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Article

Cycloartane Triterpenoid and Alkaloids from *Ameidea SPP*

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O presente trabalho descreve o isolamento do novo triterpeno 3β-O-tiglyl-24-metilcicloartanol e uma mistura contendo os ácidos ursólico e oleanólico de *Almeidea coerulea.* Além dos triterpenos foram isolados cinco alcalóides da mesma planta: 7-O-acetilhaplofilidina, dutadrupina, isodutadrupina, 7-metoxi-8-(3,3-dimetilalil)-dictamina e arborinina. De *A. rubra* foram isolados os seguintes alcalóides: arborinina, metilarborinina, evolitrina, esquimianina, cocusagina e folinina. As estruturas das substâncias isoladas foram estabelecidas com base em dados espectroscópicos. Os alcalóides 7-metoxi-8-(3,3-dimetilalil)-dictamina e 7-O-acetilhaplofilidina estão sendo descritos pela primeira vez na literatura.

A new triterpene 3-β-tiglyl-24-methylcycloartanol jointly with a mixture containing 3β-hydroxy-urs-12-ene-28-oic and 3ß-hydroxy-olean-12-ene-28-oic acids were isolated from *Almeidea coerulea*. In addition to the triterpenes five alkaloids were also isolated from the same plant: 7-O-acetylhaplophyllidine, dutadrupine, isodutadrupine, 7-methoxy-8-(3,3-dimethylallyl)-dictamnine and arborinine. From *A. rubra*, the alkaloids arborinine, methylarborinine, evolitrine, skimmianine, kokusagine and folinine were isolated. Their structures were established based on their spectral data. The alkaloids 7-methoxy-8-(dimethylallyl)-dictamnine and 7-O-acetylhaplophyllidine are described for the first time as natural products.

Keywords: Almeidea coerulea, Almeidea rubra, *Rutaceae, leaves, triterpenes, alkaloids*

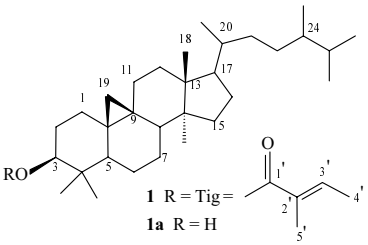
Introduction

The genus *Almeidea*, belonging to the tribe Cusparieae, is almost unknown from the chemical point of view. Few reports have been found in the literature and those that exist describe only the occurrence of flavones[1] and 2-quinolone alkaloids[2]. We have been interested in the chemistry of the South American Cusparieae for a better understanding of the circumscription of the tribe[3-6]. Here, we report the isolation and identification of triterpenoids and alkaloids from two species from the genus *Almeidea*.

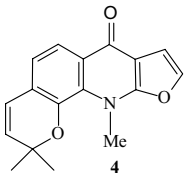
Experimental

Isolation of the constituents from A. coerulea

The leaves of *A. coerulea* collected in Espirito Santo State, southeast of Brazil, were extracted with hexane and CH_2Cl_2 yielding after evaporation of the solvent respectively 6.1 g and 8.5 g of the crude extracts. These extracts were chromatographed separately over silica gel using as eluent hexane:CH_2Cl_2:EtOAc in increasing polarity. From the hexane extract were isolated compounds **1** (178 mg), **3** (86 mg), **2** (120 mg) and **5** (98 mg). From the CH_2Cl_2



1 R = Tig =
1a R = H



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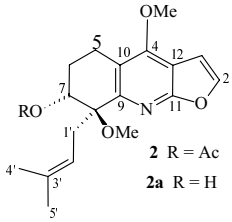
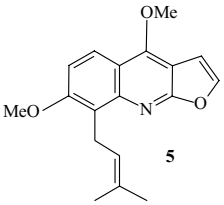


2 R = Ac
2a R = H



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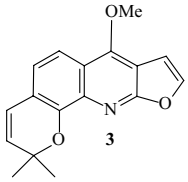
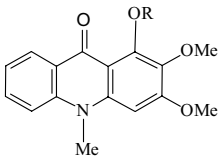
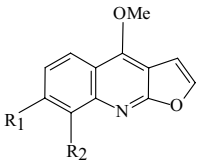


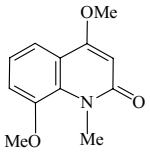
3



6 R = H
7 R = Me



8 R_1 = OMe, R_2 = H
9 R_1 = R_2 = OMe
10 R_1 = R_2 = OCH_2O



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extract were isolated compounds **3** (220 mg), **6** (680 mg) and **4** (105 mg). In a similar way the petrol extract obtained from the stems yielded a mixture of triterpenes 3β-hydroxy-urs-12-ene-28-oic and 3β-hydroxy-olean-12-ene-28-oic acids (58 mg), which were first methylated with diazomethane and then isolated as a mixture by TLC.

