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LIGNANS AND DITERPENES OF THREE *ARISTOLOCHIA* SPECIES

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Key Word Index— *Aristolochia cymbifera*; *A. esperanzae*; *A. galeata*; Aristolochiaceae; lignans; diterpenes; clerodanes; labdanes.

Abstract—Phytochemical examination of *Aristolochia cymbifera*, *A. esperanzae* and *A. galeata* led to the isolation of furofuran and dibenzylbutyrolactone type lignans, as well as of clerodane and *ent*-labdane type diterpenes. Structural assignments for two new *ent*-labdans are presented and discussed.

INTRODUCTION

In a continuation of our work on *Aristolochia* species [1, 2], we have shown that *A. cymbifera*, *A. galeata* Mart. et Zucc. and *A. esperanzae* Kuntz contain lignans of the furofuran (**1a**–**1c**) and the dibenzylbutyrolactone types (**2a**, **2d**), some labdane diterpenes (**3**, **4a**, **4b**, **5a**, **5b**) two of which appear to be new derivatives (**3**, **5b**). *Aristolochia galeata* also furnished clerodane diterpenes (**6a**–**6d**, **7a**, **8**). These compounds predominate in the roots while labdane diterpenes predominate in the leaves of the species. The chemical and spectroscopic data for the new *ent*-labdanes are presented and discussed.

RESULTS AND DISCUSSION

The roots of *A. galeata* contain β-sitosterol, the lignans asarinin (**1b**) [3], fargesin (**1c**) [4], (2*R*,3*R*)-2,3-di-(3,4-methylenedioxybenzyl)-butyrolactol (cubebin **2a**) [5], (2*R*,3*R*)-3-(3,4-dimethoxybenzyl)-2-(3,4-methylenedioxybenzyl)-butyrolactone (**2b**) [6] and the clerodane diterpenes populifolic acid (**6a**) kolavenic acid (**6b**), their corresponding methyl esters (**6g**, **6h**), 2-oxopopulifolic acid (**7a**) [2], kolavenol (**6c**), dihydrokolavenol (**6d**) [7] and kolavelool (**8**) [8]. The acetyl derivatives of **6c** and **6d** (respectively **6e** and **6f**) were also identified [7–9]. The conversions **6a**→**7a** and **6f**→**7b** [9], followed by ^{13}C NMR, reinforce the previous suggestion that the 2-oxokolavenic compounds are artifacts [2].

The leaves of *A. galeata* afforded three *ent*-labdane diterpenes: *ent*-labdan-8β-ol-15-oic acid (**4a**), *ent*-labd-13-en-8β-ol-15-oic acid (**4b**) [10–12] and copalic acid (**5a**) [13]. The latter compound and the new *ent*-labdane **3** were isolated from the leaves of *A. cymbifera*.

The ^{1}H and ^{13}C NMR spectra of **3** showed that the molecular formula was $C_{20}H_{36}O$ and revealed the presence of the following groups: four quaternary methyl groups, one tertiary methyl group, one vinyl group and one hydroxyl group. The singlets at δ_H4.45 (lost upon addition of D_2O) and δ_C73.3 in the NMR spectra as well the IR absorptions (3470, 1180 cm^{-1}) suggested the presence of a tertiary alcohol group. To determine the location of the hydroxyl group (C-8 or C-13 in a labdane system), compound **3** was submitted to dehydration under acidic conditions yielding **9a**.

The MS of **9a** contained a molecular peak at *m/z* 274, a base peak at *m/z* 191 and an additional peak at *m/z* 123. The corresponding ions were consistent with two partial structures: the side chain [$CH_2CH_2CH(Me)CH{=}CH_2$] and the AB ring system (incorporating a double bond in ring B) of a labdane type compound. Finally, the structure **9a** was assigned by comparison of the ^{1}H and ^{13}C NMR data with the analogous data reported for **9b** [14]. By similar analysis of **3** taking **4b** [10–12] and **10** [15, 16] as model systems, it was possible to establish the relative configuration of rings A and B and to place the hydroxyl at C-8 in the axial orientation.

