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Quinolizidine alkaloids from the curare adjuvant *Clathrotropis glaucophylla*

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Abstract

The bark of *Clathrotropis glaucophylla* (Fabaceae) is used as admixture of curare arrow poison by the Yanomamï Amerindians in Venezuela. A new quinolizidine alkaloid (QA), (−)-13α-hydroxy-15α-(1-hydroxyethyl)-anagyrine [(−)-clathrotropine], was isolated from the alkaloid extract of *C. glaucophylla* bark, together with eleven known QAs: (−)-anagyrine, (−)-thermopsine, (−)-baptifoline, (−)-epibaptifoline, (−)-rhombifoline, (−)-tinctorine, (−)-cytisine, (−)-*N*-methylcytisine, (−)-lupanine, (−)-6α-hydroxylupanine and (+)-5,6-dehydrolupanine. The isolation and structure elucidation were performed with the aid of chromatographic (TLC, HPLC and CC) and spectroscopic (UV and 1D/2D NMR) methods, and mass spectrometry. To our knowledge, this is the first time quinolizidine alkaloids have been isolated from an arrow poison ingredient. It is also the first report on *Clathrotropis* species being used for preparation of arrow poison.

Keywords: *Clathrotropis glaucophylla*; Fabaceae; (−)-13α-Hydroxy-15α-(1-hydroxyethyl)-anagyrine; Quinolizidine alkaloids; Curare

1. Introduction

Clathrotropis is a small genus of the Fabaceae family, tribe Sophoreae, with six species endemic to tropical South America. *C. glaucophylla* Cowan was collected in the rainforests of the upper Orinoco in Venezuela in 1999, during ethnobotanical fieldwork among the Yanomamï Amerindians. Our ethnobotanical investigation has revealed that the species *C. glaucophylla* and *C. macrocarpa* (wapu kohi) are of great economic importance to the Yanomamï, the seeds playing a significant role in alimentation, and the bark being used as ingredient of curare arrow poison.

Members of the Sophoreae are known to produce quinolizidine alkaloids, which are the largest single group of legume alkaloids. They appear to be restricted to the more primitive tribes of the Fabaceae, and have thus been shown to be of some use in establishing phylogenetic relationships at the generic and tribal level (Kinghorn and Balandrin, 1984).

To date, only three chemical studies have been carried out on *Clathrotropis* species. One concerning the silica content of *Clathrotropis* wood (Amos, 1951), the two others reporting quinolizidine alkaloids from *C. brachypetala* seeds (Hatfield et al., 1980) and *C. macrocarpa* leaves (Ricker et al., 1994), respectively. In the present paper we report on the isolation and structural elucidation of a new QA, (−)-13α-hydroxy-15α-(1-hydroxyethyl)-anagyrine (**1**). The ^{13}C NMR spectral data (in MeOD) for the known compounds (−)-anagyrine (**2**), (−)-thermopsine (**3**), (−)-baptifoline (**4**), (−)-epibaptifoline (**5**), (−)-rhombifoline (**6**), (−)-tinctorine (**7**), (−)-cytisine (**8**), (−)-*N*-methylcytisine (**9**), (−)-lupanine (**10**), (−)-6α-hydroxylupanine (**11**) and (+)-5,6-dehydrolupanine (**12**) are reported for the first time (Table 1).

2. Results and discussion

Open column chromatography (CC) and preparative HPLC of the alkaloid extract obtained from the bark of *C. glaucophylla*, yielded 12 alkaloids, of which one was new to the literature.

