Diterpenes from *Sideritis infernalis* and *S. candicans*

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Z. Naturforsch. **2008**, *63b,* 595 – 599; received November 8, 2007

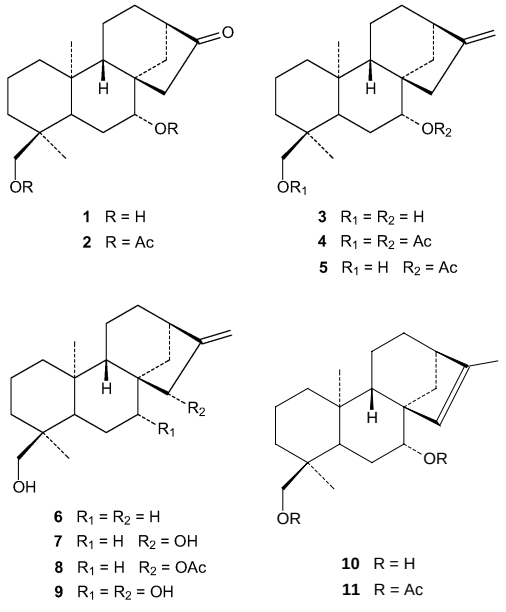
A phytochemical study of *Sideritis infernalis* led to the isolation of the new *nor*-diterpene adejone (17-*nor*-7α,18-dihydroxy-*ent*-kaur-16-one). The biosynthesis of this compound implies the decarboxylation of an epoxy-acid as the last step. In addition, three diterpenes with an *ent*-kaurene skeleton, episideridiol, candicandiol 7α-monoacetate and candidiol 15α-monoacetate, have been isolated from *S. candicans* for the first time in nature.

Key words: Sideritis, Diterpenoids, Adejone, Episideridiol

Introduction

The genus *Sideritis* (Lamiaceae) is represented in the Canary Islands by 22 species [1]. We have been interested in their phytochemistry and phylogeny for several years [2, 3].

In a continuation of this work we have now completed earlier studies of *S. infernalis* Bolle [4, 5] and reinvestigated *S. candicans* Ait [*S. oroteneriffae* L. Negrín et P. Pérez] [6]. Both species are endemic to the island of Tenerife. We have followed Sventenius's work [7] naming the species of this genus. However, we include the actual botanical name between square brackets. The whole plant of *S. candicans* has been used in Tenerife as an anticatarrhal and stimulant of the circulatory system [8].



1 R = H
2 R = Ac

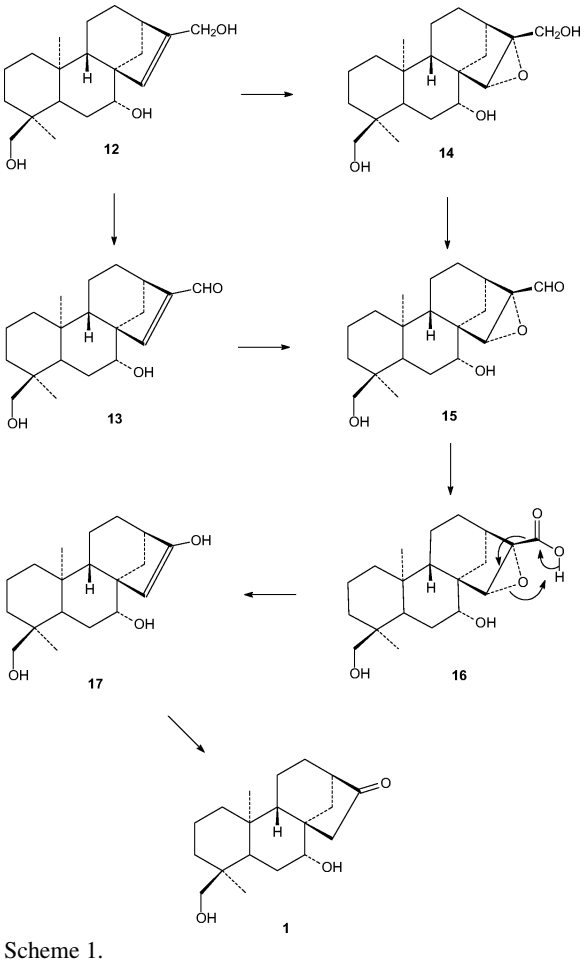
3 $R_1 = R_2 = H$
4 $R_1 = R_2 = Ac$
5 $R_1 = H$ $R_2 = Ac$

