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ALKALOIDS FROM *ARISTOLOCHIA ARCUATA*

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Key Word Index—*Aristolochia arcuata*; Aristolochiaceae; potassium nitrate; carbohydrates, aristolane sesquiterpenes; sitosterol; terahydrofuran neolignans; 13-oxidodibenzo[*a*, *g*]-quinolizinium alkaloids.

Abstract—Phytochemical studies with *Aristolochia arcuata* led to the isolation of KNO_3, fructose, sucrose, sitosterol, calarene, its 2-oxo derivative, and three neolignans. Besides which, two new 13-oxidodibenzo[*a*, *g*]-quinolizinium alkaloids were isolated. They present only one oxygenated substituent at rings A and D.

INTRODUCTION

Aristolochia arcuata Mast. (Aristolochiaceae) is a polymorphous species that responds to different habitat conditions (climatic and geographic). The species described in this paper was identified as *silvestris* Hoehne. Brazilian Aristolochia species contain allantoin, lignans, diterpenes, KNO_3 (**1**), fructose (**2**), sucrose (**3**) and sitosterol (**4**) [1–3]. We now report the isolation from *A. arcuata* of **1**–**4**; two aristolane sesquiterpenes: calarene (**5**) and its 2-oxo derivative (**6**); two tetrahydrofuran neolignans (**7** and **8**); one benzofuran neolignan (**9**) and two new 13-oxidodibenzo[*a*, *g*]-quinolizinium alkaloids (**10** and **11**). The natural occurrence of the aristolane skeleton is apparently restricted to several plant families [4, 5].

RESULTS AND DISCUSSION

Compounds **1**–**6** were identified by comparison of their physical (mp, TLC) and spectroscopic data (IR, ^1H and ^{13}C NMR) with authentic samples [1–9]. Additional proof that **1** was KNO_3 was obtained by qualitative inorganic analyses for K^+ and NO_3^- ions [1, 6]. Compound **5** was unstable, being readily oxidized to **6** under storage. It gave rise to an α,β-unsaturated product of oxidation that was identified as $\Delta^{1,10}$-aristolen-2-one [5, 9].

Compounds **7** and **8** are tetrahydrofuran neolignans, both of which can occur in six different relative configurations, i.e. four diastereomeric pairs and two *meso* or *quasi-meso* forms. The relative configuration of **7** and **8** was established by ^1H and ^{13}C NMR data, this relying mainly on chemical shifts of benzyl and methyl groups [10–13]. The chemical shift observed for methyl (1.00 ± 0.02) and benzyl (4.60 ± 0.02) protons from **7** and **8**, revealed C2-Ar and C3-Me functionalities in a *trans* relation. We could also observe from their ^1H and ^{13}C NMR spectra pairs of signals with similar chemical shifts due to symmetry elements in the molecules. Compound **8** was optically inactive ($[\alpha]_D 0°$) whereas **7** was not ($[\alpha]_D - 114°$). Based on these data, the compounds **7** and **8** were identified as zuihonin-B and (−)-galbacin, respectively [10–13].

Compound **9** was identified as eupomatenoid-7 by comparison of its ^1H and ^{13}C NMR data, as well as by comparison of its acetyl derivative data [14, 15]. Analysing the NMR data of this compound, we could establish the carbon multiplicity by DEPT-135°, and the proton multiplicity by double irradiation experiments. From CHCORR and CHCORR-LR experiments, it was possible to assign more feasible δ values for C-1,2,5,7,1′,2′ or 6′,3′,4′ and 5′ (Table 1) than those previously described in the literature [15].

