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A cytotoxic diterpene from *Alomia myriadenia*

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Abstract

An extract of the aerial parts from *Alomia myriadenia* Schultz-Bip. ex Baker (Asteraceae) showed significant cytotoxicity against a panel of human cancer cell lines in a screening of extracts from Brazilian Atlantic Forest plant species. Employing a bioassay-linked HPLC-electrospray/MS method, followed by semi-preparative HPLC, the active component was isolated and characterized as a mixture of epimers of the labdane diterpene 12*S*,16-dihydroxy-*ent*-labda-7,13-dien-15,16-olide.

Keywords: *Alomia myriadenia*; Asteraceae; 12*S*,16-dihydroxy-*ent*-labda-7,13-dien-15,16-olide; Diterpene; Cytotoxicity

1. Introduction

Alomia myriadenia Schultz-Bip. ex Baker (Asteraceae) is a tree found in the Atlantic Forest of Brazil. Although a NAPRALERT search indicated no ethnomedical information for this plant, the EtOH extract of *A. myriadenia* has shown anticrustacean activity (Zani et al., 1995). However, there have been very few previous phytochemical reports on this genus, except for the isolation of a coumarin (Pozetti & Ferreira, 1967) and a flavone (Lopes, Dias, Albuquerque & Cunha, 1997) from *Alomia fastigiata*. In a continuing search for new cytotoxic natural substances in *A. myriadenia*, we report herein the isolation and identification of a labdane diterpene and an evaluation of its cytotoxic activity against a panel of in vitro human cancer cell lines.

2. Results and discussion

The EtOH extract of the aerial part of *A. myriadenia* was partitioned successively against hexane and CH_2Cl_2. The CH_2Cl_2 extract of *A. myriadenia* was active in the KB (human oral epidermoid carcinoma) (ED_{50} 9.2 μg/ml) and Col2 (human colon cancer) (ED_{50} 2.9 μg/ml) cell lines, and was applied to the bioassay-linked LC–MS dereplication process (Cordell et al., 1997).

Fig. 1 displays the HPLC chromatogram at UV 210 nm (A), the negative total ion chromatogram (B), the extracted ion chromatogram at m/z 333 (C) and the cytotoxicity profile, using the KB cancer cell system, of the CH_2Cl_2 extract. From the activity profile, the retention time of area of strong activity was found to be at 16.9 min. The ion found in the negative extracted ion chromatogram (EIC) of the active region was at m/z 333, corresponding to a molecular weight of 334. Due to the lack of phytochemical reports on this species, this extract was chromatographed using semi-prep. HPLC to afford **1**, isolated from the cytotoxic fractions.

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Compound **1** was obtained as a white amorphous powder, and the HR–EIMS gave a $[M]^+$ at m/z 334.2134 ($C_{20}H_{30}O_4$; calcd. 334.2144 a.m.u.). IR absorptions at 3505, 1759 and 1637 cm^{-1} indicated the presence of alcohol and α,β-unsaturated γ-lactone units. The ^1H NMR spectrum of **1** in pyridine was similar to that of a labdadiene-type diterpene with three methyl groups (δ 0.79, *s*; 0.82, *s*; 0.86, *s*), two olefinic protons (δ 6.59, *s*; 5.51, *brs*) and two hydroxyl groups (δ 10.52, *brs*; 7.42, *brs*) (Zdero, Bohlmann & Niemeyer, 1991; Rodriguez, Quinoa, Riguera, Peters, Abrell & Crews, 1992; He, Kulanthaivel & Baker, 1994; Alea, Carroll & Bowden, 1994). However, the proton resonances of an *exo*-methylene group were not observed. The DEPT and APT ^{13}C NMR spectra showed 20 carbon signals, including four methyl, five methylene, six methine and five quaternary carbons. The ^{13}C NMR spectral data also showed a similar pattern to a labdadiene-type diterpene, except for several ^{13}C downfield signals. These signals were two olefinic carbons at δ 171.7 (*s*, C-13) and 117.0 (*d*, C-14), a carbonyl carbon at δ 175.4 (*s*, C-15) and a hemiacetal carbon at δ 99.2 (*d*, C-16), which led to the proposal that a hydroxy butenolide moiety was present (Alea et al., 1994). The complete assignments of the carbon signals were determined by DEPT, APT, HMBC and HMQC experiments. The ^{13}C NMR data showed that C-12 (δ 69.9) was attached to an oxygen atom. Its hydrogen, H-12 (δ 5.11), was correlated in the HMBC spectrum with C-14 (δ 117.0), C-15 (δ 175.4) and C-9 (δ 51.9). In the ^1H NMR spectrum of **1** two singlets at δ 6.84 and 6.59, assigned to H-16 and H-14, respectively, showed a weak correlation signal in the H–H COSY contour map indicating that the butenolide moiety has a β-substituent. Therefore, the proposed structure of **1** was considered to be a labdadiene diterpene containing a hydroxy butenolide. Compound **1** was acetylated using acetic anhydride/pyridine at room temperature and its physico-chemical data were shown to be identical with those described for an epimeric mixture of the labdane diterpene 12*S*,16-dihydroxy-*ent*-labda-7,13-dien-15,16-olide, isolated from *Acritopappus hagei* (Bohlmann, Zdero, Gupta, King & Robinson, 1980). The stereochemistry at C-12 of the acetate mixture was examined by MM2 calculations using PCMODEL and led us to propose the *S*-configuration ($J = 7.5$, 1.2 Hz) by comparison of the calculated coupling constants of the *R* ($J = 11.7$, 2.5 Hz) and *S* ($J = 7.1$, 2.0 Hz) iso-