6 $R_1 = R_2 = H$
7 $R_1 = H$ $R_2 = OH$
8 $R_1 = H$ $R_2 = OAc$
9 $R_1 = R_2 = OH$

10 R = H
11 R = Ac

Results and Discussion

The diterpenes candicandiol (**3**), candol B (**6**), candidiol (**7**), canditriol (**9**), sinfernol (**12**), sinfernal (**13**), epoxysinfernol (**14**), the 7-epimer of sinfernal and the flavanones 5-hydroxy-7,4'-dimethoxyflavanone and 5-hydroxy-7,3',4'-trimethoxyflavanone had been obtained from an extract of *S. infernalis* Bolle [4, 5]. Now, in this work, we give an account of other compounds later identified in this extract. One of them was a new *nor*-diterpene, which we have named adejone (17-*nor*-7α,18-dihydroxy-*ent*-kaur-16-one) (**1**). This compound was obtained in the form of its diacetate **2** by acetylation and chromatography of fractions containing it. Its high-resolution MS was in accordance with the structural formula $C_{23}H_{33}O_5$. The ^{1}H NMR spectrum showed resonances of two angular methyl groups, the H-18 as a pair of doublets at δ = 3.60 and 3.85 (J = 11.0 Hz) and the axial geminal hydrogen of the C-7 acetate at δ = 4.74 (dd, J = 11.6 and 4.2 Hz). The ^{13}C NMR spectrum showed the resonances of C-7 and C-18 at δ = 75.8 and 72.0, respectively, while the C-6-oxo group appears at δ = 220.9. Both spectra were unambigu-



Scheme 1.

ously assigned using 2D NMR data (COSY, HSQC, HMBC and NOESY). In the HMBC spectrum correlations were observed of H-5 with C-1, C-7, C-19 and C-20; H-7 with C-5, C-6, C-14 and C-15; H-9 with C-1, C-12 and C-14; H-15 with C-7, C-9, C-13 and C-16; H-18 with C-3, C-4, C-5 and C-19; H-19 with C-3, C-4, C-5 and C-18; and H-20 with C-1, C-5, C-9 and C-10.

Hydrolysis of adejone diacetate (**2**) with methanolic potassium hydroxide (3 %) at r. t. led to adejone (**1**), which is the natural diterpene formed in the plant. Their ^{1}H, ^{13}C and 2D NMR spectra were also in accordance with the assigned structures. Chemical confirmation of the structure of adejone was obtained by ozonolysis of candicandiol diacetate (**4**), which gave a product identical with adejone diacetate (**2**). This is the first time that a 16-oxo-17-*nor*-kaurane has been isolated as a natural product. The diterpene 17-*nor*-7α,19-dihydroxy-*ent*-kaur-16-one, the 4-epimer of **1**, had been prepared by 7α-hydroxylation of synthetic 17-*nor*-19-hydroxy-*ent*-kaur-16-one with the fungus *Rhizopus nigricans* [9].

A biogenetic route from sinfernol (**12**) account for the biosynthesis of adejone (**1**) (Scheme 1). The diterpenes sinfernol (**12**), sinfernal (**13**) and epoxysinfernol (**14**) involved in this route have also been isolated from this plant [4]. The same enzyme probably produces oxidations that transform the C-17 alcohol of sinfernol (**12**) into the corresponding acid **16**. The main step is a glycidic acid type decarboxylation of **16** to give the enol **17** [10]. In this reaction, decarboxylation and epoxide ring opening occur simultaneously to form the enol, which tautomerizes giving adejone (**1**). The decarboxylation of an epoxy-acid is a very rare step in the formation of a natural product.

Other compounds now identified in this species were the methyl ester of *p*-methoxycinnamic acid and a mixture of the sterols β-sitosterol, stigmasterol and campesterol.

