener PNB 7. Purification of
PNA benzoate was achieved by use of reverse phase silica column (RP-18) and methanol as eluent. The fractions corresponding to peaks showing Rt 16.5 min and 18.0 min were collected separately and labeled respectively as PNA benzoate I and PNA benzoate II.

PNA benzoate I (C₆₃H₇₆O₁₉), was obtained as amorphous powder and it showed a distinct ester carbonyl band at 1745 cm⁻¹ in its IR spectrum. Alkali hydrolysis of PNB benzoate I, furnished sucrose (identified by direct comparison) and a mixture of two acids, which on treatment with p-bromophenacyl bromide and triethyl amine and subsequent separation of the resultant product mixture by HPLC, yielded p-bromophenacyl benzoate and an ester which was identified as p-bromophenacyl ester of 5-methyl-hexanoic acid from comprehensive spectral analysis. The structure of PNA benzoate I was thus settled as 3 and the corresponding natural product PNA I as 4.

Similar alkali hydrolysis of PNB benzoate II (C₆₆H₈₂O₁₉), yielded sucrose and a mixture of acids, treatment of which with p-bromophenacyl bromide yielded, in addition to the expected p-bromophenacyl benzoate, a p-bromophenacyl ester of 6-methylheptanoic acid. The latter compound was fully characterized from comprehensive spectral analysis as well as by comparison with authentic sample7. The structure of PNA benzoate II was thus formulated as 5 and the corresponding natural product PNA II as 6.

Experimental Section

General procedure. Melting points are uncorrected. IR spectra were recorded in KBr. ¹H NMR spectra were obtained from a Jeol GSX-500 spectrometer with TMS as internal reference. Mass spectra were measured on a Jeol JMS-SX, direct inlet system. HPLC was carried out using a reverse phase (ODS) Shim-Pack column and UV detector.

Plant Material. The epigeal part of Petunia nyctaginiflora Juss were collected from the campus of Banaras Hindu University, Varanasi. A specimen of the plant material has been preserved in the Department of Medicinal Chemistry, IMS, Banaras Hindu University, Varanasi, India.

Extraction and Isolation. Chromatography of the aqueous methanol soluble portion of n-hexane extract of P. nyctaginiflora, (air dried aerial part (5 kg); silica gel column; elution with benzene-ethyl acetate (6:4)), yielded PNA (10 g) as waxy solid. IR: 3600, 3510 and 1745 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 5.65 (d, 1H, J = 3.5 Hz, anomeric-H), 5.55 (m, 1H,-CHO-), 5.14 (m, 1H, −CHO), 4.94 (dd, 1H, J = 10.2 and 3.9 Hz, −CHO), 4.34 (m, 2H,-CH₂O), 4.25 (br m, 3H, 3 ×−CHOH), 3.94 (m, 1H,−CHOH), 3.78 (m, 2H, -CH₂O-), 3.62 (dd, 2H, J = 12.2 Hz, 1′-H), 2.30 (m, 6H, −COCH₂), 2.12 (s, 3H, −OCOCH₃), 1.25 (br, methylenes), 1.10-0.88 (m, 18H, secondary-CH₃).

PNA tetraacetate. A mixture of PNA (0.2 g), Ac₂O (1.0 mL) and C₅H₅N (0.5 mL) was kept overnight under anhydrous condition. The PNA tetraacetate obtained was identical in all respect with PNB pentaacetate7.

PNA tetrabenzoate. A mixture of PNA (0.2 g), benzyol chloride (3.0 mL) and pyridine (2.0 mL) was kept overnight under anhydrous condition. PNA tetrabenzoate was obtained as waxy solid (0.25 g) after usual work-up and chromatography over silica gel column.

PNA benzoate I (3) and PNA benzoate II (5). PNA benzoate (0.2 g) was dissolved in methanol (2 mL) and was purified by reverse phase Shim-pack silica gel column (RP-18). The detector used was UV (254 nm), flow rate 6 mL/min, with MeOH as eluent. HPLC chromatogram showed two prominent peaks along with several small peaks. The fractions corresponding to peaks showing retention time 16.5 min and 18.0 min, were collected separately and designated as PNA benzoate I (3) and PNA benzoate II (5) respectively. Removal of the solvents under reduced pressure afforded 3 (43 mg) and 5 (36 mg) as waxy solid.

1 R =-(CH₂)₃-CH(CH₃)₂; R₁ = H; R₂ = H
2 R =-(CH₂)₄-CH(CH₃)₂; R₁ = H; R₂ = H
3 R =-(CH₂)₃-CH(CH₃)₂; R₁ =-COC₆H₅; R₂ =-COCH₃
4 R =-(CH₂)₃-CH(CH₃)₂; R₁ = H; R₂ =-COCH₃
5 R =-(CH₂)₄-CH(CH₃)₂; R₁ =-COOC₆H₅; R₂ =-COCH₃
6 R =-(CH₂)₄-CH(CH₃)₂; R₁ = H; R₂ =-COCH₃

NOTES
4.36 (dd, 1H), 4.08 (br, 3H), 2.15 (t, 6H), 2.12 (s, 3H), 1.53 (br m, 3H), 1.25 (br s, 12H), 1.12 (d, 18H, \( J = 6.7 \) Hz); Anal. Found: C, 66.21; H, 6.80. Calcd for C_{63}H_{76}O_{19}: C, 66.55; H, 6.69%.