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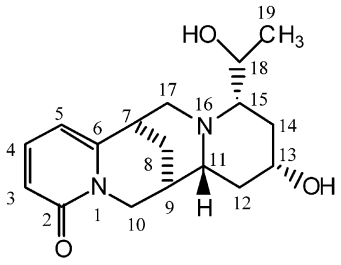

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The known compounds **2**–**12** were identified as (−)-anagyrine (**2**) (Asres et al., 1986), (−)-thermopsine (**3**) (Mikhova and Duddeck, 1998), (−)-baptifoline (**4**) (Kennelly et al., 1999), (−)-epibaptifoline (**5**) (Greinwald et al., 1990), (−)-rhombifoline (**6**) (Al-Azizi et al., 1994), (−)-tinctorine (**7**), (−)-cytisine (**8**) (El-Shazly et al., 1996), (−)-*N*-methylcytisine (**9**) (Wang et al., 2000), (−)-lupanine (**10**) (Abdel Halim et al., 1992), (−)-6α-hydroxylupanine (**11**) (Wang et al., 2000), and (+)-5,6-dehydrolupanine (**12**) (Asres et al., 1986) by their physiochemical properties ($[\alpha]_D$, MS, ^{1}H NMR and ^{13}C NMR) and by comparing these with existing literature data. For (−)-thermopsine (**3**), (−)-baptifoline (**4**), no appropriate NMR spectra could be obtained in $CDCl_3$ for comparison. For (−)-tinctorine (**7**) no complete ^{1}H and ^{13}C NMR data have previously been published. The ^{13}C NMR data for all the isolated compounds, measured in MeOD, are given in Table 1.

Compound **1** was obtained as crystals, $[\alpha]_D^{23}$ −146 (EtOH, *c* 0.1). The UV spectrum showed absorption at UV_{max} nm; 205 (2.67), 234 (2.79), 309 (2.87) (MeOH, log ϵ): suggesting the presence of a pyridone moiety (Atta-ur-Rahman et al., 1991; Al-Azizi et al., 1994). The HR-MALDI-mass spectrum showed a pseudomolecular peak at *m*/*z* 305.1845 $[M+H]^+$, compatible with the molecular formula $C_{17}H_{24}N_2O_3$. The EI–MS spectrum also showed ions at *m*/*z* 160 and 146, characteristic of lupine alkaloids containing an α-pyridone ring (Saito and Murakoshi, 1995).

The ^{1}H NMR spectrum confirmed the presence of an α-pyridone ring, showing signals at δ 6.41 (*dd*, *J* = 1.3/9.0), δ 7.47 (*dd*, *J* = 7.0/8.9) and δ 6.30 (*dd*, *J* = 1.2/7.0), corresponding to protons H-3, H-4 and H-5, respectively. The H-10α (δ 4.13) and H-10β (δ 3.92) were also characteristic for pyridone-type quinolizidine alkaloids (Atta-ur-Rahman et al., 1991). Accordingly, the ^{13}C NMR spectrum revealed the signals for 17 carbon atoms, which could be assigned as shown in Table 1. The multiplicity was attained by DEPT experiments, which revealed the signals for two quarternary carbon atoms, one CH_3 group, five CH_2 groups, and nine CH groups. The chemical shift of C-2 (δ 165.3) indicated a carbonyl group, while the chemical shifts of C-10 (δ 53.2), C-11 (δ 64.0), C-13 (δ 70.7), C-15 (δ 67.6), C-17 (δ 47.4) and C-18 (δ 67.5) suggested a position adjacent to a nitrogen or an oxygen atom.

^{1}H–^{1}H COSY and HSQC–TOCSY correlations allowed us to determine the two spin systems. The HMBC correlations between C-6 and H-4, H_2-10 and H_2-17, as well as between C-2 and H_2-10, assigned the connections of the two spin systems.

Except for two more carbon atom signals and a down field shifted value for C-15, the ^{13}C spectrum of compound **1** was very similar to that of (−)-epibaptifoline (**5**). This suggested a substitution of a hydroxyl at carbon 13 and a C_2 group at carbon 15. In the ^{1}H spectrum a large doublet integrated for 3 protons appeared at δ 0.99. The signal was assigned to CH_3-19, which showed correlations to the CH-18 at δ 67.5/3.57 in the HSQC–TOCSY and COSY spectra. H-18 further correlated

Table 1
^{13}C (75.5 MHz) NMR data of compounds **1**–**12** (in CD_3OD) (δ in ppm)

