3-Aminofurostane Alkaloids from Solanum paniculatum (“Jurubeba Verdadeira”) Roots

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The roots of Solanum paniculatum (Solanaceae) have extensively been used in folk medicine to treat liver infections and as a diuretic. Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) were used for the profiling and structural characterization of alkaloids from the roots of S. paniculatum. Sixteen 3-aminofurostane alkaloids were characterized as novel compounds. In this study, three principal alkaloids were isolated in mixture, and their structures were established by different spectroscopic methods, including 1D, 2D nuclear magnetic resonance (NMR) experiments, and the high-resolution electrospray ionization (HR-ESI)-MS analysis. The isolated alkaloids were used to explore fragmentation pathways. Compound identification was based on the exact mass and fragmentation behaviors. Two compounds were identified as new natural compounds as: (25R)-3β-amino-furost-5-en-22α,26-diol O(26)-β-D-glucopyranoside (fatimagraine) and (25R)-3β-amino-furost-22-en,26-ol O(26)-β-D-glucopyranoside (bhattacharyyaine). The unambiguous assignments of 1H and 13C NMR data and chemical correction of the structure alkaloid jurubine are reported for the first time.

Keywords: Solanum paniculatum, jurubeba, 3-aminofurostane alkaloids, UPLC-QTOF-MS

Introduction

Solanum paniculatum (Solanaceae) is popularly known in Brazil as “jurubeba verdadeira”, and “jurubeba roxa”. This species is exclusive to South America, found in Brazil, Paraguay, and Argentina. It has a wide distribution in Brazil, occurring in all regions of the country.3 The decoction of the roots is used in folk medicine for liver infections and as a diuretic.1,4

Previous phytochemical studies of S. paniculatum roots resulted in the identification of paniculidine;5 (22R,25S)-3β-amino-5α-spirostan;6 jurubine (25S)-3β-amino-5α-furostane-22α,26-diol O(26)-β-D-glucopyranoside;7 isojuripidine, isojurubidine, and isopaniculidine.8 From aerial parts and leaves were isolated steroidal saponins.9-11

As part of our chemical and pharmacological studies of Brazilian Solanum,12-24 we report the chemical investigation on S. paniculatum. The present study with S. paniculatum roots resulted in the characterization of 17 3-aminofurostane alkaloids by ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-TOF-MS/MS). Compounds 11, 12, and 17 were isolated, and their structures were established by different spectroscopic methods including 1D nuclear magnetic resonance (NMR) (1H, attached proton test (APT)), 2D NMR experiments (correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC)), and the high-resolution electrospray ionization (HR-ESI)-MS analysis. Compounds 11 and 17 were identified as new natural compounds and named (25R)-3β-amino-furost-5-en-22α,26-diol O(26)-β-D-glucopyranoside (fatimagraine) and (25R)-3β-amino-furost-22-en-26-ol O(26)-β-D-glucopyranoside (bhattacharyyaine), respectively.

Experimental

General experimental procedures

NMR spectra were obtained using Bruker DRX 500
(500 MHz for $^1$H and 125 MHz for $^{13}$C) and Bruker DPX300 (300 MHz for $^1$H and 75 MHz for $^{13}$C) spectrometers (Karlsruhe, Germany). Samples were prepared as solutions in CD$_3$OD, and tetramethylsilane (TMS) was used as an internal reference. Thin layer chromatography (TLC) was performed with pre-coated silica gel 60 PF254 plates (0.25 mm, Merck, Darmstadt, Germany). A Strata Giga Tubes (SPE C18-E 70 g/150 mL, Phenomenex Inc., Torrance, USA) cartridge was employed to obtain alkaloids (Phenomenex Co., Torrance, CA, USA). Acetonitrile was obtained from Sigma (St. Louis, MO, USA). Milli-Q water (Billerica, USA) was used for the UPLC-QTOF-MS (ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry) analysis. The XEVO-G2XSQTOF mass spectrometer (Waters, Manchester, UK) was connected to the ACQUITY UPLC system (Waters, Milford, MA, USA) via an electrospray ionization interface (ESI). The chromatographic separation of compounds was performed on the ACQUITY UPLC with a conditioned autosampler at 4 °C using the Waters ACQUITY UPLC BEH C18 (2.1 x 50 mm, 1.7 μm) (Waters, Milford, MA, USA). The mobile phase consisting of water with 0.1% formic acid in water (solvent A) and acetonitrile 0.1% formic acid (solvent B) was pumped at a flow rate of 0.4 mL min$^{-1}$. The gradient elution program was as follows: 0-5 min, 5-10% B; 5-9 min, 10-95% B. The injection volume was 5 μL. The MS analysis was performed on a Xevo G2 QTOF (Waters MS Technologies, Manchester, UK), a quadrupole time-of-flight tandem mass spectrometer coupled with an electrospray ionization source in positive ion mode. The scan range was from m/z 50 to 1200 for data acquisition. In addition, MS$^n$ experiments were carried, which allows both precursor and product ion data to be acquired in one injection. The source conditions were as follows: 3 kV capillary voltage; 120 °C source temperature; 450 °C desolvation temperature; 50 L h$^{-1}$ cone gas flow rate; 800 L h$^{-1}$ desolvation gas (N$_2$) flow rate, and 40 V cone voltage. All analyses were performed using the lockspray, which ensured accuracy and reproducibility. Leucine-enkephalin (200 pg mL$^{-1}$) was used as a standard or reference compound to calibrate mass spectrometers during the analyses. All data acquisition and analyses were controlled using the Waters MassLynx v. 4.1 software.

