# New Quinolizidine Alkaloids from *Ulex jussiaei*

Patrícia Máximo and Ana Lourenço*

*Departamento de Química, Centro de Química Fina e Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2825-114 Caparica, Portugal*

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From an acidic extract of *Ulex jussiaei* four new quinolizidine alkaloids, jussiaeiines A (**1**), B (**2**), C (**3**), and D (**4**), were isolated and characterized by spectroscopic methods together with (−)-cytisine, (−)-*N*-methylcytisine, and (−)-anagyrine. The proposed biosynthetic origin of these new alkaloids is briefly discussed.

The *Ulex* genus is included in the Papilionoideae subfamily of the Leguminosae and is widespread in Europe. In Portugal, different species grow throughout the country, and some of them are endemic.[1] *Ulex* species are shrubs that do not exceed 1−2 m. In rural areas these plants are used as beds for cattle, and some species are used as foodstuffs. In folk medicine, the flowers are commercialized and used as infusions in the treatment of liver diseases.

*Ulex* species produce quinolizidine alkaloids, with most of this phytochemical work having been performed on *U. europaeus*.[2a−c] The quinolizidine alkaloids of *Ulex* species are of chemotaxonomic significance,[3,4a,b] and serve as a chemical defense mechanism against herbivores, insects, and microorganisms and have a role in allelopathic interrelations.[5] Some plants used in oriental traditional medicine contain quinolizidine alkaloids as their pharmacologically active principles.[6]

We wish to report four new quinolizidine alkaloids, jussiaeiines A−D (**1**−**4**), from *Ulex jussiaei* Webb. These alkaloids have an unusual disubstituted pyridine ring, rather than the more common α-pyridone ring. The new structures were identified together with (−)-cytisine,[7] (−)-*N*-methylcytisine,[8] and (−)-anagyrine.[9] The new alkaloids were identified from their spectral data by comparison with similar structures from the literature.

## Results and Discussion

The GC−EIMS of jussiaeiine A (**1**) exhibited a molecular ion at *m*/*z* 236 $[M]^+$, which is in agreement with the molecular formula $C_{13}H_{20}N_2O_2$, confirmed by HREIMS. On the GC−EIMS analysis of the silylated extract of *U. jussiaei*, the molecular ion shifted to *m*/*z* 308 $[M]^+$. This value, together with a fragment at *m*/*z* 218, corresponding to the loss of one TMSOH group, indicated the presence of one hydroxyl substituent in the structure of **1**. The $^1$H NMR spectrum of **1** (see Table 1) exhibited a broad singlet at $\delta$ 2.64 that disappeared with $D_2O$ addition and two characteristic signals of a methylene group vicinal to a hydroxyl at $\delta$ 3.58 (dd, $J_1$ = 10.6 Hz, $J_2$ = 5.4 Hz) and 3.49 (dd, $J_1$ = 10.4 Hz, $J_2$ = 7.2 Hz), suggesting that **1** possesses a primary alcohol. The GC-EIMS of **1** confirmed the presence of the primary alcohol from the $[M - OH]^+$, $[M - H_2O]$, and $[M - CH_2OH]^+$ fragment peaks (see Experimental Section).

