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Two acyl sucroses from *Petunia nyctaginiflora*

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Abstract

Two new acyl sucroses were isolated from the epigeal parts of *Petunia nyctaginiflora* Juss. (Solanaceae). Their structures were determined to be 2, 3, 4-tri (5-methylhexanoyl)-α-D-glucopyranosyl-β-D-fructofuranoside (**2**) and 2, 3, 4-tri (6-methylheptanoyl)-α-D-glucopyranosyl-β-D-fructofuranoside (**4**) on the basis of chemical and spectroscopic evidence.

Keywords: *Petunia nyctaginiflora*; Solanaceae; Acyl sugars; Acyl sucroses; 2, 3, 4-tri (5-methylhexanoyl)-α-D-glucopyranosyl-β-D-fructofuranoside and 2, 3, 4-tri (6-methylheptanoyl)-α-D-glucopyranosyl-β-D-fructofuranoside

1. Introduction

Petunia nyctaginiflora Juss. (Family: Solanaceae) is an ornamental garden plant, available in India and Argentina (Oommachan, 1997). Phytochemical investigation on several *Petunia* species resulted in the isolation of a class of steroids known as petuniasterones (Elliger et al., 1988) and petuniolides (Elliger et al., 1990). However, no phytochemical work on Indian *Petunia* species, particularly on *Petunia nyctaginiflora* has yet been reported. Also, *P. nyctaginiflora* is reported to exhibit anti-stress (Padma et al., 1998a) and anti-Herpes simplex virus (Padma et al., 1998b) activities. In view of these facts and as a part of continued search for bioactive natural products, the chemical investigation of the epigeal part of *Petunia nyctaginiflora* was undertaken and two new acyl sucroses were isolated. The occurrence of glucose and sucrose esters are quite common in the members of family solanaceae. It is believed that, these esters, by virtue of their antibiotic (Chortyk et al., 1997), insect repellant (King et al., 1988, 1993), aphid resistant (Neal et al., 1990), and antifungal properties (Holley et al., 1987), protect the plants from insect predators and fungal infections.

2. Results and discussion

The *n*-hexane extract of the epigeal part of *Petunia nyctaginiflora* was partitioned with aqueous methanol (80%) and the aqueous methanol layer was concentrated and chromatographed over a bed of silica gel to afford a TLC- homogeneous viscous liquid, designated PNB. A well-dried sample of PNB showed, in its IR spectrum, broad bands at 3600 and 3510 cm^{-1} and a strong absorption band at 1745cm^{-1} indicating the presence of hydroxyl and ester carbonyl groups in the sample. The ^1H NMR spectrum of the sample showed signals for a number of oxymethylene and oxymethine hydrogens between δ3.5 and δ5.65 including a one-proton doublet at δ5.65 ($J = 3.5$ Hz), presumably due to an equatorial anomeric hydrogen of a sugar molecule. The spectrum also showed a broad envelope of signals between δ 2.2 and δ 2.5, assignable to methylene and keto-methylene groups and it was conjectured that the signals are associated with a long chain acyl function.

Acetylation of PNB with Ac_2O–pyridine, at room temperature yielded a product, the IR spectrum of which was devoid of any band in the hydroxyl region indicating acylable nature of the hydroxyl groups in PNB and complete acetylation of these hydroxyl groups under the reaction condition. The ^1H NMR spectrum of PNB acetate showed signals for five acetoxy methyl groups at δ 2.17 (6H), 2.12 (3H) and 2.10 (3H) which, in turn, indicated the presence of five acylable hydroxyl

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groups in PNB. The signals for one anomeric hydrogen and of a number of oxymethine and oxymethylene hydrogens in the ^1H NMR spectra of PNB and its acetate derivative indicated PNB to be a sugar derivative.