C	1	2	3	4	5	6	7	8	9	10	11	12
2	165.3	165.5	165.8	165.7	165.5	165.6	165.6	165.8	165.7	174.1	174.3	173.0
3	116.5	116.6	116.6	117.0	116.6	116.4	116.5	116.9	116.7	33.7	33.8	32.3
4	141.3	141.3	141.4	141.0	141.3	141.2	141.3	141.3	141.3	20.2	16.6	20.0
5	107.6	107.8	107.9	108.0	107.8	107.8	107.6	108.2	107.9	28.1	35.1	104.5
6	153.6	154.0	153.7	153.1	153.8	154.0	154.2	152.9	153.5	62.2	86.7	143.4
7	36.5	36.8	36.5	36.3	36.7	36.8	36.6	36.3	36.6	33.3	39.3	35.7
8	22.0	21.3	28.3	20.8	21.2	26.6	20.1	26.9	25.9	27.1	20.2	23.5
9	33.4	33.9	34.2	33.3	34.4	29.5	30.4	28.8	29.3	36.1	36.1	34.4
10	53.2	53.1	46.3	52.9	53.1	51.7	53.0	51.1	51.5	47.9	44.0	49.8
11	64.0	64.5	67.4	57.6	62.7	61.0	66.9	53.0	63.2	65.7	65.7	64.5
12	31.8	23.4	31.0	30.0	32.6	–	–	–	–	34.0	33.5	26.8
13	70.7	26.6	25.4	65.7	70.6	61.5	56.2	54.0	63.6	25.4	25.7	26.2
14	30.4	19.9	26.4	26.1	29.2	58.1	26.8	–	46.5	25.6	25.5	21.3
15	67.6	55.3	57.4	48.9	53.1	32.2	137.6	–	–	56.7	56.6	55.7
16	–	–	–	–	–	137.6	117.3	–	–	–	–	–
17	47.4	53.7	64.6	52.8	53.4	115.7	42.8	–	–	53.7	55.4	56.3
18	67.5	–	–	–	–	–	–	–	–	–	–	–
19	20.0	–	–	–	–	–	–	–	–	–	–	–

with H-15 in the COSY spectrum and with C-14 in the HMBC spectrum. This information confirmed the substitution of a 1-hydroxyethyl group at C-15. The configuration of compound **1** was determined to be relative 7*R*, 9*R*, 11*R*, 13*S*, 15*S* after observation of cross peaks between H-11, H-13 and H-15, between H-11 and H-10α/β, and between H-17α, H-8β, H-12α and H-14α in the ROESY spectrum. Hence, the substance was identified as (−)-13α-hydroxy-15α-(1-hydroxyethyl)-anagyrine, and was given the trivial name (−)-clathrotropine.

It is known that QAs have toxicological and pharmacological activities. They interact with ACh receptors as agonists and some inhibit Na^+ and K^+ channels, which might lead to respiratory paralysis and ventricular arrest at high doses (Kinghorn and Balandrin, 1984; Wink, 1998). This suggests that *C. glaucophylla* might be an active ingredient in curare, and therefore confirms the indigenous use of this admixture.