**Plant material**

The roots of *Solanum paniculatum* were collected in Pernambuco State, Brazil, in the municipality of Recife in December 2012. The voucher specimen (53790) has been deposited at the Herbarium Vasconcelos Sobrinho of the Universidade Federal Rural de Pernambuco (UFRPE).

**Extraction and isolation**

*S. paniculatum* dried and powdered root (981.0 g) was extracted by maceration with ethanol:NH$_2$OH 2% (9:1) at room temperature. After filtration, the combined extractive solutions were concentrated under reduced pressure to yield the crude extract (91.0 g). The ethanolic extract (50.0 g) was suspended with acetic acid 10% and filtered through a bed of Celite. The aqueous acid filtrate was basified with NH$_2$OH and left standing overnight. This solution was extracted with butanol and concentrated to produce 12.8 g of the BuOH fraction. This fraction (5.6 g) was subjected to SPE C-18 with H$_2$O, MeOH, and ethyl acetate as binary mixtures of increasing polarity, and yielded 62 fractions. Fractions 19-21 were eluted with 80% MeOH, and 35-42 with 100% MeOH resulting in the isolation of the compounds mixture 11/12 (7.8 mg), and 12/17 (43.7 mg).

**Results and Discussion**

**Identification of isolated compounds**

The alkaloidal fraction was subjected to UPLC-QTOF-MS$^5$ and was used for the profiling and structural characterization of alkaloids from the roots of *S. paniculatum*. Seventeen 3-amino furanostane alkaloids were characterized and three principal alkaloids were isolated in mixture. The isolated alkaloids were used to explore fragmentation pathways. The structures of compounds 11, 12, and 17 were identified by the combined use of 1D NMR (H and $^{13}$C NMR), 2D-NMR (H-H COSY, HSQC, HMBC), and MS spectra. The unambiguous assignments of H and $^{13}$C NMR data of compound 12 (jurubine) are reported for the first time and involved a combination of homo- and heteronuclear 2D NMR techniques (Table 1). Both compounds 11 and 17 were isolated in the mixture with jurubine, and all showed a positive Dragendorff reagent test, indicating the alkaloidal nature of the structures.

Compound 12 exhibited a protonated molecular ion at m/z 596.4156 ([M + H]$^+$; calc. 596.4156 for C$_{33}$H$_{58}$NO$_8$) in the (+)-HRESIMS. The positive ESI-MS spectra of compound 12 suggested an amino furanostane structure with one glucopyranose. Compounds 12 and 15 are isomers with the same molecular weight and same ions fragments. The MS/MS experiment (Table 2) showed a prominent fragment ion at m/z 399.3271 corresponding to the loss of glucosyl, water, and ammonia [M + H – H$_2$O – NH$_3$ – glucose$^+$. The hydroxyl at C-20 was readily eliminated as water to produce the ion at m/z 578.4054 [M + H – H$_2$O]$^+$. The amino group at C-3 was confirmed by the subsequent loss of ammonia to generate the ion at m/z 561.3792 [M + H – H$_2$O – NH$_3$]$.^+$
The ion at $m/z$ 416.3552 [M + H − H₂O − glucose]⁺ corresponded to the loss of glucose. From the fragment ion at $m/z$ 399.3271 with a proton added to the oxygen of C-16 and a positive charge in this oxygen, the bond of C-16−O was broken, and the double electrons were transferred to the oxygen. Due to the presence of the enol, tautomerization was produced at the carbonyl of C-22. The loss of one water molecule produced the ion at $m/z$ 381.3143, and finally, the losses of C₆H₈ (−96 Da) and C₈H₁₄O (−126 Da) provided the ion at $m/z$ 285.3136 and 255.2118, respectively. The proposed fragmentation pathways of compound 12 are shown in Figure 1. The ¹H NMR (Table 1) spectrum of the aglycone moiety of compound 12 showed signals of four steroid methyl groups at δ 0.79 (s, H-18), 0.85 (s, H-19), 0.79 (s, H-18), and 0.85 (s, H-19), respectively.

Table 1. ¹H and ¹³C NMR data (500 and 125 MHz, CD₃OD) for compound 12 (jurubine)