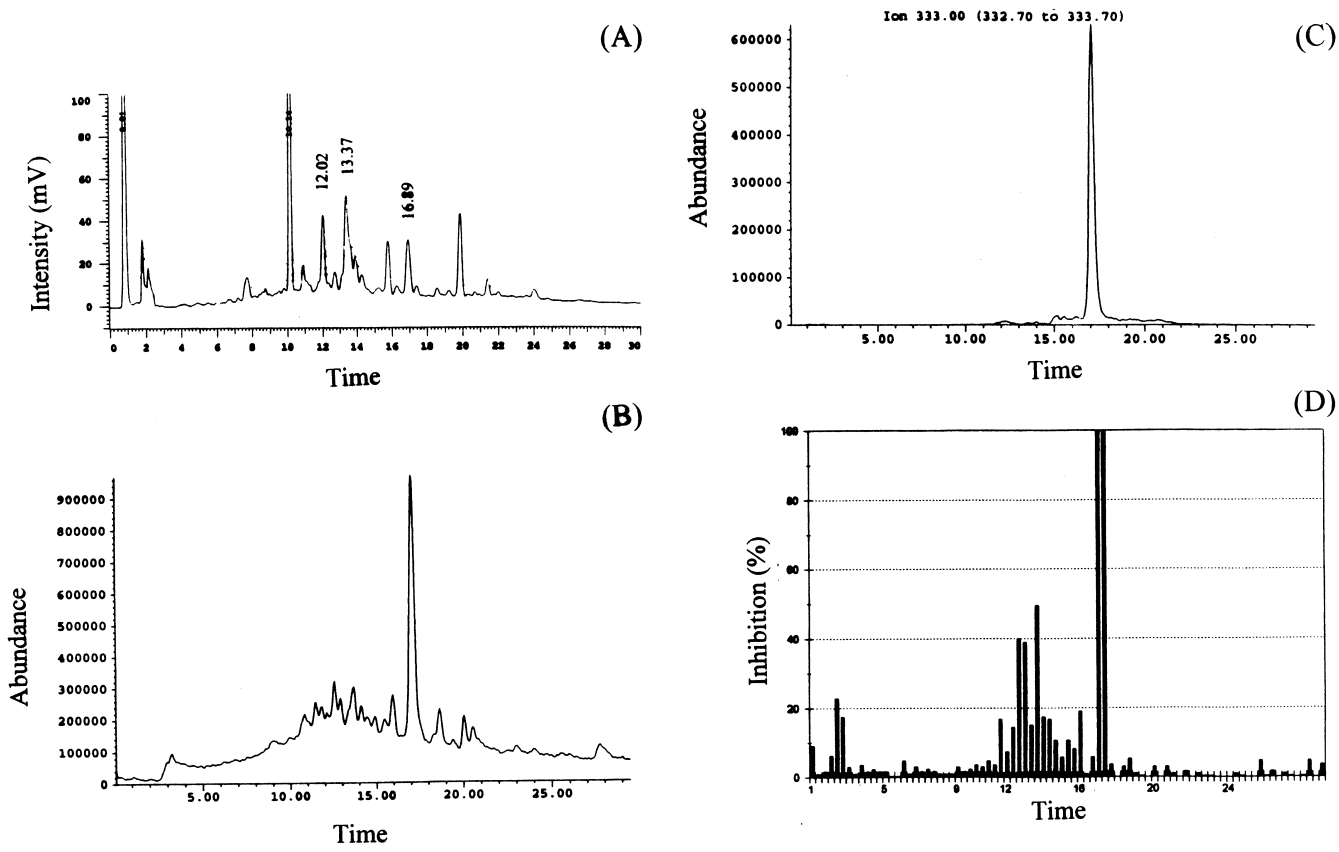
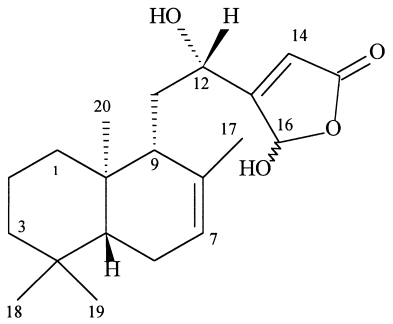


