Verbenacine and Salvinine: Two New Diterpenes from *Salvia verbenaca*

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Two new diterpenes namely verbenacine (**1**) and salvinine (**2**) have been isolated from the aerial parts of *Salvia verbenaca.* Their structures have been elucidated on the basis of chemical and spectral data as 3α-hydroxy-19-carboxykaur-15-ene and 19-hydroxy-12,14-dioxo labda-15,17-diene.

Key words: Salvia verbenaca, Verbenacine, Salvinine

Introduction

Salvia verbenaca L. (Labiatae) is widely distributed in tropical region including Saudi Arabia. The alcoholic extract of the aerial parts has been reported to potentiate smooth muscle contractions induced by acetylcholine, histamine, $BaCl_2$, and serotonin (Todorov *et al.*, 1984). A review on polyphenolics of *Salvia* species has appeared recently (Yinrong and Yeap, 2002). Previous reports on the plant have shown the presence of abietane diterpene quinone namely 6β-hydroxy-7α-acetoxy royleanone (Sabri *et al.*, 1989), flavonoids (Camarasa *et al.*, 1982; Saleh and Sabri, 1980) and flavonoid glycosides (Abdallah, 1984) from the leaves of the plant.

We now report herein the isolation of two new diterpenes characterized as 3α-hydroxy-19-carboxykaur-15-ene designated as verbenacine (**1**) and 19-hydroxy-12,14-dioxo labda-15,17-diene designated as salvinine (**2**).

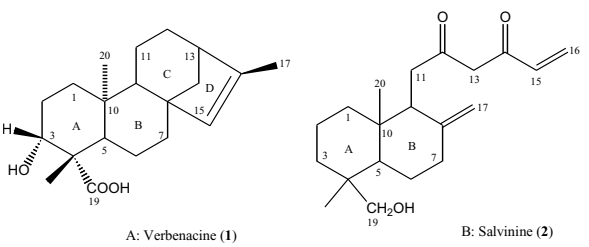

Fig. 1. Structure of Verbenacine and Salvinine.

Results and Discussion

Compound **1** named verbenacine and obtained as colourless solid, had the molecular composition $C_{20}H_{30}O_3$ as established on the basis of HR-MS (M^+ 318.2189), elemental analysis, ^{13}C NMR and DEPT spectra. The IR spectrum indicated the presence of a hydroxyl group (3374 cm^{-1}), ketonic group (1728 cm^{-1}) and double bond (1635 cm^{-1}). The ^{13}C NMR and DEPT spectra (Pegg *et al.*, 1982) showed 20 carbon atoms for the molecule consisting of three methyls, seven methylenes, five methines, and four quaternary and one carbonyl carbon atoms (in total $C_{20}H_{30}$). The sequential assignments of protons and carbon atoms were made with the help of ^1H-^1H COSY and HMQC experiments starting with the easily distinguishable olefinic proton at δ_H 5.06 (δ_C 136.1) assignable at position 15, and a carbinolic proton at δ_H 3.11 (δ_C 79.1) attributed to position 3 and further correlated with the HMBC spectrum. The ^1H NMR exhibited a doublet at δ 1.69 (J = 0.8 Hz; δ_C 15.5) due to a methyl group, which showed

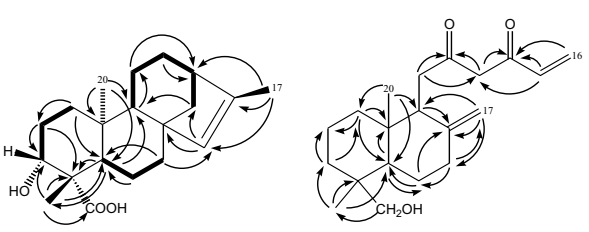

Fig. 2. Significant heteronuclear multiple bond correlations (HMBC). Arrowheads show correlations from proton to carbon, broad lines TOCSY correlations.

Table I. 1D and 2D NMR data of Verbenacine (**1**).

