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Complete ^{13}C NMR assignments for *ent*-kaurane diterpenoids from *Sideritis* species

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In this work, the detailed NMR studies and full ^{13}C NMR assignments for five diterpenoids isolated from *Sideritis caesarea* and *Sideritis athoa* are described. The assignments are based on a combination of 1D and 2D NMR techniques including ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, gHSQC [^{1}J(C,H)] and gHMBCδ_C [^{n}J(C,H) (n = 2 and 3)] and NOESY experiments.

Keywords: ^{13}C NMR; *Sideritis* species; *ent*-kaurane diterpenoids; *ent*-3β,7α-dihydroxy-kaur-16-ene

Introduction

Kaurane diterpenoids are a large group of compounds which have been isolated from Compositae and Lamiaceae plants. *Sideritis* (Lamiaceae) species in particular are an important source of these compounds. They have been widely used in traditional medicine as herbal teas especially for the treatment of inflammations, gastrointestinal disturbances, coughs, bronchitis, bronchial asthma and common cold.[1] The biological activities of *Sideritis* species have been the subject of numerous studies.[2]

As part of our search for bioactive compounds, we have up to date investigated the chemical constituents of over ten *Sideritis* species from Turkey.[3–11] Literature survey showed that ^{13}C data for the kaurane diterpenoids **1–5** (Fig. 1) are either lacking or contain ambiguities. In this study complete assignments for the ^{13}C NMR spectra of **1–5** have been presented, in order to provide a set of reference data for structurally related compounds.

Results and Discussion

The *ent*-kaurane skeleton is common among the diterpenoids present in the *Sideritis* species. All of the compounds that are described in this study possess the *ent*-kaurane skeleton with oxygenated centers at C-7, C-15 and C-18 with the exception of compound **4**, which lacks oxygenation at C-18.

The isolated compounds are, *ent*-7α-acetoxy-15β,18-dihydroxy-kaur-16-ene (eubol, **1**), *ent*-7α,15β,18-trihydroxy-kaur-16-ene (eubotriol, **2**), *ent*-7α,18-dihydroxy-15-oxokaur-16-ene (**3**), *ent*-3β,7α-dihydroxykaur-16-ene (**4**) and *ent*-7α-acetoxy-18-hydroxy-15β,16β-epoxykaurane (epoxysiderol, **5**) (Fig. 1). Compound **3** is the 7α-*epi*-isomer of the previously reported *ent*-7β,18-dihydroxy-15-oxokaur-16-ene. Compounds **3** and **4** are described for the first time in this study.

For the complete and unambiguous determination of ^{13}C NMR spectra of the diterpenoids, a combination of 1D (^{1}H, ^{13}C NMR) and 2D (COSY, HSQC, HMBC and NOESY) experiments was carried out.

Eubol (**1**) was first isolated and identified by Venturella and Bellino.[12] The ^{1}H NMR spectrum exhibits two methyl singlets at δ 0.66 (H_3-19), δ 1.02 (H_3-20) and an AB system at δ 2.95 (d, J = 10.28, H-18) and δ 3.27 (d, J = 10.28, H-18). Two one-proton singlets at δ 4.99 and 5.16 correspond to an *exo*-methylene group (H_2-17), and H-15 was observed at δ 3.96 (brs). The signal at δ 4.89 (dd, J = 2, 4 Hz) is assigned to an equatorial proton geminal to an axial OAc group at C-7. The acetyl methyl absorption is at δ 1.99.

^{13}C NMR assignments for **1** were determined as follows. The HSQC experiment indicated that the H_2-18 hydroxy-methylene protons correlated with the ^{13}C NMR absorption at δ_C 71.5 and this peak was assigned to C-18. The three-bond away HMBC correlation of the methyl protons at δ_H 0.66 with the C-18 signal at δ_C 71.5 established the C-19 methyl group which resonated at δ_C 18.1. Further HMBC studies showed cross-peaks for the following: H_3-20 (δ_H 1.02)/C-9 (δ_C 49.4), C-5 (δ_C 40.1), C-1 (δ_C 39.9) and C-10 (δ_C 39.4); H_3-19 (δ_H 0.66)/C-5 (δ_C 40.1), C-4 (δ_C 37.2) and C-3 (δ_C 35.3); H_2-17 (δ_H 5.16, s and δ_H 4.99, s)/C-13 (δ_C 42.3). Even though the expected HSQC correlation between H-13 (m) at δ_H 2.74 and C-13 at δ_C 42.3 could not be observed, the HMBC correlation between C-13 and H-17 protons was evident (Table 1).

