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LIGNANS FROM *ARISTOLOCHIA BIROSTRIS**

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Key Word Index—*Aristolochia birostris*, Aristolochiaceae, structure determination, lignans, spectroscopic analysis

Abstract—The investigation of a specimen of *Aristolochia birostris* afforded, in addition to β-sitosterol, seven lignoids *rel*-(8*R*)-$\Delta^{8'}$-3,4-methylenedioxy-5,3′,5′-trimethoxy-, *rel*-(8*R*)-$\Delta^{8'}$-3,4-methylenedioxy-3′,5′-dimethoxy-, *rel*-(8*R*)-$\Delta^{8'}$-3,3′,4,5,5′-pentamethoxy- and *rel*-(7*R*,8*R*)-$\Delta^{8'}$-3,4-methylenedioxy-3,5,5′-trimethoxy-7-hydroxy-8 0.4′-neolignan; *rel*-(8*R*,8′*R*)-3,4; 3′,4′-dimethylenedioxy-9-oxo-, *rel*-(8*R*,8′*R*)-3,4, 3′,4′-dimethylenedioxy-9β-hydroxy- and *rel*-(8*R*,8′*R*)-3,4; 3′,4′-dimethylenedioxy-9α-hydroxy-8.8′,9 0 9′-lignan and *rel*-(7*S*,8*S*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxy-7 0.7′,8 8′-lignan. The structures were elucidated by spectroscopic analysis.

INTRODUCTION

Aristolochia birostris (Aristolochiaceae), occurs in northeastern Brasil, where it is commonly called 'capivara, angilicó or jarrinha' and has been used in folk medicine [1]. An ethanol extract of the dried roots of a specimen of this plant yielded three new natural neolignans (**2**, **4**, **7**), along with other four lignoids (**1**, **3**, **5**, **6**) previously reported and β-sitosterol. The present paper describes the results obtained in this study. The nomenclature and numbering of the compounds follows the rules outlined in a recent review [2].

RESULTS AND DISCUSSION

The ethanol extract of the roots of *A. birostris* was redissolved in aqueous methanol and extracted successively with *n*-hexane and chloroform. Separation of the lignoids (**1**–**7**) was achieved by chromatography on silica gel (CC and TLC) of these extracts. Among the compounds, **1**, **3**, **5** and **6** were reported earlier. The neolignans **1** and **3** were previously isolated from *Virola carinata* [3] and *Myristica fragans* [4], respectively. The dibenzylbutirolactone **5** was reported in *A. indica* [5] and the dibenzylbutirolactols (**6a**+**b**) in *A. triangularis* [6, 7], *A. elegans* [8] and *A. indica* [9]. To the best of our knowledge, the compounds **2**, **4** and **7** are hitherto unreported as natural products and **7** has only been synthesized [10] Other 2,5-diaryl-3,4-dimethyltetrahydrofuranoid lignans (**8**–**11**) were isolated from *A. chilensis* [11] and **12** from *A. triangularis* [7] The compounds **1** and **3** are hitherto unreported from *Aristolochia* species. From the ^{1}H and ^{13}C NMR spectra of these compounds isolated from *A. birostris* the relative configurations were deduced [11–13].

The molecular formula $C_{21}H_{24}O_5$ of the new lignoid **2**, a viscous mass, was determined by a combination of low resolution mass spectrometry (*m/z* 356 (23%), $[M]^+$) and ^{1}H and ^{13}C NMR counts Functional analysis by IR and NMR allowed this formula to be expanded to $C_{18}H_{16}O(OMe)_2(OCH_2O)$ and together with the mass spectrum to $[C_6H_3(OCH_2O)(CH_2\text{–}CH\text{–}Me)]$ $[C_6H_2O(OMe)_2(CH_2\text{–}CH{=}CH_2)]$, mainly through peaks at *m/z* 194 (58%), 163 (92%), 135 (97%) and 121 (16%), which were attributed to the fragments **2a**–**d**, respectively. The ^{1}H and ^{13}C NMR spectra revealed 1,3,4-trisubstituted [δ6 67 (*br s*, H-2, 5, 6), δ_C 130.00 (C-1), 107.91 (CH-2), 147.32 (C-3, C-4), 109 94 (CH-5), 122.31 (CH-6)] and 1,3,4,5-tetrasubstituted [δ_H 6.38 (*s*, H-2′, 6′); δ_C 135 35 (C-1′), 105.80 (CH-2′, CH-6′), 153.67 (C-3′, C-5′), 132 00 (C-4′)] aromatic rings, one methylenedioxy [δ_H 5.88 (*s*), δ_C 100.58] and two methoxy [δ_H 3 70 (*s*), δ_C 56.02] groups. The signals at δ_H 3.20 (*d*, $J=7$ Hz, CH_2-7′), 6.20–5.80 (*m*, H-8′) and 5.20–4 80 (*m*, CH_2-9′) and at δ_C 40.51 (CH_2-7′), 137.32 (CH-8′) and 115 83 (CH_2-9′) were consistent with the presence of an allyl group The chemical shifts of methylene [δ_H 3 05–2 50 (*m*), δ_C 42 96], methine OCH-8 [δ_H 4.20–3.90 (*m*), δ_C 79.72] and secondary methyl [δ_H 1 10 (*d*, $J=7$ Hz), δ_C 19 51] groups and the observed multiplicity showed the attachment of the 3,4-OCH_2O C_6H_3–CH_2–CH–Me moiety to the oxygen atom of C-4′. These assignments were based on the application of the usual shift parameters, comparison with literature data and observed multiplicities of signals These spectral data provided support for the structure of neolignan **2**. This proposed structure is also supported by biogenetic arguments [2]