Position	^{1}H NMR*	^{13}C NMR	DEPT **	COSY	HMQC ***	TOCSY	HMBC****		NOESY
							$^2J_{CH}$	$^3J_{CH}$	
1a	0.99 ddd (13.6, 3.6)	40.6	CH_2	H-1b, H-2b	40.6 t	H-3, H-2a	C-2, C-10	C-5	–
1b	1.93 ddd (13.6, 7.2, 3.6)	–	–	H-1a, H-2b	–	H-3, H-2b	C-2	C-5	H-9, H-7a
2a	1.66 m	29.2	CH_2	H-2b, H-1a	29.2 t	H_2-1, H-3	C-1	C-10, C-4	H-1b
2b	2.11 ddd (13.5, 7.5, 4.4)	–	–	H-2a	–	H_2-1, H-3	C-1, C-3		Me-20
3 (β)	3.11 dd (4.8, 4.4)	79.1	CH	H-2b	79.1 d	H-1a, H-2a, H-1b, H-2b	–	–	Me-18β, Me-17β H-14β, H-5β
4	– –	50.2	C	– –	50.2 s	– –	–	– –	– –
5	0.91 m	57.1	CH	H-6a, H-6b	57.1 d		C-4, C-10	C-9, C-18	H-1a, Me-18β, Me-17β
6a (β)	1.77 ddd (14.0, 8.4, 2.4)	21.9	CH_2	H-5, H-6b, H-7a, H-7b,	21.9 t	H-5, H-7	C-5		H-5β, H-1a, Me-18β
6b (α)	1.80 ddd (14.0, 3.6, 8.4)	–	–	H-5, H-6a, H-7a, H-7b	–	H-5, H-7	C-5	– –	H-9α
7a (α)	1.49 ddd (12.6, 4.0, 3.6)	20.1	CH_2	H-7b, H-6a, H-6b	20.1 t	H_2-6	–	C-5, C-15	Me-20α
7b (β)	1.62 ddd (12.6, 7.2, 4.0)	– –	–	H-7a, H-6a, H-6b	– –	H_2-6	–	C-15, C-16	– –
8	– –	47.9	C	– –	47.9 s	– –	– –	–	–
9	0.94 m	48.3	CH	H-11a, H-11b	48.3 d	H_2-11	C-11, C-10	C-20	H-1b, H-6b
10	– –	40.5	C	– –	40.5 s	– –	–	–	--
11a	1.53 ddd (13.8, 5.2, 3.2)	40.6	CH_2	H-11b, H-9	40.6 t	H-9, H-12	– –	C-13	H-1a
11b	1.58 ddd (13.8, 5.2, 3.2)	– –	–	H-11a, H-9	– –	H-9, H-12	C-9	– –	–
12a	1.55 m	25.9	CH_2	H-11a, H-11b	25.9 t	H-13, H_2-14	–	C-14	– –
12b	1.57 m	– –	–	H-11a, H-11b	– –	H-13, H_2-14	–	C-14	– –
13	2.3 m	46.0	CH	H-14a	46.0 d	H_2-14, H_2-12, H_2-11	–	–	H-14β, Me-17β, Me-18β
14a	1.33 m	40.8	CH_2	H-13, H-14b	40.8 t	H-13, H_2-12	C-8	C-9	– –
14b	2.04 ddd (10.4, 6.0, 4.0)	–	–	H-14b	–	H-13, H_2-12	– –	–	H-3β, H-18β, Me-17β
15	5.06 s	136.1	CH	H-14b ($^3J_{HH}$) H-7b ($^3J_{HH}$)	136.1 d	Me-17	C-8	C-14	H-5β, H-7β, Me-17β
16	– –	143.6	C	– –	143.6 s		–	– –	--
17	1.69 d (0.8)	15.5	Me	– –	15.5 q	H-13, H-14	C-16	C-13	H-5β, H-3β
18	1.37 s	24.5	Me	– –	24.5 q	H-5	C-4	C-3, C-19, C-5	H-3β, H-5β
19	– –	178.7	C	– –	178.7 s	– –		– –	–
20	1.07 s	16.3	Me		16.3 q		C-10	C-1, C-5	H-2b, H-6α, H-7α