The $^1$H NMR spectrum of jussiaeiine A (**1**) exhibited an ABX pattern of three aromatic protons at $\delta$ 7.45 (t, $J$ = 7.8 Hz, 1H), 6.70 (d, $J$ = 7.2 Hz, 1H), and 6.54 (d, $J$ = 8.4 Hz, 1H). The observed *o*-coupling constants indicated that the three aromatic protons in **1** are in sequential positions. The $^1$H NMR spectrum also showed a signal at $\delta$ 2.33 (s, 3H), characteristic of a *N*-methyl group, and a singlet at $\delta$ 3.89 (s, 3H), characteristic of a methoxyl group. The IR spectrum of jussiaeiine A (**1**) did not exhibit a carbonyl vibration due to an α-pyridone ring, as commonly encountered in quinolizidine alkaloids. Jussaeiine A (**1**) possesses, instead, a substituted pyridine ring with functionality at C-6 and a OMe 2-substituent, which was deduced from the HMBC spectrum through the $^3J$ coupling of the methoxyl group and the C-2 atom of the pyridine ring. From this reasoning, and in agreement with the presence of a primary alcohol and the low-field chemical shifts of the aromatic protons, the structure of **1** could be proposed as being related to that of (+)-kuraramine.[10] Comparing the chemical shifts of the $^1$H NMR spectra of the piperidine ring protons of both alkaloids, the chemical shifts were superimposed, indicating that **1** has the same conformation for the piperidine ring as (+)-kuraramine and establishing the orientation of all substituients. The chemical shifts in the $^{13}$C NMR spectra of both compounds are similar (Table 2). The piperidine ring of **1** was assigned a chair conformation, with the primary alcohol at C-9 and the pyridine ring at C-7 with $\beta_{equatorial}$ orientations. This was confirmed by a NOESY experiment, where the connectivities H-13$\beta_{axial}$/H-11$\beta_{axial}$; H-13$\beta_{axial}$/H-8$\beta_{axial}$; H-11$\beta_{axial}$/H-8$\beta_{axial}$; H-8$\beta_{axial}$/H-10$_{proR}$; H-8$\alpha_{equatorial}$/C-10-O*H*; and H-9$\alpha_{axial}$/H-7$\alpha_{axial}$ were observed. All of the $^{13}$C NMR, HMQC, HMBC, COSY, NOESY, and MS data obtained confirmed the proposed structure **1** for jussiaeiine A.

The GC−EIMS of jussiaeiine B (**2**) exhibited a molecular ion at *m*/*z* 276, in agreement with the molecular formula $C_{16}H_{24}N_2O_2$ (confirmed by HREIMS). In the same manner as described for compound **1**, from the data obtained from the GC−EIMS of the silylated plant extract sample, it was possible to conclude that **2** has one primary hydroxyl group. The presence of the hydroxyl group at C-10 was confirmed by the analysis of its HMBC spectrum. By IR analysis, **2** did not show an α-pyridone ring, and from the $^1$H NMR spectrum it had a similar equivalent aromatic ABX pattern to **1** and a methoxyl group at $\delta$ 3.87 (s). These data indicated that jussiaeiine B (**2**) has an identical structure in rings A and B with jussiaeiine A (**1**). The difference in the EIMS between the two compounds and analysis of the NMR spectra ($^1$H NMR, $^{13}$C NMR, HMQC, HMBC, COSY, and NOESY) led us to propose that **2** has a quinolizidine system, thereby having an additional ring when compared with jussiaeiine A (**1**) (see Tables 1 and 2).

* To whom correspondence should be addressed. Tel.: 351 21 294 85 00. Fax: 351 21 294 85 50. E-mail: a.lourenco@dq.fct.unl.pt.

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**Table 1.** $^1$H NMR Data of Jussiaeiines A−D (**1**−**4**) and Jussiaeiine D Diacetate (**4a**)[a]