The ^1H and ^{13}C NMR spectra (Table 1) from the alkaloid (**10**) showed aromatic signals to 9H (δ6.7–9.4) and 16C (δ106.6–154.3), two hydroxyl groups ($\delta \sim 11.16$) and a signal at δ188.7 which indicated a carbonyl function. The ^1H–^1H COSY experiment led to the correlations: H-4 (δ7.38) with H-3 (δ6.82) and this last one with H-1 (δ8.15), as well as H-12 (δ7.72) with H-11 (δ7.19) and this last one with H-9 (δ7.66). The doublets at δ8.45 and δ8.17 were also shown to be coupled. Besides these *ortho*, *meta* and *para* interactions, it was possible to verify by ^1H–^1H NOESY experiments, the correlation between the doublet at δ8.45 with H-4 and H-3, as well as the correlation between the signals at $\delta \sim 8.16$ with H-3. Besides the direct interactions ^1H–^{13}C, the ^1H–^{13}C COSY experiments led to the following long range correlation: H-4 with C-2 (δ154.3), H-12 with C-10 (δ152.5) and C-8a (δ122.2). Finally, a methynic group (δ_H 9.39, δ_C 138.6) was shown to be correlated with a carbon that absorbs at δ115.5. The position 8 or 13 could be attributed to this group as well as to the carbonyl group. Considering that the carbonyl absorption was at too low field (δ188.7) to belong to an amide group, we suggest that it was in the 13

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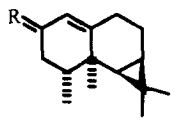
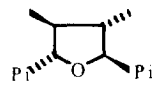


R

5 H_2

6 O



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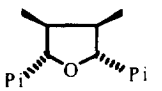

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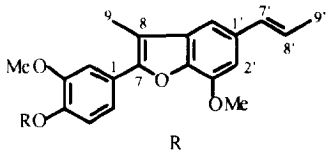
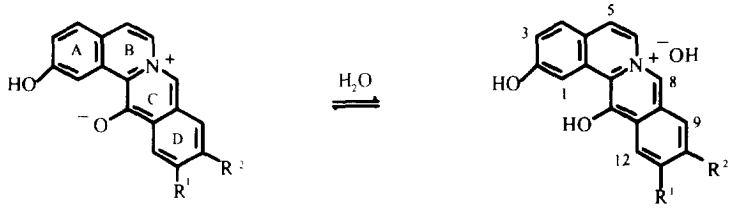


R

9 H

9a Ac



	R¹	R²
10	H	OH
10a	OH	H
11	H	Oglc

Table 1. 2D ^1H–^{13}C long range COSY experiments for **10**

^{13}C signal	Correlated ^1H signals
152.1 (C-7)	2.35 (3H-9)
147.3 (C-5)	6.94 (H-3), 3.92 (OMe)
146.4 (C-4)	7.23 (H-2)
145.5 (C-3′)	3.98 (OMe)
142.5 (C-4′)	6.99, 6.77 (H-2′, 6′)
124.3 (C-1)	6.94 (H-3)
121.3 (C-2)	7.26 (H-6)
110.9 (C-8)	2.35 (3H-9)
110.1 (C-6)	7.23 (H-2)
109.8, 105.0 (C-6′, 2′)	6.77, 6.99 (H-2′, 6′)

position. Based on these data, we can propose two alternative structures for this compound: **10** or **10a**. In order to attempt to distinguish between these possibilities, NOEDIFF experiments were undertaken with irradiation at δ9.39, signals at $\delta \sim 8.16$, 8.45 and ~ 7.7 being affected. Unfortunately, this last one did not allow us to distinguish between protons, whether it was the one that absorbed at δ7.72, (*d*, 8.7 Hz) or the one that absorbed at δ7.66 (*bd*, 2.3 Hz).

Compound **11** has ^1H and ^{13}C NMR spectra very similar to those of **10**. The main difference is the presence of a glucosyl group instead of a hydroxyl at C-10 or C-11. The mass spectra of **11** had a prominent ion $[M + H_2O]^{+\cdot}$ peak (*m/z* 457) and the ion derivatives of it. This suggested the addition of a molecule of water to the C ring (OH^- to C-8 and H^+ to O-13), leading to a dealkylation with formation of the amine and aldehyde. The fragmentation patterns also suggested that the glucosyl group was linked to the D ring. It is interesting to observe that the most abundant ions were due to glucosyl fragmentation [16], but without involving the loss of oxygen (O-1′) linked to C–Ar.

