ISOLATION AND STRUCTURES OF SESQUITERPENE LACTONES: AERIAL PARTS OF *Arctotis grandis* THUNB. SPECIES*

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Arctolide (*I*), together with seven other sesquiterpene lactones *II*, *IV—IX*, was isolated from aerial parts of *Arctotis grandis* THUNB. species. Structures, including absolute configuration, have been assigned to the isolated compounds. Compounds *VI* and *VIII* were also isolated from *Vernonia angusticeps* EKM.

Arctolide (*I*) was isolated as the main sesquiterpene lactone[1] from aerial parts of *Arctotis grandis* THUNB. species (family *Compositae*, tribe *Arctotae*). The complete revised structure of this lactone was described in our preceding communication[2]. Along with *I* we isolated other sesquiterpene lactones whose structure is the subject of the present work.

The minor sesquiterpene lactones from the aerial part of *A. grandis* were obtained from the so-called lactone fraction (see Experimental) after separation of the major part of arctolide (*I*) by crystallization. The mother liquors were then separated by repeated column chromatography on silica gel.

The least polar compound obtained was noncrystalline 10,14-deoxoarctolide (*II*), $[\alpha]_D^{20}$ +17·9°, of composition $C_{17}H_{20}O_5$. Its infrared spectrum showed the presence of a γ-lactone (1 771 cm^{-1}), an acetate (1 745 and 1 246 cm^{-1}) and a free hydroxyl

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$(3\ 480\ \text{cm}^{-1})$. The mass spectrum exhibited no molecular peak but had characteristic fragments m/z 244 (M − 60) and 226 (M − 60 − 18). In the CD spectra two maxima at 236 nm ($\Delta\varepsilon$ +0·8) and at 211 nm ($\Delta\varepsilon$ +2·8) were observed. The ^{1}H NMR spectrum (Table I) proved the presence of an acetate (δ 2·10 s, 3 H), three exo-methylene groups (δ 6·24, 5·53, 5·40, 5·30 and 5·09 (2 H)) and two protons of the type —CH—O (δ 5·71 ddt and 4·16 dt). Comparison with ^{1}H NMR spectrum of arctolide (*I*) (Table I) showed a striking similarity of δ values, coupling constants J(H, H) as well as TAI-acylation shifts* (the NH signal at δ 8·33 in the TAC-deriva-tive of *II* and the acylation shifts 0·88 ppm for the H-1 and 0·77 ppm for the H-6 proton have proven a tertiary OH group in position 5). These data led to the sug-gested structure *II* which differs from arctolide (*I*) only in that the oxirane ring in position 10(14) is replaced by an exomethylene group. This explains the significant differences in chemical shifts of the neighbouring protons, i.e. H-1, H-9α and H-9β.

  The absolute configuration of lactone *II* was derived from its CD spectrum. The $n \rightarrow \pi^*$ transition maximum of the α,β-unsaturated lactone chromophore (236 nm, $\Delta\varepsilon$ +0·8) and the NMR conclusions (γ-lactone at C-8, *trans*-annelated to a seven--membered homocycle), combined with application of the Geissman empirical rule[5], led to the absolute configuration depicted by formula *II*.
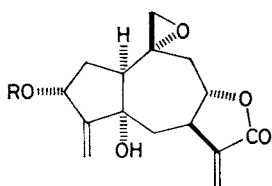
I, R = Ac
IV, R = Prop
V, R = Mebu
IX, R = H
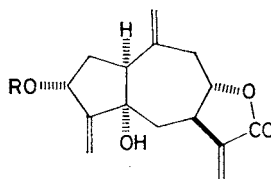
II, R = Ac
III, R = H
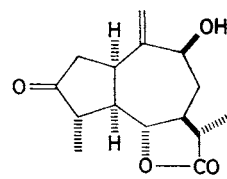
VI

VII

VIII

In formulae *I*−*V*: Ac = acetate; Prop = propionate; Mebu = 2−methylbutanoate

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*   Symbols used: TAI trichloroacetyl isocyanate, TAC trichloroacetylcarbamoyl. For a dis-cussion of the TAI-method see refs[3,4].

