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Two aromadendrane type alcohols from the liverwort *Conocephalum conicum*

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Abstract

(−)-Aromadendran-5-ol and (+)-aromadendr-4-en-12-ol were isolated from the essential oil of a chemotype of *Conocephalum conicum*. The structures of the compounds were derived by NMR investigations and by conversion of the compounds into a series of products of known configuration by hydrogenation and dehydration.

Keywords: *Conocephalum conicum*; Hepaticae; Essential oil; Aromadendran-5-ol; Aromadendr-4-en-12-ol

1. Introduction

Conocephalum conicum (a thalloid liverwort) is very commonly found in Europe, Northern Africa, Asia and Northern America. It frequently grows in moist to wet, calcareous areas and spreads out like a lichen (Frahm, & Frey, 1992). The occurrence of different chemotypes or geographic races of *C. conicum* is well known and was demonstrated by the variation in the flavonoid composition (Markham, Porter, Mues, Zinsmeister, & Brehm, 1976; Porter, 1981). A determination of different geographic races by variations in the composition of the volatile hydrocarbon fraction was reported recently (Toyota, Saito, Matsunami, & Asakawa, 1997). We have examined now four different chemotypes of *C. conicum*. The chemotype discussed in this paper was collected at an elevation of approx. 2.100 m in Vorarlberg (Austria) in July 1995. So far, this was the only case of a *C. conicum* chemotype with a composition of volatiles as reported here.

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2. Results and discussion

In addition to **1** and **2** two alcohols with biosynthetically related structures *ent*-(−)-guai-5-en-11-ol (**3**) and *ent*-(−)-viridiflorol (**4**) were identified. These constituents were recently also isolated from the liverwort *Calypogeia muelleriana* (Rücker, & Hefendehl, 1978; Faure, Ramanoelina, Rakotonirainy, Bianchini, & Gaydon, 1991; Hardt, 1994; Warmers, Wihstutz, Bülow, Fricke, & König, 1998). Furthermore, we identified (−)-ledene (**5**), (+)-isoledene (**6**), (+)-α-gurjunene (**7**) and (−)-γ-gurjunene (**8**) by enantioselective gas chromatography (GC) and by comparison of their mass spectra (Fig. 1). Since no solvent extraction of the plant material was performed, it can not be excluded that the observed sesquiterpene hydrocarbons are at least partially formed by dehydration of the related alcohols. However, in similar cases solvent extraction under mild conditions (room temperature) also indicated the presence of the corresponding hydrocarbons, which are believed to be biogenetic precursors of corresponding oxygenated compounds.

(−)-Aromadendran-5-ol (**1**) was isolated from the essential oil by preparative GC. In the mass spectrum a molecular ion signal at m/z 222 is observed ($C_{15}H_{26}O$). In the ^1H NMR spectrum signals of olefi-

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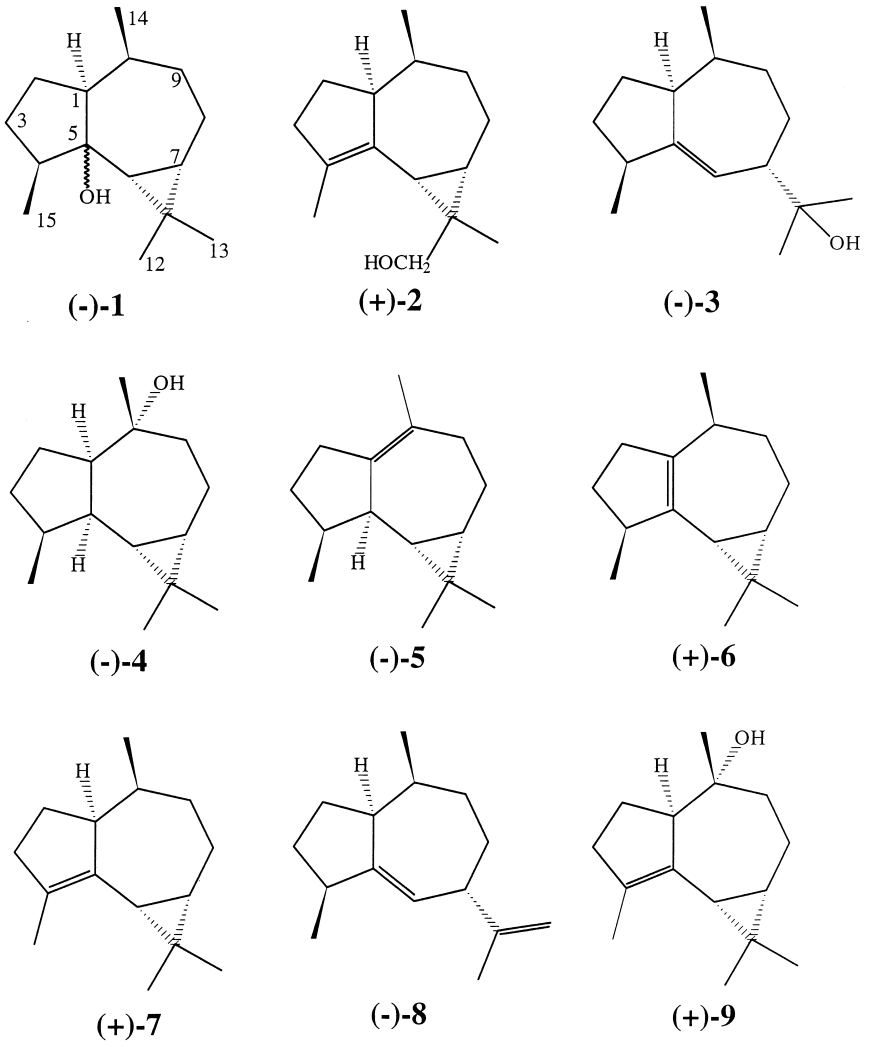