We have now reinvestigated *S. candicans* Ait. In a previous work we had isolated from this species the diterpenes *ent*-kaur-16-ene, dehydroabietane, epicandicandiol 7β-monoacetate, trachinodiol 7β-monoacetate, candicandiol (**3**) and candidiol (**7**), the sterols β-sitosterol, stigmasterol and campesterol, and the triterpenes squalene, glutinol, and ursolic and oleanolic acids [6]. Later, we indicated that the triterpene glutinol had been misidentified as a component of several species of *Sideritis*, and recognized it as its isomer rhoiptelenol [3].

We have now assigned the structure **10** to the diterpene episideridiol, which was obtained as its diacetate **11** by acetylation and chromatography of fractions containing it. Its ^{1}H NMR spectrum showed signals of three methyls, one at δ = 1.67 (d, J = 1.5 Hz) placed over a double bond, two acetates, a vinylic proton at δ = 5.10 (br s) and the two hydrogens of an acetylated primary alcohol at δ = 3.60 and 3.80 (d, J = 11.1 Hz), which was assigned to C-18 considering these chemical shifts [11]. The structure was confirmed, and unambiguously assigned by the ^{1}H and ^{13}C NMR spectra, considering 2D NMR data (HMQC, HMBC, COSY and NOESY). Basic hydrolysis of **11** led to **10**, which is the natural product found in the plant. This is the first time that episideridiol (**10**) is found in nature. It had been synthesized from its 7-epimer sideridiol [12].

Another two compounds that were isolated for the first time from a natural product were candicandiol 7α-monoacetate (**5**) (30 mg) and candidiol 15α-monoacetate (**8**) (110 mg). These diterpenes had been prepared from candicandiol (**3**) [13] and candidiol (**7**) [14], respectively, and a direct comparison of these monoacetates, and the corresponding diacetates, confirmed their structures.

Other compounds now identified for the first time in this plant were 2β-hydroxy-*ent*-13-*epi*-manoyl oxide [15], 16α,18-dihydroxy-*ent*-atisane [16], sinfernol (**12**), sinfernal (**13**) [4], sidendrodiol [17] and vierol [18]. Adejone (**1**) and epoxysinfernal (**15**) (see above) were tentatively identified in impure chromatographic fractions containing sinfernal (**13**) as the main compound.

We have shown that the Macaronesian *Sideritis* may be classified phytochemically into three groups: *Group 1*, species that contain pentacyclic triterpenes, but not diterpenes, *group 2*, characterized by possessing bicyclic diterpenes and abundance of flavones, and *group 3*, species containing tetra- and/or pentacyclic diterpenes [2, 6]. Recent morphological studies have shown that these phytochemical groups coincide with the sections *Cretica*, *Empedocleopsis* and *Marrubistrum*, respectively [1].

In accordance with their content in diterpenes, *S. infernalis* Bolle and *S. candicans* Ait [*S. orotenerifffae* L. Negrín et P. Pérez] belong to the third group. Moreover, these two species, together with *S. bolleana* Bornm. [*S. barbellata* Mend.-Heuer emend., P. Pérez et L. Negrin] [6], *S. dendrochahorra* Bolle [6, 17] and *S. ferrensis* P. Pérez et L. Negrin [19], are characterized by containing the diterpene candicandiol (**3**). In consequence, from the phytochemical viewpoint, they can form a new subgroup within our third group of *Sideritis*. Morphologically, three of these species, *S. bolleana*, *S. dendrochahorra* and *S. ferrensis*, belong to the subsection *Massoniana* (Christ) Svent [1].

Experimental Section

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500.13 and 125.77 MHz, respectively, with a Bruker AMX2-500 spectrometer. Chemical shifts are given in ppm (δ). Mass spectra were taken at 70 eV (probe) in a Micromass Autospec spectrometer. Dry column chromatographies were made on silica gel (Merck 0.02 – 0.063 mm).

C atom	**1**	**2**	**10**	**11**
1	39.7	39.6	39.7	39.7
2	17.8	17.6	17.8	17.4
3	35.0	35.6	35.0	35.7
4	37.4	36.2	37.4	36.3
5	45.8	46.3	45.7	46.6
6	28.4	24.5	27.5	25.2
7	74.1	75.8	75.0	76.0
8	47.9	46.3	55.3	53.7
9	54.0	54.0	47.9	48.1
10	39.2	39.1	38.9	38.9
11	18.3	18.2	18.4	18.1
12	30.0	29.5	25.2	24.9
13	47.2	46.8	44.0	43.4
14	28.3	29.5	34.6	35.6
15	49.7	49.1	131.9	132.0
16	222.2	220.9	145.7	143.2
17	–	–	15.5	15.3
18	71.6	72.0	71.7	78.3
19	17.6	17.5	18.3	17.7
20	18.6	18.5	17.4	18.4

Table 1. ^{13}C NMR data (δ, in ppm) of compounds **1**, **2**, **10** and **11**.

