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Diterpenoids from the fruits of *Vitex agnus-castus*

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Abstract

From the fruits of *Vitex agnus-castus* L. one new diterpene, 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene, as well as two previously described diterpenes (rotundifuran and vitexilactone) were isolated. All obtained diterpenoids belong to the labdane typ. The structures determinations were mainly based on 1D and 2D NMR spectra and MS data interpretation. 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene and rotundifuran showed an affinity to the dopamine-D_2-receptor.

Keywords: *Vitex agnus-castus* L.; Verbenaceae; Vitexilactone; Rotundifuran; 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene; Dopamine-D_2-receptor affinity

1. Introduction

The traditional use of Agni-casti-preparations in gynecology caused a high interest in *Vitex agnus-castus* L. (Verbenaceae), which is a shrub common in the Mediterranean region. The fruits of *V. agnus-castus* are mainly used against premenstrual disorder and traditionally against the symptoms of the menopause and are known to contain flavonoids, iridoid glycosides, essential and fatty oils (Abel, Goez & Wolf, 1994). From the radioligand-dopamine-D_2-receptor-in-vitro-testing the hexane fraction was shown to contain the active principle (Berger, 1998). Therefore we investigated the lipophilic fraction. This report describes the isolation, structure determination and biological evaluation of one new (**1**) and two known diterpenes (**2**, **3**). 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene (**1**) and rotundifuran (**3**) showed a dopamine-D_2-receptor affinity.

2. Results and discussion

The new diterpenoid **1** is a diacetate similar to an acetate isolated from the areal parts of *Haplopappus parvifolius* A. Gray. (Zdero, Bohlmann & Niemeyer, 1991). Its EI mass spectrum indicated a molecular formula of $C_{24}H_{38}O_5$ (*m*/*z* 406, calcd 406.56). The FTIR spectrum displayed diagnostic absorption bands of hydroxy (3473 cm^{-1}) and ester (1738 cm^{-1}) moieties.

In the ^1H-^1H COSY spectrum of compound **1** correlations between H-14/H-15, H-6/H-7, H-6/H-5, H_2-1/H_2-2, H-5/H_3-20 and H_2-11/H_2-12 were observed. Correlations between C-14/H_2-15, C-6/H-7, C-6/H-5, C-2/H_2-1, C-5/H_3-20 and C-12/H_2-11 were also found in the HMBC spectrum. Further C-1, C-2 and C-4 coupled to H_2-3, whereas C-3 and C-4 were associated to the two methyl groups H_3-18 and H_3-19. Also correlations between C-5/H_3-18 and C-5/H_3-19 were noticed in the HMBC spectrum. In addition connections between C-5/H_3-20, C-10/H-5, C-10/H_2-3 and C-10/H_3-20 were observed. Because the chemical shift values of C-6 and C-7 are at 72.75 and 66.2 ppm, respectively, it was suggested that the two acetate groups, which were indicated by MS, were attached to OH-C-6 and OH-C-7. A ^1H-^{13}C long range correlation

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between C-6 and H_3-22 established this connectivity. As a result of the coupling of the signal at 170.7 ppm to H_3-22 and H_3-24, this peak seemed to be a double signal for the two carbons with the keto functions. Furthermore in the HMBC spectrum it became obvious, that C-8 is connected on the one hand to C-9 by a double bond—because of the correlation to H_2-11—and on the other hand to H_3-17 and H-7. In turn C-9 showed a correlation to H_2-11 and H_2-12 and also to H_2-1 and H_2-2, which indicated a coupling to C-10. In the side chain connections between C-13/H-14, C-13/H_3-16 and C-13/H_2-12 were noticed. Due to the fact that the chemical shift value of C-13 is 73.0 ppm, a hydroxy group was suggested to be the fourth binding partner at C-13.

