

**NMR SPECTROSCOPIC DATA OF VERY RARE C₂₅ TERPENOIDES
(SESTERTERPENES) FROM IRANIAN SALVIA SPECIES.**

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ABSTRACT

A part from their common constituents (Terpenoides and Flavonoids) and their interesting biological activities, the genus *Salvia* is unusual as it is the only genus in the Labiate that contains Sesterterpenes (C₂₅). Since these rare and interesting compounds were, for the first time isolated and identified from Iranian *Salvia* species by the senior author, their spectroscopic data are briefly reviewed.

KEYWORD: Iranian *Salvia* Species, Lamiaceae, Sesterterpenoids, NMR Spectroscopic Data.

INTRODUCTION

Salvia is a fascinating plant genus and one of the wide-spread members of the Labiatae (Lamiaceae) family, which comprises about 900 herbs and shrubs, growing in the temperate and warmer zones of the world. Some of these species feature prominently in the pharmacopoeias of many countries throughout the world. The range of traditional applications of the herbs in domestic medicine seems to be endless: they have been used as a medication against perspiration and fever, as a carminative, a spasmolytic, an antiseptic/ bactericide, an astringent, a gargle or mouthwash against inflammation of the mouth, tongue and throat, wound- healing agent, skin and hair cure, against rheumatism and sexual debility, in treating mental and nervous conditions, and as an insecticide.^[1-4]

***Salvia hypoleuca* Benth.**

The aerial parts of *Salvia hypoleuca* Benth. afforded two crystalline compounds, molecular formula C₂₆H₄₀O₆ and C₂₅H₃₆O₅. Acetylation of the former gave a mono and a diacetate, while the IR spectrum showed the presence of an ester group (1740 cm⁻¹) and of a γ -lactone (1765 cm⁻¹). Thus the nature of all oxygen function of the first compound was established.

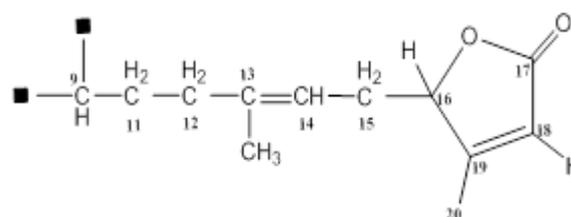
The second compound was also an alcohol as shown by the IR spectrum, which further displayed bands for two γ -lactone (1790, 1775 cm⁻¹).^[5]

The ¹H-NMR data of the two lactones were in part very similar. However, a three-fold doublet at δ 3.61 ppm was shifted to δ 4.29 ppm in the dilactone, indicating that the

second compound was most likely the lactone of a hydroxyl acid which corresponded to the ester.

The nature of the second lactone ring, present in both compounds, was evident from the downfield signals at δ 4.90 ppm and 5.89 ppm. The latter signal was due to an olefinic proton which coupled with an olefinic methyl group.^[5]

Spin decoupling of the spectrum of the ester showed that the olefinic proton was coupled with the proton which resonates as δ 4.90 ppm. The latter was further coupled with a pair of three-fold doublets which were themselves coupled with an olefinic proton (δ 5.04 ppm). Irradiation of these signals sharpened a further olefinic methyl signal at 1.67 and a broadened triplet at 2.09, which was coupled with two protons which gave rise to complex signals at 1.34 and 1.50. As these signals were altered on irradiation of a double doublet at 1.3 ppm sequence A was established.^[5]

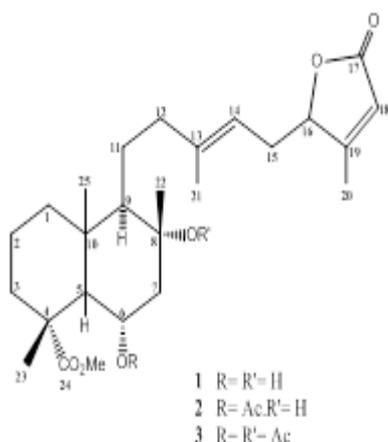


A (carbon numbering as in the final structure)

The remaining signals in the spectra of both compounds were similar to those of labdanes with a carboxyl group

at C-4. The position of the hydroxyl groups were established by the $^1\text{H-NMR}$ data of the ester, the dilactone and the acetates. The introduction of the tertiary acetoxy group caused a downfield shift of one methyl singlet and of the double doublet of H-9 indicating the presence of a tertiary hydroxyl at C-8. As two further double doublets, which were coupled with the proton under the second hydroxyl group were shifted downfield in the spectra of the acetates, a 1, 3-position of the hydroxyls was most likely.^[5]

