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Diterpene constituents of leaves from *Juniperus brevifolia*

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Abstract

The dichloromethane extract from leaves of *Juniperus brevifolia*, through chromatographic fractionations yield six compounds: 3β-hydroxy-abieta-8,11,13-trien-7-one, 18-hydroxy-sandaracopimara-8(14),15-dien-7-one, sandaracopimara-8(14),15-dien-18-yl formate; and the first examples of sandaracopimaranes and abieta-8,11,13-triene diterpenoids with a large aliphatic chain on C-18, abieta-8,11,13-trien-18-yl hexadecanoate, 7-oxoabieta-8,11,13-trien-18-yl hexadecanoate, sandaracopimara-8(14),15-dien-18-yl hexadecanoate. Moreover fifteen known compounds were also isolated, some of them for the first time identified on *Juniperus* genus. The compound abieta-8,11,13-trien-18-yl formate is reported for the first time as a natural product. All the structures were established by spectroscopic methods. 2D NMR techniques have allowed the revision of certain previously reported ^{13}C NMR assignments. Studies on the isolated new compounds showed those possessing a diterpenol ester of a long-chain fatty acid present lipophilicity very distinct from other diterpenoid compounds.

Keywords: *Juniperus brevifolia*; Cupressaceae; Cedro do mato; Abietanes; Sadaracopimaranes; Fatty acid diterpenol ester

1. Introduction

Being isolated in the middle of the Atlantic Ocean and having several natural resources, Azores becomes very interesting as a source of possible new bioactive compounds and/or with chemosystematic significance. One of these sources may be the *Juniperus brevifolia* (Seub.) Antoine (Cupressaceae), locally known as ''cedro-do-mato'' and well known for its durability and resistance to rotting. This species is the unique conifer tree endemic of Azores and it is a typical component of the primitive *laurisilva* forest (Schäfer, 2002). No evidence has been found for the use of *J. brevifolia* in traditional medicine. However, the wide range of biological activities reported for other species of this genus as well as for their constituents (Seca and Silva, 2006) stimulated our interest to study the chemical composition of *J. brevifolia*. Previous studies on this plant described the components of its essential oil (Adams, 1999; Da Silva et al., 2000) and hexane extract (Seca and Silva, in press). We report herein on the isolation and structural elucidation of six new diterpenes and fifteen known compounds from the dichloromethane extract of *J. brevifolia* leaves and on the correction of some literature ^{13}C NMR assignments for abieta-8,11,13-trien-18-yl formate.

2. Results and discussion

The analysis of the dichloromethane extract of the leaves of *J. brevifolia* led to the isolation of three new abietanes (**1**–**3**) and three new pimaranes (**4**–**6**); three of them being esters of the long-chain fatty hexadecanoic acid and other ester of formic acid (Fig. 1). Moreover, fifteen known compounds were also identified, by comparison

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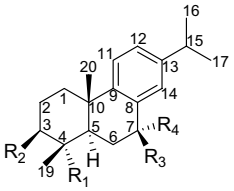
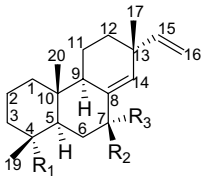


	R_1	R_2	R_3	R_4
1	$CH_2OCO(CH_2)_{14}CH_3$	H	H	H
2	$CH_2OCO(CH_2)_{14}CH_3$	H	=O	
3	CH_3	OH	=O	
7	CH_2OCHO	H	H	H



	R_1	R_2	R_3
4	CH_2OCHO	H	H
5	$CH_2OCO(CH_2)_{14}CH_3$	H	H
6	CH_2OH	=O	

Fig. 1. Structures of compounds isolated from *Juniperus brevifolia* leaves.

