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# THE SESQUITERPENE LACTONES FROM ACHILLEA FRAGRANTISSIMA, I. ACHILLOLIDE A AND B, TWO NOVEL GERMACRANOLIDES

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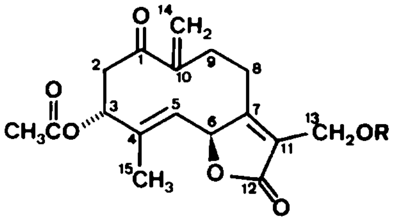
**Abstract** - With the aid of one- and two-dimensional multipulse NMR techniques the structure of two novel sesquiterpene lactones from *Achillea fragrantissima* growing in Israel were elucidated and their conformational behaviour investigated. Although both compounds are closely related in their structures their CD spectra indicate that they belong to different enantiomeric series.

## INTRODUCTION

In the course of our studies on the sesquiterpene lactones of Iraeli wild plants used in Beduin folk medicine we investigated *Achillea fragrantissima* (Forssk). Sch. Bip.. This plant grows in the wadis of the Negev Desert, the Judean Desert and the Lower Jordan Valley of Israel. It is characterized by its pleasant smell due to its high content of essential oils[1]. It is used in folk medicine for the treatment of gastro-intestinal disturbances and various infections, among them infections of the eye.

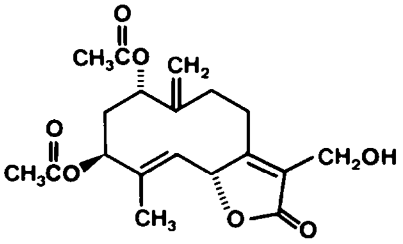
We wish to report now the isolation and identification of two new sesquiterpene lactones, designated achillolide A and B, to which the structures 1 and 3 were assigned.

## RESULTS AND DISCUSSION



1: R = H

2: R = $COCH_3$



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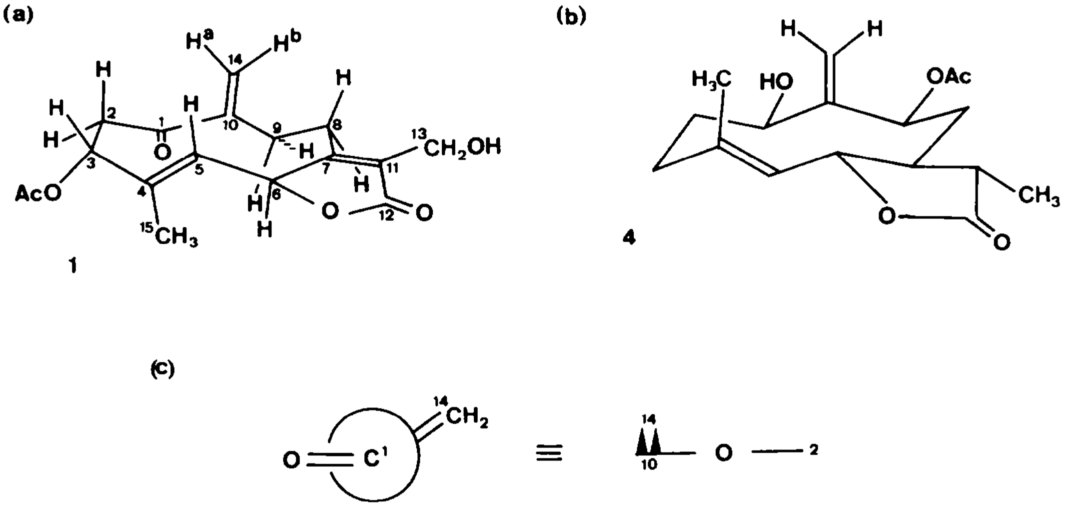
### Achillolide A (1)