Isolation of compounds from S. infernalis

We have described the isolation of several diterpenes [4] and flavanones [5] from an extract of *S. infernalis* Bolle. Continuing with the study of the components of this extract, we isolated a mixture of β-sitosterol, stigmasterol and campesterol (130 mg), adejone (**1**) and *p*-methoxycinnamic acid methyl ester (130 mg). Adejone (**1**) was obtained in form of the diacetate **2** (11 mg) by acetylation of several fractions (240 mg) of the main chromatography, which contained also candicandiol (**3**) and candidiol (**7**).

*Adejone (**1**)*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.77 (1 H, td, *J* = 13.0 and 3.9 Hz, H-1β), 0.78 (3 H, s, H-19), 1.13 (3 H, s, H-20), 1.21 (1 H, d, *J* = 8 Hz, H-9), 1.28 (1 H, dt, *J* = 13.5 and 3.0 Hz, H-3α), 1.30 (1 H, dd, *J* = 12.5 and 1.5 Hz, H-5), 1.43 (1 H, td, *J* = 13.5 and 4.4 Hz, H-3β), 1.48 (1 H, t, *J* = 12.5 Hz, H-6α), 1.73 (1 H, ddd, *J* = 12.5, 4.0 and 1.5 Hz, H-6α), 1.89 (1 H, dd, *J* = 12.0 and 3.5 Hz, H-14), 2.00 (1 H, dd, *J* = 12.0 and 5.0 Hz, H-14), 2.44 (1 H, br d, *J* = 3.5 Hz, H-13), 2.64 (1 H, dd, *J* = 18 and 1.2 Hz, H-15), 3.11 and 3.44 (each 1 H, d, *J* = 11 Hz, H-18), 3.59 (1 H, dd, *J* = 12.0 and 4 Hz, H-7). – ^{13}C NMR: see Table 1. – EIMS: *m/z* (%) = 306 (8) $[M]^+$, 275 (100), 257 (47), 193 (8), 179 (16), 175 (3), 161 (4), 147 (5), 145 (4), 133 (7), 123 (32). – HREIMS: *m/z* = 306.2194 (calcd. 306.2195 for $C_{19}H_{30}O_3$, $[M]^+$).

*Diacetate **2***

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.81 (1 H, td, *J* = 12.8 and 3.9 Hz, H-1β), 0.84 (3 H, s, H-19), 1.16 (3 H, s, H-20), 1.27 (1 H, d, *J* = 8 Hz, H-9), 1.35 (1 H, dd, *J* = 11.5 and 1.4 Hz, H-5), 1.42 (1 H, td, *J* = 13.3 and 4.4 Hz, H-3β),

1.80 (1 H, m, H-1α), 1.87 (1 H, dd, J = 18.6 and 3.1 Hz, H-15), 1.98 (1 H, dd, J = 12.0 and 5.0 Hz, H-14), 2.02 (1 H, dd, J = 12.0 and 4.0 Hz, H-14), 2.05 and 2.10 (each 3 H, s, OAc), 2.20 (1 H, dd, J = 18.6 and 1.1 Hz, H-15), 2.44 (1 H, br s, H-13), 3.60 and 3.85 (each 1 H, d, J = 11.0 Hz, H-18), 4.74 (1 H, dd, J = 11.6 and 4.2 Hz, H-7). – ^{13}C NMR: see Table 1. – EIMS: *m/z* (%) = 390 (3) $[M]^+$, 330 (25), 275 (40), 270 (97), 257 (100), 255 (58), 241 (16), 227 (9), 214 (14), 201 (12), 187 (11), 175 (15). – HREIMS: *m/z* = 390.2407 (calcd. 390.2406 for $C_{23}H_{33}O_5$, $[M]^+$).