| H | 1 | 2 | 3 | 4 | 4a |
|---|---|---|---|---|---|
| 3 | 6.54 d (8.4) | 6.52 d (8.2) | 6.55 d (8.4) | 6.55 d (8.2) | 6.55 d (8.2) |
| 4 | 7.45 t (7.8) | 7.43 t (7.7) | 7.46 t (7.8) | 7.47 t (7.7) | 7.48 dd (8.2, 7.3) |
| 5 | 6.70 d (7.2) | 6.69 d (7.2) | 6.73 d (7.2) | 6.72 d (7.2) | 6.71 d (7.3) |
| 7α | 2.96 br t (11.6) | 3.02 m | 3.08−3.02 m | 3.05−2.98 m | 2.99 tt (11.0; 3.7) |
| 8α | 1.99−1.96 m | 2.12−2.07 m | 1.99 br d (12.8) | 2.06 br d (12.0) | 2.07−2.00 m |
| 8*β* | 1.28 q (12.3) | 1.67−1.61 m | 1.81 q (12.8) | 1.65 q (12.0) | 1.58 q (12.2) |
| 9 | 1.99−1.96 m | 1.67−1.61 m | 1.55−1.47 m | 1.56 br s | 1.74−1.71 m |
| $10_{proS}$ | 3.58 dd (5.4, 10.5) | 3.71 dd (2.7, 10.8) | 3.77 dd (14.6, 11.4) | 3.73 br d (8.4) | 4.14 dd (3.3, 11.4) |
| $10_{proR}$ | 3.49 dd (7.2, 10.5) | 3.61 dd (5.1, 10.8) | 3.61 dd (2.6, 11.4) | 3.67 dd (5.3, 10.3) | 4.04 dd (5.5, 11.4) |
| 11α | 3.06 br d (10.8) | | | | |
| 11*β* | 1.70 br t (10.8) | 1.78 br s | 2.40, 2.30 m | 1.76 q (8.7) | 1.74−1.71 m |
| 12α | | 1.28−1.19 m | 1.55−1.47 m | 1.33−1.21 m | 1.35−1.27 m |
| 12*β* | | 1.95 br d (9.7) | 2.13 br d (14.0) | 2.06 br d (11.9) | 2.07−2.00 m |
| 13α | 3.06 br d (10.8) | 1.78 br s[b] | 4.20 br s | 2.06 br d[b] (11.9) | 3.81 br s |
| 13*β* | 2.09 br t (11.2) | 1.28−1.19 m[b] | | 1.33−1.21 m[b] | 2.15−2.11 m |
| 14α | | 1.67−1.61 m | 1.92 br t (13.6) | 3.81 m | 4.85 m |
| 14*β* | | 1.67−1.61 m | 1.72 br d (14.0) | | |
| 15α | | 2.86 br d (11.2) | 2.68 br d (18.8) | 3.05−2.98 m | 3.06 ddd (10.5, 4.4, 2.0) |
| 15*β* | | 2.12−2.07 m | 2.60 br t (11.4) | 1.98 br t (10.4) | 2.07−2.00 m |
| 17α | | 2.97 br d (11.2) | 3.08−3.02 m[b] | 3.05−2.98 m | 3.00 dt (10.0, 2.4) |
| 17*β* | | 2.26 br t (11.0) | 2.40, 2.30 m[b] | 2.34 br t (12.2) | 2.33 t (11.7) |
| OC*H*$_3$ | 3.89 s | 3.87 s | 3.89 s | 3.90 s | 3.90 s |
| O*H*-10 | 2.64 br s | 2.26 br t | 2.40−2.30 m | 1.86 br s | |
| O*H*-13 | | | 2.40−2.30 m | | |
| O*H*-14 | | | | 1.86 br s | |
| N-C*H*$_3$ | 2.33 s | | | | |
| COC*H*$_3$ | | | | | 2.05 s, 2.03 s |

[a] Chemical shifts are referenced to the signal of residual $CHCl_3$ ($\delta$ 7.26).Coupling constants are expressed in Hz and are presented in parentheses. [b] These assignments are interchangeable.