The high-resolution mass spectrum (HRMS) displayed a molecular peak at m/e=320 indicating a molecular formula of $C_{17}H_{20}O_6$, and the presence of one acetoxy group was suggested by the loss of 40 mass units ($CH_2CO$). In the IR spectrum a γ-lactone band appeared at 1750 $cm^{-1}$. The $^1H$ and $^{13}C$ NMR measurements including two-dimensional techniques (homonuclear and heteronuclear COSY)[2] proved the existence of the following molecular fragments: an enone, a $-CH_2-CHOAc-$ and a $-CH_2-CH_2-$ group, the two latter with quarternary carbons at each end, one $-C(CH_3)=CH-CH(OCO-)-$ fragment, a $CH_2OH$ group attached to a quaternary carbon, an exo-methylene group and one tetrasubstituted double bond. By using these fragments the constitution 1 (Scheme) was established with the help of a two-dimensional COLOC experiment[3] which can be applied to prove the existence of $^{13}C-^1H$ couplings over more than one bond. Thus, COLOC peaks were observed indicating a coupling between the two H(14) atoms and the carbonyl C(1) and a coupling between the C(15) methyl hydrogens and C(3) bearing the acetoxy group. Thus, it was proven unequivocally that C(1) is the carbonyl and the acetoxy group is at C(3) and not vice versa. Signal shifts due to acetylation of the hydroxy group (1 -> 2) confirmed the signal assignments listed in Table 1 and 2.

The final assignment of the signals of the hydrogens at C(8) and C(9) and the stereochemistry of 1 (Fig. 1a) were based on the $^1H-^1H$ coupling and on the nuclear Overhauser enhancement (NOE) information. The coupling constant J(5,6) is 10.3 Hz indicating an antiperiplanar orientation of H(5) and H(6). Since C(7) is not a chirality center we are using H(6) as reference to assign the geometries of other atoms and groups. H(6) was found by CD to be α (see below); thus H(5) is in β position. NOE effects were observed at the H(15) signal witH 7%, 2% and 2% when H(6α), H(2α) and H(9α), respectively, were irradiated proving the α orientation of C(15). Similarly, close spatial orientations between H(5β) and H(3β) (9% and 9%) as well as between H(3β) and H(14a) (2% and 2%) were also deduced from the NOE data. Furthermore, NOE's were found at H(2β) (5%) if H(14a) and at H(8β) (3%) if H(14b) was irradiated. We can therefore conclude that the acetoxy group at C(3) is in α position and C(14) and C(15) are on different faces of the ten-membered ring (Fig. 1a). This is in accordance with our findings that no NOE signals were observed for H(14) when H(15) was irradiated and vice versa. It is interesting to note that in contrast to 1 the preferred conformation of herbolide D (4, Fig.1b) containing a very similar ten-membered ring with a C-14 exomethylene group is one with both C(14) and C(15) on the same face[4]. The pairwise assignment of the H(8) and H(9) signals, respectively, is based on the $^1H-^1H$ coupling information. The

H(8β) and H(9α) signals show two coupling constants of about 12-14 Hz (one due to a geminal and one to an antiperiplanar hydrogen), whereas those of H(8α) and H(9β) display only one coupling. This assignment was confirmed by NOE experiments as well. Likewise, H(2β) was identified by the appearance of reciprocal NOE effects between this atom and H(14a) (5% and 5%, see above) and between H(2β) and H(3β) (4% and 3%). Both coupling constants between H(3β) and the two H(2) atoms are of similar magnitude (8.5-9 Hz) supporting the more or less eclipsed conformation of the C(2)-C(3) fragment as displayed by Dreiding models.