Fig. 1. The HPLC chromatogram (A), the negative total ion chromatogram (B), the extracted ion chromatogram at m/z 333 (C) and the cytotoxicity profile against KB (D) of the CH_2Cl_2 extract of *A. myriadenia*.

mers. All efforts to separate the C-16 isomers (ratio 2.1:1) of **1** by HPLC and other chromatographic methods failed (Bohlmann et al., 1980).

Compound **1** was evaluated for its cytotoxic activity against a panel of human cancer cell lines, i.e., BC-1 (human breast cancer), Lu1 (human lung cancer), Col2 (human colon cancer), KB (human oral epidermoid carcinoma), KB-VI$^-$ (vinblastine-resistant KB tested in the absence of vinblastine), KB-VI$^+$ (vinblastine-resistant KB tested in the presence of vinblastine (1 μg/ml)) and LNCaP (hormone-dependent human prostate cancer) using the procedures described previously (Likhitwitayawuid, Angerhofer, Cordell & Pezzuto, 1993). The ED_{50} values against each of the cell lines were 4.4, 3.9, 3.7, 2.4, 9.1, 8.6 and 3.0 μg/ml, respectively.



3. Experimental

3.1. General

Melting point was determined on an electrothermal apparatus Model 9100. IR spectra were obtained on a Mattson-Galaxy series FTIR 3000. The optical rotations were measured with a Perkin-Elmer 241 Polarimeter. ^{1}H, ^{13}C NMR, DEPT, HMQC and HMBC spectra were measured on a Bruker Avance DPX-300 spectrometer, using TMS as an internal standard. HR–EIMS and EIMS were recorded on a Finnigan MAT 90 mass spectrometer. HPLC-electrospray/MS chromatograms and spectra for dereplication were measured on a Hewlett Packard 5989B mass spectrometer coupled with a 59987A electrospray interface and a Hitachi HPLC L-7100 system. TLC were run on precoated silica gel plates, using CH_2Cl_2 as a developing solvent system and vanillin–sulfuric acid as a spray reagent. Analytical and semi-preparative HPLC were run on a Shimadzu chromatograph equipped with a LC-6AD pump and a UV detector set at 254 nm. Analytical (4.6 × 250 mm) and semi-prep (20 × 250 mm) columns (Shimpak prep-ODS kit) were used throughout this work.

3.2. Plant material

Aerial parts of *A. myriadenia* Schultz-Bip. ex Baker (Asteraceae) were collected by C. L. Z. near Minduri, Minas Gerais, Brazil, in June 1997. Plant identification was performed by Dr. João R. Stehmann of the Botany Department of the Federal University of Minas Gerais, in which herbarium a voucher specimen (BHCB 42865) was deposited.