Eubotriol (**2**) was also first isolated and identified by P. Venturella and A. Bellino.[12] In the present study, the ^{1}H NMR of **2** as compared with compound **1** lacks the acetyl singlet and the H-7 methine

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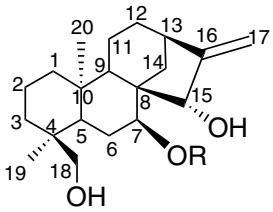

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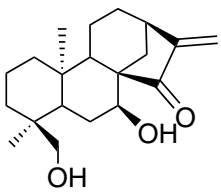
2. R = H



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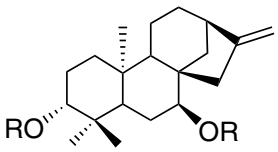
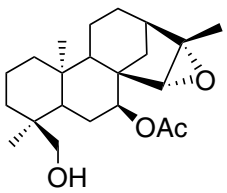


4. R = H
4a. R = Ac



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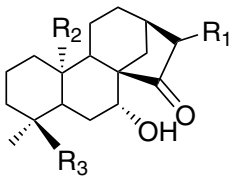


6. $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = CH_3$
7. $R_1 = CH_2$, $R_2 = CH_2OH$, $R_3 = CH_3$
8. $R_1 = CH_2$, $R_2 = CH_3$, $R_3 = CH_2OH$

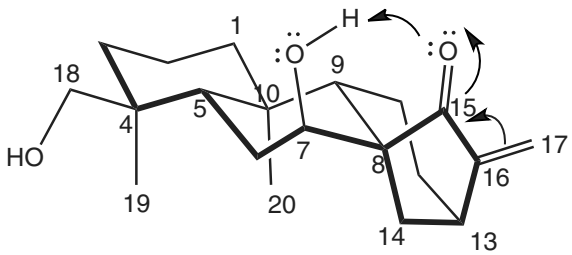
Figure 1. Structures of *ent*-kauranes **1–8**.

proton shifts from δ_H 4.89 to δ_H 3.84 (t, J = 3.2 Hz), and H-15 was observed at δ 4.05 as a singlet. The ^{13}C NMR chemical shift values for compound **2** are in close agreement with those of **1**, except for C-5, 6, 7 and 8. C-5 and C-7 show an upfield shielding effect of Δ −1.4 and Δ −2.7 ppm, respectively. The shift for C-5 is attributed to the γ-gauche effect of the hydroxyl group at C-7. The carbons C-6 and C-8 are deshielded by +2.5 and +1.1 ppm, respectively (Table 1).

As for compound **3**, the chemical shift values were assigned by comparison with **1** and **2**, as δ 0.65 (s, H-19), δ 1.08 (s, H-20), δ 2.88 (d, J = 11.30, H-18), δ 3.44 (d, J = 11.30, H-18). The vinylic H-17

Table 1. ^{13}C-NMR data for ent-kaurane diterpenoids **1–5** in $CDCl_3$

C	1	2	3	4	4a	5
1	39.9	40.0	39.6	38.5	38.2	40.1
2	18.2	18.2	17.9	27.3	24.5	17.9
3	35.3	35.4	35.1	77.2	79.7	35.4
4	37.2	37.3	37.2	45.1	39.7	37.2
5	40.1	38.7	38.1	43.5	43.5	39.7
6	23.8	26.3	25.9	27.3	23.7	23.4
7	75.9	73.2	73.0	78.8	80.6	75.6
8	50.7	51.8	53.3	48.1	46.7	46.9
9	49.4	49.7	48.8	50.2	51.1	46.8
10	39.4	40.1	40.0	38.2	37.1	39.0
11	17.9	17.8	18.3	17.8	17.8	18.1
12	33.2	33.3	32.9	33.5	33.3	27.5
13	42.3	43.0	38.3	43.7	46.7	39.2
14	34.8	35.3	29.9	38.4	39.2	31.2
15	80.4	81.4	192.4	45.1	45.0	63.7
16	159.7	159.1	149.4	154.9	154.3	78.9
17	108.8	108.9	116.3	103.5	103.8	14.3
18	71.5	71.2	71.3	28.1	28.8	71.4
19	18.1	17.9	18.0	17.4	17.4	17.7
20	17.8	17.8	17.9	15.6	16.9	17.8
Ac-Me	21.7				21.3(×2)	21.6
Ac-CO	170.8				171.8, 171	170.5