It is known that oxyberberinium and oxyprotoberberinium salts can be obtained by synthetic methods [17–23]. Generally they are obtained from natural products, which are dioxygenated in both A and D rings (at C-2,3,9,10 and C-2,3,10,11, respectively), having the L-

tyrosine and *p*-hydroxybenzaldehyde as common biogenetic precursors. The largest difference between **10**, **11** and these alkaloids is the presence of only one oxygenated function at A and D rings. Since compounds **10** and **11** are presumably biogenetically related to 8-benzylberbine and benzylisoquinoline alkaloids isolated from *Aristolochia* species [1, 24, 25], we suggest that L-tyrosine and *p*-hydroxybenzaldehyde are their biogenetic precursors. Consequently, the alternative structure in which the hydroxyl or glucosyl group is linked at R^1 rather than R^2 is excluded.

EXPERIMENTAL

General. Melting points were determined on a Kofler hot-stage microscope and are uncorred. MS were obtained on a Finnigan-G.C. MS, 70 eV, ITD-800, spectrometer coupled to a Varian Chromatograph Mod. 34100, col. DBS, 30m. IR: KBr discs. NMR spectra were measured on Bruker spectrometers, NMR: 1D (400 or 200 MHz) and 2D ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) spectra, room temp., (1D: ^{1}H and ^{13}C SFORD, DEPT-135°; 2D: COSY, CH-COSY, CH-COSY-LR) locked to the major deuterium of the solvent ($CDCl_3$, Me_2CO-d_6, MeOH-d_4, D_2O). Chemical shifts are given in ppm relative to TMS with coupling constants in Hz. Optical rotation: $[\alpha]_D$ values were recorded in units of 10^{-1} deg cm^2 g^{-1}, and were measured on a Polamat-A. UV absorptions were measured using a Hewlett Packard 8452A, Diode array spectrophotometer. TLC: Silica gel 60 PF_{254}.

Plant material. This was collected in Araraquara, SP, and identified by Dr Condorcet Aranha (Instituto Agronômico de Campinas, Campinas, SP). The material was separated by parts of plant, dried (~ 60°) and ground.

Extraction and isolation. Ground roots (350.0 g) and leaves (255.0 g) of *A. arcuata* were exhaustively extracted at room temp. with hexane, Me_2CO and EtOH successively and then concd. The hexane extract of roots (8.30 g) redissolved in hot hexane led to the crystallization of **9** (900 mg). The solution (7.16 g) was fractionated by CC (silica gel, 16.0 g, hexane–EtOAc) affording **9** (605 mg), a mixture of **7** and **8** (200 mg) and **4** (356 mg). The Me_2CO extract of roots (9.4 g) was submitted to a CC (silica gel, 40.0 g, hexane–EtOAc) yielded 5 fractions. Fraction 1 gave **5** (242 mg); fr. 2 gave **6**, **7** and **8** in a mixture (348 mg); fr. 3 gave **4** (47 mg); fr. **4** gave **9** (892 mg) and fr. 5 gave a complex mixture (6.1 g). A sample (3.7 g) of this last one was dissolved in 20% HOAc soln. Both soluble (*A*) and isoluble (*B*) fractions were neutralized with 5% NaOH soln and extracted with EtOAc. The organic phase of *B* (308 mg) yielded by prep. TLC (hexane–EtOAc, 7:3) **10** (140 mg) and a mixture of neolignans including **8** (70 mg). This mixture and fr. 2 were submitted to prep. TLC (CCl_4, 5× elution). The first one afforded **8** (23 mg), and the last one afforded **7** (92 mg) and **8** (50 mg). The aq. phases were concd. From aq. phase *B* (1.1 g) KNO_3 was obtained (209 mg) by recrystallization from Me_2CO and after that by CC (Lobar, RP-18-A, MeOH–$CHCl_3$, 300 mg) **11** (20 mg) was also obtained. A sample from aq. phase *A* by CC (Lobar, RP-18-A, MeOH–$CHCl_3$, 300 mg) afforded **2** (47 mg). The crude EtOH extract (16.0 g) prepared from dried leaves, yielded crystallized **3**, which was purified on recrystallization from MeOH (1.35 g).

