gel ($CHCl_3$–MeOH, 9:1). Prep. TLC afforded 30 mg of a mixture of cumambrin A and the new dihydrocumambrin A; the two compounds were separated from each other by HPLC (RP8, MeOH–H_2O (3:2), flow rate 3 ml/min, ca. 100 bar).

Dihydrocumambrin A. Colourless crystals, mp 174°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone) and 1740 (OAc); MS *m/z* (rel. int.): 308.162 $[M]^+$ (2) (calc. for $C_{17}H_{24}O_5$, 308.162), 290 $[M-H_2O]^+$ (6.5), 248 $[M^+-HOAc]^+$ (11), 230 $[M-HOAc-H_2O]^+$ (37), 167 (82), 107 (77), 91 (95) and 81 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 540 \quad 436\ nm}{+44 \quad +46 \quad +49 \quad +73} (CHCl_3;\ c\ 0.08).$$

REFERENCES

1. Haruna, M., Kato, M., Ito, K., Nikai, T., Sugihara, H. and Murata, H. (1981) *Phytochemistry* **20**, 2583.
2. Bloszysk, E., Geppert, B. and Drozdz, B. (1978) *Planta Med.* **34**, 79.
3. Bloszysk, E., Drozdz, B. (1978) *Acta Soc. Bot. Pol.* **47**, 3.
4. Drozdz, B. and Bloszysk, E. (1978) *Planta Med.* **33**, 379.
5. Wrang, P. A. and Jorgen, L. (1975) *Phytochemistry* **14**, 1027.
6. Öksüz, S. and Wagner, H. (1982) *J. Nat. Prod.* **45**, 374.
7. Harborne, J. B., Heywood, V. H. and Saleh, N. A. M. (1970) *Phytochemistry* **9**, 2011.
8. Chatterjee, A., Sarkar, S. and Saha, S. K. (1981) *Phytochemistry* **20**, 1760.
9. Tackholm, V. (1974) *Student's Flora of Egypt*, p. 578. Cairo University Press, Cairo.
10. Bohlmann, F. and Fritz, U. (1979) *Phytochemistry* **18**, 1888.
11. Romo, J., Romo de Vivar, A. and Díaz, E. (1968) *Tetrahedron* **24**, 5625.

Phytochemistry, Vol. 23, No. 12, pp. 2954–2956, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

SESQUITERPENE LACTONES FROM A FURTHER POPULATION OF *ARTEMISIA HERBA ALBA*

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(*Revised received* 12 *June* 1984)

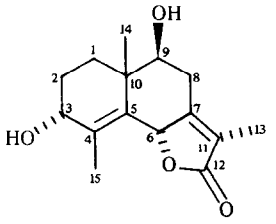
Key Word Index—*Artemisia herba alba*; Compositae; herbolides E and F; germacranolide; eudesmanolide.

Abstract—Two new sesquiterpene lactones, herbolides E and F, have been isolated from a further chemotype of *Artemisia herba alba.*

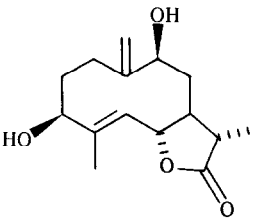
The sesquiterpene lactone constitution of five different populations of *Artemisia herba alba* growing in the Middle East has recently been reported [1–6]. Because of the extensive use of this herb in folk medicine [7, 8], the elucidation of various chemotypes is of great interest. We wish to report now the existence of a further chemotype of *Artemisia herba alba* growing in the Judean desert of Israel, based upon the constitution of its sesquiterpene lactones.

The dichloromethane extract of the inflorescences, small stems and leaves yielded on column chromatography (CC) only two fractions of sesquiterpene lactones, from which herbolide E (**1**) and herbolide F (**2**) were isolated. The structure elucidation of the two novel herbolides was based on extensive use of spectroscopic methods.