On the basis of the Carman report [17] and considering the small contribution of the asymmetric centre at C-13 to the rotatory power, it was possible to suggest **3** and **9a** as belonging to the *ent*-labdane series, since their $[\alpha]_D$ values are both negative.

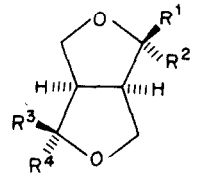
The structures of **5a** and **5b** which were isolated from the leaves of *A. esperanzae* were elucidated through their corresponding methyl esters **5c** and **5d**. Compound **5c** proved to be identical to methyl copalate [10, 13, 14].

The MS of **5d** showed $[M]^+$ at *m/z* 334 ($C_{21}H_{34}O_3$) and fragments at *m/z* 316 $[M-H_2O]^+$, 123 (ring A) and 114 $[CH_2CH_2C(Me){=}CHCO_2Me$ side chain$]^+$. The ^{1}H and ^{13}C NMR data including APT experiments, established the presence of a side chain analogous to that of **5c**, three methyl groups linked to quaternary carbons, an exocyclic double bond and a secondary alcohol. This latter was confirmed by the IR absorptions at 3550 cm^{-1} (ν_{OH}) and 1150 cm^{-1} (ν_{C-O}).

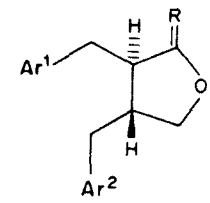
The observed differences between the corresponding chemical shifts (of protons and carbons) from **5c** and **5d** suggested that the hydroxyl group could be linked to C-6 in **5d**. Besides the expected α, β and γ effects, paramagnetic shifts were also observed on protons and carbons which are in δ relation to the hydroxyl function. Hence, it was possible to establish that this group is in the axial orientation. The proposed structure could be proved by comparison of its spectral data with those of **11** [18].

EXPERIMENTAL

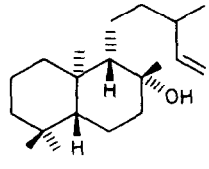
Plant material. A. galeata was collected in Itapetininga, SP, Brasil, by one of the authors (L.M.X.L.) and identified by Dr C.



	R^1	R^2	R^3	R^4
1a	H	Ve	Ve	H
1b	H	Pi	Pi	H
1c	Pi	H	H	Ve



	R	Ar^1	Ar^2
2a	<OH H	Pi	Pi
2b	=O	Pi	Ve
2c	<OH H	Pi	Ve
2d	=O	Pi	Pi



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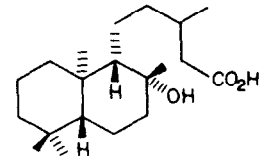
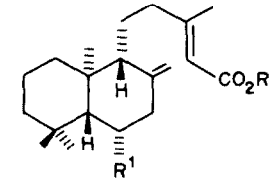
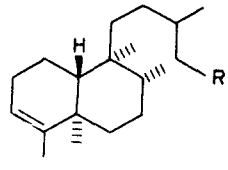


4a

4b $\Delta^{13,14}$

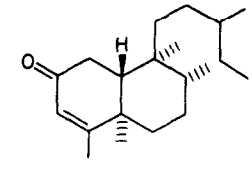


	R^1	R^2
5a	H	H
5b	OH	H
5c	H	CH_3
5d	OH	CH_3



	R
6a	CO_2H
6b	CO_2H , $\Delta^{13,14}$
6c	CH_2OH , $\Delta^{13,14}$
6d	CH_2OH

	R
6e	CH_2OAc , $\Delta^{13,14}$
6f	CH_2OAc
6g	CO_2Me
6h	CO_2Me , $\Delta^{13,14}$

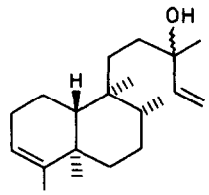


	R
7a	CO_2H
7b	CH_2OAc

Aranha (IAC, Campinas, SP, Brasil). *A. cymbifera* was collected in Governador Valadares, MG, Brasil, by Mrs L. S. Espinola (SMS, Uberlândia, MG, Brasil) and furnished by Dr G. L. Pozetti (UNESP, Araraquara, SP, Brasil). *A. esperanzae* was collected in Araraquara, SP, Brasil, by Dr G. L. Pozetti and identified by Dr C. Aranha.