3.3. Bioactivity-guided fractionation and isolation

Dried leaves of *A. myriadenia* (300 g) were powdered and macerated in 500 ml of EtOH (3 × 24 h) to afford, after solvent removal, a crude extract (20.73 g). This extract was active in the panel of human tumor cells. A portion (10.1 g) was suspended in MeOH–H_2O (9:1, 50 ml) and extracted five times with hexane (20 ml) to afford the fraction AMY84A (1.03 g). The hydro-alcoholic phase was then adjusted to 1:1 by the addition of H_2O (40 ml) and extracted with CH_2Cl_2 (3 × 50 ml) to yield AMY84B (3.23 g) and a hydro-alcoholic fraction (AMY84C, 0.99 g). The cytotoxicity was concentrated in fraction AMY84B. Bioassay-linked HLPC-electrospray/MS indicated that the major component was responsible for the observed activity. This component was isolated using the following procedure: AMY84B (100 mg) was sonicated in CH_3CN–H_2O (2:1) to afford, after centrifugation (10.000 × *g*, 5 min) an insoluble (AMY15H, 12.1 mg) and a soluble fraction (AMY15G, 87.7 mg). Part of the latter fraction (82 mg) was injected onto a Shimpak Prep-ODS column (RP-18, 5 μm, 20 × 250 mm) and eluted with CH_3CN–H_2O (7:3) at 15 ml/min, with detection at 254 nm. Six fractions were collected from the column: AMY15A (0–6 min, 11.7 mg), AMY15B (6–8 min, 7.7 mg), AMY15C (8–11.7 min, 4.9 mg), AMY15D (11.7–13 min, 5.0 mg), AMY15E (13–23 min, 4.6 mg), AMY15F (23–33 min, 8.9 mg). AMY15D corresponded to the most intense peak and also to the most cytotoxic fraction to Col2 and KB cells. The semi-prep. separation was repeated several times and the AMY15D recrystallized from CH_2Cl_2 to afford AMY15Dc (32 mg), which was used to obtain the spectral data.

12S,16-Dihydroxy-ent-labda-7,13-dien-15,16-olide (**1**). White amorphous powder. mp 151–152°C; HR–EIMS *m/z*: 334.2134 for $C_{20}H_{30}O_4$ (calcd. 334.2144); EIMS (probe) 70 eV, *m/z* (rel. int.): 334 (3), 316 (26), 301 (17), 283 (10), 255 (7), 205 (72), 190 (65), 175 (59), 137 (28), 109 (100); IR (KBr) cm^{-1}: 3505 (hydroxyl group), 1759, 1637 (α,β-unsaturated γ-lactone); ^{1}H NMR spectral data (300 MHz, pyridine-d_5): δ 0.79 (3H, *s*, H-19), 0.82 (3H, *s*, H-18), 0.86 (3H, *s*, H-20), 2.01 (3H, *s*, H-17), 5.11 (1H, *m*, H-12), 5.51 (1H, *brs*, H-7), 6.59 (1H, *s*, H-14), 6.84 (1H, *s*, H-16), 7.42 (1H,

brs, 16-OH), 10.52 (1H, *brs*, 12-OH); ^{13}C NMR spectral data (75 MHz, pyridine-d_5): δ 175.4 (*s*, C-15), 171.7 (*s*, C-13), 135.3 (*s*, C-7), 122.8 (*d*, C-6), 117.0 (*d*, C-14), 99.2 (*d*, C-16), 69.9 (*d*, C-12), 51.9 (*d*, C-9), 50.4 (*d*, C-5), 42.3 (*t*, C-3), 39.6 (*t*, C-1), 37.5 (*s*, C-10), 33.2 (*t*, C-11), 32.9 (*q* C-19), 32.9 (*s*, C-4), 24.3 (*t*, C-6), 23.2 (*q*, C-17), 21.8 (*q*, C-18), 18.9 (*t*, C-2), 13.9 (*q*, C-20).