Herbolide E, which emerged first on CC, has an empirical formula of $C_{15}H_{22}O_4$, which was established by high-resolution mass spectrometry (HRMS). In its IR spectrum herbolide E showed bands for a hydroxyl function (3400 cm^{-1}) and a γ-lactone group (1760 cm^{-1}). No further carbonyl band, for example for an acetate, was observed. The eudesmanolide structure with two hydroxyl substituents was assigned to herbolide E on the basis of the following data. The 300 MHz ^1H NMR spectrum showed a three-proton singlet at δ1.19 (Me-14), indicating an angular position of the methyl group. The signal of a second methyl group appeared as a narrow doublet at δ2.00 (J = 1.3 Hz, Me-15). The presence of a Δ^4-double bond was deduced from the appearance of the H-6 proton signal as a doublet at δ4.82 (J = 11.5 Hz). Irradiation at the H-7 multiplet (δ2.25) turned this doublet into a singlet. One hydroxyl group was assigned to the 9β-position because of the chemical shift of the H-9 signal (δ3.83) and the similarity of its shape compared with the corresponding H-9 signals in other herbolides [2, 6] and their eudesmanolide derivatives [9]; the total coupling constants of 16 Hz suggested an antiperiplanar orientation of H-9 and H-8β. The other hydroxyl must be in the 3α-position because of its appearance as a narrow multiplet at δ4.02 ($\Delta\nu_{1/2} \approx 8$ Hz) which apparently does not contain an antiperiplanar hydrogen–hydrogen three-



1



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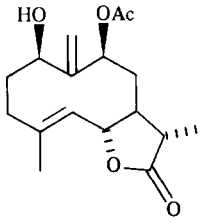

3

Table 1. ^{13}C NMR chemical shifts of herbolides D (3), E (1) and F (2), downfield from internal TMS*

Carbon	1	2	3
1	21.2 *t*	—†	74.8 *d*
2	29.8 *t*	—†	31.4 *t*
3	72.9‡ *d*	75.4‡ *d*	37.8‡ *t*
4	126.9 *s*	—§	145.5 *s*
5	134.3 *s*	122.9 *d*	121.7 *d*
6	81.6 *d*	79.9 *d*	80.4 *d*
7	48.1 *d*	48.5 *d*	51.5 *d*
8	42.3 *t*	42.7 *t*	37.0‡ *t*
9	71.8‡ *d*	75.9‡ *d*	79.3 *d*
10	38.2 *s*	152.0 *s*	153.7 *s*
11	37.9 *d*	40.7 *d*	41.9 *d*
12	179.5 *s*	179.9 *s*	177.7 *s*
13	9.9 *q*	11.8 *q*	12.8 *q*
14	17.4‡ *q*	110.6 *t*	114.7 *t*
15	18.0‡ *q*	10.6 *q*	17.8 *q*

*1 and 3 were recorded in $CDCl_3$, 2 in $(CD_3)_2CO$.

†Overlapped by solvent signals.

‡In the same column, these assignments may be interchanged.

§Not observed.

bond coupling. This assignment was confirmed by the observation of a significant NOE effect at this signal on irradiation of the H-15 protons. This experiment excluded the attachment of the hydroxyl at C-2. A three-proton doublet at δ1.21 (J = 7.5 Hz) belongs to the C-11 methyl group. The ^{13}C NMR data (Table 1) are in full accordance with this structure.

The structure of the second sesquiterpene lactone, herbolide F, was determined as **2** on the basis of the following data. The empirical formula $C_{15}H_{22}O_4$ was deduced from HRMS. The IR spectrum indicated the presence of hydroxyl groups (3400 cm^{-1}) and a γ-lactone ring (1760 cm^{-1}). No further carbonyl band, for an acetate, was observed. In the 300 MHz ^1H NMR spectrum, the narrow three-proton doublet at δ1.64 (J = 1.4, Me-15) turned into a sharp singlet when the H-5 signal at δ4.93, which is partially overlapped with the H-14a signal (δ4.94), was irradiated. In $(CD_3)_2CO$ at 80 MHz these two signals were separated: H-5 at δ5.26 (*d*, J = 10.1 Hz) and H-14a at δ5.13 (*d*, J = 1.8 Hz). The signal of H-14b appeared as a broadened singlet at δ4.84 both in $CDCl_3$ and $(CD_3)_2CO$. The sequence and the shape of these two H-14 signals are analogous to the corresponding ones of herbolide D (**3**) [6]. The presence of the Δ^4-double bond emerged from the simplification of the H-5 signal when either H-15 (δ1.64) or H-6 at δ4.58 (*dd*, both couplings are *ca* 10 Hz) was irradiated. In $CDCl_3$ solution only one further signal in the low-field region could be identified as an unresolved multiplet at δ4.19. In the 80 MHz spectrum in $(CD_3)_2CO$, a well-resolved signal was obtained at δ4.19 (X-part of an ABX sub-spectrum, $\Delta\nu_{1/2}$ = 15–16 Hz). Decoupling of this hydrogen nucleus led to simplification of the H-5 signal so that it was assigned to H-3 in the α-position. The signal of the second hydrogen attached to a hydroxylated carbon (H-9) was found at δ3.88 (again X-part of an ABX sub-spectrum, $\Delta\nu_{1/2}$ = 14–15 Hz). These total coupling constants also suggest an antiperiplanar orientation of the H-9 and H-8β atoms. The existence of an hydroxyl group in the 3β-position was unequivocally indicated by the chemical shift of the C-15 carbon atom (δ10.6). The corresponding value in herbolide D (**3**) is 17.8 [6]. This high-field shift in herbolide F (**2**) is due to the γ-gauche substituent effect of the hydroxyl group on C-15 (cf. Table 1) [10].