|  δc  / ppm |  δh  / ppm (mult., J in Hz) |  ²JCH |  ³JCH |
|------------|----------------------------|-------|-------|
|  1         | 36.9 1.50 (m), 1.21 (m)     | 2H-2  | 3H-19 |
|  2         | 30.0 1.72 (m), 1.12 (m)     |       |       |
|  3         | 52.0 2.70 (m)               |       |       |
|  4         | 38.7 1.46 (m), 1.03 (m)     | H-9   | 2H-6  |
|  5         | 46.9 1.17 (m)               |       | 3H-19 |
|  6         | 31.5 1.75 (m), 1.38 (m)     | 2H-7  |       |
|  7         | 32.8 1.70 (m), 0.97 (m)     | 2H-6  |       |
|  8         | 36.6 1.58 (m)               | 2H-7, H-9 |       |
|  9         | 55.9 0.71 (m)               |       | 3H-19 |
| 10         | 38.2                           |       |       |
| 11         | 22.2 1.53 (m), 1.38 (m)     | H-9   |       |
| 12         | 41.2 1.70 (m), 1.11 (m)     |       | 3H-18 |
| 13         | 42.3                           | 2H-12, 3H-18 | H-20, H-16, 2H-15 |
| 14         | 57.9                           |       | 3H-18 |
| 15         | 32.2 1.95 (dd, J 12.0, 10.0, 5.0 Hz) 1.24 (m) | H-14, H-16 | H-17 |
| 16         | 82.6 4.35 (q, J 7.0 Hz)      | 2H-15, H-17 |       |
| 17         | 65.3 1.70 (m)               | H-16  | 3H-18, 3H-21, H-20 |
| 18         | 17.2                           | H-17  | H-20  |
| 19         | 12.9                           |       | 2H-1, H-9 |
| 20         | 41.3 2.15 (q, J 7.0 Hz)      | 3H-21 | H-16, 2H-23 |
| 21         | 16.2                           |       |       |
| 22         | 116.0                          | H-20, 2H-23 | 3H-21, 2H-24 |
| 23         | 31.5 1.65 (m), 1.82 (m)     | 2H-24 | H-20, 2H-25 |
| 24         | 29.1 1.15 (m), 1.62 (m)     | H-25  | 3H-27 |
| 25         | 35.1 1.75 (m)               | 2H-26, 2H-24, 3H-27 |       |
| 26         | 76.1 3.72 (dd, J 10.0, 7.0 Hz), 3.37 (dd, J 10.0, 7.0 Hz) | H-25 | 2H-24, H-1’ |
| 27         | 17.6 0.94 (d, J 7.0 Hz)     |       | 2H-24, 2H-26 |

Glucose

|  δc  / ppm |  δh  / ppm (mult., J in Hz) |  ²JCH |  ³JCH |
|------------|----------------------------|-------|-------|
|  1’        | 104.7 4.23 (d, J 8.0 Hz)   | H-2’  | H-3’, 2H-26 |
|  2’        | 75.3 3.18 (dd, J 9.0, 8.0 Hz) | H-1’, H-3’ | H-4’ |
|  3’        | 78.3 3.31 (dd, J 9.0, 8.0 Hz) | H-2’  | H-1’, H-5’ |
|  4’        | 71.9 3.27 (m)              | H-3’, H-5’ | 2H-6’ |
|  5’        | 78.1 3.24 (m)              | H-4’, 2H-6’ | H-3’ |
|  6’        | 62.9 3.85 (dd, J 12.0, 2.0 Hz), 3.65 (dd, J 12.0, 5.0 Hz) | H-5’ |       |
| Compound | t<sub>b</sub> (min) | [M + H]<sup>+</sup> (m/z) calculated | [M + H]<sup>+</sup> (m/z) measured | MS/MS fragments (m/z) calculated | Tentative identification |
|----------|-------------------|-------------------------------------|----------------------------------|-------------------------------|-------------------------|
| 1        | 2.84              | 626.3898                            | 626.3898                         | 3-amino-dihydroxy-dehydro-furostane-22-ol | 608.3860 [M + H – H_2O]^+ , 591.2657 [M + H – H_2O – NH_3]^+ , 573.3503 [M + H – 2H_2O – NH_2]^+ , 464.3369 [M + H – glucose]^+ , 429.3031 [M + H – H_2O – NH_ – glucose]^+ , 411.2908 [M + H – 2H_2O – NH_ – glucose]^+ , 393.2814 [M + H – 3H_2O – NH_ – glucose]^+ , 269.1812 [M + H – 3H_2O – NH_ – glucose – C_6H_8O]^+ |
| 2        | 2.99              | 774.4477                            | 774.4477                         | 3-amino-dihydroxy-dehydro-furostane-22-ol | 754.4370 [M + H – H_2O]^+ , 610.3963 [M + H – glucose]^+ , 593.3848 [M + H – NH_ – glucose]^+ , 431.3193 [M + H – NH_ – 2glucos]e]^+ , 413.3193 [M + H – H_2O – 2glucose – NH_3]^+ , 395.2957 [M + H – 2H_2O – 2glucose – NH_3]^+ , 281.2274 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ , 271.2069 [M + H – H_2O – 2glucose – NH_ – C_6H_8O]^+ , 253.1966 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ |
| 3        | 3.11              | 774.4634                            | 774.4634                         | 3-amino-dihydroxy-furostane-22-ol | 756.4547 [M + H – H_2O]^+ , 738.4454 [M + H – 2H_2O]^+ , 594.4022 [M + H – H_2O – glucose]^+ , 432.3488 [M + H – H_2O – 2glucose]^+ , 415.3056 [M + H – H_2O – 2glucose – NH_3]^+ , 397.3122 [M + H – 2H_2O – 2glucose – NH_3]^+ , 379.2999 [M + H – 3H_2O – 2glucose – NH_3]^+ , 283.2423 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ , 353.1966 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ |
| 4        | 3.22              | 628.4055                            | 628.4055                         | 3-amino-dihydroxy-furostane-22-ol | 610.3965 [M + H – H_2O]^+ , 593.3704 [M + H – H_2O – NH_3]^+ , 575.3593 [M + H – H_2O – glucose]^+ , 444.3441 [M + H – H_2O – glucose]^+ , 341.3163 [M + H – H_2O – NH_ – glucose]^+ , 313.3058 [M + H – 2H_2O – NH_ – glucose]^+ , 395.2949 [M + H – 3H_2O – NH_ – glucose]^+ , 377.2850 [M + H – 4H_2O – NH_ – glucose]^+ , 281.2280 [M + H – 4H_2O – NH_ – glucose – C_6H_8O]^+ , 269.2440 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ , 251.1802 [M + H – 4H_2O – NH_ – glucose – C_6H_8O]^+ |
| 5        | 3.29              | 774.7634                            | 774.7634                         | 3-amino-dihydroxy-furostane-22-ol | 756.4643 [M + H – H_2O]^+ , 738.4458 [M + H – 2H_2O]^+ , 594.4016 [M + H – H_2O – glucose]^+ , 432.2822 [M + H – H_2O – 2glucose]^+ , 415.3064 [M + H – H_2O – 2glucose – NH_3]^+ , 397.3122 [M + H – 2H_2O – 2glucose – NH_3]^+ , 379.2999 [M + H – 3H_2O – 2glucose – NH_3]^+ , 283.2423 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ , 353.1966 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ |
| 6        | 3.58              | 612.4106                            | 612.4106                         | 3-amino-dihydroxy-furostane-22-ol | 594.4011 [M + H – H_2O]^+ , 577.3746 [M + H – H_2O – NH_3]^+ , 559.3644 [M + H – 2H_2O – NH_3]^+ , 432.3482 [M + H – H_2O – glucose]^+ , 341.3163 [M + H – H_2O – NH_ – glucose]^+ , 313.3058 [M + H – 2H_2O – NH_ – glucose]^+ , 397.3113 [M + H – 3H_2O – NH_ – glucose]^+ , 379.3657 [M + H – 4H_2O – NH_ – glucose]^+ , 383.2972 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ , 271.2612 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ , 253.2489 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ |
| 7        | 3.90              | 772.4477                            | 772.4477                         | 3-amino-dihydroxy-dehydro-furostane-22-ol | 754.4370 [M + H – H_2O]^+ , 610.3963 [M + H – glucose]^+ , 593.3848 [M + H – NH_ – glucose]^+ , 431.3193 [M + H – NH_ – 2glucos]e]^+ , 413.3193 [M + H – H_2O – 2glucose – NH_3]^+ , 395.2957 [M + H – 2H_2O – 2glucose – NH_3]^+ , 281.2274 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ , 271.2069 [M + H – H_2O – 2glucose – NH_ – C_6H_8O]^+ , 253.1966 [M + H – 3H_2O – 2glucose – NH_ – C_6H_8O]^+ |
| 8        | 4.12              | 612.4106                            | 612.4106                         | 3-amino-hydroxy-furostane-22-ol | 594.4010 [M + H – H_2O]^+ , 577.3746 [M + H – H_2O – NH_3]^+ , 559.3651 [M + H – 2H_2O – NH_3]^+ , 432.3492 [M + H – H_2O – glucose]^+ , 341.3207 [M + H – H_2O – NH_ – glucose]^+ , 315.3108 [M + H – 2H_2O – NH_ – glucose]^+ , 379.3657 [M + H – 3H_2O – NH_ – glucose]^+ , 383.2972 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ , 271.2612 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ , 253.2489 [M + H – 2H_2O – NH_ – glucose – C_6H_8O]^+ |
| 9        | 4.21              | 758.4689                            | 758.4689                         | 3-amino-furostane-22-ol | 740.4604 [M + H – H_2O]^+ , 578.4096 [M + H – H_2O – glucose]^+ , 417.3568 [M + H – NH_ – 2glucos]e]^+ , 416.3518 [M + H – H_2O – 2glucose]^+ , 399.3269 [M + H – H_2O – NH_ – 2glucose]^+ , 285.2586 [M + H – H_2O – NH_ – 2glucose – C_6H_8O]^+ , 381.3177 [M + H – H_2O – NH_ – 2glucose]^+ , 255.2120 [M + H – 2H_2O – NH_ – 2glucose – C_6H_8O]^+ |
Table 2. Characterization of 3-aminofurostane alkaloids from S. paniculatum roots by UPLC-QTOF-MS® (cont.)