Irradiation of a doublet at $\delta 2.09$ ppm collapsed the signal at 3.61 ppm to a double doublet indicating that the former signal most likely was that of H-5. Similar results



Furthermore, the $^{13}\text{C-NMR}$ spectrum of **1** was in good agreement with the proposed stereochemistry. Also we have named the free acid which corresponds to **1** and **4** salvileucolide.^[5]

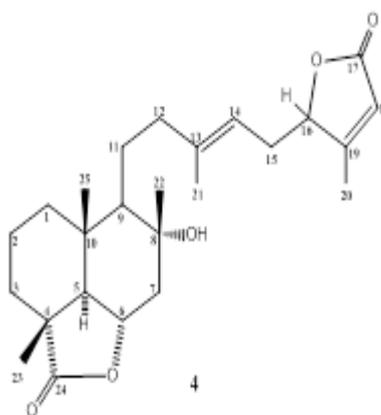
Further Sesterterpenes from *Salvia hypoleuca* Benth.

The polar fractions of *Salvia hypoleuca* afforded several further sesterterpene lactones, a hydroperoxide, three isomeric epoxides and a monolactone with an additional carbocyclic ring, all derived from salvileucolide lactone, as well as several salvileucolide methyl ester derivatives with a hydroperoxide group.

We have studied the more polar fractions of *S. hypoleuca* which afforded several further sesterterpenes, the salvileucolide methyl ester derivatives **8**, **9a**, **9b** and **10** as well as the isomeric epoxides **6a-6c** and the hydroperoxide **5** derived from salvileucolide-6, 23-lactone and a sesterterpene with a further new carbon skeleton, the ketone **7**.^[6]

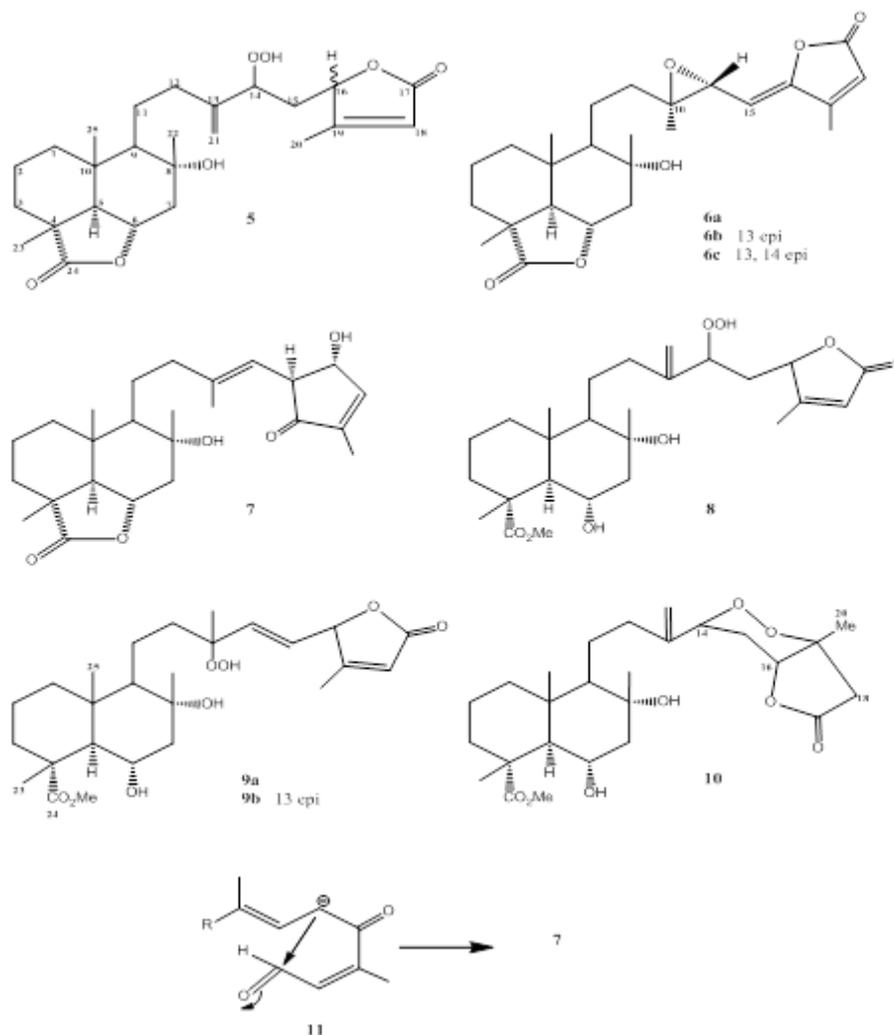
The structure of **5** followed from the $^1\text{H-NMR}$ spectrum which was in part close to that of salvileucolide-6, 23-lactone.^[5] However, one of the olefinic methyl singlets was replaced by a pair of broadened singlets at $\delta 5.21$ ppm and 5.06, a broadened singlet at $\delta 9.47$ ppm and a double doublet at $\delta 4.73$ ppm. In addition the CIMS showed the highest peak at 449 m/z. Therefore the presence of a hydroperoxide was likely. Spin decoupling