Basic hydrolysis of PNB and subsequent work-up furnished an oily liquid, presumably a mixture of fatty acids, from the less polar fraction and a sugar from the polar fraction. The sugar was identified as sucrose [$C_{12}H_{22}O_{11}$; mp 183–185 °C, $[\alpha]_D + 66.1^0$ (H_2O); octa-acetate, $C_{28}H_{38}O_{19}$ mp 88 °C, $[\alpha]_D + 58.3°$ (C_2H_5OH)] by direct comparison with authentic sample of sucrose and its octa-acetyl derivative. The isolation of sucrose by alkali hydrolysis of PNB and the formation of a penta acetate derivative of PNB, taken in conjunction with the observed hydroxyl and ester bands in the IR spectrum of the compound clearly suggest that PNB is a sucrose molecule with three of its eight hydroxyl groups remaining as esters. This assumption gained credence from an analysis of 500 MHz ^1H NMR spectrum of PNB which showed the anomeric hydrogen signal at δ 5.65 (1H, *d*, $J = 3.4$ Hz) and three oxymethine hydrogen signals at a relatively lower field at δ 4.95, 5.16 and 5.55. That the presence of three acyl-functions of PNB in pyranose ring occupying three contiguous positions was verified by a careful analysis of ^1H–^1H COSY spectrum, which showed connectivity of the anomeric hydrogen at δ 5.65 with the low- field carbinyl hydrogen at δ 4.94, which, in turn showed connectivity with the carbinyl hydrogen signal at δ 5.55. Again, the carbinyl hydrogen signal at δ 5.55 was found to be flanked between the hydrogens showing signals at δ 4.94 and δ 5.16. Hence the part structure of PNB may include a sucrose molecule with three same or different ester groups.

To ascertain the nature of the acyl functions, PNB was subjected to trans-esterification with NaOMe in MeOH to yield methyl esters, GLC analysis of which showed it to be a mixture of methyl esters of different fatty acids and this led us to believe that either the ester functions at C-2, C-3 and C-4 of the pyranose ring are different or PNB is a mixture of structurally related compounds. The purity of PNB was checked by HPLC analysis of its benzoate derivative, prepared by