The relative stereoconfigurational structure of **1** was based on the optical rotation and on a 2D ROESY experiment. The optical rotation value of **1** was positive and suggested a labdane nucleus (Zdero et al., 1991). ROE interactions between H-5/H_3-19, H-5/H-6 and H-5/H-7 implied that they are on the same side of the diterpene (α), while correlations between H_3-20/H-12a, H_3-20/H_3-18 and H_3-18/H-12a indicated their β position.

Comparing the ^{1}H NMR and ^{13}C NMR values of compound **1** with the values of the 6β-acetoxy-7β,13-dihydroxy-labda-8,14-diene in (Zdero et al., 1991), some clear differences were noticed resulting from the presence of the second acetate group. Based on the obtained results the structure of **1** was established as 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene.

Compound **2,** a labdane diterpenoid, was identified as vitexilactone by 1D and 2D NMR data and the MS spectrum and comparison of its spectroscopic data with those published in Taguchi (1976) and Kondo, Sugiyama and Nozoe (1986). Compound **2** was isolated before from the leaves of *V. cannabifolia* Sieb. et Zucc. (Taguchi, 1976) and the fruits of *V. rotundifolia* L. (Kondo et al., 1986).

By comparing the NMR chemical shift values of **2** with the values found in the literature (Taguchi, 1976; Kondo et al., 1986), it became obvious that the ^{1}H NMR data reported in Taguchi (1976) are the same, but the assignment of the ^{13}C values requires a revision. HMQC, HMBC and ^{1}H-^{1}H COSY experiments showed that the C-1/C-11, the C-3/C-12, the C-7/C-10 and the C-19/C-22 values had to be exchanged. Comparison of these data with those of rotundifuran **(3)**, obtained from the same fraction, showed that **2** differed from **3** only in the presence of a butenolide ring as the substituent in position 12 instead of a furan ring. In the ^{1}H-^{1}H COSY spectrum of compound **2** the H_2-16 coupled to H-14 and from the HMBC spectrum the quaternary carbon C-15 is correlated to both H_2-16 and H-14. Further the chemical shift of C-15 (170.85 ppm) indicated a lactone group at this carbon. Moreover a connection between C-14/H_2-12 and C-16/H_2-12 was extracted from the HMBC spectrum. The chemical shift value of C-14 indicated a double bond. From the HMBC spectrum a correlation between the carbon signal at 170.9 ppm and H_2-12 and H_2-11 was seen. As a connection between C-15 and H_2-12 or even H_2-11 is not realistic, the signal at 170.9 ppm must belong to C-15 and C-13.

The relative stereochemistry of the asymmetric carbons within **2** was accomplished by means of 2D ROESY measurement. Diagnostic ROEs between H_3-18/H_3-22, H_3-18/H_3-20, H-8/H_3-20 and H_3-20/H_2-11 attested them to be all on one side (β), while interactions between H_3-19/H-5, H-5/H_{eq}-6 and H_3-17/H-5 disclosed these to be in the α position. The ROE noticed between the H_3-17 and H_2-16 protons aligned it with the configuration shown in **2**.

Compound **3** was isolated before from the leaves of *V. rotundifolia* L. (Asaka, Kamikawa & Kubota, 1973). Compound **3** was identified as rotundifuran by 1D and 2D NMR data and the MS spectrum and comparison of its spectroscopic data with those published (Asaka et al., 1973).