**Table 2.** $^{13}$C NMR Spectra Data of Jussiaeiines A−D (**1**−**4**) and Jussiaeiine D Diacetate (**4a**)[a]

| | 1 | | 2 | | 3 | | 4 | | 4Ac |
|---|---|---|---|---|---|---|---|---|---|
| C | $\delta_C$ | HMBC | $\delta_C$ | HMBC | $\delta_C$ | HMBC | $\delta_C$ | HMBC | $\delta_C$ |
| 2 | 163.5 s | H-4, OC*H*$_3$ | 163.5 s | H-4,O-C*H*$_3$ | 163.5 s | H-4,O-C*H*$_3$ | 163.5 s | H-4,O-C*H*$_3$ | 163.7 s |
| 3 | 107.8 d | H-5 | 107.8 d | H-5 | 107.9 d | H-5 | 107.8 d | H-5 | 108.0 d |
| 4 | 138.8 d | | 138.7 d | | 138.8 d | | 138.8 d | | 138.8 d |
| 5 | 114.1 d | H-3 | 114.1 d | H-3 | 114.0 d | H-3 | 114.1 d | H-3 | 114.1 d |
| 6 | 160.7 s | H-4 | 160.9 s | H-4 | 160.7 s | H-4 | 160.6 s | H-4 | 160.5 s |
| 7 | 43.6 d | H-5 | 43.3 d | | 43.4 d | | 43.5 d | H-5, H-17 | 43.3 d |
| 8 | 32.6 t | H-10, H-11, H-13 | 33.8 t | H-17 | 33.6 t | H-10, H-17 | 33.7 t | H-17 | 33.9 t |
| 9 | 39.2 d | | 43.8 d | | 43.6 d | | 43.6 d | H-8 | 41.0 d |
| 10 | 66.1 t | H-8, H-11 | 64.4 t | | 63.9 t | | 64.7 t | | 66.2 t |
| 11 | 58.8 t | H-8, H-10, H-13, N-C*H*$_3$ | 63.8 d | H-15, H-17 | 56.8 d | H-10, H-15, H-17 | 62.5 d | H-15, H-17 | 62.7 d |
| 12 | | | 29.7 t | | 36.2 t | | 28.2 t | | 28.1 t |
| 13 | 61.1 t | H-8, H-11, N-C*H*$_3$ | 24.5 t | H-15 | 64.4 d | H-15 | 33.8 t | | 30.1 t |
| 14 | | | 25.6 t | | 32.3 t | | 67.3 d | H-15 | 69.6 d |
| 15 | | | 56.7 t | H-17 | 50.1 t | | 63.6 t | H-17 | 59.6 t |
| 17 | | | 61.5 t | H-15 | 61.2 t | | 61.3 t | H-15 | 61.2 t |
| O-C*H*$_3$ | 53.1 q | | 53.1 q | | 53.1 q | | 53.2 q | | 53.2 q |
| N-C*H*$_3$ | 46.3 q | H-11, H-13 | | | | | | | |
| C*O*CH$_3$ | | | | | | | | | 171.0 s |
| C*O*CH$_3$ | | | | | | | | | 170.2 s |

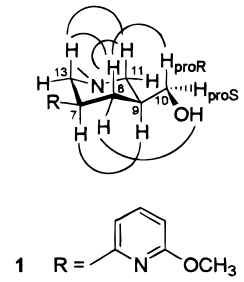
[a] Chemical shifts are referenced to the signal of residual $CHCl_3$ ($\delta$ 77.0).

Comparing the $^1$H and $^{13}$C NMR data of jussiaeiine B (**2**) with those of mamanine,[11] it was inferred that **2** also has a *trans* junction between the two rings of the quinolizidine nucleus, so that they are in chair conformation and their substituients have an equatorial orientation. On this basis, the connectivities observed in a NOESY experiment were as follows, and permitted the assignment of the orientation of each proton of the methylene groups: H-11$\beta_{axial}$/H-17$\beta_{axial}$; H-15$\alpha_{equatorial}$/H-17$\alpha_{equatorial}$; H-8$\beta_{axial}$/H-11$\beta_{axial}$; and H-9$\alpha_{axial}$/H-12$\alpha_{axial}$. With the information obtained from the 2D NMR experiments, all of the $^1$H and $^{13}$C NMR signals of structure **2** would be assigned (see Tables 1 and 2), and jussiaeiine B was proposed as having structure **2**.

The GC−EIMS of jussiaeiines C (**3**) and D (**4**) exhibited the same molecular ion at *m*/*z* 292, in agreement with the molecular formula $C_{16}H_{24}N_2O_3$ (both confirmed by HREIMS) and exhibited the fragments $[M - OH]^+$, $[M - H_2O]$, and $[M - CH_2OH]^+$, conferring on each compound the presence of a primary alcohol. From the GC−EIMS analysis of the silylated extract of *U. jussiaei*, the fragment peak at *m*/*z* 436 $[M]^+$, together with mass fragment at *m*/*z* 346 corresponding to the loss of two TMSOH groups, in each case suggested that a second hydroxyl group is present in both **3** and **4**.