nic protons are missing; in fact, all the signals are found in the range of δ 0.2–2.0 confirming a tricyclic alcohol. The typical signals of a cyclopropane ring system appear at δ 0.22 as a doublet (H-6) and at δ 0.70 as a doublet of triplets (H-7). A coupling correlation for these protons is observed in the $^1H^1H$-COSY NMR spectrum. Two additional doublets at δ 0.94 and at 0.90, both corresponding to three protons, are observed. They are representing the methyl groups C-14 and C-15 and, as proved by 2D NMR, are coupled with the methine protons H-4 and H-10, respectively. H-4 absorbs in the region of δ 1.73–1.82 and H-10 in the region of δ 1.84–1.96, both as multiplets. Finally, two singlets at δ 1.28 and 1.07, both corresponding to three protons, are observed. They represent the geminal methyl groups C-12 and C-13. The protons are coupled to each other as shown in the $^1H^1H$-COSY NMR spectrum.

A sample of (−)-**1** was dehydrated with phosphoryl chloride and the resulting products were identified as (+)-α-gurjunene (**7**), (−)-γ-gurjunene (**8**) and (+)-isoledene (**6**) by GC-mass spectrometry (MS) and comparison with reference compounds. The absolute configuration of (+)-α-gurjunene (**7**) was identified by coinjection with a racemic standard onto a capillary GC column with heptakis(6-O-*t*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin as a chiral stationary phase. On this column the (−)-enantiomer is eluted before the (+)-enantiomer with a separation factor (α) of 1.34. As (+)-**7** is a product of the dehydration of (−)-aromadendran-5-ol (**1**) the absolute configuration of the new compound is verified with the exception of the C-5 position.

(+)-Aromadendr-4-en-12-ol (**2**) was also isolated from the essential oil by preparative GC. The mass spectrum exhibits a molecular ion signal at $m/z = 220$ ($C_{15}H_{24}O$). In the ^1H NMR spectrum two dd signals, each standing for one proton, appear at δ 3.48 and δ 3.57. Both protons couple with each other as shown in the $^1H^1H$-COSY NMR spectrum and represent the methylene protons at C-12 in the neighborhood of a hydroxy group. This signal exhibits an additional coupling correlation with an unusually high coupling constant $J = 5$ Hz to a multiplet signal in the range of

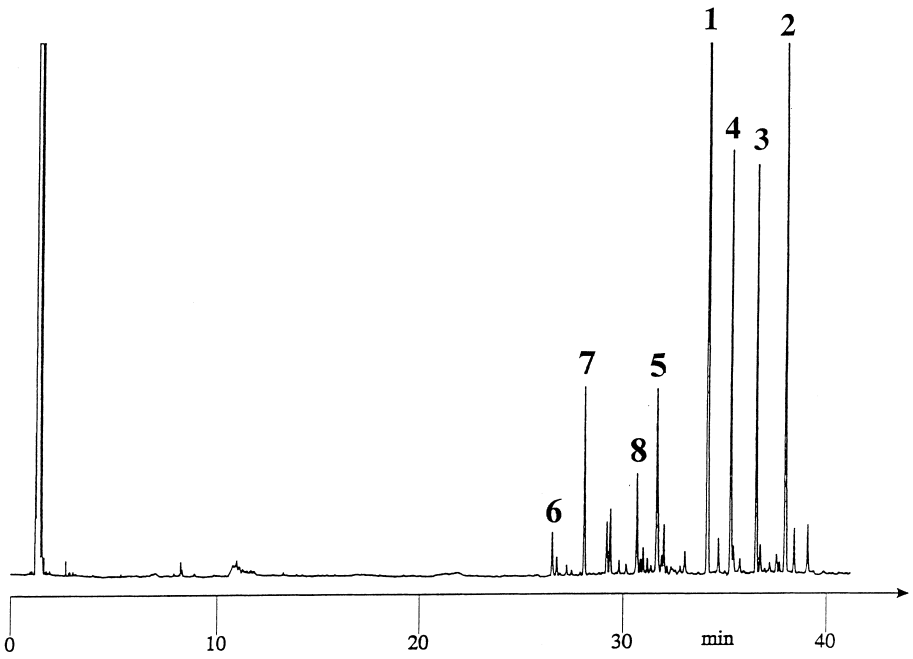

Fig. 1. Gas chromatographic separation of the hydrodistillate of *C. conicum*. 25 m fused silica capillary with CpSil 5; column temperature 50°C, 3°C min^{-1} to 230°C.