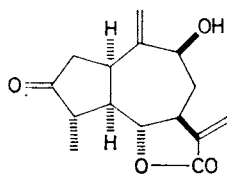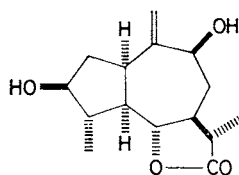Compounds *I* and *II*, with an erroneous configuration of the acetoxy group at C-3 and the oxirane ring in *I* and without absolute configurations, were described by Halim and Zaghloul[6].

In the light of the complete revised structures of arctolide (*I*) and 10,14-deoxy-arctolide (*II*), the configuration at C-3 in deacetyl-10,14-deoxoarctolide (compound *3* in ref.[8]) should be corrected, its absolute configuration being as depicted by formula *III*.

Another isolated lactone, 3-deacetyl-3-propionylarctolide (*IV*), $C_{18}H_{22}O_6$, $[\alpha]_D^{20}$ +46·8°, was also not crystalline. Its IR spectrum showed the presence of a γ-lactone $(1\ 770\ cm^{-1})$, a saturated ester group $(1\ 730\ cm^{-1})$, a double bond $(1\ 670\ cm^{-1})$ and a free hydroxyl $(3\ 525\ cm^{-1})$. In the mass spectrum no molecular peak was present but characteristic fragments m/z 260 (M − 74), 242 (M − 74 − 18), 57 $(C_2H_5CO^+)$ and 29 $(C_2H_5^+)$ were observed. The CD maximum of *IV* was located at 209 nm $(\Delta\varepsilon\ +4\cdot9)$. The ^1H NMR spectrum was almost identical with that of arctolide (*I*) (cf signals of H-1 to H-15 in Table I) and so was its ^{13}C NMR spectrum (cf C-1 to C-15 signals in Table II). The only difference is that the lactone *IV* contains a propionate (^1H NMR: 2·35 q (2 H) and 1·15 t (3 H); ^{13}C NMR: 174·1 (C=O), 27·7 (CH_2), 9·0 (CH_3); for reference data of esters see ref.[7]) instead of acetate. All this evidence leads unequivocally to the structure *IV*.

The assignment of absolute configuration for 3-deacetyl-3-propionylarctolide (*IV*) is discussed at the end of this work.

In the subsequent chromatographic fractions we found arctolide (*I*), $C_{17}H_{20}O_6$, m.p. 142−144°C, $[\alpha]_D^{20}$ +55·6°. Its identity with a standard sample was proven by comparison of IR, NMR and mass spectra and by mixture melting point. The ^1H and ^{13}C NMR data of arctolide (discussed in detail in ref.[2]) are given for comparison in Tables I and II.

Further we isolated noncrystalline 3-deacetyl-3-(2'-methyl)butyrylarctolide (*V*) of composition $C_{20}H_{26}O_6$. According to the IR spectrum the compound contains a γ-lactone $(1\ 770\ cm^{-1})$, an ester $(1\ 731\ cm^{-1})$, a double bond (1 626 and $1\ 670\ cm^{-1})$ and free hydroxyl $(3\ 525\ cm^{-1})$. Its mass spectrum contains no molecular peak but only characteristic fragments m/z 260 (M − 102), 242 (M − 102 − 18), 85 $(C_4H_9CO^+)$ and 57 $(C_4H_9^+)$. The CD spectrum exhibits a maximum at 260 nm $(\Delta\varepsilon\ +0\cdot1)$ and at 205 nm $(\Delta\varepsilon\ +6\cdot4)$. The ^1H and ^{13}C NMR data of *V* (Tables I and II) are also strikingly similar to those for arctolide (*I*) and the propionyl derivative *IV*. We have proven that the only difference exists in the character of the acyl group on C-3 which in compound *V* is 2-methylbutyryl group (^1H NMR: 2·35 m (CO—CH), 1·67 m and 1·48 m (CH_2), 0·91 t and 1·15 d $(2 \times CH_3)$; ^{13}C NMR: 176·2 (C=O), 41·0 (CH), 26·7 (CH_2), 11·6 and 16·6 $(2 \times CH_3)$; for reference NMR data of esters see ref.[7]). Its structure is thus unequivocally expressed by formula *V*.