* Assignments were based on COSY, TOCSY and HMQC experiments; coupling constants in Hertz are given in parentheses; s, singlet; d, doublet; m, multiplet; t, triplet.
** DEPT chemical shifts are presented at $\theta = 3\pi/4$ when methylene groups reach negative maximum.
*** C-multiplicities were established by DEPT experiment: s = C, d = CH, t = CH_2, q = CH_3.
**** The correlations in HMBC have been shown from protons to carbons.

long-range couplings in HMBC spectrum with C-16 (δ_C 143.6, quaternary carbon), C-13 (δ_C 46.0) and C-15 (δ_C 136.1, olefinic carbon) allowing to place it at position 17 of ring D (Fig. 1A). The TOCSY experiment exhibited correlations of H-13 with H_2–14, H_2–12 and H_2–11; H_2–14 with H-13 and H_2–12; H_2–12 with H_2–11, H-13 and H_2–14; and H_2–11 with H_2–12 and H-9 indicating the positions of C-11, C-12, C-13 and C-14 in the ring C, which in turn was linked with Ring D as shown by HMBC-long-range correlations of H-15 with C-8 (δ_C 47.9), C-14 (40.8) C-16 (143.6) and C-7 (20.1), whereas Me-17 with C-13, C-15 and C-16 (Table I, Fig. 2A). The carbinolic proton at posi-

tion 3 displayed correlations in TOCSY spectrum with H_2–1 and H_2–2, whereas HMBC spectrum showed long-range correlations of Me-18 (δ_H 1.37, δ_C 25.5) with C-3 (carbinolic carbon), C-19 (carboxylic group), C-4 (δ_C 50.2) and C-5 (57.1) indicating the locations of Me-18, COOH-19 and hydroxyl group in ring A. Moreover, H-1a (δ_H 0.999, ddd) exhibited HMBC correlations with C-2 (δ_C 29.2), C-5 and C-10 (40.5); H-1b (δ_H 1.93, ddd) with C-2 and C-5; H-2a (δ_H 1.66, m) with C-1 (δ_C 40.6), C-10 and C-4; H-2b (δ_H 2.11, ddd) with C-1 and C-3; whereas H-5 (δ_H 0.91, m) displayed with C-4, Me-18 and C-9 (0.94, m) substantiating the proposed structure of ring A. Further, H_2-6 exhibited TOCSY and COSY correlations with H_2-7 and H–5 and long-range correlations in HMBC spectrum of H_2–6 with C-5; H-7a (δ_H 1.49, ddd) with C-5 and C-15; H-7b (1.62, ddd) with C-15 indicating that ring B was linked between ring A and ring C/D. The Me-20 (δ_H 1.07, s) showed long-range correlations in HMBC spectrum with C-1, C-10, C-5 and C-9; whereas H-9 with Me-20 and C-11, which allowed the placement of Me-20 at position 10 (Table I, Fig. 2A).

The compound **1** on acetylation with Ac_2O/pyridine yielded mono-acetate (δ_H 2.10, 3H, s, CO-*Me*; δ_C 24.04, CO-*Me*; 171.0, *CO*-Me; H-3 shifted down field from δ_H 3.11 to 4.56) indicating only one hydroxyl group in the molecule, which has been assigned at position 3. It also gave a monomethyl derivative (δ_H 3.63, 3H, s, CO-O*Me*) on methylating with diazomethane indicating the presence of only one carboxylic group in the molecule, which has been assigned at position 19. The coupling constant of carbinolic proton at δ_H 3.11 (dd, J = 4.8, 4.4) indicated the equatorial-equatorial and equatorial-axial couplings with H_2–2, which confirmed the β-orientation of H-3 and α-orientation of hydroxyl group (Silverstein *et al.*, 1981). The NOESY spectrum exhibited correlations of H-3β with Me-18β, Me-17β and H-5β; Me-18β and Me-17β with H-13β; whereas Me-20α displayed correlations with H-6α and H-7α, and H-6α with H-9α indicating the *trans*-configuration of H-5 and Me-20; Me-17 and H-13; and *cis*-configuration of H-9 and Me-20. Other NOESY correlations have been given in Table I.