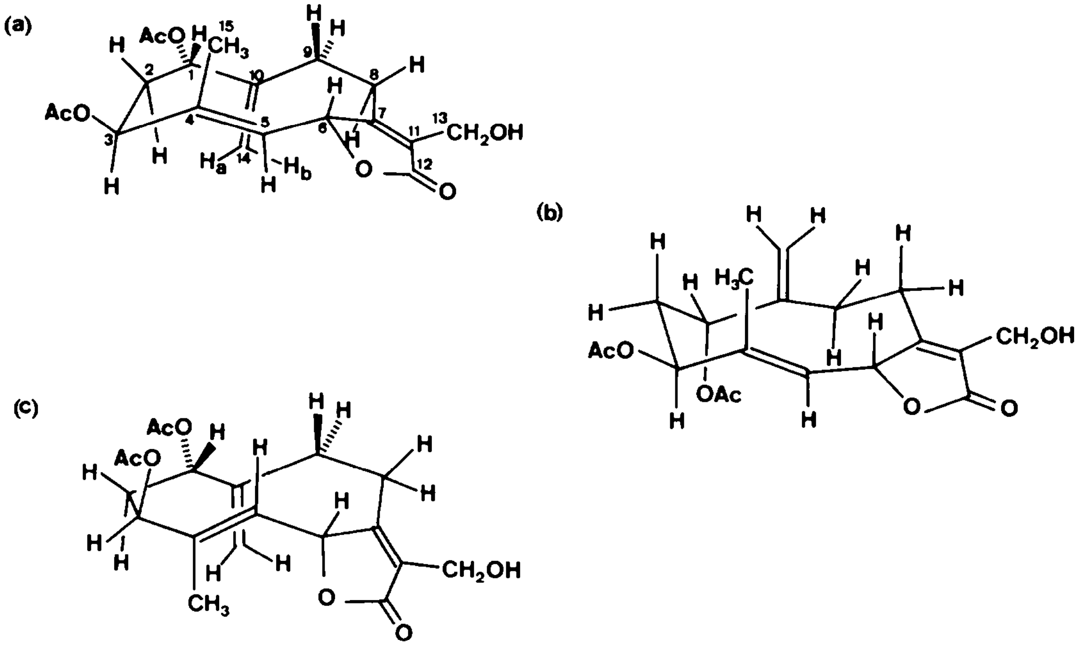
Fig. 1: Structures of achillolide A (1) and herbolide D (4)



## Achillolide B (3)

The mass spectrum of 3 displayed a molecular peak at m/e 364 which is consistent with the molecular formula $C_{19}H_{24}O_7$ and the presence of two acetoxy groups. In the IR spectrum a broad carbonyl signal at 1745-1775 cm$^{-1}$ indicated the presence of a γ-lactone and a C=C-C-OCOR function. A comparison of the $^1$H and $^{13}$C NMR data with those of 1 (Tables 1 and 2) suggests that the structures of 1 and 3 are very similar and that the second acetoxy group in 3 is at C(1); indeed, there is no ketone signal at about $\delta$ = 195-200 in the $^{13}$C NMR. NOE experiments again prove that the C(14) methylene and the C(15) methyl are at different faces of the molecule so that we conclude that 1 and 3 adopt similar conformations (for a more detailed analysis see below). A CD measurement of 3 indicated that - in contrast to 1 - H(6) is in β position (see below). The relative configuration of C(1) was determined by NOE measurements as well. Irradiation of H(15) gave NOE effects at H(6β) (8%) and H(1β) (1%) and irradiation of H(9β) gave NOE effects at H(1β) (1%) and H(15) (3%) (cf. Fig. 2a). Furthermore, NOE's were observed at H(5α) (3%) and H(14a) (4%) when the proton at C(3) was irradiated. Thus, the acetoxy groups at C(1) and C(3) are α and β, respectively.

Fig. 2: Conformations of achillolide B (3)