Extraction of A. galeata. The air-dried and powdered plant material was successively extracted with *n*-hexane, Me_2CO and EtOH at room temp. A further EtOH extract was prepared in a Soxhlet apparatus. The Me_2CO extract (43.4 g) of the roots (860 g) was treated with aq. MeOH yielding a ppt. (1.4 g). This precipitate was fractionated by filtration CC (silica gel, 15 g, $CHCl_3$) into a mixture of **6a**+**6b** (300 mg) and cubebin (**2a**, 579 mg). The soluble fraction was extracted with *n*-hexane. The resulting *n*-hexane soln was evapd and the residue (17 g) was submitted to CC (silica gel–10% H_2O, 40 g). Elution with *n*-hexane–EtOAc yielded seven fractions. Fraction 1 (8 g) contained a mixture of **6a** + **6b** (3:1). Fraction 2 (0.6 g), after prep. TLC (silica gel, CCl_4–EtOAc, 9:1) afforded **6a** (228 mg) containing a small amount of **6b** and **6b** (180 mg) which were identified by direct comparison with authentic samples respectively of populifolic and kolavenic acids [2]; in addition, 2-oxopopulifolic acid was isolated (**7a**, 20 mg).

After methylation (CH_2N_2, standard conditions) and CC (silica gel, 8 g, CCl_4–EtOAc), fraction 3 (3.7 g) yielded **6g**+**6h** (3.0 g, 3:1), **6c** (73 mg), **6d** (219 mg) and **8** (100 mg). Compounds



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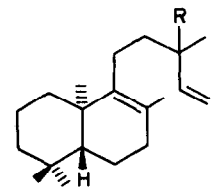
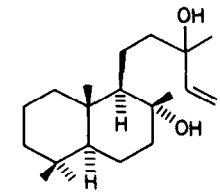


R

9a H

9b OH



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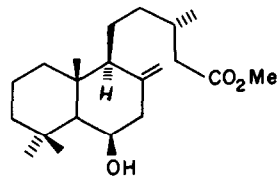

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6c and **6d** were submitted to acetylation (Ac_2O, pyridine). Those compounds were identified by comparison of their chemical and spectral data with those reported in the literature [2,7–9].

From fraction 4 (1.0 g) were obtained by prep. TLC (silica gel C_6H_6–EtOAc, 4:1) β-sitosterol (168 mg) and asarinin (**1b**, 132 mg), the properties of which were identical to those previously reported [3].

Fractions 5 (1.0 g), 6 (0.6 g) and 7 (0.3 g) after recrystallization from MeOH yielded respectively fargesin (**1c**, 563 mg), cubebin (**2a**, 227 mg) and (2*R*,3*R*)-3-(3,4-dimethoxybenzyl)-2-(3,4-methylenedioxybenzyl)-butyrolactone (**2b**, 90 mg). The mp, IR, MS and ^{1}H NMR data of these compounds were identical to those of authentic samples [1, 3–6].

The Me_2CO crude extract (3.5 g) from leaves (130 g) of *A. galeata* on filtration CC (silica gel, 19 g, *n*-hexane) afforded three compounds which were identified by comparison of their data with those previously reported for *ent*-labdan-8β-ol-15-oic acid (**4a**, 238 mg), *ent*-labd-13-en-8β-ol-15-oic acid (**4b**, 200 mg) [10, 11] and copalic acid (**5a**, 1.15 g) [12]. The hitherto unreported ^{13}C NMR data of compounds **4a**, **6f**, **7a**, **7b** and **8** are presented here.

ent-*Labdan-8β-ol-15-oic acid* (**4a**). ^{13}C NMR (20 MHz, $CDCl_3$): δ 177.9 (*s*, C-15), 73.3 (*s*, C-8), 59.3 (*d*, C-9), 55.9 (*d*, C-5), 42.1 (*t*, C-3, C-12), 41.3 (*t*, C-7), 40.5 (*t*, C-14), 39.1 (*t*, C-1), 38.9 (*s*, C-10), 33.3 (*q*, C-19), 33.1 (*s*, C-4), 30.9 (*d*, C-13), 30.5 (*q*, C-17), 22.3 (*t*, C-11), 21.5 (*q*, C-18), 19.5 (*q*, C-16), 18.2, 18.1 (2 × *t*, C-2, C-6).