EXPERIMENTAL

Stems including leaves and heads (capitulae) of *Artemisia herba alba* were harvested in the Judean desert, 23 km south of Jerusalem and 15 km northeast of Hebron, Voucher No. Eden 15.4.1982. Voucher specimens have been deposited at the Herbarium of the Hebrew University of Jerusalem. The air-dried flowers, leaves and small stems (350 g) were crushed and soaked overnight in petrol; the extract was discarded. The residue was soaked three times overnight in CH_2Cl_2 and filtered. Evapn of the combined filtrates yielded a thick tar (20 g). The tar was separated on a Florisil column, eluting first with CH_2Cl_2 and then with CH_2Cl_2–MeOH (1–4%). The first lactone fraction which emerged from the column (1.2 g) was purified by chromatography on a silica gel column; elution was with *i*-PrOH (6–12%) in petrol. Rechromatography on silica gel with Me_2CO (0.18%) in EtOAc yielded 230 mg crude herbolide E (**1**), which was crystallized from Me_2CO to yield pure herbolide E (60 mg), mp 162–164°; $[\alpha]_D^{22}$ 154° ($CHCl_3$; *c* 0.2); HRMS *m/z* 266.1518 (calc. for $C_{15}H_{22}O_4$: 266.152); MS 70 eV *m/z* (rel. int.): 266 (28) $[M]^+$, 251 (15) $[M-Me]^+$, 248 (40) $[M-H_2O]^+$, 233 (27) $[M-H_2O-Me]^+$. The second more polar lactone fraction (900 mg) was crystallized 2 × from $CHCl_3$ to yield 110 mg pure herbolide F (**2**), mp 179–181°; $[\alpha]_D^{22}$ 198° ($CHCl_3$; *c* 0.5); HRMS *m/z*: 266.1517 (calc. for $C_{15}H_{22}O_4$: 266.152); MS 70 eV *m/z* (rel. int.): 266 (1) $[M]^+$, 251 (1) $[M-Me]^+$, 248 (3) $[M-H_2O]^+$, 233 (2) $[M-Me-H_2O]^+$. All NMR spectra were measured in $CDCl_3$ unless otherwise stated, using Bruker WP-80, WH-90, WM-250 and WH-300 spectrometers; the chemical shifts are reported in ppm relative to internal TMS.

Acknowledgements—R. S. wishes to thank the Alexander-von-Humboldt-Stiftung for a fellowship supporting this research.

H. D. is grateful to the Fonds der Chemischen Industrie for financial support. We thank Professor G. Snatzke for helpful discussions.

REFERENCES

1. Khafagy, S. M., Gharbo, S. A. and Sarg, T. M. (1971) *Planta Med.* **20**, 90.
2. Segal, R., Sokoloff, S., Haran, B., Zaitschek, D. V. and Lichtenberg, D. (1977) *Phytochemistry* **16**, 1237.
3. Zalkow, L. H. and Gordon, M. M. (1979) *J. Nat. Prod.* **42**, 681.
4. Zalkow, L. H., Gordon, M. M., Dickinson, C. and Gelbaum, L. T. (1980) *Planta Med.* **39**, 265.
5. Gordon, M. M., van Derveer, D. and Zalkow, L. M. (1981) *J. Nat. Prod.* **44**, 432.
6. Segal, R., Feuerstein, I., Duddeck, H., Kaiser, M. and Danin, A. (1983) *Phytochemistry* **22**, 129.
7. Taeckholm, V. (1939) *Hortic. Rev. Egypt Hortic. Soc.* **27**, 22.
8. Bailey, C. and Danin, A. (1981) *Econ. Botany* **35**, 145.
9. Sokoloff, S. and Segal, R. (1977) *Tetrahedron* **33**, 2837.
10. Stothers, J. B. (1972) *Carbon-13 NMR Spectroscopy*. Academic Press, New York.