were obtained by spin decoupling of the dilactone. In this case addition of $\text{Eu}(\text{fod})_3$ and spin decoupling allowed the assignment of the remaining signals of H-1 – H-3 clearly establishing the presence of a labdane with an additional isoprene unit at C-15. Irradiation of the signals of H-12 and H-5 further showed W-coupling with the corresponding methyl groups. All the data and the observed $\text{Eu}(\text{fod})_3$ -induced shifts agreed with the structures **1** and **4** for the new sesterterpenes. The stereochemistry at C-5 and C-6 followed from the couplings observed, while that at C-8 was proposed following the chemical shifts of H-6 β , H-7 β and H-9 which favoured an equatorial hydroxyl at C-8. Also the $\text{Eu}(\text{fod})_3$ -induced shifts supported this assignment.^[5]



starting with H-16 confirmed this assumption. However, the relative configuration at C-8, C-14 and C-16 could not be determined.^[6]

The $^1\text{H-NMR}$ spectra of **6a** and **6b** also were close to that of salvileucolide-6, 23-lactone. Again the signals of the olefinic double bond were replaced by methyl singlets at $\delta 1.37$ ppm and 1.43 and doublets at $\delta 3.82$ ppm and 3.79 respectively. Furthermore, new olefinic signals at $\delta 5.09$ ppm and 5.14 respectively were visible. In agreement with the molecular formula, presence of epimeric epoxides derived from the dehydro derivative of salvileucolide-6, 23-lactone was very likely. The relative configuration at C-13 and C-14 was determined by the observed NOEs. Thus in the case of **6b** NOE between H-21 and H-14 was observed. Further NOEs established that the remaining stereochemistry was unchanged from that of salvileucolide-6, 23-lactone and also allowed the assignment of the methyl signals. Thus clear effects were present between H-24, H-25 and H-6 and between H-22, H-25 and H-7 β in both isomers (**6a** and **6b**). The Z-configuration of the 15, 16-double bond followed from the NOE between H-20 and H-15 which were observed in all three isomers. The $^1\text{H-NMR}$ data and the optical rotation of **6c** differed slightly, from those of **6a** and the NOE between H-21 and H-15 indicated a presence of a 13, 14-bis-epi-isomer of **6a**. The relative configuration at C-13 and C-14 in the isomers **6a-6c** could not be determined.^[6]



The $^1\text{H-NMR}$ spectrum of **7** again was close to that of salvileucolide-6, 23-lactone. However, the second lactone moiety was now missing. Spin decoupling led to a sequence which required a cyclopentenone group. Thus doublet quartet at $\delta 5.09$ ppm was coupled with the olefinic methyl ($\delta 1.72$ *d*) and with a double doublet at $\delta 3.18$ ppm which itself collapsed to a doublet ($J = 9$ HZ) on irradiation of the broadened singlet at $\delta 4.61$ ppm. This irradiation also made the doublet quartet at $\delta 7.15$ ppm collapses to a quartet. The latter signal and the singlet at $\delta 4.61$ ppm were both coupled with a methyl triplet at $\delta 1.82$ ppm. The couplings together with the chemical shifts required the presence of the proposed cyclopentenone residue which most likely was formed by aldol condensation via the intermediate **11**. This latter could easily be formed from the 19, 16-lactone by hydrolysis, oxidation at C-16 and reduction at C-19. The ketone **7** has been named salvileucolidone.^[6]

The $^1\text{H-NMR}$ spectrum of **8** clearly indicated that a hydroperoxide similar to **5** was again present. However, the 6, 23-lactone ring was replaced by the corresponding hydroxyl ester. Accordingly, the signals were in part identical with those of salvileucolide methyl ester.