Dihydrokolavenol acetate (**6f**). ^{13}C NMR (20 MHz, $CDCl_3$): δ 178.6, 20.8 (Ac), 144.4 (*s*, C-4), 120.4 (*d*, C-3), 62.8 (*t*, C-15), 46.4 (*d*, C-10), 38.3, 38.1 (2 × *s*, C-5, C-9), 36.5 (*t*, C-14), 36.1 (*d*, C-8), 35.9, 35.5, 35.4 (3 × *t*, C-6, C-11, C-12), 30.6 (*d*, C-13), 27.5 (*t*, C-2), 26.8 (*t*, C-7), 19.6 (*q*, C-16), 19.3 (*q*, C-19), 18.3 (*q*, C-18), 17.8 (*q*, C-20), 17.3 (*t*, C-1), 15.7 (*q*, C-17).

2-*Oxopopulifolic acid* (**7a**). ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.60 (1H, *br s*, COOH), 5.70 (1H, *br s*, H-3), 1.87 (3H, *br s*, H-18), 1.10 (3H, *s*, H-19), 0.96 (6H, *d*, J = 6.0 Hz, H-16, H-17), 0.80 (3H, *s*, H-20); ^{13}C NMR (20 MHz, $CDCl_3$): δ 201.2 (*s*, C-2), 178.9 (*s*, C-15), 173.4 (*s*, C-4), 125.5 (*d*, C-3), 45.7 (*d*, C-10), 41.5 (*t*, C-14), 40.0, 38.6 (2 × *s*, C-5, C-9), 36.1 (*d*, C-8), 36.0 (2 × *t*, C-6, C-12), 35.6 (*t*, C-1), 34.9 (*t*, C-11), 30.8 (*d*, C-13), 27.0 (*t*, C-7), 19.9 (*q*, C-16), 18.9 (*q*, C-19), 18.4 (*q*, C-18), 17.9 (*q*, C-20), 15.7 (*q*, C-17).

2-*Oxodihydrokolavenol acetate* (**7b**). ^{13}C NMR (20 MHz, $CDCl_3$): δ 178.9, 20.8 (Ac), 200.2 (*s*, C-2), 171.0 (*s*, C-4), 125.5 (*d*, C-3), 63.0 (*t*, C-15), 45.7 (*d*, C-10), 38.8, 38.6 (2 × *s*, C-5, C-9), 36.9 (*t*, C-14), 36.1 (*d*, C-8), 35.6, 35.5, 34.9, 34.7 (4 × *t*, C-1, C-6, C-11, C-12), 30.4 (*d*, C-13), 26.9 (*t*, C-7), 19.9 (*q*, C-16), 18.7 (*q*, C-19), 18.5 (*q*, C-18), 18.0 (*q*, C-20), 15.8 (*q*, C-17).

Kolavelool (**8**). ^{13}C NMR (20 MHz, $CDCl_3$): δ 145.2 (*d*, C-14), 144.4 (*s*, C-4), 120.3 (*d*, C-3), 111.6 (*t*, C-15), 73.3 (*s*, C-13), 46.3 (*d*, C-10), 38.3, 38.1 (2 × *s*, C-5, C-9), 36.8 (*d*, C-8), 36.1, 35.2 (2 × *t*, C-6, C-12), 31.8 (*t*, C-11), 27.7 (*t*, C-2), 27.4 (*q*, C-16), 26.8 (*t*, C-7), 19.8 (*q*, C-19), 18.3 (*q*, C-18), 18.1 (*t*, C-1), 17.8 (*q*, C-20), 15.8 (*q*, C-17).