3. Experimental

3.1. General experimental procedures

CC: silica gel *60,* 40–63 and 63–200 μm (Merck); aluminium oxide 60 type 507C neutral, activity III 50–150 μm (Fluka). HPLC: Merck-Hitachi L-7150 pump connected to a Merck-Hitachi L-7200 Injector, a Merck-Hitachi L-7400 UV detector, and a Knauer HPLC column (LiChrosorb Si60, 5 μm; 250 × 16 mm); Merck-Hitachi L-6200 pump connected to a Rheodyne 7125 Injector, a Merck-Hitachi L-4000 UV detector, a Merck D-2500 Chromato-integrator, and a Knauer HPLC column (LiChrosorb Si60, 5 μm; 250 × 8 mm). TLC: Silica gel *60* F_{254} precoated aluminium plates (0.2 mm, Merck); aluminium oxide 60 F_{254} aluminium sheets (Merck). Detection: Dragendorff's reagent. Optical rotation: Perkin-Elmer 241 polarimeter. UV: Uvikon 930 spectrophotometer, HR-MALDI–MS: Ionspec Ultima FTMS spectrometer with 2,5-dihdyroxybenzoic acid (DHB) as matrix. DEI-MS: micromass Tribrid double focusing mass spectrometer at 70 eV. ^{13}C NMR, DEPT-135, DEPT-90 for all compounds and ^1H, [^1H,^1H] COSY, [^{13}C,^1H] HSQC, [^{13}C,^1H] HMBC and [^1H,^1H] ROESY for compounds **6**, **9** and **11** were measured on Bruker AMX-300 at 295 K (operating at 300.13 MHz for ^1H, and 75.47 MHz for ^{13}C), ^1H, [^1H,^1H]-COSY, [^{13}C,^1H]-HSQC, [^{13}C,^1H]-HSQC-TOCSY, [^{13}C,^1H]-HMBC and [^1H,^1H]-ROESY experiments for all the other compounds were measured on a Bruker DRX-500 at 295 K (operating at 500.13 MHz for ^1H, and 125.77 MHz for ^{13}C), chemical shifts δ were given in ppm and coupling constants J in Hz. The spectra were measured in CD_3OD for all the compounds and also in $CDCl_3$ for compound **2**, **5**, **6**, **8**, **9**, **10**, **11** and **12** to compare with the literature. The spectra were referenced against residual non-deuterated solvent.

3.2. Plant material

The bark of *C. glaucophylla* Cowan was collected in Venezuela in 1999, in the surroundings of the Yanomamï village Hasupïweitheri (primary forest). An herbarium sample (JG-134) exists in VEN (Herbario Nacional de Venezuela) and in MYF (Herbario Ovalles, Universidad Central de Venezuela). The plant material was collected during an ethnobotanical project based on an international contract (Contrato de Acceso a los Recursos Fitogenéticos) between the Ministry of Environment of Venezuela (MARNR) and Swiss Federal Institute of Technology (No. 2–1-99), according to the Andean Pact Desicion 391. Prior informed consent from the Yanomamï and permits from INPARQUES, D.A.I, as well as ORPIA and the Guardia Nacional were obtained in 1999.

3.3. Extraction and isolation

Air-dried and ground material (250 g) was macerated to exhaustion at room temperature with EtOH 75%. The concentrated EtOH extract (32.7 g) was suspended in HCl (0.1 N) and extracted with CH_2Cl_2 (three times). The acidic solution was brought to pH 9 with 25% NH_4OH and extracted with CH_2Cl_2 (four times). The remaining water extract was brought to pH 11 with 25% NH_4OH and again extracted with CH_2Cl_2 (four times). The two CH_2Cl_2 extracts were combined and evaporated to dryness, yielding a dark brown syrup (alkaloid extract *1*: 750 mg). For primary fractionation the alkaloid extract *1* (730 mg) was submitted to open column (CC) on silica gel, using mixtures of dichloromethane and methanol of increasing polarity as mobile phase. This gave fractions 1–14. Compounds **2** (3 mg) and **10** (9 mg) were isolated from fraction 8 and 10, respectively, using CC with aluminium oxide as stationary phase and hexane–ethyl acetate–ethanol of increasing polarity as mobile phase.

Air-dried and ground material (750 g) was successively macerated to exhaustion with CH_2Cl_2, MeOH and MeOH–H_2O (80:20) at room temperature. The MeOH extract (71.1 g) and the MeOH–H_2O extract (11.4 g) were subsequently subjected to an alkaloid extraction as described above. The resulting alkaloid extract was added to the remaining fractions of alkaloid extract *1* to give alkaloid extract *2* (1.5 g).

Alkaloid extract *2* was submitted to CC on silica gel, using CH_2Cl_2–MeOH–25% NH_4OH[1] of increasing polarity as mobile phase. Eight fractions were obtained,

[1] 1.2 ml NH_3/500 ml CH_2Cl_2–MeOH.

of which one was pure **2** (16 mg). Compounds **6** (9 mg), **7** (1.2 mg) and **3** (3 mg) were isolated by normal phase HPLC from fractions 2, 3, and 4, respectively. The mobile phase was CH_2Cl_2–MeOH–25% NH_4OH[1] (97:3). Fractions 6 and 7 were further fractionated using the same stationary and mobile phases as the primary fractionation. Purification of fractions 6.2, 6.3, 6.7 and 7.2 by normal phase HPLC, using CH_2Cl_2–MeOH–25% NH_4OH[1] (96:4) as mobile phases, resulted in the isolation of compounds **1** (9 mg), **2** (20 mg), **4** (3.5 mg), **5** (9 mg), **8** (7.5 mg), **9** (15 mg) and **12** (18 mg). Compound **11** (8 mg) was obtained from fraction 6.5 after an open column with aluminium oxide as stationary phase and hexane–ethyl acetate–ethanol of increasing polarity as mobile phase.