| Compound | t_R / min | [M + H]^+ (m/z) | [M + H]^+ (m/z) calculated | MS/MS fragments (m/z) | Tentative identification |
|----------|-----------|----------------|--------------------------|----------------------|-------------------------|
| **10**   | 4.28      | 610.3948       | 610.3949                 | 3-amino-hydroxy-dehydro-furostane-22-ol |                         |
|          |           |                |                          | 592.3856 [M + H – H_2O]^+; 574.3755 [M + H – 2H_2O]^+; 430.3345 [M + H – H_2O – glucose]^+; 413.3070 [M + H – H_2O – NH_2 – glucose]^+; 395.2962 [M + H – 2H_2O – NH_2 – glucose]^+; 377.2246 [M + H – 3H_2O – NH_2 – glucose]^+; 269.2612 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; 251.1813 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **11**   | 4.54      | 594.4001       | 594.4000                 | (25R)-3β-aminofurost-5-en-22α,26-diol O(26)-β-D-glucopyranoside (fatimagrainé) | 576.3913 [M + H – H_2O]^+; 559.3636 [M + H – H_2O – NH_2]^+; 414.3391 [M + H – H_2O – glucose]^+; 397.3105 [M + H – H_2O – NH_2 – glucose]^+; 379.3011 [M + H – 2H_2O – NH_2 – glucose]^+; 283.2433 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; 253.1956 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **12**   | 4.63      | 596.4156       | 596.4156                 | 3-amino-dehydro-furostane-22-ol-26-glucopyranoside | 738.4423 [M + H – H_2O]^+; 594.4001 [M + H – glucose]^+; |                         |
|          |           |                |                          | 577.3755 [M + H – NH_2 – glucose]^+; 559.3644 [M + H – H_2O – NH_2 – glucose]^+; | 432.3481 [M + H – 2glucose]^+; 414.3373 [M + H – H_2O – 2glucose]^+; 413.3073 [M + H – NH_2 – 2glucose]^+; | 397.3119 [M + H – H_2O – NH_2 – glucose]^+; 253.1961 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **13**   | 4.79      | 756.4528       | 756.4528                 | fatimagrainé isomer | 576.3906 [M + H – H_2O]^+; 559.3669 [M + H – H_2O – NH_2]^+; |                         |
|          |           |                |                          | 414.3371 [M + H – H_2O – glucose]^+; 397.3110 [M + H – H_2O – NH_2 – glucose]^+; | 283.1955 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; 253.1955 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **14**   | 5.08      | 594.4001       | 594.4000                 | jurubine isomer | 578.4072 [M + H – H_2O]^+; 561.3793 [M + H – H_2O – NH_2]^+; |                         |
|          |           |                |                          | 416.3518 [M + H – H_2O – glucose]^+; 399.3274 [M + H – H_2O – NH_2 – glucose]^+; 381.3143 [M + H – 2H_2O – NH_2 – glucose]^+; 285.2583 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; | 255.2118 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **15**   | 5.17      | 596.4156       | 596.4156                 | dehydro-bhattacharyyina | 559.3630 [M + H – NH_2]^+; 397.3109 [M + H – NH_2 – glucose]^+; |                         |
|          |           |                |                          | 414.2479 [M + H – glucose]^+; 379.3002 [M + H – H_2O – NH_2 – glucose]^+; 283.2439 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; 253.1955 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **16**   | 6.04      | 576.3894       | 576.3894                 | (25R)-3β-aminofurost-22-en-26-glucopyranoside (bhattacharyyina) | 561.3789 [M + H – NH_2]^+; 399.3263 [M + H – NH_2 – glucose]^+; 416.3539 [M + H – glucose]^+; 381.3161 [M + H – H_2O – NH_2 – glucose]^+; 285.2591 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
|          |           |                |                          | 255.2121 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |
| **17**   | 6.15      | 578.4051       | 578.4051                 |                         | 561.3789 [M + H – NH_2]^+; 399.3263 [M + H – NH_2 – glucose]^+; 416.3539 [M + H – glucose]^+; 381.3161 [M + H – H_2O – NH_2 – glucose]^+; 285.2591 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; 255.2121 [M + H – 2H_2O – NH_2 – glucose – C_4H_10O]^+; |                         |