δ 0.96–1.04, representing the cyclopropyl proton at C-7. The second proton of the three-membered ring absorbs in the range of δ 1.25–1.27, which shows a long-range coupling to the broad singlet at δ 1.70 representing the methyl group C-15. Furthermore the ^{1}H NMR spectrum shows a doublet at δ 0.91, which is assigned to the methyl group C-14 and couples to the methine proton at C-10, which appears as a multiplet in the region of δ 1.75–1.83. Another singlet of three protons is observed at δ 1.24 and represents the methyl group C-13.

Hydrogenation of (+)-**2** resulted in a simultaneous dehydration to fully saturated diastereoisomeric guaianes (mass 208), which were identical to the hydrogenation products of (+)-4,5-dehydroviridiflorol (**9**), recently isolated from *Calypogeia muelleriana* (Warmers et al., 1998). Investigations by enantioselective GC proved that (+)-aromadendr-4-en-12-ol (**2**) and (+)-4,5-didehydroviridiflorol (**9**) have identical absolute configuration. The configuration at C-12 of **2**, however, could not be determined because the isolated sample amount was insufficient for NOE measurements.

3. Experimental

3.1. Collection of plant material and preparation of essential oils

C. conicum was collected in Vorarlberg (Austria) in August 1995 and identified by one of the authors (H.M.). A specimen of *C. conicum* is deposited in the Botanical Institute of the University of Ulm. Volatile constituents of the plants were prepared by hydrodistillation (2 h) of aq. homogenates of 120 g of fresh plant material using *n*-hexane as collection solvent. The amount of essential oil was too small to be weighed.

3.2. Dehydration reactions

Ca. 1 mg of **1** was taken up in 0.5 ml pyridine and 1 drop of phosphoryl chloride was added under ice cooling. After 1 h of stirring at room temp. the reaction was quenched by adding a few drops of water and the mixture was extracted 3 times with *n*-hexane. The organic phase was washed several times with water and dried over Na_2SO_4.

3.3. Hydrogenation

To a stirred solution of ca. 1 mg of sample in 1 ml *n*-hexane 0.5 mg Pd/C was added. The suspension was treated with H_2 and stirred under H_2 at room temp for 1 h. The reaction mixture was filtered and the reaction products were analyzed by GC-MS and by GC on several capillary columns with cyclodextrin derivatives.

3.4. Preparative GC

Isolation of **1** and **2** was achieved by prep. GC on a Varian 1400 instrument, equipped with a stainless steel

column (Silcosteel, Amchro) of 10% polysiloxane SE-30 on Chromosorb W-HP (1.85 m × 4.3 mm) (Hardt, & König, 1994).

3.5. NMR-spectroscopy

NMR measurements were performed with an instrument WM 400 (400 MHz) (Bruker) using TMS as int. standard.

3.6. GC-MS

Electron impact (70 eV) GC-MS measurements were carried out on a Hewlett Packard HP 5890 gas chromatograph coupled with a VG Analytical VG 70-250S mass spectrometer.

3.7. Polarimetry

Optical rotation measurements were performed with a Perkin Elmer 341 polarimeter. Because of the small sample amounts only the sense of rotation and not the specific optical rotation was determined.

3.7.1. (−)-Aromadendran-5-ol (**1**)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.22 (1H, d, $J = 10$ Hz, H-6), 0.70 (1H, dt, $J = 10$ Hz, $J = 6$ Hz, $J = 2$ Hz, H-7), 0.90, 0.94 (each 3H, d, each $J = 7$ Hz, H-14, H-15), 1.07, 1.28 (each 3H, s, H-12, H-13); EIMS (70 eV), m/z (rel. int.): 222 (2), 204 (49), 189 (44), 179 (9), 161 (75), 149 (29), 147 (29), 133 (37), 119 (54), 105 (78), 91 (66), 81 (61), 67 (41), 55 (62), 41 (100).

3.7.2. (+)-Aromadendr-4-en-12-ol (**2**)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, d, $J = 7$ Hz, H-14), 0.96–1.04 (1H, m), 1.24 (3H, s, H-13), 1.25–1.27 (1H, m), 1.70 (3H, bs, H-15), 2.85 (1H, m), 3.48 (1H, dd, $J = 11$ Hz, $J = 5$ Hz), 3.57 (1H, dd, $J = 11$ Hz, $J = 5$ Hz); EIMS (70 eV), m/z (rel. int.): 220 (12), 202 (63), 189 (58), 173 (18), 159 (44), 145 (77), 131 (71), 119 (58), 105 (100), 91 (95), 79 (46), 77 (44), 67 (25), 55 (41), 41 (61).

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