2,10-*Dihydroxy*-13-*oxidodibenzo*[a, g]-*quinolizinium* (**10**). Amorphous red solid, 250° decomp. (MeOH). (Found: C, 61.7; H, 5.6; N, 4.6. $C_{17}H_{13}NO_4 \cdot 2H_2O$ requires: C 61.6; H, 5.1; N, 4.2%). UV λ_{max}^{MeOH} nm (log ε): 218 (4.68), 302 (4.39), 394 (4.08) sh. $\lambda_{max}^{MeOH+NaOH}$ nm (log ε): 228 (4.66), 310 (4.39) sh. IR ν_{max}^{KBr} cm^{-1}: 3409, 2928, 2855, 1633, 1514, 1464, 1385, 1215, 1124, 1036. EIPOS 70 eV *m/z* (rel. int.): 295 $[M + H_2O]^{+\cdot}$ (4), 278 $[M + H]^+$ (2), 265 (10), 250 (9), 193 (14), 177 (18), 165 (21), 149 (30), 129 (32), 91 (63), 83 (39), 77 (43), 69 (55), 57 (81), 56 (50), 55 (100). ^{1}H NMR (200 MHz, Me_2CO-d_6): δ11.17 (1H, *s*, OH), 11.15 (1H, *bs*, OH), 9.39 (1H, *s*, H-8), 8.45 (1H, *d*, J = 5.0 Hz, H-6), 8.17 (1H, *d*, J = 5.0 Hz, H-5), 8.15 (1H, *d*, J = 2.2 Hz, H-1), 7.72 (1H, *d*, J = 8.7 Hz, H-12), 7.66 (1H, *bd*, J = 2.3 Hz, H-9), 7.38 (2H, *d*, J = 8.6 Hz, H-4), 7.19 (1H, *dd*, J = 8.7, 2.3 Hz, H-11), 6.82, (1H, *dd*, J = 8.6, 2.2, H-3). ^{13}C NMR (50 MHz, Me_2CO-d_6): δ188.7 (*s*, C-13), 154.3 (*s*, C-2), 152.5 (*s*, C-10), 138.6 (*d*, C-8), 137.2 (*d*, C-6), 136.6, 131.7, 131.4 (3C, 3*s*, C-4a, 12a, 14a), 122.2 (*s*, C-8a), 119.4 (*d*, C-11), 118.3 (*d*, C-5), 115.5 (*s*, C-14), 114.0 (*d*, C-12), 113.4 (*d*, C-3), 113.0 (*d*, C-4), 107.9 (*d*, C-1), 106.6 (*d*, C-9).

2-*Hydroxy*-10-O-[*glucopyranosyl*]-13-*oxidodibenzo*[a, g]-*quinolizinium* (**11**). Amorphous red solid, 248° decomp. (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3652, 3393, 1654, 1514, 1266. EIPOS 70 eV *m/z* (rel. int.): 457 $[M + H_2O]^{+\cdot}$ (2), 456 (3), 440 $[M + H]^+$ (2), 428 $[M + H_2O\text{-}CHO]^+$ (3), 414 (5), 400 (3), 343 (3), 327 (4), 326 (6), 313 $[M + H_2O - AB]^{+\cdot}$ (3), 296 $[M + H_2O - AB - OH]^{+\cdot}$ (4), 281 $[M + H_2O - AB - CO]^+$ (4), 265 (6), 256 (5), 250 (6), 239 (6), 227 (6), 215 (7), 203 (8), 202 (9), 193 (11), 192 (10), 191 (9), 189 (10), 178 (15), 167 (23), 165 (27), 142 (31), 141 (45), 115 (92), 91 (100). ^{1}H NMR (400 MHz, MeOH-d_4): δ8.88 (1H, *s*, H-8), 8.42 (1H, *d*, J = 5.0 Hz, H-6), 8.14 (1H, *d*, J = 5.0 Hz, H-5), 8.00 (1H, *d*, J = 1.2 Hz, H-1), 7.56 (1H, *d*, J = 1.2 Hz, H-9), 7.53 (1H, *d*, J = 8.6, H-12), 7.31 (1H, *d*, J = 8.6 Hz, H-4), 7.13 (1H, *dd*, J = 8.6, 1.2 Hz, H-11), 6.81 (1H, *dd*, J = 8.6, 1.2 Hz, H-3), 4.1–3.4 (5H, *m*, H-glc), signals of H-1′ and H-5′ were totally obscured by the solvent signals.

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