Spectral comparison of **2** and **4** ($[M]^+$ 402: $C_{22}H_{26}O_7$) allowed us to classify these compounds into the same

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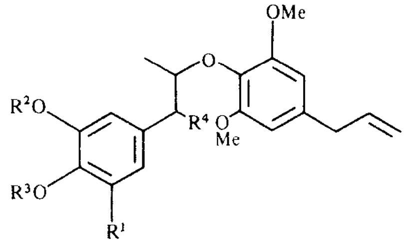


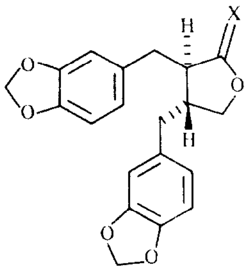
1 R^1 = OMe, R^2,R^3 = CH_2, R^4 = H

2 R^1 = R^4 = H, R^2 R^3 = CH_2

3 R^1 = OMe, R^2 = R^3 = Me, R^4 = H

4 R^1 = OMe, R^2,R^3 = CH_2, R^4 = OH

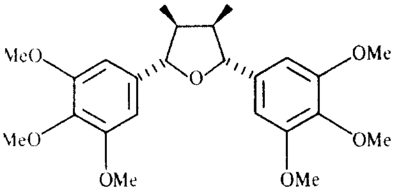
4a R^1 = OMe, R^2,R^3 = CH_2, R^4 = OAc



5 X = O

6a X = β-OH,H

6b X = α-OH H



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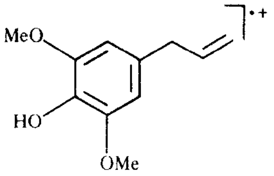
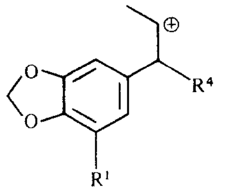
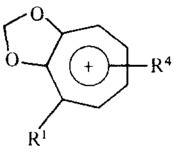


2a *m/z* 194 (58 %)



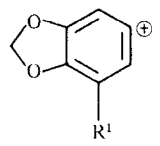
2b R^1 = R^4 = H *m/z* 163 (92 %)

4a R^1 = OMe R^4 = OH *m/z* 209 (7 %)



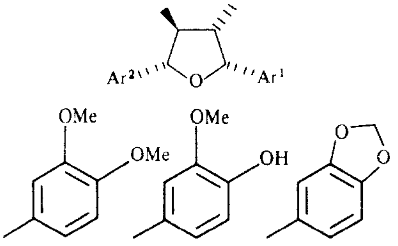
2c R^1 = R^4 = H *m/z* 135 (97 %)

4b R^1 = OMe, R^4 = OH *m/z* 181 (7 %)

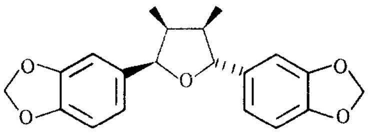


2d R^1 = H *m/z* 121 (16 %)

4c R^1 = OMe *m/z* 151 (12 %)