Isolation of alkaloids from A. rubra

A. rubra was collected in Espirito Santo State, southeast of Brazil. The leaves were extracted with CH_2Cl_2 and MeOH successively. The CH_2Cl_2 extract was chromatographed on a silica gel column deactivated with 10% water, followed by column chromatography on silicagel, using as eluent mixtures of CH_2Cl_2:acetone to get increasing polarities. The fractions collected were further purified yielding compounds **10** (6 mg), a mixture of **6** and **10** (45 mg), **6** (385 mg), **7** (21 mg), and a mixture of **9** and **11** (198 mg). The MeOH extract was submitted to solvent partition MeOH/$H_2$0/CH_2Cl_2. The MeOH fraction was chromatographed on a silica gel column using as eluent mixtures of CH_2Cl_2/acetone of increasing polarities affording **6** (21 mg) and **7** (15 mg). A second CH_2Cl_2 extract from the same plant was submitted to filtration chromatography using CH_2Cl_2, EtOAc and MeOH successively. The CH_2Cl_2 fraction after column chromatography yielded **8** (135 mg), **11** (7 mg) and a mixture of **8** and **10** (2 mg). The EtOAc fraction after chromatography afforded **8** (8 mg) and **11** (9 mg). The MeOH fraction was submitted to DCCC, using as stationary phase the aqueous layer of the mixture petroleum eter/MeOH/H_2O/ EtOAc (5:4:1:2) and the organic layer as mobile phase, affording **8** (69 mg) and **11** (4 mg).

*3β-Tiglyl-24-methyl-cycloartenol (**1**)*

Amorphous solid, IR ν_{max} (cm^{-1}): 2980, 1730, 1460, 1370. ^{1}H-NMR ($CDCl_3$, 80 MHz, δ): 6.8 (m, H-3'), 4.65 (dd, J 11.0, 4.0 Hz, H-3), 1.82 (s, Me-5'), 0.50 and 0.20 (d, J 5.0 Hz, 2H-19). ^{13}C-NMR ($CDCl_3$, 20 MHz, δ): 167.7 (s, C-1'), 136.1 (s, C-2'), 129.1 (d, C-3'), 78.5 (d, C-3), 52.1 (d, C-17), 48.8 (d, C-14), 46.8 (d, C-5), 46.6 (d, C-8), 45.2 (s, C-13), 41.6 (s, C-4), 38.4 (d, C-24), 36.9 (d, C-20), 35.2 (t, C-12), 33.1 (t, C-22), 32.7 (t, C-15), 31.8 (t, C-1), 31.3 (t, C-23), 30.9 (d, C-25), 26.4 (t, C-2), 29.4 (t, C-19), 28.1 (t, C-7), 26.9 (s, C-10), 26.4 (t, C-16), 26.4 (t, C-11), 25.1 (q, C-29), 20.9 (t, C-6), 20.9 (d, C-9), 19.1 (q, C-26), 19.0 (q, C-4'), 18.5 (q, C-28), 18.5 (q, C-21), 17.7 (q, C-18), 17.6 (q, C-5'), 17.4 (q, C-27), 14.2 (q, C-31), 14.1 (q, C-30). EIMS *m/z* (rel. int.): 524 (8), 424(100), 409(65), 283(80), 43 (70). Hydrolysis of **1** under basic conditions afforded *24-methylcicloartenol* (**1a**): amorphous solid. ^{13}C-NMR ($CDCl_3$, 20 MHz, δ): 76.6 (d, C-3), 52.5 (d, C-17), 49.0 (s, C-14), 46.7 (d, C-5), 46.6 (d, C-8), 44.9 (s, C-13), 39.8 (d, C-24), 39.8 (s, C-4), 36.5 (s, C-20), 35.4 (t, C-12), 33.1 (t, C-22), 32.9 (s, C-15), 30.8 (d, C-25), 30.9 (t, C-1), 30.9 (t, C-2), 30.6 (t, C-23), 29.6 (t, C-19), 28.4 (t, C-7), 26.9 (t, C-16), 26.2 (t, C-11), 26.1 (s, C-10), 25.5 (q, C-29), 20.6 (t, C-6), 20.9 (q, C-26), 19.9 (d, C-9), 19.0 (q, C-28), 18.5 (q, C-21), 17.6 (q, C-27), 17.3 (q, C-18), 14.4 (q, C-31), 14.0 (q, C-30).