**PNA benzoate II (5).** \( \alpha \)[D] +69.4° (c, 0.25, CHCl_3); 1H NMR (CDCl_3, 270 MHz): \( \delta \) 8.04 (m, 8H), 7.45 (m, 12H), 6.01 (d, 1H), 5.92 (m, 1H), 5.85 (m, 1H), 5.16 (m, 1H), 4.93 (dd, 1H), 4.63 (m, 5H), 4.36 (dd, 1H), 4.07 (br, 3H), 2.15 (t, 6H), 2.12 (s, 3H), 1.50 (br m, 3H), 1.25 (br s, 18H), 1.12 (d, 18H, \( J = 6.7 \) Hz); Anal. Found: C, 67.98; H, 7.03. Calcd for C_{66}H_{82}O_{19}: C, 67.23; H, 6.96%.

**Basic hydrolysis of PNA benzoate I (3).** A solution of PNA benzoate I (20 mg) in 5 mL of 10 % methanolic KOH was refluxed for 1 hr. It was neutralized with dil. HCl and diluted with H_2O (5 mL). The resultant solution was extracted with Et_2O (3 \times 10 mL) and then methanol was evaporated under reduced pressure. The concentrated solution was then extracted with \( n \)-BuOH and identified as sucrose, C_{12}H_{22}O_{11}, m.p. 183-185°C. The ether soluble portion was dried and evaporated to give acid mixture.

The acetonic solution of the above acid mixture (7 mg in 2 mL) was added to a solution of \( p \)-bromophenacyl bromide (35 mg) and triethyl amine (2 drops) in acetone (2 mL). The resultant mixture was stirred at 50°C for 0.25 hr. The acetone was evaporated and the residue was passed through silica-gel. The solvents of combined eluates were evaporated under reduced pressure. The residue was purified by prep-HPLC using CH_3CN-H_2O (9:1), as a mobile phase on a reverse phase Shim-pack silica column, flow rate 6 mL/min, detector used was UV, wavelength 254 nm. The fractions corresponding to peaks A and B, retention time 5.3 and 7.1 min respectively, were collected separately. Removal of solvent obtained from peak A yielded a residue (6 mg), which was identified as \( p \)-bromophenacyl benzoate, C_{15}H_{11}BrO_{3}; MS: m/z at 318/320 (M+), 196/198, 183/185, 155/157, 105, 77, 51, 50.

Evaporation of solvent obtained from peak B, Rt 7.1 min, yielded a solid (2.1 mg), which was characterized as \( p \)-bromophenacyl ester of 5-methylhexanoic acid, C_{15}H_{19}BrO_{3}, IR: 3030, 2960, 2930, 2060, 1742, 1703, 1595, 1412, 1400 cm\(^{-1}\);

\( ^1 \)H NMR (CDCl_3, 500 MHz): \( \delta \) 7.77 (d, 2H, \( J = 8.3 \) Hz, Ar-H), 7.63 (d, 2H, \( J = 8.3 \) Hz, Ar-H), 5.28 (s, 2H, ArCOCH_2), 2.47 (t, 2H, \( J = 7.4 \) Hz, 2-H_2), 1.68 (quintet, 2H, \( J = 7.4 \) Hz, 3-H_2), 1.53 (m, 1H, \(-CH(CH_3)_2\)), 1.36 (m, 2H), 1.22 (2H), 0.88 (d, 6H); MS: m/z at 340/342, 256/258, 198/200, 183/185, 169/171, 155/157, 143, 127, 109, 78, 57, 43, 41, 29.

**Basic hydrolysis of PNA benzoate II (5).** The same procedure was followed as before which yielded \( p \)-bromophenacyl benzoate, C_{15}H_{11}BrO_{3}, M+ at m/z 318/320 (Rt 5.3 min). The eluates corresponding to Rt 7.9 min was then characterized as \( p \)-bromophenacyl ester of 6-methylheptanoic acid, C_{16}H_{21}BrO_{3}. 1H NMR (CDCl_3, 500 MHz): \( \delta \) 7.77 and 7.63 (2H each), 5.28 (s, 2H), 2.47 (t, 2H), 1.68 (quintet, 2H), 1.53 (m, 1H), 1.36 (m, 2H), 1.22 (2H), 0.88 (d, 6H); MS: m/z at 340/342, 256/258, 198/200, 183/185, 169/171, 155/157, 143, 127, 109, 83, 76, 57, 55, 43, 41, 29.

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**References**