8	Ar^1	—	Ar^2
9	—	Ar^1	Ar^2
10	Ar^1	Ar^2	—
12	—	—	Ar , Ar^2



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group of neolignans. The major distinction between the two natural products was the presence of a hydroxyl (ν_{max} 3480 cm^{-1}) and an additional methoxyl group in **4** (Table 1) The ^{1}H NMR spectrum indicated the existence of a 3,4-methylenedioxy-5-methoxyphenyl group [δ_H 6 50 (*br s*, 2H, H-2, 6)] and one benzylic oxymethine proton [δ_H 4 30 (*d*, $J = 8$ Hz, 1H, H-7) which was shifted by 1.4 ppm to lower field upon acetylation [**4a** δ_H 5 70 (*d*, $J = 8$ Hz, 1H, H-7)]. Thus, the additional OMe and the OH groups can only be located as shown in the formula **4**. Further support for this proposal was obtained from the mass spectrum, mainly through ions at *m/z* 209 (7%), 194 (100%), 181 (7%) and 151 (12%) corresponding to the fragments **4a**, **2a**, **4b** and **4c**, respectively The relative *threo*-configuration for **4** was proposed on the basis of the coupling constant observed for the H-7 signal [δ_H 4.30 (*d*, $J = 8$ Hz)], which interacts with H-8 [δ_H 4 10–3 80 (*m*)] In the *erythro*-configuration this coupling constant is *ca* 3 Hz [14]

The spectral data of the other new natural product **7** ([M]$^+$ 432 (10%) $C_{24}H_{32}O_7$) are in good agreement with those reported for compound previously synthesized by phenol oxidation [10] The ^{1}H NMR spectrum (Table 1) showed only two signals for the four protons of the tetrahydrofuran ring [δ_H 4 34 (*d*, $J = 6$ Hz, H-7, 7′), 2 50–1 90 (*m*, H-8, 8′)] and one doublet for the two methyl groups [δ_H 1 02 (*d*, $J = 6$ Hz)] These chemical shifts and the coupling constant are in accordance with the symmetry shown in formula **7** [10] The chemical shift of the methyl group (δ_H 1 02) indicated that the aryl/Me substituents are *trans* oriented In the *cis* aryl/Me configuration this chemical shift is *ca* 0 65 ppm [10, 15, 16] The relative

Table 1. ^1H and ^{13}C NMR data of compounds **2** [^1H 80 MHz ($CDCl_3$); ^{13}C 20 MHz ($CDCl_3$)], **4** (60 MHz, CCl_4), **4a** (80 MHz, $CDCl_3$) and **7** (60 MHz, CCl_4) [chemical shifts (δ) are expressed in ppm from internal TMS, coupling constants (J) in Hz]

	δ_C	δ_H			
C	**2**	**2**	**4**	**4a**	**7**
1	130.00	—	—	—	—
2	107.91	6.67 (*br s*)	6.50 (*br s*)	6.40 (*s*)	6.48 (*s*)
3, 4	147.32	—	—	—	—
5	109.94	6.67 (*br s*)	—	—	—
6	122.31	6.67 (*br s*)	6.50 (*br s*)	6.40 (*s*)	6.48 (*s*)
7	42.96	3.05–2.50 (*m*)	4.30 (*d*, $J=8$)	5.70 (*d*, $J=8$)	4.34 (*d*, $J=6$)
AcO-7	—	—	—	1.90 (*s*)	—
8	79.72	4.20–3.90 (*m*)	4.10–3.90 (*m*)	4.30–3.80 (*m*)	2.50–1.90 (*m*)
9	19.51	1.10 (*d*, $J=7$)	1.10 (*d*, $J=7$)	1.20 (*d*, $J=7$)	1.02 (*d*, $J=6$)
3,4-OCH_2O	100.58	5.88 (*s*)	5.88 (*s*)	5.93 (*s*)	—
3-OMe	—	—	—	—	3.74 (*s*)
4-OMe	—	—	—	—	3.67 (*s*)
5-OMe	—	—	3.80 (*s*)	3.80 (*s*)	3.74 (*s*)
1′	135.35	—	—	—	—
2′,6′	105.80	6.38 (*s*)	6.30 (*s*)	6.20 (*s*)	6.48 (*s*)
3′,5′	153.67	—	—	—	—
4′	132.00	—	—	—	—
7′	40.51	3.20 (*d*, $J=7$)	3.20 (*d*, $J=7$)	3.25 (*d*, $J=7$)	4.34 (*d*, $J=6$)
8′	137.32	6.20–5.80 (*m*)	6.20–5.70 (*m*)	6.20–5.80 (*m*)	2.50–1.90 (*m*)
9′	115.83	5.20–4.80 (*m*)	5.40–4.80 (*m*)	5.30–4.60 (*m*)	1.02 (*d*, $J=6$)
3′,5′-$(OMe)_2$	56.02	3.70 (*s*)	3.80 (*s*)	3.89 (*s*)	3.74 (*s*)
4′-OMe	—	—	—	—	3.67 (*s*)

configuration of compound **7** is different from that observed for other lignoids (**8**–**12**) isolated from *Aristolochia* species [11, 12].