*7-O-Acetylhaplophyllidine (**2**)*

Mp. 144-7°, IR ν_{max} (cm^{-1}): 2990, 1730, 1600, 1550, 1380. ^{1}H-NMR ($CDCl_3$, 80 MHz, δ): 7.55 (d, J 3Hz, H-2), 6.95 (d, J 3Hz, H-3), 5.20 (m, H-2', H-7), 4.28 (s, OMe-4), 3.13 (s, OMe-8), 1.98 (s, OAc-7), 1.66, 1.60 (s, Me-4', Me-5'). EIMS *m/z* (rel. int.): 359 (5), 344 (20), 328 (37), 311 (56), 248 (100), 69 (98). ^{13}C-NMR: Table 1.

*7-Methoxy-8-(3,3-dimethylallyl)-dictamnine (**5**)*

Mp.86-88° IR ν_{max} (cm^{-1}): 3100, 1595, 1450, 1260. ^{1}H-NMR ($CDCl_3$, 80 MHz, δ): 8.12 (d, J 9.0 Hz, H-5), 7.52 (d, J 3.0 Hz, H-2), 7.15 (d, J 9.0 Hz, H-6), 7.00 (d, J 3.0 Hz, H-3), 5.32 (br.t, J 6.0 Hz, H-2'), 4.38 (s, OMe-4), 3.92 (s, OMe-7), 1.90, 1.65 (s, Me-4', Me-5'). ^{13}C-NMR ($CDCl_3$, 20 MHz, δ): 142.4 (d, C-2), 104.5 (d, C-3), 157.3 (s, C-4),

Table 1. ^{13}C-NMR data for compounds 2 and haplophyllidine (2a)[8].

C	2	2a
2	161.5	161.8
3	116.4	116.8
4	157.6	157.8
5	17.9	18.4
6	20.7	23.9
7	71.7	69.3
8	76.9	78.8
9	149.6	150.1
10	104.4	104.7
11	141.9	142.6
12	104.0	104.2
1'	28.5	29.7
2'	118.3	119.5
3'	132.6	133.1
4'	17.3	17.8
5'	25.2	25.8
4-OMe	57.7	58.1
8-OMe	49.8	50.3
OAc	169.2/20.7	

120.9 (d, C-5), 110.8 (d, C-6), 145.4 (s, C-7), 124.1 (s, C-8), 156.9 9 (s, C-9), 101.3 (s, C-10), 163.9 (s, C-11), 113.9 (s, C-12), 23.7 (t, C-1'), 123.5 (d, C-2'), 130.7 (s, C-3'), 17.9 (q, C-4'), 25.6 (q, C-5'), 58.7 (q, OMe-4), 56.2 (q, OMe-7).

Results and Discussion

Working separately on two species *A. coerulea* and *A. rubra* we have isolated from the leaves of the first a new triterpenoid 3β-O-tiglyl-24-methylcycloartanol (**1**), together with the alkaloids 7-O-acetylhaplophyllidine (**2**), dutadrupine (**3**), isodutadrupine (**4**), 7-methoxy-8-(3,3-dimethylallyl)-dictamnine (**5**) and arborinine (**6**). *A. rubra* has yielded the alkaloids arborinine (**6**), methylarborinine (**7**), evolitrine (**8**), skimmianine (**9**), kokusagine (**10**) and folinine (**11**).