## Conformational Behaviour

The conformational behaviour of partially unsaturated ten-membered rings[5] (especially in sesquiterpenes) is very delicate. In dimethylcyclodecadienes energetically favoured conformations were observed with the two methyl groups on the same[6] and on opposite[7] faces of the ring. Most of the $^1H$ NMR signals of **3** at room temperature are broad and so are some of the $^{13}C$ signals, that of C(8) is even nearly undiscoverable. Heating the sample to +50°C, however, afforded signals with the usual resolution whereas cooling reveals the typical coalescence behaviour. From the $^1H$ signals, especially from those of H(15) (Fig. 3), the following conclusions can be drawn: (a) the conformational interconversion barrier at the coalescence temperature ($T_c \approx 250$ K) is ca 51-50 kJ/mol; (b) by integration of the low-temperature signals a ratio of major to minor conformer of ca 5.5 was obtained, i.e. the major conformer (Fig. 2a) is present with about 85% and is ca 3.5 kJ/mol more stable than the minor one at T = 250 K. The structure of the minor component can be deduced as follows. In an NOE experiment at 313 K we observed small signals for H(14a) and H(14b) (ca 0.5 %) when H(15) was irradiated. Moreover, when H(14b) is irradiated a small NOE effect (0.5-1 %) is observed at H(8β) besides a larger one (3%) at H(8α) which is due to the major conformer. These findings are consistent with a C(14)/C(15)-syn orientation, both at the β face (Fig. 2b), but not with the second possibility with C(14)/C(15)-syn at the α face (Fig. 2c). This observation was to be expected since the second syn-conformer (Fig. 2c) exhibits a boat-type conformation of the C(5)-C(10) part of the ten-membered ring.

Table 1: $^1$H chemical shifts of achillolide A (**1**), its acetate **2** and achillolide B (**3**)[a]

| | **1** | **2** | **3** |
|---|---|---|---|
| H-1 | - | - | 4.99 |
| H-2α | 2.57 | 2.62 | 1.98 |
| H-2β | 3.66 | 3.72 | 1.98 |
| H-3 | 5.49 | 5.53 | 4.11 |
| H-5 | 4.81 | 4.86 | 4.99 |
| H-6 | 5.27 | 5.32 | 5.42 |
| H-8α | 2.99 | 3.08 | 3.21 |
| H-8β | 2.25 | 2.19 | 2.44 |
| H-9α | 2.07 | 2.07 | 2.02 |
| H-9β | 2.76 | 2.83 | 2.35 |
| H-13 | 4.35 | 4.86 | 4.73 |
| | 4.33 | 4.79 | 4.73 |
| H-14a | 5.77 | 5.81 | 5.14 |
| H-14b | 5.70 | 5.68 | 4.77 |
| H-15 | 1.71 | 1.77 | 1.76 |
| OH | 3.15 | - | 2.3 |
| Ac | 2.03 | 2.08 | 1.99 |
| | | 2.07 | 1.98 |

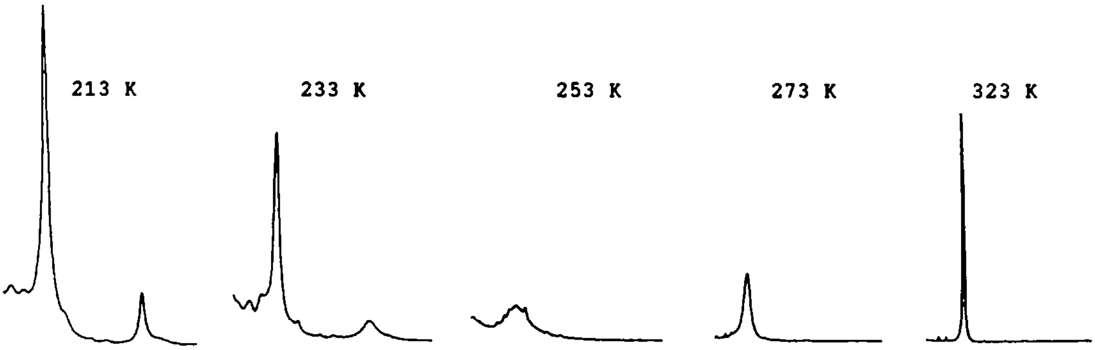
[a] In ppm; on δ scale; 400 MHz; solvent: $CDCl_3$; standard: $CHCl_3$, δ = 7.24.