Comparing the $^1$H NMR spectra of jussiaeiines C (**3**) and D (**4**) with that of jussiaeiine B (**2**), the overall similarity of these three compounds was apparent, although **3** and **4** contained one more hydroxyl group in their structures (see Tables 1 and 2). The difference in Kovats retention indexes (RI) and the fragmentation of the GC−EIMS spectra led us to assume that jussiaeiines C (**3**) and D (**4**) are isomers.

The fragments at *m*/*z* 83 and 99 in the mass spectrum of jussiaeiine C (**3**) mean that the hydroxyl group should



**1** R = 

**Figure 1.** Connectivities observed from the NOESY experiment of compound **1**.

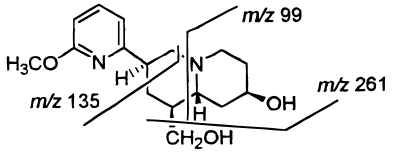


**Figure 2.** Proposed mass spectral fragmentation pattern of jussiaeiine C (**3**).

be at either C-12, C-13, C-14, or C-15. The hydroxyl group was proposed to be at C-13$\beta_{axial}$, by comparing the $^{13}$C NMR spectrum of **3** and that of the known 13$\beta$-hydroxymamanine.[12] To prove this hypothesis, the $^{13}$C NMR data were analyzed. The signals corresponding to the carbon atoms of ring B of **3** coincided with those of jussiaeiine B (**2**), meaning that rings B and C both have a chair conformation at the *trans* junction, and the orientation of their substituents is equatorial. The signals corresponding to ring C of **3** were in agreement with the introduction of a hydroxyl group at C-13 due to the $\alpha$(on C-13), $\beta$ (on C-12/C-14), and $\gamma$ gauche (on C11/C-15) shifts observed. Considering that **3** has a chair conformation for rings B and C, the hydroxyl group must be 13$\beta_{axial}$. This hypothesis was further confirmed by 2D NMR experiments of jussiaeiine C (**3**). The NOESY spectrum showed interactions between H-13$\alpha_{equatorial}$ ($\delta$ 4.20) and H-14$\alpha$ ($\delta$ 1.92) and H-12$\alpha$ ($\delta$ 1.55−1.47). It should be noted that the coupling constants $J_{10,9\alpha}$ of protons H-10$_{proS}$ and H-10$_{proR}$ of jussiaeiine C (**3**) ($J_{10proS,9\alpha}$ = 4.6 Hz; $J_{10proR,9\alpha}$ = 2.6 Hz) have inverse magnitudes similar to those observed for the corresponding protons of jussiaeiine B **2** ($J_{10proS,9\alpha}$ = 2.7 Hz; $J_{10proR,9\alpha}$ = 5.1 Hz). This can be explained because the hydroxyl group at the C-13$\beta_{axial}$ position induces a preferred quasi-axial position to the hydroxyl group at C-10$\beta_{equatorial}$. The fragmentations of the mass spectrum of jussiaeiine C (**3**) at *m*/*z* 261 and 135 (Figure 2) also confirmed that jussiaeiine C has structure **3**.