3.4. (−)-Clathrotropine (1)

$[\alpha]_D^{23}$ −146 (EtOH, *c* 0.1). UV λ_{max}^{MeOH} nm (log ϵ): 205 (2.67), 234 (2.79), 309 (2.87). HR-MALDI-MS (pos. mode): 305.1845 $[M+H]^+$ (calculated for $C_{17}H_{24}N_2O_3$, 305.1865). DEI-MS *m/z* (rel. int.): 302 (21), 286 (39), 268 (24), 259 (62), 245 (100), 215 (23), 160 (21), 146 (28), 44 (44). ^{13}C NMR in Table 1. ^1H NMR (MeOD, 500.1 MHz) δ 7.47 (1H, *dd*, *J* = 7.0, 8.9, H-4), δ 6.41 (1H, *dd*, *J* = 1.3, 9.0 Hz, H-3), δ 6.30 (1H, *dd*, *J* = 1.2, 7.0, H-5), δ 4.13 (1H, *d*, *J* = 15.4, H-10α), δ 3.92 (1H, *dd*, *J* = 6.3, 15.4, H-10β), δ 3.75 (1H, *m*, H-13), δ 3.57 (1H, *m*, H-18), δ 3.12 (1H, *bs*, H-7), δ 3.06 (1H, *dd*, *J* = 2.0, 11.2, H-17α), δ 2.96 (1H, *bd*, *J* = 12.5, H-11), δ 2.74 (1H, *dd*, *J* = 2.1, 11.4, H-17β), δ 2.36 (1H, *ddd*, *J* = 2.1, 8.5, 12.3, H-15), δ 2.29 (1H, *m*, H-9), δ 2.15 (1H, *bd*, *J* = 13.2, H-8β), δ 1.94 (1H, *m* (*pseudo q*), *J* = 12.5, H-12α), δ 1.85 (1H, *bd*, *J* = 13.2, H-8α), δ 1.58 (1H, *m**, H-14β), δ 1.56 (1H, *m**, H-12β), δ 1.36 (1H, *m* (*pseudo q*), *J* = 12.5, H-14α), δ 0.99 (3H, *d*, *J* = 6.2, H-19). *overlap.

3.5. (−)-Tinctorine (7)

$[\alpha]_D^{23}$ −59 (EtOH, *c* 0.1). ^{13}C NMR in Table 1. ^1H NMR (MeOD, 500.1 MHz) δ 7.46 (1H, *dd*, *J* = 7.0, 8.9, H-4), δ 6.41 (1H, *dd*, *J* = 1.3, 8.9, H-3), δ 6.26 (1H, *dd*, *J* = 1.1, 7.0, H-5), δ 5.81 (1H, *m*, H-15), δ 5.14 (1H, *m*, *J* = 17.0, H-16a), δ 5.08 (1H, *td*, *J* = 1.3, 10.1, H-16b), δ 3.93 (2H, *m**, H-10α, H-10β), δ 3.02 (1H, *bd*, *J* = 2.2, H-7), δ 2.91 (2H, *m**, H-11, H-13a), δ 2.51 (1H, *m*, *J* = 11.4, H-13b), δ 2.45 (2H, *m**, H-9, H-14a), δ 2.33 (1H, *m**, H-14b), δ 2.23 (3H, *s*, N-CH_3), δ 2.07 (1H, *td*, *J* = 2.8, 13.4, H-8a), δ 1.70 (1H, *brdd*, *J* = 1.3, 13.3, H-8b). *overlap.

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