Table 2: $^{13}$C chemical shifts of achillolide A (**1**), its acetate (**2**) and achillolide B (**3**)[a]

| | **1** | **2** | **3** |
|---|---|---|---|
| C-1 | 198.4 | 198.2 | 75.1 |
| C-2 | 44.1 | 44.2 | 37.0 |
| C-3 | 75.5 | 75.5 | 74.8 |
| C-4 | 136.2 | 136.7 | 137.9 |
| C-5 | 126.9 | 126.6 | 123.1 |
| C-6 | 79.3 | 79.3 | 79.2 |
| C-7 | 165.4 | 167.9 | 167.7 |
| C-8 | 33.4 | 33.4 | 27.0 |
| C-9 | 23.4 | 23.7 | 24.5 |
| C-10 | 148.2 | 148.3 | 149.5 |
| C-11 | 127.5 | 124.0 | 123.2 |
| C-12 | 173.8 | 172.3 | 172.2 |
| C-13 | 54.5 | 55.5 | 54.8 |
| C-14 | 125.3 | 125.1 | 114.1 |
| C-15 | 10.6 | 10.8 | 11.2 |
| Ac-CO | 169.8 | 170.5 | 170.5 |
| | | 169.7 | 169.7 |
| Ac-$CH_3$ | 20.8 | 21.0 | 20.9 |
| | | 20.8 | 20.5 |

[a] In ppm on δ scale; 100.6 MHz; solvent: $CDCl_3$; standard: central peak of $CDCl_3$ δ = 77.0.

Fig. 3: H-15 signals of **3** at different temperatures



The NMR signals of 1 do not display line broadening at room temperature but on cooling they show coalescence behaviour, too, the maximal broadening being also at about 250 K. Even at 208 K, however, no minor conformer can be detected so that the ground state energy difference between the conformers must be larger than 6.5 kJ/mol. Apparently, it is the H(1ß) atom directed inwards in 3 (Fig. 2a) that destabilizes this molecule, an effect which does not exist in 1.

## Determination of absolute configurations

The absolute configurations of 1 and 3 were determined by circular dichroism measurements. In the CD spectrum of 1 three bands appear at 317 nm ($\Delta\epsilon$=-0.43), 243 nm ($\Delta\epsilon$= +10.4) and 193 nm ($\Delta\epsilon$=-51). The first two bands can be assigned to the $n\pi^*$ and the $\pi\pi^*$ transitions, respectively, of the conjugated enone chromophore. The $n\pi^*$ band is certainly originating from the non-coplanarity of the enone moiety and, according to the well established rule[8], its negative sign reflects the positive helicity of the chromophore shown in Fig. 1c. Though the positive sign of the second band is also in accordance with this conclusion, the Cotton effect of the $\pi\pi^*$ transition must also contain strong contributions from exciton interactions with the other olefinic chromophores. The latter mechanism is responsible for the very intensive third band[9]. Because of the complex nature of these exciton interactions the two stronger CD bands could hardly be used for an unequivocal stereochemical assignment[10]. The Dreiding model of 1 suggests that the carbonyl oxygen and the methylene group are bent outwards. This geometry is supported by the observation that NOE influences between the two H(14) and H(5ß) are existent but minute (<1%). As the molecular model shows, the positive helicity of the enone moiety and the features of the relative geometry established by NMR results are only consistent with the 3R,6S configuration of 1 (Fig. 1a).

This assignment may be considered an indirect one since the configurational chirality of C(6) has been deduced from the conformational chirality of the enone chromophore being rather far remote from this chirality center. A more direct method, however, was not possible because the weaker bands expected to come from the butenolide chromophore are completely hidden by the strong Cotton effects due mainly to the enone moiety. Nevertheless, the absolute geometry of the whole molecule, and thus the helicity of the enone cannot be determined except by the configurations of C(6).

In the CD spectrum of 3 lacking a carbonyl group the weaker positive band at 256 nm ($\Delta\epsilon$=+0.78) and the stronger negative one at 223 nm ($\Delta\epsilon$=-7.5) are assigned unambiguously to the $n\pi^*$ and the $\pi\pi^*$ transitions, respectively, of the lactone ring with the endocyclic $\alpha$,ß-double bond[11]. Since the Dreiding model shows the butenolide ring to be almost planar, the $n\pi^*$ band cannot be used with confidence to assign the absolute configuration. The negative sign of the $\pi\pi^*$ band, however, indicates, according to the rule of Uchida and Kuriyama[12], that the absolute configuration of the chirality center C(6) in 3 is R. The above rule must be considered an empirical one only which has not yet been verified by theoretical calculations. However, there are many examples in the literature which are in agreement with it[12]. The sesquiterpene lactone confertolide, also with an endocyclic double bond in the lactone ring, exhibited a CD spectrum almost identical to that of 3 and, accordingly, was assigned to the 6R absolute configuration[13]. Our configurational assignment is thus based on a number of analogies. Taking into account the relative positions of the groups established by NMR, the absolute configuration of **3** must be 1S,3S,6R.

