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THE SESQUITERPENE LACTONES FROM TWO POPULATIONS OF *ARTEMISIA HERBA ALBA*

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Abstract—The sesquiterpene lactones of two additional Israeli *Artemisia herba alba* populations, one from Elat and the other from Mizpe Ramon, were investigated While the Elat population was devoid of any sesquiterpene lactones, the Mizpe Ramon variety contained herbolides A, B and C and desacetylherbolide A Furthermore, it was found to contain the hitherto unknown herbolide D On the basis of these and previous results the presence of at least five different chemotypes in the Middle Eastern populations of *A herba alba* is established

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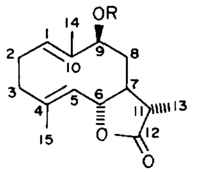
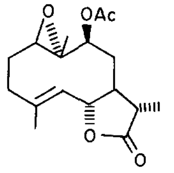
INTRODUCTION

Artemisia herba alba is a characteristic plant of the steppes of the Middle East and North Africa In Israel it abounds in vast areas of the Negev and the Judean Desert This plant is of special interest since it is one of the best known medicinal herbs used by the local population for a great diversity of ailments [1-3]

Phytochemical investigations of *A herba alba* showed that samples collected at three localities in the Middle East contain very different sesquiterpene lactones A sample from Sede Boqer (Negev, Israel) contained herbolides A(**1**), B(**2**) and C(**3**) [4] A sample from Gebel Katherina (Sinai) contained 11β-*H*-tatridin D(**4**), 3-oxo-11β-*H*-tatridin D(**5**) and 11α-*H*-gallicin (**6**) [5-7] while a sample from Ras El Hikma (Egypt) contained santonin (**7**) [2] It was thus suggested that *A herba alba* comprises various chemotypes [7, 8] We wish to report the results of our investigations concerning the presence and constitution of sesquiterpene lactones from two further populations growing in Israel One was collected near Mizpe Ramon



1 R = COMe
9 R = H



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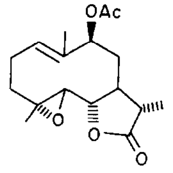

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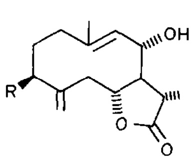
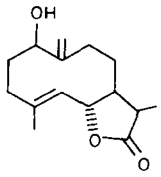


4 R = β - OH
5 R = =O



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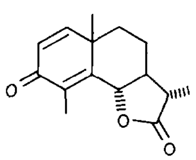

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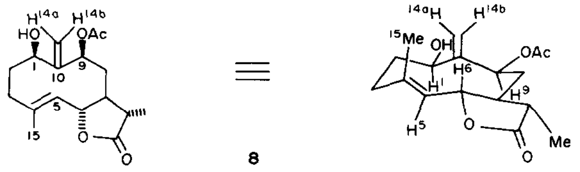

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and the other near Elat From the Mizpe Ramon population a new sesquiterpene lactone was isolated and its configuration determined

RESULTS AND DISCUSSION

Mizpe Ramon population

The chloroform extract of the inflorescences, small stems and leaves yielded on CC several fractions of sesquiterpene lactones from which herbolides A(**1**), B(**2**) and C(**3**) were isolated in the quantitative ratio of 100 10 1, respectively Medium pressure chromatography of the more polar fractions yielded two further sesquiterpene lactones, herbolide D(**8**) and desacetylherbolide A(**9**), which could not be crystallized

The determination of structure **8** for herbolide D was based on extensive use of spectroscopic methods High resolution mass spectrometry established the empirical formula $C_{17}H_{24}O_5$ In the IR spectrum herbolide D showed bands for a hydroxyl function (3450 cm^{-1}), a γ-lactone group (*ca* 1760 cm^{-1}), and an ester group (1720 cm^{-1}) which may be attributed to an acetate because of a strong $[M - 60]^+$ peak in the mass spectrum and the ^1H NMR spectral data summarized below