Experimental results have suggested that the anterior lobe of the pituitary gland is the point of attack for the Agni-casti-preparations. The prolactin secretion, which is regulated by dopamine and often pathologically higher during prementrual disorders, is inhibited and this effect is mediated by dopamine-D_2-receptors (Jarry, Leonhardt, Wuttke & Gorkow, 1994). 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene and rotundifuran inhibited the in-vitro-binding of ^{3}H-spiroperidol in a dopamine-D_2-receptor test system with fresh calf striatal membrane. Compared to the standard (+)-butaclamol HCL (IC_{50}-value of 5.6 nmol/ml) the IC_{50}-values of 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene and rotundifuran with 194 nmol/ml (79 μg/ml) and 124 nmol/ml (45 μg/ml) respectively were still in the range, where biological effects are possible. Five ethanolic extracts [Ze 440] containing 0.17–0.80% 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene and 1.04–2.25% rotundifuran showed a similar affinity comparable to the pure compounds within the range of IC_{50}-values of 40–70 μg/ml. A crude hexane extract, which was used afterwards to isolate the diterpenes, showed an IC_{50}-value of 15 μg/ml. Therefore a synergistic effect of the diterpenes and other not investigated compounds of the complex extract matrix was postulated (Berger, 1998).

3. Experimental

3.1. General

^{1}H NMR and ^{13}C NMR: 300 MHz and 75 MHz, re-

Table 1
NMR data of compounds **1** and **2**

	^{13}C NMR of **1**, (75.5 MHz, $CDCl_3$)	^{1}H NMR of **1**, (300 MHz, $CDCl_3$)	^{13}C NMR of **2**, (75.5 MHz, $CDCl_3$)
1	25.9 *t*	2.01 *m*	33.5 *t*
2	19.4 *t*	1.63 *m*	18.5 *t*
3	39.4 *t*	1.54 *m*	43.5 *t*
4	34.6 *s*	–	33.9 *s*
5	36.4 *d*	2.09 *d* (6.9)	47.6 *d*
6	72.8 *d*	4.87 *dd* (3.2/3.3)	69.6 *d*
7	66.2 *d*	5.62 *d* (3.0)	35.9 *t*
8	132.3 *s*	–	32.0 *d*
9	141.5 *s*	–	77.1 *s*
10	42.9 *s*	–	43.7 *s*
11	29.3 *t*	1.46 *m*	31.5 *t*
12	38.5 *t*	1.49 *m*	25.3 *t*
13	73.0 *s*	–	170.9 *s*
14	144.5 *d*	5.84 *q* (17.3/10.6)	115.0 *d*
15	112.1 *d*	5.16 *dd* (17.4/11.3)	170.9 *s*
16	28.2 *q*	1.26 *s*	73.0 *d*
17	27.8 *q*	0.90 *s*	16.0 *q*
18	29.4 *q*	1.08 *s*	33.5 *q*
19	28.1 *q*	1.08 *s*	23.6 *q*
20	11.8 *q*	0.92 *s*	18.9 *q*
$OCO(21)CH_3$	170.7 *s*	–	170.3 *s*
$OCO(23)CH_3$	170.7 *s*	–	–
$OCOCH_3(22)$	21.4 *q*	2.04 *s*	21.8 *q*
$OCOCH_3(24)$	20.9 *q*	1.99 *s*	–

spectively. Solvent resonances were used as int. refs and chemical shifts (δ) are reported in ppm. EIMS: 70 eV. VLC: silica gel 40–60 μm (Chromagel).

3.2. Plant material

The fruits of *V. agnus-castus* were obtained from Zeller AG, Romanshorn, Switzerland. They were distributed by Paul Müggenburg AG, Hamburg, Germany, Lot. No. PR-00-348-95.

3.3. Extraction and isolation of compounds *1* and *2*

The powdered fruits (80 g) were extracted with 100% hexane by turbo extraction. The obtained extract was evaporated to dryness to yield 7.25 g of a residue, which was fractionated by VLC on a silica gel column (6.5 × 22 cm) and eluted with hexane followed by a gradient of EtOAc up to 100%. A part, 50 mg, of the VLC fraction obtained with hexane–EtOAc (40:60) was separated over Spherisorb S ODS 2 (5 μm, 250 × 16 mm) with UV detection at 220 nm with acetonitrile–H_2O (7:3) as mobile phase. 5 mg of compound **1** were obtained. 47.5 mg of the VLC fraction obtained with hexane–EtOAc (60:40) were separated over Spherisorb S ODS 2 (5 μm, 250 × 16 mm) with MeOH–H_2O (7:3) and UV detection at 220 nm; 3 mg of **2** were obtained.