The proposed structure of compound **1** was also confirmed by the fragmentation pattern of mass spectrum, which displayed prominent peaks at *m/z* 198 and 120 due to rupture of ring C via C-9 (C-10) and C-7 (C-8); 134 due to rupture via C-9 (C-10) and C-6 (C-7); 125 via C-9 (C-10) and C-5 (C-6) and peak at *m/z* 79 due to elimination of ring D. Other peaks at *m/z* 300, 303, 285, 273, 257, 240, 225, 107, 105 and 91 were also supportive of the proposed structure (Fig. 3A).

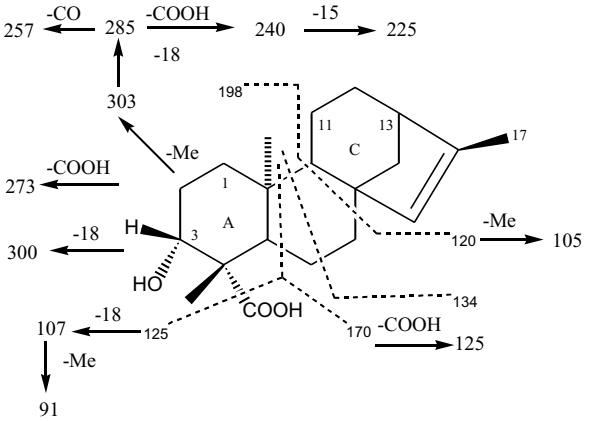

Fig. 3. Fragmentation during mass spectroscopy of verbenacine (**1**), $C_{20}H_{30}O_3$ (M^+ 318).

Thus on the basis of above chemical and spectral data the structure of compound **1** was elucidated as 3α-hydroxy-19-carboxykaur-15-ene, and has been designated as verbenacine.

Compound **2** named salvinine and obtained as colourless solid, had the molecular composition $C_{20}H_{30}O_3$ as established on the basis of HR-MS (M^+ 318.2189), elemental analysis, ^{13}C NMR and DEPT spectra. The IR spectrum indicated the presence of a hydroxyl group (3445 cm^{-1}), ketonic groups (1773, 1743 cm^{-1}) and double bond (1636 cm^{-1}). The ^{13}C NMR and DEPT spectra (Pegg *et al.*, 1982) showed 20 carbon atoms for the molecule consisting of two methyls, ten methylenes, three methines, and three quaternary and two carbonyl carbon atoms (in total $C_{20}H_{30}$). The sequential assignments of protons and carbon atoms were made with the help of ^1H-^1H COSY and HMQC experiments starting with easily distinguishable olefinic protons at δ_H 5.94 (δ_C 115.4) assignable at position 15, 4.90 and 4.93 (δ_C 75.2) due to = CH_2-16, and methine protons at δ_H 1.40 (δ_C 57.7) attributable to H-5 and 1.75 (58.0) displayed by H-9, which were further correlated with long-range couplings in HMBC spectrum. The ^1H NMR exhibited two doublets (J = 9.2 Hz each) at δ_H 3.81 and 4.26 (δ_C 71.5) due to hydroxy methylene group, which showed long-range correlations in HMBC spectrum with C-4 (δ_C 38.8) and Me-18