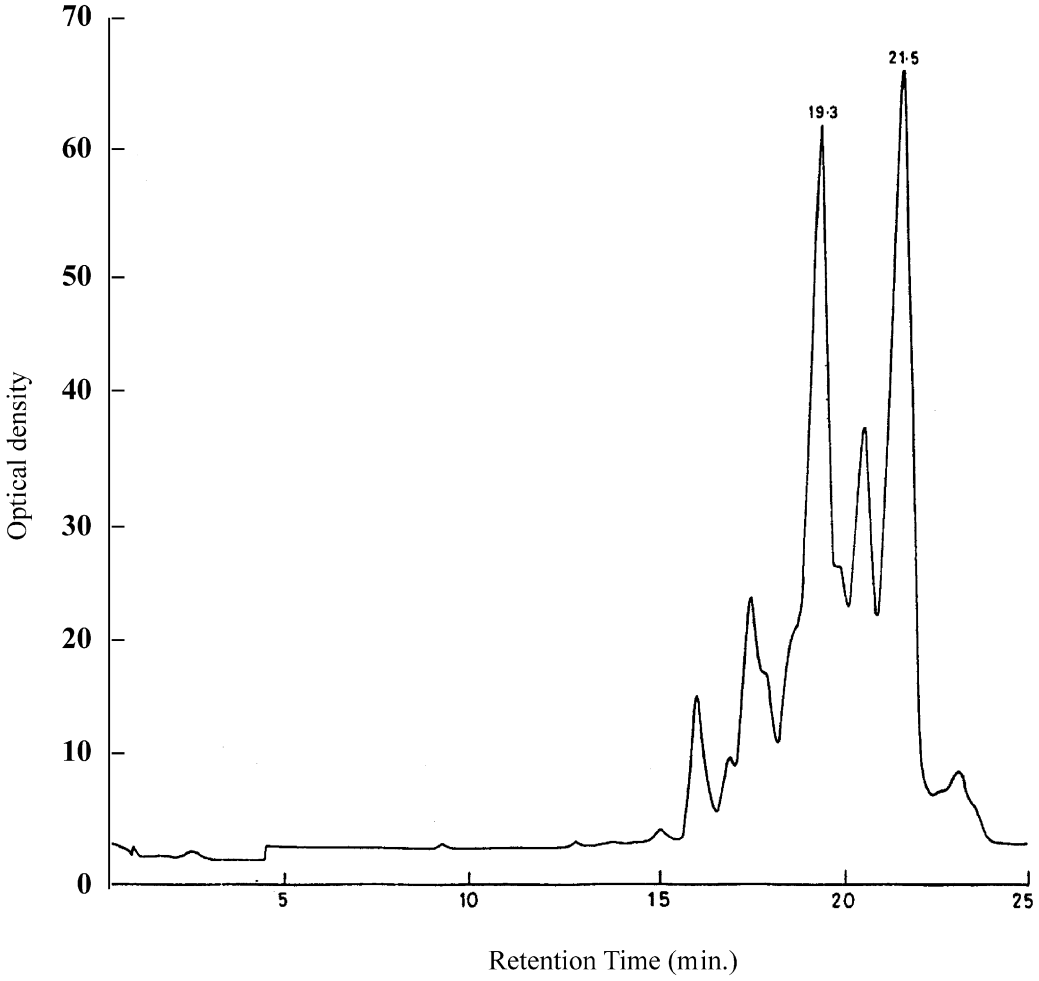


Fig. 1. HPLC profile of PNB benzoate. Conditions: column, Shim-pack CLC-ODS (15 cm×6 mm i.d.); solvent, MeOH; UV detector (254 nm); flow rate 6 ml/min.

benzoylation with benzoyl chloride and pyridine, and it was found to be a mixture of several compounds of close elution time (Fig. 1). Purification of PNB benzoate was achieved by use of reverse phase silica column (RP-18) and methanol as eluent. The fractions corresponding to the peaks showing Rt. 19.3 min and 21.5 min. were collected separately and labeled, respectively as PNB benzoate I and PNB benzoate II.

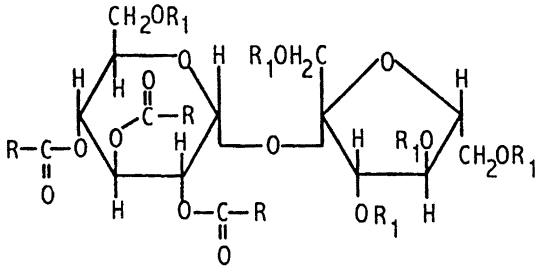
PNB benzoate I, ($C_{68}H_{78}O_{19}$), was obtained as amorphous powder and it showed a distinct ester carbonyl band at 1743 cm^{-1} in its IR spectrum. Alkali hydrolysis of PNB benzoate I, furnished sucrose and a mixture of two acids, which on treatment with *p*-bromophenacyl bromide and Et_3N and subsequent separation of the mixture by HPLC, yielded *p*-bromophenacyl benzoate and an ester which was identified as *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 PNB benzoate I was thus settled as **1** and the corresponding natural product PNB I as **2**.

Similar alkali hydrolysis of PNB benzoate II, $C_{71}H_{84}O_{19}$, 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- methyl heptanoic acid. The latter compound was fully characterized from comprehensive spectral analysis. The structure of PNB benzoate II was thus formulated as 3 and the corresponding natural product PNB II as **4** (Fig. 2).

3. Experimental

3.1. General

Mps are uncorr. IR spectra were recorded in KBr. ^1H NMR spectra were obtained from a Jeol GSX-500 spectrometer with TMS as int. ref. 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.