3.4. Extraction and isolation of *3*

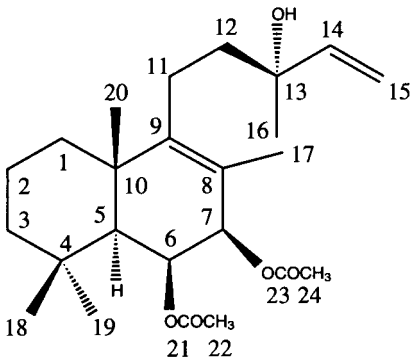
The powdered fruits (80 g) were extracted with 80% MeOH by turbo extraction. The extract was evaporated in vacuo to yield 4.7 g of a residue. The residue was solved in 200 ml 90% MeOH. The solution was fractionated with 300 ml hexane for three times by a Kupchan partition (Kupchan, Britton, Ziegler & Sigel, 1973). The collected hexane fraction was evaporated in vacuo to yield 1.4 g of a residue, which was brought on a silica gel column (4.5 × 22 cm) and eluted with hexane, followed by a gradient of EtOAc up to 100%. 101 mg of the fraction obtained with 80% hexane and 20% EtOAc, were separated on a HPLC column over Spherisorb S ODS 2 (5 μm, 250 × 16 mm) with acetonitrile–H_2O (95:5) using refractometric detection; 4 mg of **3** were obtained.

3.5. Bioassays

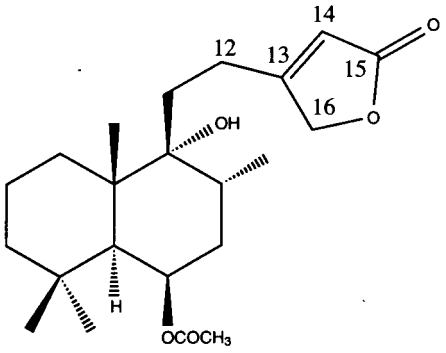
The dopamine-D_2-receptor testing was carried out by D.B. within the scope of his thesis at the University of Basel. For detailed experimental data see Berger (1998).

3.6. 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene (*1*)

$[\alpha]_D^{20} + 1.1^\circ$ ($CDCl_3$, *c* 1); UVλ_{max}^{MeOH} (logε) nm: 205 (1.0), IR$\nu_{max}^{CHCl_3}$ cm $^{-1}$: 3473, 2965, 2931, 1738, 1368, 1248, 1029; ^{1}H NMR ($CDCl_3$): Table 1; ^{13}C NMR



1



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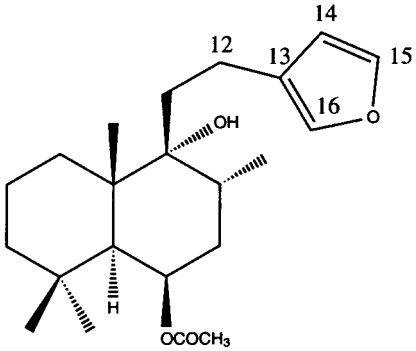

3

(CDCl$_3$): Table 1, EIMS *m/z* (rel. int.): 346 [M−$C_2H_4O_2$] (3), 286 [M−$2C_2H_4O_2$] (17), 247 (14), 205 (100), 187 (18), 135 (37), 199 (8), 84 (6).

3.7. Vitexilactone (**2**)

All of the physical and spectroscopical data were identical with those of vitexilactone described in the literature (Taguchi, 1976) except the ^{13}C NMR data; ^{13}C NMR (CDCl$_3$): Table 1.

3.8. Rotundifuran (**3**)

All of the physical and spectroscopical data were identical with those of rotundifuran described in the literature (Asaka et al., 1973).

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