Extraction of A. cymbifera. The Me_2CO extract from the leaves of *A. cymbifera* worked-up in the usual fashion was treated with aq. MeOH. The residue afforded lignans [1]. From the MeOH soluble fraction, a portion (1.0 g) was submitted to filtration CC (silica gel, 20 g, *n*-hexane–EtOAc) to give **5a** (65 mg) and **3** (333 mg). A soln of **3** (140 mg) and of TsOH (10 mg) in C_6H_6 (50 ml) was heated under reflux (90 min.). After cooling to room temp., it was washed with satd $NaHCO_3$ soln, dried ($MgSO_4$), filtered and evapd to yield **9a** (130 mg).

ent-*Labd-8β-ol-14-ene* (**3**). Colourless oil $[\alpha]_D^{25}$ −5° ($CHCl_3$, *c* 0.60); IR ν_{max}^{film} cm^{-1}: 3470, 1640, 1180, 905; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.928 (1H, *ddd*, J = 17.34, 10.34, 8.00 Hz, H-14), 5.232 (1H, *br dd*, J = 17.34, 1.08 Hz, H-15$_t$), 5.084 (1H, *br d*, J = 10.34,

H-15$_c$), 1.130 (3H, *s*, H-17), 1.009 (3H, *d*, $J = 6.60$ Hz, H-16), 0.956 (3H, *s*, H-20), 0.873 (3H, *s*, H-19), 0.831 (3H, *s*, H-18); ^{13}C NMR (20 MHz, $CDCl_3$): δ144.7 (*d*, C-14), 111.8 (*t*, C-15), 73.3 (*s*, C-8), 58.2 (*d*, C-9), 55.8 (*d*, C-5), 41.9 (2 × *t*, C-3, C-12), 40.5 (*t*, C-7), 39.0 (*t*, C-1), 38.8 (*s*, C-10), 33.3 (*s*, C-4), 33.1 (*q*, C-19), 30.9 (*d*, C-13), 30.4 (*q*, C-17), 22.4 (*t*, C-11), 21.5 (*q*, C-18), 19.5 (*q*, C-16), 18.2, 18.1 (2 × *t*, C-2, C-6).

ent-*Labd*-8,14-*diene* (**9a**). Colourless oil, $[\alpha]_D^{25}$ −89° ($CHCl_3$, c = 1.48); MS *m/z* (rel. int.): 274 $[M]^+$ (< 1), 206 (10), 191 (100), 123 (45), 108 (38); ^{1}H NMR (60 MHz, $CDCl_3$): δ1.50 (3H, *br s*, H-17), 1.02 (3H, *d*, $J = 6.5$ Hz, H-16), 0.90 (3H, *s*, H-20), 0.87 (3H, *s*, H-19), 0.82 (3H, *s*, H-18); ^{13}C NMR (20 MHz, $CDCl_3$): δ145.9 (*d*, C-14), 140.4 (*s*, C-9), 125.4 (*s*, C-8), 112.0 (*t*, C-15), 51.8 (*d*, C-5), 41.2, 41.7 (2 × *t*, C-3, C-12), 37.2 (*s*, C-10), 36.9 (*t*, C-1), 33.5 (*t*, C-7), 33.2 (*s*, *q*, C-4, C-19), 31.1 (*d*, C-13), 25.3 (*t*, C-11), 21.5 (*q*, C-18), 20.0, 19.4, 19.3 (3 × *q*, C-16, C-17, C-20), 19.0 (2 × *t*, C-2, C-6).

Extraction of A. esperanzae. The Me_2CO extract from the leaves of *A. esperanzae* was partitioned as described above. The *n*-hexane portion afforded **2a** [1] and two diterpenoid fractions containing mainly **5a** (2g) and *ent*-labd-6β-ol-8(17),13-dien-15-oic acid (**5b**, 60 mg). Methylation of these fractions (CH_2N_2 standard conditions) followed by prep. TLC (silica gel, *n*-hexane–EtOAc, 7:3) yielded pure samples of **5c** (1.7 g) and **5d** (43 mg).