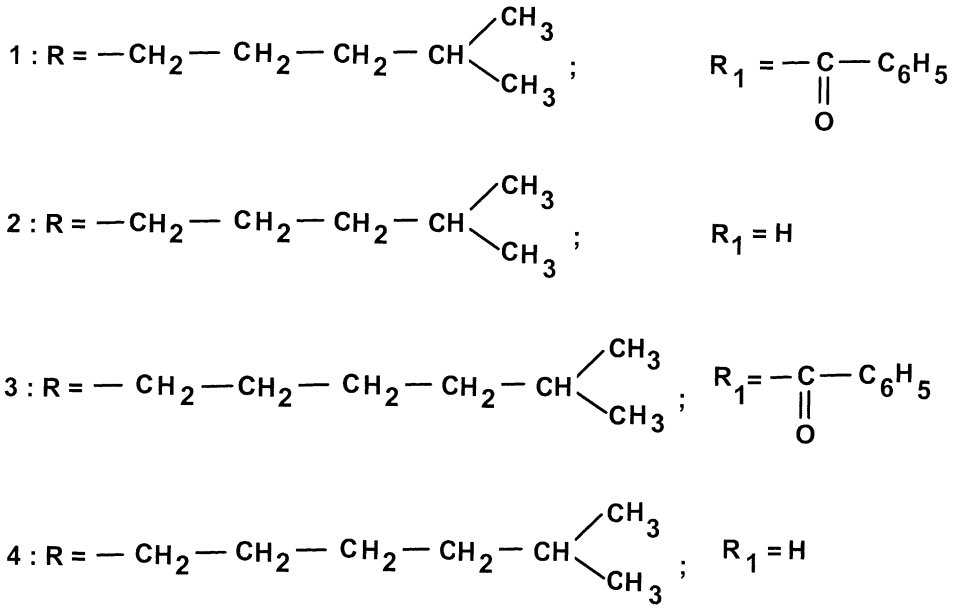


Fig. 2. PNB benzoate I (**1**) and PNB I (**2**), PNB benzoate II (**3**) and PNB II (**4**).

3.2. 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.

3.3. Extraction and isolation

Air-dried epigeal part of *Petunia nyctaginiflora* (5 kg) was milled and soxhletted with *n*-hexane. The *n*-hexane extract (20 l) was concentrated to 2.5 l and partitioned against MeOH–H_2O (1:4) mixture. The aqueous MeOH soluble portion obtained was evaporated under reduced pressure to a residue (19.5 g), which was chromatographed over silicagel (60–120 mesh) column and eluted with solvents of increasing polarity. C_6H_6–EtOAc (4:6) eluates yielded PNB (7 g).

IR (γ_{max}) 3600, 3510 and 1745 cm^{-1}.^{1}H NMR (500 MHz; $CDCl_3$): δ 5.65 (1H, *d*, $J=3.5$ Hz, anomeric-H), 5.62 (1H, *m*, –C<u>H</u>OR), 5.15 (1H, *m*, –C<u>H</u>OR), 4.96 (1H, *dd*, $J=10.2$ and 3.9 Hz, –C<u>H</u>OR), 4.20 (4H, *m*), 3.78 (2H, *m*), 3.45 (4H, *m*, 2×–C<u>H</u>$_2$OH), 2.30 (*m*, –COC<u>H</u>$_2$), 1.25 (*br*, methylenes), 1.10–0.98 (doublets, secondary –CH_3).

3.4. Acetylation of PNB to PNB acetate

A mixture of PNB (0.2 g), Ac_2O (1 ml) and pyridine (0.5 ml) was kept overnight under anhydrous conditions at room temperature. The reaction mixture after usual work up yielded PNB penta acetate (0.22 g) as a gummy residue.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (3H, *m*), 5.22 (1H, *d*, $J=3.5$ Hz, anomeric –H), 5.1 7 (1H, *m*), 4.86 (1H, *dd*, $J=10.2$ and 3.9 Hz), 4.30 (3H, *m*), 4.15 (5H, *m*), 2.17 (6H, *s*), 2.14 (3H, *s*), 2.09 (3H, *s*), 2.07 (3H, *s*), 1.22 (*br s*, methylenes), 1.08 (*d*, methyls).