their spectra data with those reported in the literature, as 15,16-bisnor-13-oxo-labda-8(17),11*E*-dien-19-oic acid (**8**) (Inoue et al., 1985; Muhammad et al., 1996), *E*- and *Z*-communic acid (**9**) (Muhammad et al., 1995), hinokiol (**10**) (Fang et al., 1996; Wang et al., 2002), 18-hydroxy-dehydroabietane (pomiferin A) (**11**) (San Feliciano et al., 1992; Fraga et al., 2003), sugiol (**12**) (Ara et al., 1988; Fang et al., 1993), sandaracopimara-8(14),15-dien-18-ol (**13**) (San Feliciano et al., 1992; Barrero et al., 2004), sandaracopimaric acid (**14**) (Wenkert and Buckwalter, 1972; Sakar and San Feliciano, 1994), nootkatone (**15**) (Miyazawa et al., 2000; Schneider et al., 2004), stigma-4-en-3-one (**16**), β-sitosterol (**17**) (Seca et al., 2000); and for the first time in *Juniperus* species, sandaracopimara-8(14),15-diene (**18**) (Kenmoku et al., 2004), methyl ester of 15-agathic acid (**19**) (Richomme et al., 1991), 7-oxo-abieta-8,11,13-trien-18-ol (**20**) (Tanaka et al., 1997) and eicosanyl-*trans*-*p*-coumarate (**21**) (Mahmood et al., 2003). Abieta-8,11,13-trien-18-yl formate (**7**) is a synthetic dehydroabietane derivative with gastroprotective effect (Sepúlveda et al., 2005) which is identified for the first time as a natural product. A detailed analysis of the COSY, HSQC and HMBC spectra of **7** have shown that the literature assignment resonances of C-4 and C-10 (Sepúlveda et al., 2005) must be interchanged. In fact, the HMBC spectrum of **7** showed correlations between the proton resonance at δ 7.18 (1H, *d*, $J = 8.2$ Hz, H-11) with the quaternary carbons at δ_C 37.3 (C-10), 134.6 (C-8) and 145.7 (C-13) and also between that of the methyl group at δ_H 0.96 (3H, *s*, H-19) with the carbons at δ_C 71.8 (C-18), 43.9 (C-5), 36.7 (C-4) and 35.4 (C-3). These data allowed the unequivocal assignment of C-4 and C-10 which proves the previous assignments must be interchanged.

The HR-ESIMS data of **1** exhibited a sodiated molecular ion peak at *m*/*z* 547.4463 establishing the molecular formula $C_{36}H_{60}O_2$. The IR spectrum showed absorption bands at ν_{max} 1738, 1166 and 2924 cm^{-1}, suggesting the presence of an ester group of an aliphatic long-chain acid. This was also supported by the resonance at δ_C 174.1 and a large number of signals between δ_C 29.2 and 29.7 in its ^{13}C NMR spectrum. The ^1H NMR spectrum of **1** showed the presence of three protons in a trisubstituted aromatic ring at δ_H 7.18 (1H, *d*, $J = 8.2$ Hz), 7.00 (1H, *dd*, $J = 8.2$, 1.8 Hz) and 6.89 (1H, *brs*), two quaternary methyl groups at δ_H 1.22 and 0.93, and one hydroxymethylene group at δ_H 3.96 and 3.69 (AB system, 1H each, *d*, $J = 11.0$ Hz). The presence of an isopropyl group linked to a quaternary carbon was supported by the signals at δ_H 2.83 (1H, *sept*, $J = 6.9$ Hz) and 1.23 (6H, *d*, $J = 6.9$ Hz). Comparison of the ^{13}C NMR data of **1** and those of abieta-8,11,13-trien-18-yl formate (**7**) showed a good agreement except for the chemical shift of C-1′ (shifted downfield, $\Delta\delta$ +12.9 ppm, due to the substitution of H–COO to RCOO group) and the presence of one methyl and fourteen methylene additional groups (confirmed by ^{13}C DEPT NMR spectra). The presence of a long-chain aliphatic acid esterified with the dehydroabietane derivative on C-18 was confirmed by the important connectivities found in the HMBC spectrum (as shown in Fig. 2) and the NOE cross peaks observed in the NOESY spectrum between the signal at δ_H 0.93 (3H, *s*, H-19) with that at 1.22 (3H, *s*, H-20). The equatorial position of the hydroxymethylene group was confirmed by the shift of the 4-*C*H_3 at δ_C 17.5 corresponding to axial position (Chamy et al., 1987; Ulubelen and Topcu, 1992). All of these data established the structure of **1** (Fig. 1) as abieta-8,11,13-trien-18-yl hexadecanoate.

Compound **2** was shown to have the molecular formula $C_{36}H_{58}O_3$ according to the HR-ESIMS quasi-molecular ion peak observed at *m*/*z* 539.4446 $[M+H]^+$. Its IR spectrum revealed absorptions for two types of carbonyl groups (ν_{max} 1683 and 1737 cm^{-1}). The ^{13}C NMR spectrum (Table 1) was similar to those of **1**, being the presence of a carbonyl group (δ_C 199.0), lack of one methylene group and the deshielding effect on C-6 (from δ_C 18.9 to 36.0) the major differences. The downfield shift in the resonances of the 1,2,4-trisubstituted aromatic ring (δ_H 7.31, *d*, $J = 8.2$ Hz, H-11; 7.42, *dd*, $J = 2.1$ and 8.2 Hz, H-12; 7.88, *d*, $J = 2.1$ Hz, H-14) of **2** compared to those of **1** are the main differences in their ^1H NMR spectra. These data were compatible with the presence of a carbonyl group at C-7 conjugated with the aromatic ring which resulted in a mesomeric deshielding effect on H-12, and H-14, and mainly anisotropic deshielding effect on H-14 and H-6. This was confirmed by the connectivities found in the HMBC spectrum (Fig. 2). The stereochemistry of **2** was established based on chemical shift resonances of the 4-methyl group