Methyl ent-*labd*-6β-*ol*-8(17),13-*dien*-15-*oate* (**5d**). Colourless oil, $[\alpha]_D^{25}$ −11° ($CHCl_3$, *c* 0.35); MS *m/z* (rel. int.): 334 $[M]^+$ (< 1), 316 (< 1), 203 (3), 153 (9), 133 (9), 123 (11), 119 (11), 114 (49), 109 (26), 95 (24), 93 (17), 69 (60), 41 (100); IR ν_{max}^{film} cm^{-1}: 3550, 1720, 1640, 1150; ^{1}H NMR (60 MHz, $CDCl_3$): δ5.65 (1H, *br s*, H-14), 5.10 (1H, *br s*, H-17), 4.80 (1H, *br s*, H-17), 4.40 (1H, *m*, H-6), 3.75 (3H, *s*, OMe), 2.25 (3H, *br s*, H-16), 1.20 (3H, *s*, H-19), 1.10 (6H, *s*, H-18, H-20); ^{13}C NMR (20 MHz, $CDCl_3$): δ167.3 (*s*, C-15), 160.7 (*s*, C-13), 144.0 (*s*, C-8), 115.2 (*d*, C-14), 110.3 (*t*, C-17), 69.4 (*d*, C-6), 57.5, 56.7 (2 × *d*, C-5, C-9), 50.8 (*q*, OCH_3), 47.7 (*t*, C-7), 43.9, 42.0 (2 × *t*, C-1, C-3), 40.9 (*s*, C-10), 39.5 (*t*, C-12), 34.5 (*s*, C-4), 33.7 (*q*, C-19), 23.6 (*q*, C-18) 21.6 (*t*, C-11), 19.5 (*t*, C-2), 19.0 (*q*, C-16), 17.1 (*q*, C-20).

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REFERENCES

1. Lopes, L. M. X., Bolzani, V. da S. and Trevisan, L. M. V. (1988) *Rev. Latinoam. Quim.* **19**, 000.
2. Lopes, L. M. X., Bolzani, V. da S. and Trevisan, L. M. V. (1987) *Phytochemistry* **26**, 2781.
3. Becker, E. D. and Beroza, M. (1962) *Tetrahedron Letters* 157.
4. Lopes, L. M. X., Yoshida, M. and Gottlieb, O. R. (1984) *Phytochemistry* **23**, 2647.
5. Koul, S. K., Taneja, S. C., Dhar, K. L. and Atal, C. K. (1983) *Phytochemistry* **22**, 999.
6. Lopes, L. M. X., Yoshida, M. and Gottlieb, O. R. (1983) *Phytochemistry* **22**, 1516.
7. Kitagawa, I., Kamigauchi, T., Yonetani, K. and Yoshihara, M. (1980) *Chem. Pharm. Bull.* **28**, 2403.
8. Misra, R., Pandey, R. C. and Dev, S. (1979) *Tetrahedron* **35**, 985.
9. Kusumoto, S., Okazaki, T., Ohsika, A. and Kotake, M. (1969) *Bull. Chem. Soc. Jpn* **42**, 812.
10. Hugel, G., Oehlschlager, A. C. and Ourisson, G. (1966) *Tetrahedron, Suppl.* **8**, Pt I, 203.
11. Marsaioli, A. J., de Freitas Leitão Filho, H. and de Paiva, Campello, J. (1975) *Phytochemistry* **14**, 1882.
12. Imamura, P. M., Marsaioli, A. J., Barata, L. E. S. and Rúveda, E. C. (1977) *Phytochemistry* **16**, 1842.
13. Zinkel, D. F., Toda, J. K. and Rowe, J. W. (1971) *Phytochemistry* **10**, 1161.
14. Bastard, J., Do Khac Duc, Fetizon, M., Francis, M. J., Grant, P. K., Weavers, R. T., Kaneko, C., Baddeley, G. V., Bernassau, J. M., Burfitt, I. R., Wovkulich, P. M. and Wenkert, E. (1984) *J. Nat. Prod.* **47**, 592.
15. Almqvist, S. O., Enzell, C. R. and Wehrli, F. W. (1975) *Acta Chem. Scand.* **B29**, 695
16. Forster, P. G., Ghisalberti, E. L. and Jefferies, P. R. (1985) *Phytochemistry* **24**, 2991.
17. Carman, R. M. (1966) *Aust. J. Chem.* **19**, 629.
18. Pascual Teresa, J. de, Urones, J. G. and Montes Sanchez, A. (1978) *An. Quim.* **74**, 959.