3.5. Basic hydrolysis of PNB

A solution of PNB (0.2 g) in 10 ml of 5% methanolic KOH was refluxed for 1 h. The basic solution was cooled, neutralized carefully with 2 N HCl and diluted with water (10 ml). The resultant solution was extracted with Et_2O (3×10 ml) and then MeOH was evaporated under reduced pressure. The solution was then extracted with *n*-BuOH. The *n*-BuOH soluble portion was evaporated to dryness. The residue obtained was divided into two parts. Part I was crystallised from aqueous alcohol to colourless cubes; $C_{12}H_{22}O_{11}$; mp 183–185 °C; $[\alpha]_D+66.1°$ (H_2O). Part II was left overnight with Ac_2O/pyridine at room temperature. The usual work up of the reaction mixture yielded sucrose octa acetate, $C_{28}H_{38}O_{19}$: mp 88 °C; $[\alpha]_D+58.3°$ (C_2H_5OH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.61 (1H, *d*, $J=3.5$Hz, anomeric –H), 5.38 (1H, *d*, $J=5.5$Hz, –C<u>H</u>OAc), 5.30 (1H, *t*, $J=6.0$ Hz, –C<u>H</u>OAc), 5.00 (1H, *t*, $J=11$ Hz, –C<u>H</u>OAc), 4.80 (1H, *dd*, $J=10.0$ and 4.0 Hz, –C<u>H</u>OAc), 4.04–4.30 (8H, *m*, 3×–C<u>H</u>$_2$OAc, 2×C<u>H</u>O), 2.12 (3H, *s*, –OCOC<u>H</u>$_3$), 2.01–2.05 (15H, *s*, 5×–OCOC<u>H</u>$_3$), 1.94 (3H, *s*, –OCOC<u>H</u>$_3$), 1.92 (3H, *s*, –OCOC<u>H</u>$_3$).

3.6. Benzoylation of PNB

A mixture of PNB (0.2 g), benzoyl chloride (2 ml), pyridine (0.5 ml) was kept overnight under anhydrous conditions. The reaction mixture was poured over crushed ice, stirred for 0.5 h and then extracted with $CHCl_3$, washed, dried, freed from pyridine in a vacuum and chromatographed over silicagel. Elution with *n*-hexane–EtOAc (8:2), yielded PNB benzoate (0.25 g).

3.7. Isolation of PNB benzoate I (*1*) and PNB benzoate II (*3*)

PNB benzoate (0.21 g) was dissolved in MeOH (2 ml) and was purified by reverse phase Shim-pack silicagel column (RP—18). The detector used was UV (254 nm), flow rate was 6 ml/min., with MeOH as eluant. HPLC chromatogram showed two prominent peaks along with several small peaks. The fractions corresponding to peaks showing retention time 19.3 min and 21.5 min were collected separately and designated as PNB benzoate I (**1**) and PNB benzoate II (**3**) respectively. The solvent was removed under reduced pressure. PNB benzoae I (**1**): $C_{68}H_{78}O_{19}$; ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.04 (10H, *m*), 7.45 (15H, *m*) 6.0 (1H, *d*), 5.92 (1H, *m*), 5.85 (1H, *m*), 5.15 (1H, *m*), 4.95 (1H, *dd*), 4.63 (3H, *m*), 4.36 (1H, *dd*), 4.08 (2H, *br*), 2.15 (6H, *t*), 1.53 (3H, *br m*), 1.25 (12H, *br s*), 1.12 (18H, *d*, $J=6.7$ Hz).

PNB benzoae II (**3**): $C_{71}H_{84}O_{19}$; ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.05 (10H, *m*), 7.45 (15H, *m*), 6.01 (1H, *d*), 5.92 (1H, *m*), 5.85 (1H, *m*), 5.16 (1H, *m*), 4.93 (1H, *dd*), 4.63 (3H, *m*), 4.36 (1H, *dd*), 4.07 (2H, *br*), 2.15 (6H, *t*), 1.50 (3H, *br m*), 1.25 (18H, *br s*), 1.12 (18H, *d*, $J=6.7$ Hz).