The germacranolide structure with an exocyclic methylene group and one additional double bond was assigned to herbolide D(**8**) on the basis of the following data The ^1H NMR spectrum showed a narrow three proton doublet at δ1 55 (J = 1 5 Hz, Me-15) which turned into a sharp singlet when the H-5 signal (δ5 11, *dd*, $J_{5\,6}$ = 10 2 Hz, $J_{5\,15}$ = 1 5 Hz) was irradiated Two narrow one proton doublets for H-14a at δ5 39 (*d*, $J_{14\,9}$ = 1 2Hz) and for H-14b at δ5 32 (*d*, $J_{14\,1}$ = 0 7Hz), were attributed to the exocyclic methylene group The assignment of this group to position 10, and of the hydroxyl and acetyl groups to positions 1 and 9, was done by decoupling experiments Irradiation at the frequency of H-9 (δ4 75, *br dd*, $J_{9\,8\beta}$ = 8–9 Hz), turned the doublet centered at 5 39 (H-14a) into a sharp singlet, irradiation at H-1 (δ3 91, *dd* $J_{1\,2\alpha}$ = 4 Hz, $J_{1,2\beta}$ = 9–10 Hz), turned the doublet centered at δ5 32 (H-14b) into a singlet The allylic nature of H-6 (δ4 32, *dd*, $J_{5\,6}$ = 10 2 Hz, $J_{6,7}$ = 8 8 Hz) and of the C-15 methyl group was determined by comparing their chemical shifts with those obtained for herbolides A(**1**) and B(**2**) [4] and were confirmed by double irradiation It should be noted that the chemical shifts of H-5 and H-6 recorded for compound **1** (herbolide A) in ref [4] should be at δ4 55 instead of 5 55

The conformation of the 10-membered ring was proved by NOE measurements Irradiation of the H-15 atoms considerably enhanced the intensity of H-6 and, to a lesser extent, the low-field H-14 signal, whereas the other H-14β and all other protons remained nearly unaffected Thus, both C-14 and C-15 are on the β side of the molecule

Furthermore, one of the two H-14 atoms is closer to C-15 than the other thus indicating a slight twist of this group Inspection of the splitting of the two H-14 signals, before and after decoupling experiments showed that both are due to transoid allylic couplings [9] The low-field signal with the larger coupling belongs to H-14a (see formula **8**) since it is more or less coplanar with H-9 However, H-1 is significantly distorted out of the H-14b–C-14–C-10-plane thus giving rise to the smaller H-1/H-14 coupling constant These considerations also provide the signal assignment for H-1 and H-9 An additional argument for the location of the hydroxyl group at C-1 is the similarity of the H-1 chemical shift in herbolide D and the corresponding proton of verlotorin [10, 11] In order to prove unequivocally the position of the acetate group at C-9, we irradiated the acetate methyl protons and found NOE effects for the nearby H-9 only The configuration of the acetate should be β because of the appearance of the H-9 signal which indicates the presence of an axial proton split by both one axial and one equatorial proton

The configuration at C-1 was also determined by a NOE experiment When H-1 was irradiated, signal enhancements were found for H-5 and H-9 to a similar extent, but practically none was observed for the H-14 atoms This result is consistent with the β position of the hydroxyl group

The similarity in the chemical shifts and the coupling constants of the Me-13 group (δ1 26, 3H, *d*, $J_{13\,11}$ = 6 6 Hz) with those of the other herbolides (Table 1 in ref [4]) shows that the methyl group in herbolide D is also α-oriented

Thus, the configuration and conformation of herbolide D is determined to be as shown in the projection formula **8** This is the most plausible structure to be expected if we assume that herbolide D is biogenetically derived from herbolide B (**2**)

In addition to herbolides A,B,C and D, desacetylherbolide A(**9**) was isolated and identified by its ^1H and ^{13}C NMR spectra, and by its conversion to herbolide A on acetylation

The ^{13}C NMR data for herbolides A(**1**) and B(**2**) have not yet been published and are presented in Table 1 together with the data for herbolide D(**8**) and desacetylherbolide A(**9**) The signal assignments were based on off-resonance spectra and on data reported previously for similar compounds [10, 11] Use was also made of the observation of well-known acetylation shifts [12] when the spectra of **1** and **9** were compared Among the two

Table 1 ^{13}C NMR chemical shifts of compounds **1**, **2**, **8** and **9** downfield from internal TMS (solvent $CDCl_3$)

Carbon	**1**	**2**	**8**	**9**
1	131 3(*d*)	65 3(*d*)	74 8(*d*)	129 3(*d*)
2	25 4(*t*)	23 0(*t*)	31 4(*t*)	25 5(*t*)
3	39 3(*t*)	36 1(*t*)	37 8(*t*)*	39 4(*t*)
4	140 4(*s*)	142 7(*s*)	145 5(*s*)	140 1(*s*)
5	127 0(*d*)	124 1(*d*)	121 7(*d*)	127 2(*d*)
6	81 0(*d*)	80 7(*d*)	80 4(*d*)	80 9(*d*)
7	51 4(*d*)	51 1(*d*)	51 5(*d*)	51 5(*d*)
8	34 2(*t*)	33 0(*t*)	37 0(*t*)*	36 5(*t*)
9	80 8(*d*)	80 0(*d*)	79 3(*d*)	79 6(*d*)
10	134 8(*s*)	61 3(*s*)	153 7(*s*)	138 9(*s*)
11	42 0(*d*)	42 4(*d*)	41 9(*d*)	42 0(*d*)
12	177 9(*s*)	177 5(*s*)	177 7(*s*)	178 2(*s*)
13	13 3(*q*)	12 8(*q*)	12 8(*q*)	13 3(*q*)
14	11 5(*q*)	12 8(*q*)	114 7(*t*)	10 7(*q*)
15	17 3(*q*)	17 4(*q*)	17 8(*q*)	17 3(*q*)
Acetate C=O	169 9(*s*)	170 1(*s*)	171 9(*s*)	—
Me	21 3(*q*)	21 1(*q*)	21 4(*q*)	—