The $^1\text{H-NMR}$ spectra of **9a** and **9b** nicely agreed with the presence of 13-epimeric hydroperoxides derived from salvileucolide methyl ester. The configuration of the $\Delta^{1,4}$ -double bond followed from the coupling. The relative configurations of C-8 and C-13 could not be determined. Triphenylphosphine reduction of **9a** gave the corresponding alcohol with the expected shifts in the $^1\text{H-NMR}$ spectrum.^[6]

The last compound (**10**) was also derived from salvileucolide methyl ester as followed from the $^1\text{H-NMR}$ spectrum. However, the butenolide part was now changed as the olefinic proton (H-18) was replaced by a pair of doublets at $\delta 2.64$ ppm and 2.51 ppm. As the olefinic methyl signal was replaced by a singlet at $\delta 1.58$ ppm the compound must be formed by addition of an oxygen function at the conjugated double bond. Spin decoupling indicated that the allylic proton (H-14) was coupled with a pair of threefold doublet at $\delta 2.24$ ppm and 2.07 ppm which themselves were coupled further with H-16 (4.46 *t*). This data as well as the CIMS could only be rationalized by a cyclized hydroperoxide. Irradiation of H-20 gave a clear NOE with H-18 and H-16. The latter showed a NOE with H-15 α while H-14 gave no NOE with H-16.

The isolation of all these sesterterpenes may be an indication that these compounds are characteristic for this genus *Salvia*. However, further investigations are needed to establish whether these compounds are widespread in this genus.^[6]

Salvia syriaca L.

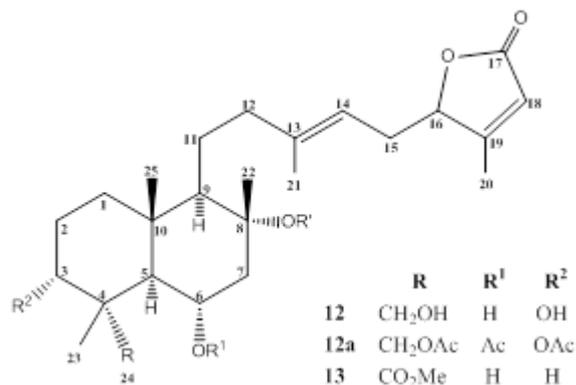
The extract of the aerial parts of *Salvia syriaca* L. afforded a polar sesterterpene lactone with four hydroxyl groups. Acetylation gave a triacetate. The structure was elucidated by high field NMR spectroscopy.

We have studied a further species from Iran. *Salvia syriaca* L. The polar fractions were separated by TLC and HPLC. Finally colorless oil was obtained. CIMS indicated the presence of a sesterterpene with the molecular formula $C_{25}H_{40}O_6$. This was supported by CIMS of the corresponding triacetate obtained by mild acetylation (m/z 563 corresponding to $C_{31}H_{46}O_9 + 1$) and by the ^{13}C -NMR spectrum of **12** which showed 25 carbon signals. The 1H -NMR spectrum as well as the IR spectrum (1765 cm^{-1}), indicated the presence of a butenolide. This was established by spin decoupling.

Saturation of a narrowly split signal at $\delta 5.86$ ppm changed the methyl doublet at $\delta 2.07$ ppm to a singlet and sharpened the broadened signal at $\delta 4.90$ ppm. The proton corresponding to the latter signal was further coupled with allylic protons which showed threefold doublet at $\delta 2.80$ ppm and 2.29 ppm. These signals and the coupling partners nicely agreed with the corresponding signals of salvileucolide methyl ester (**13**) isolated from *S. Hypoleuca*.^[5] Several further signals also were similar. However, a hanged substitution pattern was indicated by the absence of the methoxy signal which was replaced by a broadened two proton singlet at $\delta 3.69$ ppm which was shifted down field in the corresponding triacetate ($\delta 4.09$ *d* and 3.59 *d*). Accordingly, a hydroxymethyl group at C-4 was very likely. Furthermore a double doublet at $\delta 8.58$ ppm and a threefold doublet at $\delta 3.91$ ppm required two secondary hydroxyl groups. Both signals were shifted downfield in the spectrum of the triacetate.

