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The Isolation from *Nicandra physalodes* and Identification of the 3-O-β-D-glucopyranoside of 1α,2β,3α,6α-tetrahydroxy-*nor*-tropane (Calystegine B_1).

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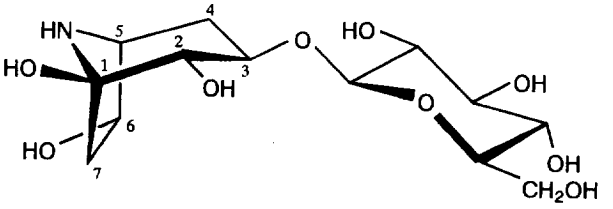
Abstract: The isolation and identification of 3-O-β-D-glucopyranosyl-1α,2β,3α,6α-tetrahydroxy-*nor*-tropane from *Nicandra physalodes* Boehm. fruits (Solanaceae) is reported.

Polyhydroxylated mono- and bicyclic nitrogen heterocycles are an important class of glycosidase inhibitors[1]. Polyhydroxy-*nor*-tropane alkaloids are the most recent naturally-occurring class of these inhibitors to be discovered and they have been shown to be potent inhibitors of β-glucosidases and β-galactosidases[2]. These alkaloids were first found in bindweeds[3] (Convolvulaceae) and given the trivial name calystegines but have since been found in human foods such as potato tubers (*Solanum tuberosum*) and aubergine fruits (*Solanum melongena*)[4]. Calystegines are clearly widespread and their significance in the human diet remains to be explored. We now report the first isolation and identification of a glucoside of a calystegine.

Nicandra physalodes Boehm. (Solanaceae) fruits (230g fresh weight) were homogenised in 70% aqueous ethanol. The filtrate was applied to the cation exchange resin Dowex 50W-X2 (H^+ form) and the bound compounds displaced with 2M ammonia solution. The tropane alkaloid calystegine B_1 (1α,2β,3α,6α-tetrahydroxy-*nor*-tropane) and a glycoside of it were determined to be the major alkaloids present by GC-MS of the trimethysilyl-derivatives. The alkaloids were readily separated from amino acids in the extracted material by ion exchange chromatography using Amberlite CG120 (NH_4^+ form) with the glycoside displaced with 2M pyridine and the aglycone eluted before arginine with 0.1M ammonia solution. The glycoside was then purified on the anion exchange resin Dowex 1-X2 (OH^- form) and washed off with water (yield 2.1mg).

The structure of the glycoside was determined to be 3-O-β-D-glucopyranosylcalystegine B_1 **1** on the basis of ^1H and ^{13}C NMR data, including 2D HMQC and HMBC spectral data[5]. The complete carbon and hydrogen atom connectivity of both the aglycone and glycone was defined. From comparison with previously reported NMR data[2,6], the aglycone was identified as calystegine B_1. The large vicinal J values of the glycone H-2', H-3', and H-4' and coupling constant of the anomeric proton (H-1', δ 4.50, $J_{1',2'}$ = 7.8 Hz) indicate that the glycone part of this glycoside is the pyranose form of β-glucose. It was shown that D-glucose is contained in the filtrate after acid hydrolysis of this glycoside using Dowex 50W-X2 (H^+) resin by the D-glucose-oxidase peroxidase method. The aglycone part was eluted with 0.5M ammonia solution from the resin, concentrated to dryness, and confirmed as calystegine B_1 by GC-MS of the trimethylsilylated eluate. The HMBC spectrum showed a correlation peak between the anomeric proton

of the glucone and the aglycone C-3 carbon, defining the linkage site. The ^{13}C-NMR data for the calystegine component shows a 7.6 ppm downfield shift for C-3 and 2.0 and 2.7 ppm upfield shifts for C-2 and C-4 respectively, compared to the free calystegine, also consistent with a 3-O- linkage.



3-O-β-D-glucopyranosyl-(calystegine B_1) 1

3-O-β-D-Glucopyranosylcalystegine B_1: ^{1}H-NMR (400 MHz, D_2O); δ: 1.40 (m, 1H, H-7exo); 1.49 (ddd, 1H, $J_{3,4ax}$=10.7, $J_{4ax,4eq}$=13.4, $J_{4ax,5}$=3.9Hz, H-4ax); 2.19 (ddd, 1H, $J_{3,4eq}$=6.4, $J_{4ax,4eq}$=13.4, $J_{4eq,5}$=2.7 Hz, H-4eq); 2.53 (dd, 1H, $J_{6,7endo}$=7.3, $J_{7endo,7exo}$=14.4 Hz, H-7endo); 3.26 (dd, 1H, $J_{1',2'}$=7.8, $J_{2',3'}$=9.5 Hz, H-2'); 3.29 (m, 1H, H-5); 3.37 (dd, 1H, $J_{3',4'}$=9.0, $J_{4',5'}$=9.8 Hz, H-4'); 3.44 (ddd, 1H, $J_{4',5'}$=9.8, $J_{5',6'a}$=6.1, $J_{5',6'b}$=2.2 Hz, H-5'); 3.45 (dd, 1H, $J_{2,3}$=8.5, $J_{2,7exo}$=1.7 Hz, H-2); 3.47 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.0 Hz, H-3'); 3.63 (ddd, 1H, $J_{2,3}$=8.5, $J_{3,4ax}$=10.7, $J_{3,4eq}$=6.4 Hz, H-3); 3.70 (dd, 1H, $J_{5',6'a}$=6.1, $J_{6'a,6'b}$=12.2 Hz, H-6'a); 3.92 (dd, 1H, $J_{5',6'b}$=2.2, $J_{6'a,6'b}$=12.2 HZ, H-6'b); 4.09 (dd, 1H, $J_{6,7endo}$=7.3, $J_{6,7exo}$=2.7 Hz, H-6); 4.50 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1'); ^{13}C-NMR (100 MHz, D_2O); δ: 36.2 (C-4); 43.5 (C-7); 62.7 (C-5); 63.6 (C-6'); 72.5 (C-4'); 75.7 (C-6); 75.8 (C-2'); 78.4 (C-3'); 78.7 (C-5'); 79.3 (C-2); 80.3 (C-3); 93.7 (C-1), 102.9 (C-1'). HRFAB-MS[7] *m/z* 338.1446 [M + H] ($C_{13}H_{24}O_9N$ requires 338.1451) measured on a Jeol JMS-SX 102A spectrometer with glycerol matrix[8].

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5. NMR abbreviations: HMQC, heteronuclear multiple quantum correlation spectroscopy; HMBC, heteronuclear multiple bond correlation spectroscopy.
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7. MS abbreviations: HR, high resolution; FAB, fast atom bombardment.
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