Compound **1** is an amorphous solid and was identified as 3β-O-tiglyl-24-methylcycloartanol (**1**). The IR spectrum of **1** showed a characteristic carbonyl band (1730 cm^{-1}). From the mass spectrum the parent peak (*m/z* 524) allowed us to propose the following elementary formula $C_{36}H_{60}O_2$ for compound **1**, as well as, the fragment (*m/z* 424, M-100) indicating the loss of a tiglic acid or angelic acid ($C_5H_8O_2$ = 100 daltons). The ^{1}H-NMR of **1** showed two doublets at δ 0.70 and 0.48 (J 5.0Hz) attributed to two protons in a cyclopropane ring, as well as, 42H in the region between δ 0.80 and 1.65 characterizing 18H and 8Me. Furthermore, a singlet at δ 1.82 attributed to the vinylic dimethyl in the tiglate moiety. Two other signals at δ 4.65 and 6.80 were assigned to the carbinolic and an olefinic protons respectively. The ^{13}C-NMR showed besides the characteristic high field signals of the triterpene skeleton, three signals (δ 167.7, 136.1, 129.1) indicating the presence of a carbonyl and two other C-sp^2. Compound **1** was hydrolyzed under basic conditions, affording one acid and the known 24-methylcycloartanol **1a**[7]. The ^{13}C-NMR of the alcoholic moiety did not show the signals at low field indicating that the acidic moiety should contain one double bond. The mass spectrum obtained for **1a** showed a parent peak (*m/z* 424), which corresponds to the loss of a fragment $C_5H_7O_2$ from **1**. These results lead us to propose the structure of 3-O-tiglyl-24-methylcycloartanol (**1**) to compound **1**. The corresponding isomer angeloyl was excluded based on the ^{1}H-NMR of **1** which showed a signal for the olefinic proton (δ 6.80) indicating the presence of tigloyl moiety. The same signal would be expected at δ 5.90 in the case of angeloyl group.

7-O-Acetylhaplophyllidine (**2**) showed to be a furoquinoline alkaloid, however it has a reduced tetrahydroquinoline ring. ^{1}H-NMR of **2** showed a pair of doublets (δ 7.53 and 6.87) referring to the furan ring, two overlapping signals due to a vinylic and O-acetylcarbinolic proton signal (δ 5.10-5.30), two methoxyl groups (δ 4.28 and 3.13) and one acetyl group (δ 1.98). The presence of the 3,3-dimethylallyl moiety linked to C-8 was also deduced from ^{1}H-NMR (δ 5.15, 1.66 and 1.60). Compound **2** was hydrolyzed in basic conditions yielding the corresponding alcohol haplophyllidine (**2a**). The most significant differences between the ^{1}H NMR of both compounds **2a** and **2** was the shielding observed for the carbinolic proton signal (δ 4.15) and the lack of the acetyl (δ 1.98) signal in the former. The ^{13}C-NMR also confirmed the presence of the prenyl chain for compound **2** (δ 118.3, 132.6, 28.5, 25.2 and 17.3) and two methoxyl groups (δ 57.7 and 49.8). The similarity between the chemical shifts observed for all carbons when compared with the model compound haploplyllidine[8] and the data reported for haplophyllidine and its epimer at C-7[9] (Table 1) led us to propose a *cis* relationship between the acetoxy and prenyl groups.

The ^{1}H-NMR of compound **5** showed signals due to a prenyl (3,3-dimethylallyl) substituent (δ 5.32, t, H-2' and 1.90, 1.65, br s, H-4'/H-5'), two methoxy groups (4.38, 3.92), two *ortho* hydrogens in a quinoline system (δ 8.12, 7.15) and a 2,3-substituted furan ring (δ 7.52, 7.00) leading to a furoquinoline alkaloid structure. The ^{13}C-NMR confirmed the structure of a furoquinoline alkaloid for **5**, which was identified as 7-methoxy-8-(3,3-dimethylallyl)-dictamnine (**5**). The possibility of the isomer 7-(3,3-dimethylallyl)-8-methoxydictamnine for compound **5** was ruled out

on the basis of the chemical shift for the methoxyl (δ 56.2), this methoxy group at C-8 would display a downfield chemical shif (δ ~60)[11].

The other alkaloids dutadrupine (**3**)[10], isodutadrupine (**4**)[10], arborinine (**6**), methylarborinine (**7**)[11,12], evolitrine (**8**)[13], skimmianine (**9**)[11], kokusagine (**10**)[14] and folinine (**11**)[15] had their structures determined through comparison of their ^{1}H- and ^{13}C-NMR data with the literature. Isodutadrupine has already been known as a reaction product obtained from dutadrupine, however this is the first time that it has been isolated as natural product.

The chemical evidence to hand for *Almeidea*, strongly reinforces its position as a member of the Cusparieae. However, up to now, the occurrence of dihydro and tetrahydrofuroquinoline alkaloids has been only described for the genus *Haplophyllum* belonging to the allied tribe Ruteae.

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