t_R: retention time.

0.99 (d, J 7.0 Hz, H-21), and 0.94 (d, J 7.0 Hz, H-27), two methylene protons bearing an oxygen function at δ 3.72 (dd, J 10.0, 7.0 Hz) and 3.37 (dd, J 10.0, 7.0 Hz, H-26), one methine proton bearing an oxygen function at δ 4.35 (q, J 7.0 Hz, H-16), and one β-amino group at δ 2.70 (m, H-3). The 'H NMR spectrum (Table 1) of the sugar moiety of compound 12 showed signals of one methylene of glucopyranose (δ 3.85 (dd, J 12.0, 2.0 Hz) and 3.65 (dd, J 12.0, 5.0 Hz, H-6’)). The β-configuration of D-glucose was determined by the J^2 value of 8.0 Hz. These results suggested that compound 12 consists of a furostane type aglycone with one amino group and one sugar moiety of a glucopyranose. From the ^13C NMR spectrum (Table 1), the results were further supported by the presence of signals of four methyl groups of steroid at δ 17.2 (C-18), 12.9 (C-19), 16.1 (C-21), and 17.6 (C-27), two methylene carbons bearing an oxygen function at δ 76.1 (C-26) and 62.9 (C-6’), one methine carbon bearing an oxygen function at δ 82.6 (C-16), three quaternary carbon at δ 38.2 (C-10), 42.3 (C-13), and 116.0 (C-22), one anomic carbons of sugar at δ 104.7 (C-1’), and one signal for a carbon-bearing nitrogen at δ 52.0 (C-3). This pattern of proton
chemical shifts, the $^{13}$C NMR spectrum of compound 12 with the presence of anomeric at $\delta$ 104.7 (C-1') and the hemiacetal at $\delta$ 116.0 (C-22) carbons signal suggested the furostane nature of the aglycone of compound 12. All the above proton resonances were unambiguously associated with relevant carbon atoms by using the HSQC spectrum. The steroid and glucoside moiety were assigned from their proton-proton correlations in the $^1$H-$^1$H COSY spectrum, and the long-range correlations between protons and carbons by the HMBC spectrum. In particular, it was observed HMBC cross-peaks H$_3$-19 ($\delta$ 0.85) with C-10 ($\delta$ 38.2), C-1 ($\delta$ 36.9), C-5 ($\delta$ 46.9), and C-9 ($\delta$ 55.9); H$_2$-18 ($\delta$ 0.79) with C-13 ($\delta$ 42.3), C-12 ($\delta$ 41.2), and C-14 ($\delta$ 57.9); and H$_2$-23 ($\delta$ 1.65 and 1.82) with C-20 ($\delta$ 41.3) and the hemiacetal carbon C-22 ($\delta$ 116.0). Conversely, the HMBC cross-peak of H$_2$-26 ($\delta$ 3.72 and 3.37) with the anomeric carbon at $\delta$ 104.74 (C-1') allowed us to identify C-26 as a further glycosidic linkage site. The relative stereochemistry of compound 12 indicated the A/B trans-ring fusion due to the signals corresponding to carbons C-5 and C-9 at $\delta$ 46.9 and 55.9, respectively.26 The B/C trans and C/D trans-ring fusion geometry evidences the usual furostane ring junctions with rings.27 The 22α orientation of compound 12 was established on the basis of the $^1$H NMR resonances of H-21 ($\delta$ 0.99) and H-16 ($\delta$ 4.35) suggesting that, most likely, they are deshielded by the cis-oriented OH-22 group.26 The 25R configuration was determined on the basis of differences in chemical shifts of the geminal protons at H$_2$-26 that exhibit pronounced dependence; the difference ($\Delta$ab = $\Delta$a − $\Delta$b) between their chemical shifts ($\Delta$ab ≤ 0.48 for 25R; $\Delta$ab ≥ 0.57 for 25S) seems to be of general applicability for ascertaining the 25R/25S orientation of the 27-methyl group of furostane-type steroidal saponins.27 Consequently, based on the results described above, the structure of compound 12 was established as (25R)-3β-amino-5α-furostane-22α,26-diol O(26)-β-D-glucopyranoside. Analogously, as a result of the inspection of the $^1$H and $^{13}$C NMR spectra of compound 12 (Table 1), the same gross structure of jurubine29 was identified. Therefore, we argue that these molecules should differ only in the stereochemistry of one chiral carbon. This was readily identified as the C-25 carbon. Compound 12 have the 25R configuration as shown above, and jurubine has been reported to have the 25S configuration.7 Since there are no NMR data in the literature that prove the absolute stereochemistry of C-25S for jurubine, it is possible that the correct structure of jurubine is presented in this work, i.e., jurubine has actually the C-25R configuration. Jurubine was only identified in nature in two plant species: roots of Solanum paniculatum7 and Solanum torvum.30 The unambiguous assignments of $^1$H and $^{13}$C NMR data of jurubine (12) are reported for the first time in this study.