The observed coupling constants required equatorial orientation of these groups. Spin decoupling indicated that one hydroxyl group was at C-6 while the other one

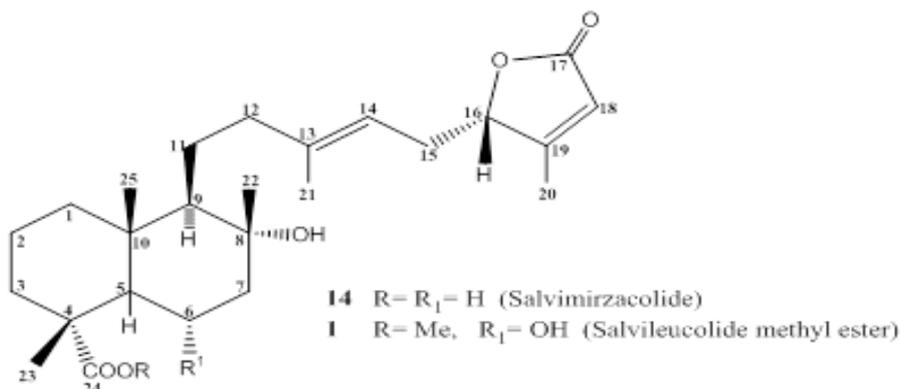
was at C-1 or C-3. A third hydroxyl group was most probably at C-8. All data therefore agreed with the structure **12** and for the corresponding triacetate had structure **12a**. NOE difference spectroscopy with a **12a** allowed the assignment of the methyl signals, the stereochemistry and also indicated that one hydroxyl group was at C-3. Clear effects were observed between H-24, H-6 (8%), H-23(5%) and H-23' (5%), between H-25 and H-6 (10%), between H-9 and H-5 (8%), between H-22, H-6 (8%) and H-25 (8%) as well as between H-3 and H-5 (4%). Compound **12** has been named salvisyriacolide.

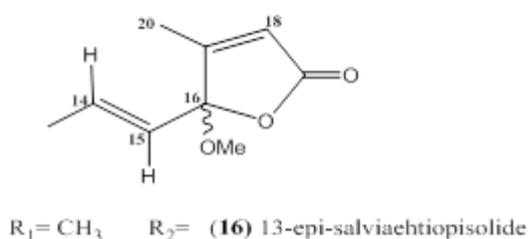
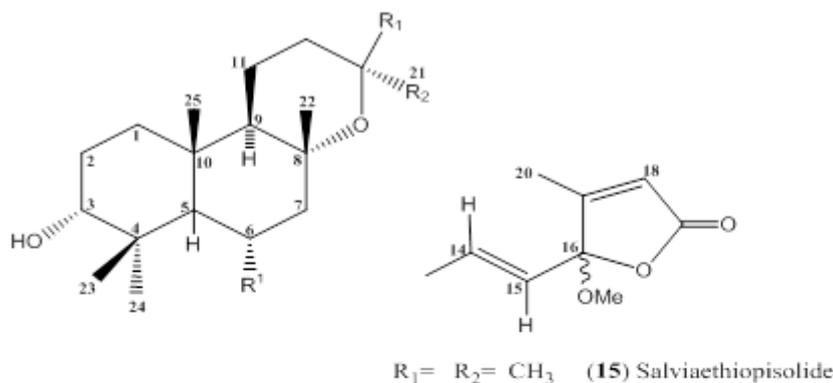


The isolation of sesterterpenes from two *Salvia* species may be of chemotaxonomic interest. Further investigations may show whether this rare type of natural product in higher plants^[7] is more widespread in the large genus *Salvia*.

Salvia mirzayanii Rech.f. & Esfand.

A sesterterpene, salvimirzacolide (**14**) was isolated from the aerial parts of *S. mirzayanii* Rech.f. & Esfand. and its structure established by high field NMR spectroscopy and X-ray diffraction analysis.^[8] Comparison of the 1H -NMR spectrum of **14** with the revised data of **1**, isolated from *S. sahendica*^[9], 13 years after its first report from *S. hypoleuca*^[5] indicated a great similarity between these two compounds. Two sesterterpene lactones with a partial structure of manoyl oxide (**15**) and 13-epi-manoyl oxide (**16**), both as epimeric pairs at the anomeric carbon atom C-16 of a γ -methoxybutenolide were isolated from the aerial parts of *S. aethiopsis*.^[10]





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