Phytochemistry, Vol. 23, No. 12, pp. 2956–2958, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

9α,15-DIHYDROXYGERMACRA-1(10),4-DIEN-11β,13-DIHYDRO-6α,12-OLIDE, A GERMACRANOLIDE ISOLATED FROM *CENTAUREA ASPERA* SUBSP. *STENOPHYLLA*

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(*Revised received* 4 *June* 1984)

Key Word Index—*Centaurea aspera* subsp. *stenophylla*; Compositae; 11,13-dihydrostenophyllolide; sesquiterpene lactone; germacranolide.

Abstract—A new germacranolide, isolated from *Centaurea aspera* subsp. *stenophylla*, was identified as 9β,15-dihydroxygermacra-1(10),4-dien-11β,13-dihydro-6α,12-olide by spectroscopic evidence and partial synthesis.

INTRODUCTION

Centaurea aspera L. subsp. *stenophylla* (Dufour) Nyman has been examined in our laboratory and we have isolated from the hexane extract α-amyrin, β-amyrin, taraxasterol and lupeol [1] and from the alcoholic extract apigenine, 6-methoxyluteoline, ethyl-7-*O*-apigenin-glucuronate, melitensin, 11,13-dehydromelitensin and 9β,15-dihydroxygermacra-1(10),4,11-trien-6α,12-olide (stenophyllolide) [2]. The alcoholic mother liquors remaining after crystallization of stenophyllolide contained material which displayed an IR absorption of a γ-lactone (1760 cm^{-1}). One of the lactones present was converted into a diacetate and was isolated by chromatography as a crystalline compound and identified as 9β,15-dihydroxygermacra-1(10)-4,dien-11β,13-dihydro-6α,12-olide.

RESULTS AND DISCUSSION

Stenophyllolide, isolated as the major sesquiterpene lactone from *C. aspera* subsp. *stenophylla*, was shown to be the 9β,15-dihydroxygermacra-1(10),4,11-trien-6α,12-olide (**1**) by spectroscopic and X-ray analysis [3]. Stenophyllolide was crystallized from ethanol, and the mother liquors from this crystallization still contained further sesquiterpene lactones which did not crystallize. These lactones were converted into their acetates and the mixture was chromatographed on silica gel, from which hexane–ether eluted a crystalline material identified as 9,15-diacetoxy-1(10)-4-dien-11β,13-dihydro-6α,12-olide (**2b**) on the basis of the following evidence. The molecular formula determined by low- and high-resolution mass spectrometry was $C_{19}H_{26}O_6$. Of the six oxygens, two formed part of a γ-lactone ring, as indicated by an IR absorption at 1760 cm^{-1} [4], and four were acetoxyl groups, as revealed by the IR spectrum (1735 cm^{-1}), the ^1H NMR spectrum which showed two singlets at δ2.05 and 2.00 for 6H (signal of two Me groups and the mass spectral peaks at *m/z* 350 $[M]^+$, 291 $[M-59]^+$ and 232 $[M-2\times59]^+$. Double bonds are placed at $\Delta^{1(10)}$ (since the signal for H-1 at δ5.24 was sharpened by irradiation of H-14) and $\Delta^{4(5)}$ (since the signal for H-5 at δ4.79, was modified by irradiation of H-6). Acetoxyl groups were placed at C-9 (H-9, δ5.12, modified by irradiation of H-8) and C-15 (H-15, δ4.58). There were two methyl groups, one at C-11 (H-13, δ1.27, *d*, for 3H). The unusual upfield position of the last C-10 vinylic methyl may be explained by the reduced anisotropic diamagnetic deshielding of the decadiene ring at the C-10 methyl group; a similar value