(28.2), and thus could be assigned at position 19. The Me-18 (δ_H 1.10, s) displayed long-range correlations with C-4, C-3 (37.1) and C-5. The H_2–3 (1.11, m, H-3a; 1.98, m, H-3b), in turn correlated with C-2 (δ_C 20.2) and C-1 (40.3), and consequently H-2b with C-4; H-1a (δ_H 1.18, ddd) with C-2 and C-5; H-1b (1.92, m) with C-2, C-10 (δ_C 40.9) and C-5 indicating the structure of ring A. The consecutive assignments of methylene groups at positions 1, 2 and 3 were also confirmed by proton-proton correlations in COSY spectrum. The other methyl group (δ_H 0.81, s) showed long-range correlations in HMBC spectrum with C-1, C-5, C-9 (58.0) and C-10, which allowed its placement at position 20. The ^{1}H NMR spectrum displayed broad signals at δ_H 4.61 and 4.95 (δ_C 107.5) due to an exocyclic methylene group, which exhibited long-range couplings with C-8 (149.2), C-9 and C-7 (39.8), and thus could be placed at position 17. H-9 showed a ^{1}H-^{1}H correlation in COSY spectrum with H_2-11 (δ_H 1.76, m, H-11a; 1.89, m, H-11b). The H-11a showed long-range correlations with C-9, C-12 (δ_C 175.1) and C-13 (28.7), whereas H-11b and H_2-13 (2.36, dd, H-13a; 2.65, dd, H-13b; δ_C 28.7) with C-12 indicating the location of one carbonyl group at position 12. The H-13b, H-15 and H_2–16 also exhibited correlation with another carbonyl group (δ_C 177.3), which could place it at position 14. Other correlations in HMBC and COSY spectra were also in accordance with the proposed structure (Table II, Fig. 2B).

The mass spectrum also supported the proposed structure of compound **2**, which showed prominent peaks at *m/z* 55 due to elimination of side chain via C-13 (C-14), 97 and 221 via C-11 (C-12) and 207 via C-9 (C-11) confirming the structure of side chain. The prominent peak at 109 was obtained due to rupture of ring A through collapse of ring B via C-9 (C-10) and C-5 (C-6), which supported the structure of rings A and B. Other peaks at *m/z* 300, 303, 287, 285, 277, 189, 176, 161, 146, 133 and 119 were also consistent with the proposed structure of the compound (Fig. 4).

Thus on the basis of above chemical and spectral data the structure of compound **2** was elucidated as 19-hydroxy-12,14-dioxo labda-15,17-diene and has been designated as salvinine.

Experimental Section

General

Melting points were determined on a Mettler 9100 electro thermal apparatus by open capillary method and are uncorrected. The IR spectra were recorded as KBr pellets on a PYE UNICAM spectrophotometer. The optical rotation was recorded in chloroform by a Perkin-Elmer 241 MC Polarimeter. The mass spectra were recorded on a Finnegan MAT 300 mass spectrometer, and relative intensities have been given in parentheses. The ^{1}H (400 MHz) and ^{13}C (and DEPT 90 and 135) NMR (100 MHz) and 2D NMR (COSY, TOCSY, HMBC, HMQC and NOESY) were recorded on Bruker DRX 400 spectrometer in $CDCl_3$ and MeOH-d_4 using TMS as internal standard reference, chemical shifts are in δ (ppm) and coupling constants (J values) in Hz. The elemental analysis was performed on a Perkin-Elmer CHNSO analyzer, model no. 2400. Column chromatography was performed using silica gel (0.04–0.063 mm, 230–400 mesh) as an adsorbent. The Centrifugal Preparative TLC was performed on a Chromatotron of Harrison Research Inc. (Palo Alto, California, USA) using 4 mm rotor and silica gel PF-254 with $CaSO_4 \cdot 0.5H_2O$ as an adsorbent. TLC were performed on silica gel 60 F-254 Merck plates and sprayed with vanillin/H_2SO_4 reagents for visualization of the spots.

Plant material

The aerial parts of *Salvia verbenaca* L. were collected on 18th February 2001 from Fifa Mountains, Southern Province (Assir), Saudi Arabia and identified by a taxonomist of the center, where a voucher specimen (No. 14182) has been deposited at the herbarium of College of Pharmacy, King Saud University for future reference.

Extraction and isolation

The dried aerial parts (700 g) were crushed to coarse powder and extracted exhaustively with 95% alcohol in a percolator. The alcoholic extract was concentrated and dried under reduced pressure to get a viscous residue (35.0 g). It was then extracted with hexane and subsequently with acetonitrile. The CH_3CN fraction (11.0 g) was chromatographed on a column of silica gel, and successively eluted with petroleum ether, dichloro-

Table II. 1D and 2D NMR data of Salvinine (**2**).