As mentioned above, jussiaeiine D (**4**) is structurally similar to jussiaeiine C (**3**). From the $^{13}$C NMR data (see Table 2), the chemical shifts of the carbon atoms of rings A and B were almost coincident, so that the conformations and orientations of the rings of jussiaeiine D (**4**) are the same as those of compound **2** (rings B and C in chair conformation, a *trans* junction between them, and equatorial orientation of the substituents at C-7 and C-9). Comparing the $^{13}$C NMR spectra of jussiaeiines B (**2**) and D (**4**), it was possible to verify the $\alpha$ effect on C-14, the $\beta$ effect on C-13 and C-15, and the $\gamma$-*trans* effect on C-12, which indicated the $\beta$ orientation of the hydroxyl group. Further confirmation of the orientation of this hydroxyl group at C-14 was obtained by the analysis of the $^1$H NMR spectrum of the diacetyl derivative of jussiaeiine D (**4a**) (Table 1). In this spectrum the signal of the geminal proton to the hydroxyl group was shifted downfield, and its multiplicity and *J* values indicated that the hydroxyl group should be located at the C-14$\beta_{equatorial}$ position (see Table 1). A similar conclusion was made in the identification of (+)-9$\beta$-hydroxylamprolobine,[13] a quinolizidine alkaloid from *Sophora velutina* var. *zimbadweenis*. For this compound, the $\gamma$-*trans* effect observed in the $^{13}$C NMR spectrum is also of the same magnitude as that observed for jussiaeiine D (**4**).

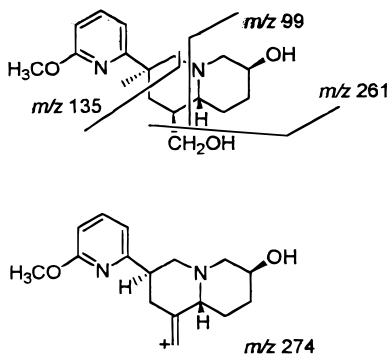
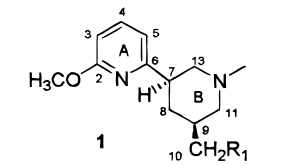
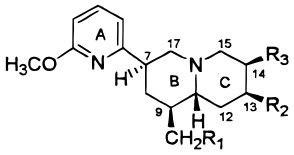


**Figure 3.** Proposed mass spectral fragmentation pattern of jussiaeiine D (**4**).



**1**



**2** $R_1$=OH, $R_2$=H, $R_3$=H

**3** $R_1$=OH, $R_2$=OH, $R_3$=H

**4** $R_1$=OH, $R_2$=H, $R_3$=OH

**4a** $R_1$=OCOCH$_3$, $R_2$=H, $R_3$=OCOCH$_3$

From HMQC, HMBC, and COSY spectra of jussiaeiine D (**4**), it was possible to assign all the chemical shifts in the $^1$H NMR and $^{13}$C NMR spectra. The connectivities observed in the NOESY spectrum resulted in assignments for H-17$\beta$, H-15$\beta$, and H-12$\alpha$ as follows: H-11$\beta_{axial}$/H-17 ($\delta$ 2.34); H-17$\beta_{axial}$/H-15 ($\delta$1.98); and H-9$\alpha_{axial}$/H-12 ($\delta$ 1.33−1.21). It should be noted that the coupling constants of proton H-10$_{proR}$ are of the same magnitude as those observed for the corresponding proton of jussiaeiine B (**2**). This can be explained by the fact that the hydroxyl group at C-10 adopts a preferential position that does not depend on the influence of an extra hydroxyl group on ring C, as shown for jussiaeiine C (**3**). The mass spectrum was also in agreement with structure **4** for jussiaeiine D and some important fragments at *m*/*z* 274, 261, 135, and 99 are presented in Figure 3.

The molecules of jussiaeiines A (**1**), B (**2**), C (**3**), and D (**4**) could be formed by oxidative cleavage of the bond N-1−C-10 between rings A and B of the quinolizidine skeleton, so that the nitrogen atom is not fused. This hypothesis has been described for the biosynthetic pathway for kuraramine, mamanine, and 13$\beta$-hydroxymamanine.[12] The following steps would involve the aromatization and methoxylation of ring A of the alkaloid structures. Jussiaeiines A (**1**), B (**2**), and C (**3**) could be formed from *N*-methylcytisine,

anagyrine, and baptifoline, respectively. Jussiaeiine D (**4**) would, in turn, be formed from 14$\beta$-hydroxyanagyrine, although this alkaloid has never been isolated to date.