The absolute configuration of *V* follows from the positive Cotton effect of the $n \rightarrow \pi^*$ transition of its α-methylene-γ-lactone chromophore (260 nm, $\Delta\varepsilon\ +0\cdot1$).

TABLE I

Proton NMR parameters (chemical shifts in ppm, coupling constants in Hz) of C-8 lactones I, II, IV, V, IX and their TAI-acylation shifts in CDCl$_3$

Proton	I		II		IV		V		IX	
				Chemical shifts and multiplicity (TAI-acylation shifts)						
H-1	1·91 bdd	(1·16)	≈2·62 bt	(0·88)	1·91 bdd	(1·16)	1·90 bdd	(1·17)	1·87 dd	(1·26)
H-2α	2·52 ddd	(0·05)	2·57 dt	(0·18)	2·52 ddd	(0·05)	2·52 ddd	(0·05)	2·40 ddd	(0·29)
H-2β	1·51 ddd	(0·01)	≈1·65 m	(0·09)	1·51 ddd	(0·01)	1·56 m	(≈0)	1·47 ddd	(0·24)
H-3	5·65 ddt	(0·04)	5·71 ddt	(0·07)	5·66 ddt	(0·04)	5·66 ddt	(0·04)	4·71 tt	(1·11)
H-6α	2·28 dt	(0·60)	2·12 bd	(0·77)	2·28 dt	(0·63)	2·28 dt	(0·68)	2·28 dt	(0·58)
H-6β	1·88 dd	(0·03)	1·78 dd	(0·03)	1·87 dd	(0·05)	1·88 dd	(0·04)	1·94 dd	(0·03)
H-7	3·40 dtt	(−0·05)	3·06 dtt	(0·10)	3·40 dtt	(−0·03)	3·41 m	(−0·04)	3·40 bt	(−0·03)
H-8	4·27 ddd	(−0·12)	4·16 dt	(−0·19)	4·26 dt	(−0·11)	4·24 dt	(−0·10)	4·30 ddd	(−0·14)
H-9α	2·77 dd	(−0·20)	3·13 ddt	(−0·02)	2·77 dd	(−0·21)	2·79 dd	(−0·20)	2·80 dd	(−0·24)
H-9β	1·87 dd	(0·17)	2·59 dd	(−0·09)	1·87 dd	(0·17)	1·86 dd	(0·19)	1·86 dd	(0·21)
H-13	6·28 d	(0·00)	6·24 d	(0·02)	6·29 d	(0·02)	6·29 d	(0·03)	6·27 d	(0·06)
H-13′	5·62 d	(0·03)	5·53 d	(0·04)	5·62 d	(0·03)	5·62 d	(0·04)	5·60 d	(0·05)
H-14	2·65 d	(0·03)	5·09 m	(0·08)	2·65 d	(0·04)	2·65 d	(0·04)	2·65 d	(0·12)
H-14′	2·62 d	(−0·02)	5·09 m	(0·04)	2·62 d	(−0·02)	2·62 d	(−0·02)	2·63 d	(0·01)
H-15	5·40 d	(0·06)	5·40 d	(0·09)	5·40 d	(0·05)	5·40 d	(0·06)	5·36 d	(0·27)
H-15′	5·27 d	(0·18)	5·30 d	(0·19)	5·27 d	(0·17)	5·25 d	(0·18)	5·24 d	(0·32)
Ester	2·09 s		2·10 s		2·35 q		2·35 m		—	
					1·15 t		1·67 dq			
							1·48 dq			
							0·91 t			
							1·15 d			