Positions	^{1}H NMR*	^{13}C NMR	DEPT**	COSY	HMQC***	HMBC****	
						$^2J_{CH}$	$^3J_{CH}$
1a	1.18 ddd (13.4, 9.2, 3.9)	40.3	CH_2	H-1b, H-2b, H-2a	40.3 t	C-2,	C-5
1b	1.92 m	–	–	H-1a, H-2b	–	C-2, C-10	C-5
2a	1.57 ddd (11.7, 9.8, 3.6)	20.2	CH_2	H-2b, H-1a,	20.2 t	– –	–
2b	1.71 ddd (12.0, 9.5, 3.6)	– –	–	H-2a, H-1a	– –	–	C-4
3a	1.11 m	37.1	CH_2	H-3a, H-2a	37.1 t	C-2	C-1
3b	1.98 m	– –	–	H-3a, H-2b	– –	C-2	C-1
4	–	38.8	C	– –	38.8 s	– –	–
5	1.40 m	57.7	CH	H-6a	57.7 d	C-10, C-6	– –
6a	1.48 ddd (14.4, 8.8, 4.0)	25.8	CH_2	H-6b, H-5, H-7a	25.8 t	C-5	C-10, C-8
6b	1.99 m	– –		H-6a, H-7b	– –	C-5	C-8
7a	2.04 m	39.8	CH_2	H-7b	39.8 t	C-6	C-17
7b	2.49 ddd (12.4, 8.8, 3.6)	– –	–	H-7a, H-6a	– –	C-6	C-5, C-17
8	– –	149.2	C	– –	149.2 s	– –	–
9	1.75 m	58.0	CH_2	H-11a, H-11b	58.0 t	C-8, C-10	– –
10	– –	40.9	C–	–	40.9 s	C-11	– –
11a	1.76 m	22.8	CH_2	H-9, H-13b	22.8 t	C-12, C-9	C-13
11b	1.89 m	– –	–	H-9, H-13b ($^3J_{HH}$)	– –	C-12	– –
12	– –	175.1	C	– –	175.1 s	– –	–
13a	2.36 dd (16.4, 2.0)	28.7	CH_2	H-13b, H-11a, H-11b	28.7 t	C-12	C-11
13b	2.65 dd (16.4, 2.0)	–	– –	H-13a, H-11a, H-11b.	–	C-12, C-14	– –
14	– –	177.3	C	– –	177.3 s	– –	–
15	5.94 dd (8.8, 2.0)	115.4	CH	H_2-13 ($^3J_{HH}$), H-16b	115.4 d	C-14, C-16	C-13
16a	4.90 ddd (12.0, 8.8, 2.0)	75.2	CH_2	H-16b	75.2 t	C-15	C-14
16b	4.93 ddd (12.0, 8.8, 2.0)	–	–	H-16a, H-15	–	C-15	C-14
17a	4.61 brs	107.5	CH_2	H-17b, H-9 ($^3J_{HH}$)	107.5 t	C-8	C-9, C-7
17b	4.95 brs	– –	–	H-17a, H-9 ($^3J_{HH}$)	– –	C-8	– –
18	1.11 s	28.2	Me	H-19b ($^3J_{HH}$)	28.2 q	C-4	C-3, C-5
19a	3.81 d (9.2)	71.5	CH_2	H-19b	71.5 t	C-4	C-18
19b	4.26 d (9.2)	– –	–	H-19a, Me-18 ($^3J_{HH}$)	– –	C-4	C-18
20	0.81 s	15.9	Me		15.9 q	C-10	C-1, C-5, C-9

* Assignments were based on COSY and HMQC experiments; coupling constants in Hertz are given in parentheses. s, Singlet; d, doublet; m, multiplet; t, triplet; brs, broad singlet.
** DEPT chemical shifts are presented at $\theta = 3\pi/4$ when methylene groups reach negative maximum.
*** C-multiplicities were established by DEPT experiment; s = C, d = CH, t = CH_2, q = CH_3.
**** The correlations in HMBC have been shown from protons to carbons.

methane (DCM) and methanol with increasing order of polarity. The fraction obtained at the eluent MeOH/DCM (1:9 v/v) was further purified by chromatotron using acetone/hexane (1:9) as eluting solvent, which afforded verbenacine (**1**) (113 mg). Further elution of the column with MeOH/DCM (1:4 v/v) yielded a fraction, which was re-chromatographed on a chromatotron using MeOH/DCM (5:95) to obtain salvinine (**2**) (40 mg).