3.8. Basic hydrolysis of PNB benzoate I

Basic hydrolysis of PNB benzoate I (**1**) was carried out as described in case of PNB. The sugar part obtained was identified as sucrose, $C_{12}H_{22}O_{11}$, mp 183–185 °C, while the Et_2O soluble portions were dried and evaporated to give acid mixture.

3.9. p-Bromophenacyl ester of acids

The acetonic solution of the acid mixture (5 mg in 2 ml) obtained from the basic hydrolysis of **1** was added to a solution of *p*-bromophenacyl bromide (27.8 mg) and triethylamine (2 drops) in acetone (2 ml). The

resultant mixture was stirred on an oil bath at 50 °C for 0.25 h. The acetone was evaporated under N_2 stream and the residue was passed through a silicagel column and eluted with ether. The eluates were combined and concentrated under reduced pressure, giving a mixture of *p*-bromophenacyl ester of benzoic acid and 5-methyl hexanoic acid. A preparative HPLC was carried out using acetonitrile–H_2O (9:1), as a mobile phase on a reverse phase Shim-pack silica column at a flow rate of 6 ml/min, UV wavelength 254 nm was used as a detector. The fractions corresponding to peaks A and B, retention time 5.3 and 7.1 min respectively were collected. Evaporation of the solvent obtained from peak A under reduced pressure yielded a residue (5 mg), which was identified as *p*-bromophenacyl benzoate, $C_{15}H_{11}BrO_3$. MS (*m*/*z*): 318/320 (M^+), 196/198, 183/185, 155/157, 105, 77, 51, 50.

Evaporation of the solvent obtained from peak B, Rt. 7.1 min yielded a solid (1.8 mg), which was characterized as *p*-bromophenacyl ester of 5-methyl hexanoic acid, $C_{15}H_{19}BrO_3$; IR (γ_{max}): 3030, 2960, 2930, 2060, 1742, 1703, 1595, 1412, 1400, 1375, 1230, 1180, 1110, 1075, 1050, 975, 820 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ 7.77 (2H, *d*, $J=8.3$ Hz, Ar H), 7.63 (2H, *d*, $J=9.3$ Hz, Ar H), 5.28 (2H, *s*, Ar COC$\underline{H}_2$), 2.47 (2H, *t*, $J=7.4$ Hz, 2–H_2), 1.68 (2H, quintet, $J=7.4$ Hz, 3-H_2), 1.55 (1H, *m*, –C$\underline{H}$ $(CH_3)_2$), 1.29 (2H, quintet, –C$\underline{H}_2$–), 0.88 (6H, *d*, $J=6.6$ Hz, –CH (C$\underline{H}_3)_2$). MS (*m*/*z*): 326/328, 256/258, 198/200, 183/185, 169/171, 155/157, 143, 127, 109, 90, 75, 57, 43, 41, 29.

3.10. Basic hydrolysis of **3**

The same procedure was followed as before which yielded *p*-bromophenacyl benzoate, $C_{15}H_{11}BrO_3$, M^+ at *m*/*z* 318/320 (R_t 5.3 min). The eluates corresponding to R_t 7.9 min was then characterized as *p*-bromophenacyl ester of 6-methyl heptanoic acid, $C_{16}H_{21}BrO_3$, ^1H NMR (500 MHz, $CDCl_3$): 7.77 and 7.63 (2H, each,), 5.28 (2H, *s*), 2.47 (2H, *t*), 1.68 (quintet), 1.53 (1H, *m*), 1.36 (2H, *m*), 1.22 (2H), 0.88 (6H, *d*). MS (*m*/*z*): 340/342, 316/312, 256/258, 198/200, 183/185, 167/171, 155/157, 157, 143, 127, 109, 90, 83, 76, 57, 55, 43, 41, 29.

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