*Ozonolysis of candicandiol diacetate (**4**)*

The diacetate **4** (12 mg) was dissolved in dichloromethane (3 mL) and cooled to −78 °C. A stream of ozone was bubbled through the solution for 20 min, until it turned to a light blue color. Excess of ozone was removed by bubbling nitrogen through the solution. Triphenylphosphine (16 mg) was added, and the solution was stirred for 1 h at r. t. Usual work up, extraction with EtOAc, and purification by column chromatography afforded adejone diacetate (**2**).

Isolation of compounds from S. candicans

The aerial parts of *S. candicans* Ait (*S. oroteneriffae* L. Negrín et P. Pérez) (4.1 kg) were collected in May at the higher parts of Arafo, near of the end of the road from Arafo to La Cumbre (TF-523) on Tenerife Island. The plant was identified by Prof. Pedro Pérez de Paz, Botanical Department, University of La Laguna (Tenerife). A general description of the procedure to isolate the compounds of *Sideritis* species has been published previously [6]. The following substances were isolated: Candol B (**6**) (30 mg), rhoiptelanol (59 mg), a mixture of β-sitosterol, stigmasterol and campesterol (1.3 g), 2β-hydroxy-*ent*-13-*epi*-manoyl-oxide, trachinodiol 7β-monoacetate, candidiol 15α-monoacetate (**8**), candicandiol 7α-monoacetate (**5**) and epicandicandiol 7β-monoacetate (obtained by acetylation as their corresponding diacetates, the yields being 21, 480, 30, 110 and 360 mg, respectively), oleanolic and ursolic acids (8 mg), sinfernal (**13**), sinfernol (**12**), 16α,18-dihydroxy-*ent*-atisane, sidendrodiol, vierol and episideridiol (**10**) (identified as their corresponding diacetates, the yields being 28, 2, 8, 6, 5 and 20 mg, respectively), candidiol (**7**) (505 mg) and candicandiol (**3**) (620 mg). Adejone (**1**) and epoxysinfernal (**15**) were also identified, as traces, in impure fractions containing sinfernal (**13**) as the main compound.

*Episideridiol (**10**)*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.74 (3 H, s, H-19), 0.99 (1 H, d, J = 7.8 Hz, H-9), 1.06 (3 H, s, H-20), 1.17 (1 H, dd, J = 12.2 and 1.7 Hz, H-5), 1.71 (3 H, s, H-17), 1.78 (1 H, dt, J = 12.9 and 3.1 Hz, H-1α), 2.38 (1 H, br s, H-13), 3.08 and 3.42 (each 1 H, d, J = 10.8 Hz, H-18), 3.65 (1 H, dd, J = 11.3 and 4.2 Hz, H-7), 5.12 (1 H, br s, H-15). – ^{13}C NMR: see Table 1. – EIMS: *m/z* (%) = 304 (100) $[M]^+$, 286 (10), 268 (7), 255 (18), 164 (10), 147 (9), 131 (8), 123 (38). – HRMS: *m/z* = 304.2439 (calcd. 304.2402 for $C_{20}H_{32}O_2$, $[M]^+$).

*Diacetate (**11**)*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.73 (1 H, td, J = 12.9 and 4.2 Hz, H-1β), 0.80 (3 H, s, H-19), 0.99 (1 H, d, J 7.6 Hz, H-9), 1.08 (3 H, s, H-20), 1.21 (1 H, dd, J 12.1 and 1.6 Hz, H-5), 1.67 (3 H, d, J = 1.5 Hz, H-17), 2.00 and 2.08 (each 3 H, s, OAc), 2.31 (1 H, br s, H-13), 3.60 and 3.80 (each 1 H, d, J = 11.1 Hz, H-18), 4.86 (1 H, dd, J = 11.3 and 4.2 Hz, H-7), 5.10 (1 H, br s, H-15). – ^{13}C NMR: see Table 1. – EIMS *m/z* (%) = 388 (11) $[M]^+$, 346 (21), 328 (13), 313 (7), 268 (27), 255 (31), 240 (15), 225 (14), 197 (6), 185 (11), 171 (7). – HREIMS *m/z* = 388.2596 (calcd. for 388.2614 $C_{24}H_{36}O_4$, $[M]^+$).

Acknowledgements

This work has been supported by the Ministry of Education and Science, Spain (BQU2002-765). C. Bressa thanks this institution for a fellowship.

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