3

Figure 2. Representation of the spatial interaction in compound **3**.

singlets at δ 5.26 and δ 5.93 showed a much larger chemical shift difference than either **1** or **2**, which is associated with a spatial interaction with the neighboring carbonyl moiety at C-15. The carbon shift values for **3** are consistent with those for compounds **1** and **2**, except for the one at δ_C 192.4 which is attributed to a carbonyl at C-15. The signals at δ_C 149.4 and δ_C 116.3 are assigned to C-16 and C-17, respectively (Table 1).

The ^{13}C data for **3** have been compared with values from the literature. The ^{13}C NMR spectra of the related compounds *ent*-7β-hydroxy-kauran-15-one (**6**) and *ent*-7β,20-dihydroxy-16-kauren-15-one (**7**) have been described by Buchanan *et al.*[13] (Fig. 1). In the former compound, the carbonyl carbon at C-15 resonates at δ_C 224. In the latter compound, which contains an exocyclic methylene moiety at C-16, conjugated to the carbonyl, C-15 is shielded to δ_C 209. This is due to a resonance effect that imparts a partial single bond character to the carbonyl function (Fig. 2). However, the carbonyl carbon in **3** is observed at even higher field at δ_C 192.4. This can be attributed to hydrogen bonding to the 7-OH, which would stabilize the above resonance structure.

Molecular modeling (Fig. 2) suggests that H-bonding would exist only in the case of an axial 7-OH and not in the case of the equatorial orientation reported for the above compounds **6** and **7**. The reported ^{1}H NMR value for H-7 of compound **6** is δ_H 3.94 (dd, J = 12.4, 4.6 Hz) and for H-7 of compound **7** is δ_H 4.12 (dd, J = 11.7, 5.1 Hz), confirming the axial orientation of H-7 and the equatorial orientation of 7-OH in both compounds.[13] On the contrary, our values for compound **3** exhibit a pattern for an equatorial H-7 at δ_H 4.14 (dd, J = 3.12 and 2.34 Hz) and thus verify the axial conformation of the C-7 hydroxyl group.

In a study by Minh *et al*.[14] the structurally related compound *ent*-7β,18-dihydroxy-15-oxo-kaur-16-ene (**8**) was isolated and identified. The reported ^{13}C NMR values are consistent with our data except for the large difference in C-15. The ^{13}C NMR resonance for C-15 is reported as δ_H 210.5. In the ^{1}H NMR, axial H-7 is reported at δ_H 4.14 (dd (J = 11.5, 4.8 Hz; *aa* and *ae* couplings). These data suggest that **3** is the 7α-OH epimer of **8**. Thus, compound **3** is determined to be *ent*-7α,18-dihydroxy-15-oxokaur-16-ene.

Compound **4** was isolated for the first time by our group, from *S. athoa* extract. However, we erroneously reported it as a known compound.[11] Prof. B. M. Fraga reported that this should be the new compound *ent*-3β,7α-dihydroxykaur-16-ene.[15] No ^{1}H and ^{13}C NMR data have yet been published for **4**. Hence, we now include its ^{13}C NMR data in Table 1. In its ^{1}H NMR spectrum (in $CDCl_3$), three methyl singlet signals were observed at δ 0.77 (H_3-19), 0.96 (H_3-18) and 1.01 (H_3-20). Exocyclic methylene protons were observed at δ 4.78 and 4.81, and the characteristic H-13 signal resonated at δ 2.67 as a multiplet. A triplet δ 3.60 (J = 2.5 Hz, H-7) and a doublet of doublets at δ 3.25 (J = 6.2, 10 Hz, H-3) indicated presence of two secondary hydroxyl substituents. Acetylation of **4** at room temperature yielded compound **4a**, which exhibited methyl singlets at δ 0.78, 0.84 and 1.01 along with two acetyl methyl signals at δ 2.05 and 2.07. The neighboring protons H-7 and H-3 to the acetyl groups moved to δ 4.76 (t) and 4.52 (dd), respectively, showing almost the same J values. The EI-MS spectrum also verified the structure of the compound exhibiting a molecular ion peak at m/z 304, and fragment ions at m/z 286 $[M\text{-}H_2O]^+$ and m/z 268 $[M\text{-}2H_2O]^+$.