H_iH_j	Coupling constants[a]				
1, 2α	7·7	≈8·0	7·8	8·0	7·4
1, 2β	11·8	≈8·0	11·6	11·6	12·3
1, 6	1·2	≈1·0	0·9	1·3	≈0
2α, 2β	13·7	13·5	13·6	13·6	13·3
2α, 3	8·6	8·4	8·4	8·4	8·2
2β, 3	6·5	6·4	6·4	6·4	6·8
3, 15	2·1	2·1	2·0	2·0	2·1
3, 15′	1·8	1·8	1·8	1·8	1·8
6α, 6β	15·0	14·8	14·7	15·2	15·0
6α, 7	1·5	1·5	1·3	1·3	1·6
6β, 7	10·9	10·7	10·8	10·6	10·7
7, 8	9·8	9·8	9·7	9·8	10·0
7, 13	3·5	3·4	3·5	3·5	3·5
7, 13′	3·1	3·1	3·0	3·1	3·1
8, 9α	7·4	7·2	7·4	7·4	7·4
8, 9β	9·4	9·3	9·3	9·4	9·2
9α, 9β	14·6	14·2	14·7	14·9	14·9
14, 14′	4·3	≈0	4·4	4·3	4·4

[a] Absolute values of coupling constants are given only.

Application of the Geissman rule[6] leads to S-configuration at C-7 and R-configuration at C-8. The formula V depicts thus also the correct absolute configuration.

Further chromatographic fractions afforded compound VI, m.p. 134—136°C, $[\alpha]_D^{20}$ +90·6°, of composition $C_{15}H_{20}O_4$. Its IR spectrum proved the presence of a γ-lactone $(1\,777\,cm^{-1})$, a five-membered ketone $(1\,742\,cm^{-1})$, a double bond $(1\,648\,cm^{-1})$ and a free hydroxyl $(3\,490\text{ and }3\,605\,cm^{-1})$. The mass spectrum exhibited a molecular peak m/z 264 and a characteristic fragment 246 $(M-18)$. The compound exhibited one CD maximum at 297 nm $(\Delta\varepsilon\ +1·0)$. In the ^{1}H NMR spectrum (Table III) we detected signals due to two secondary methyl groups (δ 1·24 d, $J=6·8$ Hz and δ 1·30 d, $J=6·9$ Hz), one exomethylene group (δ 5·43 d and 4·83 bs) and two CH—O protons (δ 3·95 dd and 4·23 bdd). The TAI-acylation shifts (0·04 and 1·06 ppm, respectively) of the CH—O protons have shown that the

TABLE II

Carbon-13 chemical shifts of compounds I, $IV-VI$ and $VIII$ in $CDCl_3$

Carbon	I	IV	V	VI	$VIII$
C-1	52·2	52·4	52·5	47·1	48·1
C-2	30·4	30·6	30·7	44·0	38·0
C-3	72·3	72·2	71·9	219·0	78·3
C-4	153·9	154·0	154·1	36·5	39·9
C-5	79·6	79·7	79·9	44·2	46·8
C-6	38·8	39·1	39·1	88·0	85·4
C-7	40·6	40·6	40·6	50·7	50·6
C-8	81·3	81·3	81·3	40·5	40·3
C-9	39·3	39·3	39·4	74·3	74·4
C-10	55·6	55·8	56·0	152·7	153·7
C-11	138·8	138·8	138·7	41·4	41·8
C-12	169·8	169·8	169·7	177·9	^{a}
C-13	120·4	120·5	120·6	13·2	13·1
C-14	50·6	50·9	51·2	109·4	109·7
C-15	111·4	111·7	111·5	13·7	17·5
Ester:					
C-1'	170·6	174·1	176·2	—	—
C-2'	21·1	27·7	41·0	—	—
C-3'	—	9·0	26·7	—	—
C-4'	—	—	11·6	—	—
C-5'	—	—	16·6	—	—

^{a} The signal was not detected probably due to very low intensity.