The structure of compounds 11 and 17 was readily elucidated on the basis of their considerable similarities to compound 12. The (+)-HRESIMS spectrum of compound 11 exhibited a protonated molecular ion at
m/z 594.4001 ([M + H]^+), calcld. 594.4000 for C_{17}H_{35}NO_{3}), compound 17 showed a protonated molecular ion at m/z 578.4051 ([M + H]^+), calcld. 578.4051 for C_{13}H_{36}NO_{3}) in accordance with the ^13C-APT spectra. A good level of similarity in the chemical shifts of the glucose was observed for the two compounds 11 and 17, however, with some differences in the aglycone. The molecular weight of compounds 11 and 17 that have mass units lesser than those of compound 12 indicates that 11 has two hydrogen atoms less, and 17 has one oxygen atom less than 12, respectively. The MS/MS experiment of compound 11 (Table 2) showed a profile similar to that of 12, with the difference in all ions from the spectra of 1 H and 13 C NMR, 1 H-1 H COSY, HSQC, and HMBC (Table 3) as (25-amino-furost-22-en-26-ol) and named batthacharyyaine.

The 3-Aminofurostane Alkaloids from Solanum paniculatum (“Jurubeba Verdadeira”) Roots

| δ_c / ppm | δ_h / ppm | J_{CH} / Hz | J_{CH} / Hz |
|-----------|-----------|-------------|-------------|
| 85.8      | 1.23 (m)  | H-18        | H-18        |
| 82.5      | 4.36 (m)  | H-15, H-17  |             |
| 65.9      | 1.75 (m)  | H-16        | 3H-18, 3H-21|
| 16.9      | 0.83 (s)  | H-9         | H-9         |
| 41.3      | 2.17 (m)  | 3H-21       |             |
| 26.3      | 0.98 (d, J 7.0 Hz) | H-20 |          |
| 23.5      | 1.81 (m)  | 1.65 (m)    | H-25        |
| 28.8      | 1.20-1.40 (m) | H-25 | 3H-27     |
| 35.1      | 1.72 (m)  | 3H-27       |             |
| 76.1      | 3.73 (m)  | 3.35 (m)    | H-1'        |
| 37.5      | 2.17 (m)  | 3H-21       | 2H-24, 2H-26|
| 21.6      | 0.98 (d, J 7.0 Hz) | H-20 |          |
| 114.0     | 1.23 (m)  | 1.65 (m)    | H-25        |
| 24.8      | 1.20-1.40 (m) | H-25 | 3H-27     |
| 35.1      | 1.72 (m)  | 3H-27       |             |
| 76.1      | 3.73 (m)  | 3.35 (m)    | H-1'        |
| 17.4      | 0.95 (d, J 7.0 Hz) | 2H-24, 2H-26|

Table 3. ^1H and ^13C NMR data (500 and 125 MHz, CD_{3}OD) for compound 11 (fatimagraine)

The lack of finding spirostane alkaloids type compounds in the roots of S. paniculatum in this study, despite of
Table 4. $^1$H and $^{13}$C NMR data (500 and 125 MHz, CD$_3$OD) for compound 17 (bhattacharyyaine)