## Experimental Section

**General Experimental Procedures.** Melting points were measured on a Reichert thermovar apparatus and are uncorrected. A Perkin-Elmer 241MC polarimeter was used to obtain optical specific rotation values. IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT-IR spectrometer and UV spectra on a Milton Roy-spectronic 1201 instrument. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker ARX400 NMR spectrometer at 400 and 100 MHz, respectively. All NMR spectra were recorded in $CDCl_3$ solution and referenced by the residual chloroform signal ($\delta$ 7.26 ppm and 77.0 ppm); $\delta$ values are expressed in parts per million (ppm) and $J$ couplings in hertz (Hz). The GC−EIMS and GC−HREIMS studies were performed on a fused-silica OV-1 capillary column (i.d. = 0.32 mm; film thickness = 0.25 $\mu$m; length = 30 m) and recorded on Finnigan MAT 4500 and Finnigan MAT 302 instruments at 45 eV. The GC operating conditions employed were as follows: He as carrier gas; split 1:10; injection port temperature, 250 °C; temperature program, 120 °C held for 3 min, increased 6 °C/min to 312 °C, held for 10 min. The Kovats RI values were calculated by co-injection with a linear alkane standard mixture.

**Plant Material.** *Ulex jussiaei* was collected at Terras do Risco, Arrábida, Portugal, in April 1996, and a voucher specimen is deposited in the Herbário, Museu, Jardim Botânico, Faculdade de Ciências, Universidade de Lisboa (TO42516a).

**Extraction and Isolation.** Dried and finely powdered aerial parts of *U. jussiaei* (600 g) were extracted with HCl 0.5 M, as previously described by Wink et al.[14] The alkaloid extract (606 mg) was then chromatographed on a Si gel 60 (Merck no. 7734) column with $CHCl_3$−MeOH−$NH_4OH$ mixtures of increasing polarity (97.5:2.5:1, 95:5:1, and 90:10:1). From the fraction eluted with $CHCl_3$−MeOH−$NH_4OH$, 95:5:1, after purification by Si gel 60 (Merck no. 5554 or no. 5744), preparative TLC eluted with $CHCl_3$−MeOH−$NH_4OH$ and/or $Et_2O$−MeOH−$NH_4OH$ mixtures, (−)-anagyrine[9] (6.1 mg), (−)-*N*-methylcytisine[8] (4.9 mg), and jussiaeiine B (**2**, 17.7 mg) were obtained, in increasing order of polarity. From the fraction eluted with $CHCl_3$−MeOH−$NH_4OH$ (90:10:1), after purification by preparative TLC in a manner similar to that discussed above, (−)-cytisine[7] (24.5 mg), jussiaeiine A (**1**, 22.8 mg), jussiaeiine D (**4**, 79.9 mg), and jussiaeiine C (**3**, 6.7 mg) were obtained, in increasing order of polarity.

**Jussiaeiine A (1):** colorless oil; $[\alpha]^{25}_D$ +3.3° (*c* 0.26, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 219 (3.34), 267 (3.56), 274 (sh) nm; IR (KBr) $\nu_{max}$ 3368, 2944, 2794, 1578, 1466, 1440, 801, 756, 740 cm$^{-1}$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2; EIMS *m/z* 236 $[M]^+$ (81) 221 (10), 219 (7), 218 (10), 205 (43), 192 (28), 178 (24), 162 (29), 149 (83), 136 (100), 135 (57), 134 (53), 123 (49), 101 (49), 84 (24), 58 (99), 43 (98); GC−MS RI 1871; HREIMS *m/z* 236.1517 (calcd for $C_{13}H_{20}O_2N_2$, 236.1525).

**Jussiaeiine B (2):** colorless oil; $[\alpha]^{25}_D$ +27.2° (*c* 0.27, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 217 (3.26), 268 (3.38) 273 (sh) nm; IR (KBr) $\nu_{max}$ 3181, 2929, 2806, 1578, 1465, 1441, 804 cm$^{-1}$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2; EIMS *m/z* 276 $[M]^+$ (17), 261 (5), 259 (10), 258 (8), 245 (23), 218 (44), 136 (91), 135 (100), 134 (81), 83 (81); GC−MS RI 2241; HREIMS *m/z* 276.1839 (calcd for $C_{16}H_{24}O_2N_2$, 276.1838).