* May be interchanged

signals belonging to C-2 and C-3 the one at higher field was attributed to C-2 in all cases, since C-2 experiences two shielding γ-gauche interactions with C-14 and C-15

Elat population

Identical work-up of the plant material collected near Elat yielded a chloroform extract which showed no typical lactone band in the IR region In order to ascertain that such a band was not obscured by the presence of a very strong carbonyl band at *ca* 1720 cm^{-1}, the extract was fractionated by CC and all fractions were re-examined by IR spectroscopy In none of them could the presence of γ-lactones be detected The results of our investigation of the *A herba alba* population from Mizpe Ramon induced us to reinvestigate the compositions of the sesquiterpene lactones from the Sede Boqer population, which was described in a previous publication [4] Very thorough chromatographic fractionation of the chloroform extract confirmed our previous results concerning the quantitative yields of herbolides A, B and C Furthermore, neither herbolide D nor desacetylherbolide A could be detected in this population

Table 2 summarizes the composition of the sesquiterpene lactones from the various *A herba alba* populations investigated so far These results show that there exist at least five different chemotypes in the Middle Eastern populations

The sesquiterpene lactone composition of the various chemotypes leads to the conclusion that the Sede Boqer and the Mizpe Ramon populations are most closely related The high content of sesquiterpene lactones in the Mizpe Ramon population, especially herbolide A, is remarkable In both populations the sesquiterpene lactones are of the germacranolide-type possessing a 9β-oxygen function This obviously indicates that they have a common biogenetic precursor, most probably herbolide A

Table 2 Composition of the sesquiterpene lactones in various *A herba alba* populations

Site of growth	Sesquiterpene lactones	Reference
1 Elat	—	—
2 Ras El Kikma	Santonin (**7**)	[2]
3 Sinai	11β-*H*-Tatridin D (**4**)	[5–7]
	3-Oxo-11β-*H*-tatridin D (**5**)	[5–7]
	11α-*H*-Gallicin (**6**)	[5–7]
4 Sede Boqer	Herbolide A (**1**) (0 003%)	[4]
	Herbolide B (**2**) (0 02%)	[4]
	Herbolide C (**3**) (0 002%)	[4]
5 Mizpe Ramon	Herbolide A (**1**) (0 16%)	—
	Herbolide B (**2**) (0 015%)	—
	Herbolide C (**3**) (0 0016%)	—
	Herbolide D (**8**) (0 02%)	—
	Desacetylherbolide A (**9**) (0 02%)	—

EXPERIMENTAL

Plant material Stems including leaves and heads (capitulae) of *A herba alba* were harvested in October–December 1979 in the three following localities (1) near Sede Boqer (42 km south of Be'er Sheva), voucher No 1-79-9-1, (2) near Mizpe Ramon, voucher No 1-79-16-1, (3) near Elat (12 km north-west of Elat) voucher No 1-79-20-1 Voucher specimens are deposited in the Herbarium of the Hebrew University of Jerusalem The air dried plant material was crushed and soaked overnight in petrol, the extract was discarded The residue was allowed to stand for several days covered with $CHCl_3$ Evaporation of the solvent yielded a thick tar The tar was separated on a Florisil column, eluting with $CHCl_3$–MeOH (95 5) The fractions containing herbolides A, B and C were further purified as described [4]

Mizpe Ramon population The extract from 300 g dry plant material yielded herbolide A (500 mg), herbolide B (45 mg) and herbolide C (5 mg) The more polar fraction from the Florisil column was purified by medium pressure chromatography (elution with 0 5% MeOH in $CHCl_3$) to yield herbolide D (60 mg) Herbolide D could not be crystallized, $[\alpha]_D^{22} - 6\ 5°$ ($CHCl_3$, *c* 0 24), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3450 (OH), 1760 (γ-lactone), 1720 (acetate), high resolution EIMS *m/z* 308 1606 (calcd for $C_{17}H_{24}O_5$ 308 1623), EIMS 70 eV *m/z* (rel int) 308 (0 2), 248 $[M - AcOH]^+$ (3), 230 $[M - AcOH - H_2O]^+$ (3) The ^1H and ^{13}C NMR spectra were recorded using Bruker WP-80 and WM-250 spectrometers

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