Verbenacine (**1**): Colourless solid (113 mg). – M.p. 229–30 °C. – $[\alpha]_D$ – 30.83° (*c* 0.06, MeOH). – R_f 0.37 (acetone/hexane = 1:9). – IR (KBr): ν_{max} = 3374 (OH), 2932 (CH_3), 2849 (CH_2), 1728 (C=O), 1635 (C=C), 1469, 1436, 1372, 1166, 1139, 1015 (C-O, alcoholic), 990, 928, 809, 701 cm^{-1}. – 1D and 2D NMR data, see Table I. – EIMS: *m/z* (rel. int.) 318 [M^+, $C_{20}H_{30}O_3$] (100), 300 (45), 303 (10), 285 (28), 273 (15), 257 (18), 240 (10), 225 (28), 198 (20), 187 (35), 159 (25), 134 (35), 125 (38), 120

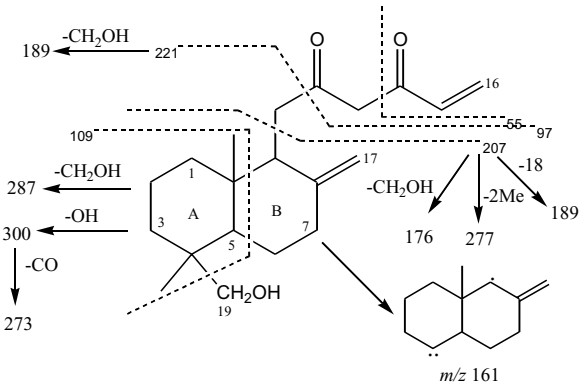

Fig. 4. Fragmentation during mass spectroscopy of salvinine (**2**), $C_{20}H_{30}O_3$ (M^+ 318).

(72), 107 (88), 105 (92), 94 (95), 91 (83), 79 (40), 55 (25). – HRMS: *m/z* (M^+ 318.2189) (calcd. for $C_{20}H_{30}O_3$ 318.2195). – Elemental analysis: Found C 75.60%, H 7.05%, O 17.35%; required for $C_{20}H_{30}O_3$: C 75.43%, H 9.50%, O 15.07%.

Salvinine (**2**): Colourless solid (40 mg). – M.p. 189–90 °C. – $[\alpha]_D$ 10.0° (*c* 0.04, MeOH); R_f 0.38 (MeOH/DCM 1:4). – IR (KBr): ν_{max} = 3445 (OH), 2925 (CH_3), 2850 (CH_2), 1773 (C=O), 1743 (C=O), 1636 (C=C), 1443, 1235, 1167, 1128, 1065 (C-O, alcoholic), 980, 904, 885, 831 cm^{-1}. – 1D and 2D NMR data: see Table II. – EIMS: *m/z* (rel. int.) = 318 [M^+, $C_{20}H_{30}O_3$] (20), 300 (35), 303 (5), 287 (42), 285 (45), 273 (18), 221 (40), 207 (40), 189 (98), 176 (40), 161 (25), 146 (42), 133 (44), 119 (75), 109 (100), 97 (80), 91 (62), 79 6(48), 55 (43). – HRMS: *m/z* (M^+ 318.2189) (calcd. for $C_{20}H_{30}O_3$ 318.2195). – Elemental analysis: Found C 75.65%, H 7.15%, O 17.20%; required for $C_{20}H_{30}O_3$: C 75.43%, H 9.50%, O 15.07%.

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