The last *ent*-kaurane (**5**) was initially identified by Venturella *et al*.[16] ^{13}C NMR data for **5** have been previously published.[17] The reported values are in close agreement with ours, except for two resonances, the one for C-17 and the one for the acetoxy carbon attached to C-7. The methyl resonance at δ_H 1.43 belongs to methyl neighboring the epoxy group. The HSQC experiment correlates the methyl protons at δ_H 1.43 with the methyl carbon (C-17) at δ_H 14.3. The previously reported value for C-17 was at δ_H 17.4. Also, the acetyl carbonyl shift which has been reported at δ_H 178.8 has been observed at δ_H 170.5 in our present study (Table 1).

Experimental

Plant material

In this study the diterpenoids **1–3** and **5** were isolated from *S. caesarea*. Among the Turkish *Sideritis* species, we have isolated compounds **1** and **2** also from *S. arguta*[3] and *S. stricta*,[4] compounds **1, 2** and **5** from *S. leptoclada*[5] and *S. dichotoma*,[6] while *S. congesta* afforded only **5**[7] and compound **4** was obtained only from *S. athoa*.[11]

S. caesarea was collected in its flowering stage from Balıkesir, in June 2006. It was authenticated by Dr. Tuncay Dirmenci, at the Department of Botany, University of Balıkesir. A voucher specimen has been deposited in the herbarium of T. Dirmenci. The acetone extract of the aerial parts was analyzed in this study. Information on extraction and isolation procedures for other plant extracts have been given in former publications.[3–11]

NMR experimental details

The NMR experiments were carried out on a 400-MHz Mercury-Vx Varian instrument with the following parameters: ^{1}H NMR spectrum: spectral width 6800 Hz, acquisition time 2.6 s, relaxation delay 1.0 s and 512 number of transients.

^{13}C NMR spectrum: spectral width 40 000 Hz, acquisition time 1.3 s, 16 000 numbers of transients and relaxation delay 3.0 s. Line broadening of 0.5 Hz was used for processing of the spectrum.

Gradient-selected phase-sensitive COSY spectrum: spectral width 6200 Hz, acquisition time 0.60 s, 1024 data points were collected for 512 t_1 increments of 16 transient each. Data were processed with $\pi/2$ shifted sine-bell in both dimensions and presented in phase-sensitive mode. Zero-filling once in t_1 resulted in digital resolution of 2.3 Hz/pt in each dimension.

Gradient-selected HSQC experiments were measured with the following parameters: spectral width of proton dimension 6234 Hz, spectral width of the carbon dimension 17 097 Hz, relaxation delay 1.0 s, acquisition time 0.6 s and heteronuclear coupling ($j1 \times h$) 140 Hz. 1024 data points were collected for 512 t_1 increments of 32 transient each. Data were processed with $\pi/2$ shifted sine-bell in both dimensions and presented in phase-sensitive mode. Zero-filling once in t_1 resulted in digital resolution of 4 and 40 Hz/pt in $f2$ and $f1$, respectively.

Gradient-selected HMBC spectrums were recorded with the following parameters: spectral width of the proton dimension 6230 Hz, spectral width of the carbon dimension 20 115 Hz, relaxation delay 1.5 s, acquisition time 0.6 s, heteronuclear coupling ($j1 \times h$) 140 Hz and long-range heteronuclear coupling ($jn \times h$) 8 Hz. 1024 t_2 data points were collected for 512 t_1 increments of 32 transient each, and the data processed with unshifted sine-bells in both dimensions, followed by magnitude calculation. After zero-filling once in t_1 the digital resolution was 4 and 80 Hz/pt in $f2$ and $f1$, respectively.

Conclusion

In this work, complete ^{13}C NMR chemical shift assignments were made for five diterpenoids with the *ent*-kaurane skeleton and C-7, 15 and 18-oxygenation, except for compound **4**. No unambiguously assigned ^{13}C NMR spectra have yet been published for **1–5**. Compounds **3** and **4** have been described for the first time in this study.

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