|   | $\delta_{\text{C}}$ / ppm | $\delta_{\text{H}}$ / ppm (mult., $J$ in Hz) | $^J_{CH}$ | $^J_{CH}$ |
|---|------------------|------------------------------------------|----------|----------|
| 1 | 36.9             | 1.50 (m), 1.21 (m)                          | 2H-2     | 3H-19    |
| 2 | 30.0             | 1.72 (m), 1.12 (m)                          |          |          |
| 3 | 52.0             | 2.70 (m)                                   |          |          |
| 4 | 38.7             | 1.46 (m), 1.03 (m)                          | H-9      | 2H-6     |
| 5 | 46.9             | 1.17 (m)                                   | 3H-19    |          |
| 6 | 31.5             | 1.75 (m), 1.38 (m)                          | 2H-7     |          |
| 7 | 32.8             | 1.70 (m), 0.97 (m)                          | 2H-6     |          |
| 8 | 36.6             | 1.58 (m)                                   | 2H-7, H-9|          |
| 9 | 55.9             | 0.71 (m)                                   | 3H-19    |          |
| 10| 38.2             |                                          |          |          |
| 11| 22.2             | 1.53 (m), 1.38 (m)                          | H-9      |          |
| 12| 41.2             | 1.70 (m), 1.11 (m)                          |          | 3H-18    |
| 13| 44.7             |                                          | 3H-18    | H-16, 2H-15 |
| 14| 56.2             | 1.23 (m)                                   | 3H-18    |          |
| 15| 32.2             | 1.95 (ddd, $J$ 12.0, 10.0, 5.0 Hz), 1.65 (m) | H-14, H-16 | H-17    |
| 16| 85.8             | 4.70 (dt, $J$ 8.0, 2.0 Hz)                  | 2H-15, H-17 |          |
| 17| 65.3             | 2.44 (d, $J$ 7.5 Hz)                        | H-16     | 3H-18, 3H-21 |
| 18| 14.9             | 0.69 (s)                                   | H-17     |          |
| 19| 12.9             | 0.85 (s)                                   |          | 2H-1, H-9 |
| 20| 104.8            |                                          |          | 3H-21    |
| 21| 12.0             | 1.58 (s)                                   |          |          |
| 22| 153.0            |                                          |          | H-17    |
| 23| 32.2             | 1.81 (m), 1.65 (m)                          |          | H-25     |
| 24| 29.1             | 1.15 (m), 1.62 (m)                          | H-25     | 3H-27    |
| 25| 35.1             | 1.75 (m)                                   | 2H-26, 2H-24, 3H-27 |          |
| 26| 76.1             | 3.72 (dd, $J$ 10.0, 7.0 Hz), H-25           | 2H-24, H-1’ |          |
| 27| 17.6             | 0.94 (d, $J$ 7.0 Hz)                        |          | 2H-24, 2H-26 |

Glucose

|   | $\delta_{\text{C}}$ / ppm | $\delta_{\text{H}}$ / ppm (mult., $J$ in Hz) | $^J_{CH}$ | $^J_{CH}$ |
|---|------------------|------------------------------------------|----------|----------|
| 1’ | 104.7            | 4.23 (d, $J$ 8.0 Hz)                       | H-2’     | H-3’, 2H-26 |
| 2’ | 75.3             | 3.18 (dd, $J$ 9.0, 8.0 Hz)                 | H-1’, H-3’ | H-4’    |
| 3’ | 78.3             | 3.31 (dd, $J$ 9.0, 8.0 Hz)                 | H-2’     | H-1’, H-5’ |
| 4’ | 71.9             | 3.27 (m)                                   | H-3’, H-5’ | 2H-6’   |
| 5’ | 78.0             | 3.24 (m)                                   | H-4’, 2H-6’ | H-3’   |
| 6’ | 62.9             | 3.85 (dd, $J$ 12.0, 2.0 Hz), 3.65 (dd, $J$ 12.0, 5.0 Hz) | H-5’ |          |

Figure 2. The proposed fragmentation pathways of 16 and battacharyyaine (17) using ESI-qTOF-MS/MS.
being reported in the literature,⁶ may be due to the direct analysis of the alkaloidal fraction. According to Li et al.,⁵ the conventional methods present disadvantages because of the cyclization of furostanol to spirostanol aglycone, which can yield several structures that are alternatives to that of the original compound. These probable artifacts have been previously reported as natural alkaloids.⁶,⁸

Characterization of compounds by UPLC-QTOF-MS/MS

The UPLC-QTOF-MS⁹ experiments were performed to identify alkaloids in Solanum paniculatum roots. Only aglycone structure skeleton present in steroidal alkaloid glycoside as jurubine [3-amino-furostan-26-β-D-glucopyranoside] (or its isomer) was detected in all compounds. The compounds exhibited the presence of unsaturation, hydroxyls, or additional glucose in the structure as variations. The common sugar moiety is D-glucose; it is linked to the aglycone through the hydroxy group typically at C-26 and C-3-linked to an amino group.

The alkaloidal fraction was subjected to UPLC-QTOF-MS⁹; the profile of the base peak ion (BPI) chromatograms showed 17 main peaks (Figure 3). In ESI⁺, alkaloids always produced a protonated ion [M + H]⁺; the common characteristics for all compounds comprise glycosylation pattern at C-26 and furostanol-type aglycone structure.³¹ The hydroxyl group was characterized by the neutral loss of 18 Da (loss of water). The presence of additional glycosyl residues was observed by the loss of 162 Da.

The evidence that all detected structures are glycoalkaloids was based on the protonated peak in the positive mode [M + H]⁺ to the molecular ion with even mass (compounds contain one nitrogen atom). The furostane-type compounds with the 22-OH group (compounds 1-15) resulted in an exclusive detection of [M + H − H₂O]⁺ in the positive mode. Only compounds 16 and 17 with unsaturation between C-20 and C-22 did not show [M + H − H₂O]⁺; however, they presented [M + H − NH₃]⁺. Table 2 shows the high-resolution mass data and fragments data in the positive ion mode of the 3-aminofurostane alkaloids from Solanum paniculatum roots. Their analyses and comparisons with structures reported in the literature⁸ showed that out of the seventeen identified glycoalkaloid, only compound 12 (jurubine) had been previously reported, all other 16 are new compounds.