It is rather surprising that two sesquiterpene lactones found in the same plant are of different configurations at the two chirality centers[14] and thus are of almost enantiomeric geometry. Such a case, however, is not unique in literature[15]. The configuration of **3**, as established here, is in closer analogy with that of the majority of sesquiterpene lactones than is the configuration of **1**. It should, however, be mentioned that the absolute configurations of the glaucolides which contain a similar butenolide ring with an endocyclic double bond and which can therefore be considered the closest relatives of the achillolides, are not yet known; the X-ray studies[16] have established only their relative geometries. This intriguing problem can be solved by studying further derivatives.

## EXPERIMENTAL

Stems including leaves and heads (capitulae) of *Achillea fragrantissima* were harvested in the vicinity of Nahal Beer-Sheva in June 1984. Voucher specimens have been deposited at the Herbarium of the Hebrew University of Jerusalem. The air dried flowers, leaves and small stems (950 g) were crushed and soaked three times overnight in $CH_2Cl_2$. Evaporation of the combined filtrates yielded a thick tar which was taken up with ethanol and processed with $Pb(OAc)_2$ as described by Fisher et al.[17]. The crude material obtained (28 g) was separated on a silica gel column eluted with $CH_2Cl_2$-MeOH (0.5-5%). The two lactones emerged at 2% MeOH (1.5 g). Rechromatography on a silica gel column with petrol ether (60-80°) - ethyl acetate (1 : 1) yielded achillolide A (**1** , 400 mg). Twice crystallized from acetone - petrol ether (40-60°) pure crystals with m.p. 144-5° were obtained, $[\alpha]_D^{22}$ -37.4° (EtOH, c=0.5). High-resolution mass spectrum (HRMS): m/e 320.1260 (calcd. for $C_{17}H_{20}O_6$ 320.1259). MS (70 eV): m/e (relative intensity) 320 (4) ($M^+$), 278 (8) ($M^+$-$C_2H_2O$), 260 (45) ($M^+$-acetate).

The second polar lactone (200 mg) was rechromatographed on silica gel with petrol ether (60-80°) - acetone (3 : 1), to yield 50 mg achillolide B (**3**) which could not be crystallized, $[\alpha]_D^{22}$ = -1.4° (EtOH, c=0.5). MS (70 eV): m/e (relative intensity) 364 (0.7) ($M^+$), 322 (1.1) ($M^+$-$C_2H_2O$), 304 (2.3) ($M^+$-acetate), 262 (9) ($M^+$-$C_2H_2O$-acetate), 244 (20) ($M^+$-2 acetates).

All NMR spectra were recorded in $CDCl_3$ using a Bruker AM-400 spectrometer (400.1 MHz $^1H$ and 100.6 MHz $^{13}C$) and a dual probe-head. Chemical shifts refer to the solvent signal ($^1H$: $\delta$ = 7.24 for $CHCl_3$; $^{13}C$: $\delta$ = 77.0 for the center peak of $CDCl_3$). The carbon signal assignments were supported by DEPT experiments[2d]. For the two-dimensional homo- and heteronuclear COSY and the NOE-difference experiments Bruker standard software was employed; details about the COLOC experiment have been published elsewhere[3]. The 70 eV electron impact mass spectra were recorded on Varian MAT CH-5 and CH-7 instruments. The CD spectra were obtained on a Jobin-Yvon III dichrograph in ca $10^{-4}$ molar acetonitrile solution. Quartz cells of 0.05 and 1 cm length were used.

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