**Jussiaeiine C (3):** colorless oil; $[\alpha]^{25}_D$ +32.2° (*c* 0.19, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 218 (3.29), 269 (3.43), 273 (sh) nm; IR (KBr) $\nu_{max}$ 3350, 2925, 1578, 1466, 1439, 803, 736 cm$^{-1}$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2; EIMS *m/z* 292 $[M]^+$ (26), 275 (3), 274 (4), 261 (12), 234 (49), 136 (59), 135 (100), 134 (57), 99 (8); GC−MS RI 2464; HREIMS *m/z* 292.1802 (calcd for $C_{16}H_{24}O_3N_2$, 292.1787).

**Jussiaeiine D (4):** colorless oil; $[\alpha]^{25}_D$ +14.5° (*c* 0.64, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 218 (3.20), 269 (3.29) nm; IR (KBr) $\nu_{max}$ 3340, 2927, 2805, 1599, 1578, 1466, 1439, 804, 736 cm$^{-1}$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2; EIMS *m/z* 292 $[M]^+$ (8), 275 (21), 274 (84), 261 (20), 234 (27), 136 (81), 135 (100), 134 (62), 99 (11); GC−MS RI 2474; HREIMS *m/z* 292.1794 (calcd for $C_{16}H_{24}O_3N_2$, 292.1787).

**Silylation of 1−4.** The acidic extract of *U. jussiaei* (4.2 mg) was silylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (Aldrich) (100 $\mu$L) at room temperature for 24 h under $N_2$. This sample was analyzed by GC−EIMS, and the EIMS spectra of the silyl derivatives of jussiaeiines A−D (**1**−**4**) follows.

**TMS derivative of jussiaeiine A (1):** EIMS *m/z* 308 $[M]^+$ (44), 293 (35), 218 (31), 205 (56), 178 (16), 173 (37), 162 (23), 149 (67), 136 (72), 135 (42), 134 (35), 123 (32), 84 (8), 73 (81), 58 (100), 43 (15).

**TMS derivative of jussiaeiine B (2):** EIMS *m/z* 348 $[M]^+$ (37) 333 (32), 258 (29), 245 (47), 218 (62), 136 (61), 135 (100), 134 (88), 83 (60), 73 (55).

**TMS derivative of jussiaeiine C (3):** EIMS *m/z* 436 $[M]^+$ (32), 421 (23), 346 (21), 333 (21), 306 (53), 170 (16), 136 (50), 135 (100), 134 (65), 73 (70).

**TMS derivative of jussiaeiine D (4):** EIMS *m/z* 436 $[M]^+$ (38), 421 (58), 346 (100), 333 (51), 306 (75), 170 (22), 136 (76), 135 (89), 134 (78), 73 (94).

**Acetylation of jussiaeiine D (4):** Jussiaeiine D diacetate (**4a**) was obtained from jussiaeiine D (**4**, 9.9 mg) and dissolved in 1 mL of pyridine with the addition of 1 mL of acetic anhydride. The mixture was left at room temperature for 25 h. After the reaction was complete, 5 mL of water were added, and the pyridine was removed by distillation under reduced pressure. The residue was purified by TLC giving pure jussiaeiine D diacetate (**4a**, 8.0 mg), which was obtained as a colorless oil; $[\alpha]^{25}_D$−9.9° (*c* 0.73, $CHCl_3$); IR (KBr) $\nu_{max}$ 2950, 2854, 1739, 1580, 1467, 1441, 1242, 1035, 758 cm$^{-1}$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2; EIMS *m/z* 377 $[M]^+$, 317 (9), 316 (14), 257 (4), 243 (2), 176 (5), 111 (24), 97 (54), 83 (76), 82 (59), 69 (70), 67 (43), 57 (79), 55 (74), 43 (100).

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