EXPERIMENTAL

Mps are uncorr. NMR (^1H 60, 80 and 100 MHz, ^{13}C 20 and 25.2 MHz) were recorded in $CDCl_3$ or CCl_4 soln with TMS as int std MS were measured by direct inlet at 70 eV ionization TLC was carried out on silica gel (PF-254)

Plant material Roots of *A. birostris* Duchtre, were collected at Caldas Brandão, Paraiba State The specimen was identified by Maria de F. Agra, Universidade Federal da Paraíbia, João Pessoa

Isolation of constituents After drying, roots were reduced to powder (3 kg) and extracted with EtOH. The residue obtained (97 g) was dissolved in $MeOH–H_2O$ (9:1) and extracted successively with *n*-hexane and $CHCl_3$ The hexane extract (30 g) was chromatographed on a silica gel column Elution with hexane–C_6H_6 mixts of the indicated composition gave frs A (hexane), B, C (9:1), D (1:1) and E (C_6H_6) Fr A (907 mg) was recrystallized from MeOH yielding sitosterol (80 mg). The remaining soln was evapd and the residue purified by TLC with C_6H_6, furnishing **1** (400 mg) and **2** (21 mg). Fr. B (928 mg) was purified by TLC with C_6H_6, affording **5** (26 mg). Fr C (500 mg) was chromatographed on a silica gel column, elution with hexane–C_6H_6 (3:2) and C_6H_6 giving frs C-1 and C-2, respectively. TLC of C-1 with $CHCl_3$ yielded **3** (16 mg), C-2 was treated with activd charcoal to furnish **4** (14 mg). Fr D (650 mg) was chromatographed on a silica gel column The fr eluted with hexane–C_6H_6 (1:1) was purified by TLC with C_6H_6–$CHCl_3$–EtOAc (4:5:1) affording **6a**+**b** (32 mg) Fr E (830 mg) was chromatographed on a silica gel column. The fr. eluted with hexane–C_6H_6 (1:1) was purified by TLC with C_6H_6–$CHCl_3$–EtOAc (9:10:1), furnishing **7** (22 mg) The $CHCl_3$ ext was also chromatographed and furnished **4** (94 mg) and **7** (64 mg).

rel.-(8R)-$\Delta^{8'}$-3,4-*Methylenedioxy-3′,5′-dimethoxy-*8.O.4′*-neolignan* (**2**) Oil. IR ν_{max}^{Nujol} cm^{-1} 1615, 1595, 1505, 1255, 1140, 1100, 1050. EIMS *m/z* (rel int.) 356 (23, $[M]^+$), 194 (58), 163 (93), 147 (20), 135 (97), 133 (40), 121 (16), 105 (100), 104 (24), 103 (39) ^1H (80 MHz, $CDCl_3$). ^{13}C NMR (20 MHz, $CDCl_3$) see Table 1

rel -(7R,8R)-$\Delta^{8'}$-3,4-*Methylenedioxy-3′,5,5′-trimethoxy-7-hydroxy-*8.O.4′*-neolignan* (**4**). Oil IR ν_{max}^{KBr} cm^{-1}: 3480, 1635, 1590, 1505, 1240, 1130, 1040. EIMS *m/z* (rel int) 402 (2, $[M]^+$), 221 (8), 220 (5), 209 (7), 208 (6), 205 (5), 194 (100), 193 (10), 181 (7), 179 (20), 165 (9), 163 (10), 161 (7), 151 (12), 147 (13), 137 (11), 135 (10), 133 (15), 131 (13), 123 (15), 121 (11) ^1H NMR (60 MHz, CCl_4) see Table 1 *Monoacetate* (**4a**) Compound **4** (16 mg) was dissolved in a mixt. of Ac_2O (0.5 ml) and dry pyridine (0.3 ml) and kept at room temp. for 8 hr Usual work-up afforded the monoacetyl derivative **4a** (15 mg). ^1H NMR (80 MHz, $CDCl_3$) see Table 1

rel -(7S,8S,7′R,8′R)-3,3′,4,4′,5,5′-*Hexamethoxy-*7.O.7′,8.8′*-lignan* (**7**). Mp, IR, ^1H NMR (see Table 1) and MS data are in agreement with lit. values [10]

rel.-(8R,8′R)-3,4; 3′,4′-*Dimethylenedioxy-9β-hydroxy-*(**6a**) and rel.-(8*R*,8*R*′)-3,4; 3′,4′-*dimethylenedioxy-9α-hydroxy-*8.8′,9.O.9′-*lignan* (**6b**). Spectral data of this mixt are in agreement with lit values [6, 7, 9]

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