Each identified glycoalkaloid showed its pair with only one additional unsaturation such as compounds [M + H]⁺:

![Figure 3. ESI base peak ion (BPI) chromatogram of the Solanum paniculatum roots analyzed by UPLC-QTOF-MS.](image-url)
1/4 (m/z 626.3898/628.4055), 2/3 (m/z 772.4477/774.4634), 6/10 (m/z 612.4106/610.3948), 9/13 (m/z 578.4689/576.4528), 11/12 (m/z 594.4001/596.4156), and 16/17 (m/z 576.3894/578.4051). Furthermore, compounds 2, 7 (m/z 772.4477); 3, 5 (m/z 774.4634); 6, 8 (m/z 612.4106); 11, 14 (m/z 594.4001); and 12, 15 (m/z 596.4156) are isomers (Table 2).

Compounds 1, 2, 3, 4, 5, and 7 exhibited two hydroxyl groups, which may be attached at the carbons of the A-D rings (perhydrocyclopentenophenanthrene nucleus). Likewise, compounds 6, 8 and 10 showed only one hydroxyl group. Compounds 9 and 13 showed two more glycosides. Analyses of the mass spectra of these compounds supported the identification. The MS² experiment showed fragment ions characteristics of consecutive losses of water (−18 Da) and/or glucose (−162 Da). Compounds 1-8 and 10 eliminated at least two molecules of water whereas compounds 2, 3, 5, 7, 9 and 13 showed the loss of two glucose units. Compounds 11 and 12, and 17 were isolated and identified by ¹H and ¹³C NMR.

The ESI-QTOF-MS/MS investigation of the fragmentation mechanism of each 3-aminofurostane alkaloids was performed, and the characteristic fragment ion derived from the fission of aglycone with crucial fragment ions are presented in Table 2. Compounds 3, 5, 6, 8, 9, 12, 15, and 17 (no unsaturation in the A-D rings) produced fragment ions at m/z 415/416, except compound 4 (m/z 413). The unsaturated compounds (A-D rings) 2, 7, 10, 11, 13, 14, and 16, present characteristic fragments at m/z 414/413, except compound 1 (m/z 411). These ions

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**Figure 4.** Flowchart for structural analysis of 3-aminofurostane alkaloids with ESI positive experiment.
are derived from the sequential loss of sugar moieties, water, and ammonia groups from the molecular weight of protonated aglycone. The predominant ions were detected at \( m/z \) 399/397 and 381/379 (Figures 1, 2 and 4) due to the loss of ammonia and one molecule of water from the ion product at \( m/z \) 416/414, respectively. At this time, a proton added to the oxygen of C-16 and leading to the formation of a positive charge on the oxygen allowed the electron pair transfer from C-16 to the oxygen and the fission of the C-16–O bond. Due to the presence of enol,

**Figure 5.** (a) Product ion spectrum of the ion \([\text{M + H}]^+ \ m/z \ 772.4477 \) (2); (b) product ion spectrum of the ion \([\text{M + H}]^+ \ m/z \ 612.4106 \) (6).
the carbonyl of C-22 was produced by the tautomeration. From this tautomer, the elimination of H₂O produced the ion at m/z 381/379. The ion at m/z 285/283 was formed by the hydrogen transfer from, C-23 to C-20, and the fission of bond C-20–C-22. The carbonyl on C-22 played the key role in the loss of C₃H₆O. The fission of bond C-17–C-20 led to the formation of the ion at m/z 253/255 (Figures 4 and 5). This pathway is attributable to the hydrogen transfer from C-16 to the carbonyl via the McLafferty rearrangement. Table 2 summarizes the characteristic fragments observed from C-16 to the carbonyl via the McLafferty rearrangement.

This pathway is attributable to the hydrogen transfer from C-16 to the carbonyl via the McLafferty rearrangement. Table 2 summarizes the characteristic fragments observed from C-16 to the carbonyl via the McLafferty rearrangement. Table 2 summarizes the characteristic fragments observed from C-16 to the carbonyl via the McLafferty rearrangement.

Although shown to be a powerful tool for the assignment of 3-amino-furostane alkaloids, the presented method of mass spectra interpretation cannot completely solve the structure of newly detected molecules (positions of hydroxyl and sugar residues, unsaturation in the A-D rings, and stereochemistry). The correct order of sugar residues can only be partially determined, and the exact position of substituents/double bonds on the aglycones results are not completely clear. However, such information is very helpful in discriminating between the (often) large numbers of possible structures obtained.

Conclusions

The analysis of Solanum paniculatum roots by UPLC-TOF-MS/MS resulted in the characterization of 17 3-amino-furostane alkaloids; out of these, only jurubine had previously been reported in the literature. The NMR analysis of the main isolated compounds allowed the identification of two new compounds: (25R)-3β-amino-furost-5-en-22α,26-diol O(26)-β-D-glucopyranoside (fatimagraine) and (25R)-3β-amino-furost-22-en-26-ol O(26)-β-D-glucopyranoside (bhattacharyyaine). The unambiguous assignments of ¹H and ¹³C NMR data of jurubine are reported for the first time with rectification of the structure data.

Supplementary Information

The NMR and MS (11, 12, and 17) spectra of isolated compounds are shown in the Supplementary Information, available free of charge at http://jbcs.sbq.org.br as PDF file.

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