3.4. Acetylation of AMY15Dc

AMY15Dc (16.5 mg) was stirred at room temperature with acetic anhydride (1 ml) and pyridine (1 ml). After 17 h, TLC indicated completion of the reaction. The mixture was worked up as usual and after flash chromatography using a Pasteur pipette filled with 400 mesh silica gel, afforded pure AMY15Dac (20 mg, yield 96.9%). Acetate of **1**: amorphous gum, CIMS (methane) *m*/*z* (rel. int.): 419 (4) $[M + H]^+$, 359 (35), 299 (100), 109 (10); ^{1}H NMR spectral data (300 MHz, $CDCl_3$): 5.48, 5.50 (1H, *brs*, 7-H), 5.70, 5.71 (1H, *dt*, $J = 7.5$, 1.2 Hz, 12-H), 6.99, 7.05 (1H, *d*, $J = 0.82$, 1.0 Hz, 14-H), 6.09, 6.18 (1H, *t*, $J = 1.1$, 0.82 Hz, 15-H), 1.71 (3H, *brs*, 17-H), 0.88 (3H, *s*, 18-H), 0.86 (3H, *s*, 19-H), 0.77 (3H, *s*, 20-H), 2.20, 2.16, 2.14, 2.09 (3H, *s*, OAc); $[\alpha]_D^{25} - 13.4^\circ$ (*c*, 1.0 in $CHCl_3$) ($[\alpha]_D^{24} - 10.7^\circ$ (*c*, 5.7 in $CHCl_3$ (Bohlmann et al., 1980)).

3.5. Dereplication method using HPLC-electrospray/MS

The cytotoxic $CHCl_3$ extract (AMY84B) of the aerial parts of *A. myriadenia*, prepared as indicated above, was subjected to dereplication analysis, employing a previously published protocol, using standard chromatographic conditions, and the KB cytotoxicity assay to monitor activity (Cordell et al., 1997).

3.6. Evaluation of cytotoxic potential

The crude CH_2Cl_2 extract of the aerial part of *A. myriadenia*, sub-fractions of the extract, and compound **1** were tested according to standard procedures (Likhitwitayawuid et al., 1993) against the BC1 (human breast cancer), Lu1 (human lung cancer), Col2 (human colon cancer), LNCaP (human prostate cancer), KB (human oral epidermoid carcinoma), and KB-VI [KB in the presence and absence of vinblastine (1 μg/ml)] cell lines.

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References

Alea, G. V., Carroll, A. R., & Bowden, B. F. (1994). Coscinoquinol, a new cytotoxic sesterterpene from a dictyoceratid sponge *Coscinoderma sp. Australian Journal of Chemistry*, *47*(1), 191.

Bohlmann, F., Zdero, C., Gupta, R. K., King, R. M., & Robinson, H. (1980). Diterpenes and tetranorditerpenes from *Acritopappus* species. *Phytochemistry*, *19*(12), 2695.

Cordell, G. A., Beecher, C. W. W., Kinghorn, A. D., Pezzuto, J. M., Constant, H. L., Fang, L., Seo, E.-K., Long, L., Cui, B.-L., & Barrilos, K. S. (1997). In Atta-ur-Rahman, *Studies in natural products chemistry*, *vol. 19* (pp. 749–791). Amsterdam: Elsevier Science.

He, H. Y., Kulanthaivel, P., & Baker, B. J. (1994). New cytotoxic sesterterpenes from the marine sponge *Spongia* sp. *Tetrahedron Letters*, *35*(39), 7189.

Likhitwitayawuid, K., Angerhofer, C. K., Cordell, G. A., & Pezzuto, J. M. (1993). Traditional medicinal plants of Thailand. 20. Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Stephania erecta*. *Journal of Natural Products*, *56*(1), 30.

Lopes, J. L. C., Dias, D. A., Albuquerque, S., & Cunha, W. R. (1997). Trypanomicidal activity of *Alomia fastigiata*. *Fitoterapia*, *68*(2), 182.

Pozetti, G. L., & Ferreira, P. C. (1967). Identification of a coumarin from *Alomia fastigiata*. *Revista Faculdade de Farmacia Bioquimica Universidade Sao Paulo*, *5*(1), 253.

Rodriguez, J., Quinoa, E., Riguera, R., Peters, B. M., Abrell, L. M., & Crews, P. (1992). The structures and stereochemistry of cytotoxic sesquiterpene quinones from *Dactylospongia elegans*. *Tetrahedron*, *48*(32), 6667.

Zani, C. L., Chaves, P. P. G., Queiroz, R., De Oliveira, A. B., Cardoso, J. E., Anjos, A. M. G., & Grandi, T. S. M. (1995). Brine shrimp lethality assay as a prescreening system for anti-*Trypanosoma cruzi* activity. *Phytomedicine*, *2*(1), 47.

Zdero, C., Bohlmann, F., & Niemeyer, H. M. (1991). An unusual dimeric sesquiterpene and other constituents from Chilean *Baccharis* species. *Phytochemistry*, *30*(5), 1597.