first belongs to a lactone methine (CH—O—CO) and the second to a hydroxyl methine (CH—OH). The J(H, H) coupling constants were consistent with the guai-10(14)-en-6,12-olide skeleton and with the carbonyl in position 3 (high $^2J(2,2')$ value (19·2 Hz) characteristic of a —CH$_2$—C=O grouping; the fragment —CH$_2$— —C(O)—CH(CH$_3$)— was determined from $^4J(2, 4) = 1·3$ Hz). The relative configurations of protons in positions 1, 4, 5, 6, 7, 9 and 11 were derived from the vicinal coupling constants J(H, H) (Table III) and from analysis of Dreiding models. All these data have shown that the compound is 1αH, 4βH, 5αH, 6βH, 7αH, 11βH-3-oxo- -9β-hydroxyguai-10(14)-en-6,12-olide described by formula *VI*. Its absolute configuration follows from the positive CD maximum at 220 nm which, according to the sector rule[9,10], indicates the S-configuration at C-11. As has already been discussed earlier[11], the sign and intensity of the Cotton effect at 297 nm reflect the absolute configuration of the cyclopentanone part of the molecule. The absolute configuration of the whole molecule of *VI*, based either on the determination of absolute configuration at C-11, and/or the absolute configurations at C-1, C-4 and C-5 is the same. The compound is thus (1*R*,4*S*,5*S*,7*S*,9*S*,11*S*)-3-oxo-9-hydroxyguai-10(14)-en-6,12-olide, depicted by formula *VI*.

The compound *VI* was isolated by us also from the *Vernonia angusticeps* Eкм. species (family *Compositae*, tribe *Vernonieae*). Its isolation and structure (without absolute configuration) has been published in 1980 by Halim and coworkers[12] and the published ^{1}H NMR data agree well with our values, excepting the H-4 and H-5 signals.

Since the substitution with hydroxyl on C-9 in the guaianolide series is relatively rare, we checked the structure of the lactone *VI* using X-ray diffraction analysis[13] which fully confirmed our conclusions obtained from the NMR and CD spectra. The set of vicinal coupling constants J(H, H), interrupted only by the C-3 and C-10 quaternary carbon atoms, allows to compare the conformation of compound *VI* in solution with that in crystal. Table IV presents dihedral angles between the protons, Φ, obtained from the X-ray analysis (in crystal)[13], together with the angles derived from the ^3J(H, H) values using a Karplus-like equation[14] (in solution). On the average, the angles Φ differ by 14°, the maximum difference being 30°. If we estimate that the error in determination of angles Φ from ^3J(H, H) $= f(\Phi)$ is about 10° and assume that the molecule in solution is at least partially flexible, we can say that the preferred conformation of *VI* in solution is very similar to that found[13] in crystal.

Since we have obtained further compounds of this structural type, we suggest that the hitherto hypothetical compound (1*R*,4*R*,5*S*,6*S*,7*S*,9*S*,11*S*)-9-hydroxyguai- -10(14)-en-6,12-olide from which the natural compounds are formally derived, should be named grandolide. This would be along the lines of IUPAC Nomenclature Commission concerning nomenclature rationalization. For the compound *VI* we thus suggest the name 3-oxograndolide, used already in ref.[13].

TABLE III

Proton NMR parameters (chemical shifts in ppm, coupling constants in Hz) of C-6 lactones *VI— VIII* and their TAI-acylation shifts in $CDCl_3$

Proton	VI		VII		VIII	
	Chemical shifts and multiplicity (TAI-acylation shifts)					
H-1	2·97 dt	(0·11)	3·01 m	(0·11)	2·71 bq	(0·18)
H-2α	2·62 dd	(0·05)	2·65 dd	(0·05)	2·06 t	([a])
H-2β	2·48 ddd	(0·03)	2·50 bdd	(0·02)	2·06 t	([a])
H-3	—		—		4·26 dt	(0·92)
H-4	2·32 ddq	(0·01)	2·33 ddq	([a])	2·37 m	(0·31)
H-5	2·16 m	(0·09)	2·26 m	([a])	2·19 dt	([a])
H-6	3·95 dd	(0·04)	3·92 dd	(0·11)	4·06 dd	(0·05)
H-7	2·05 m	(0·07)	2·98 m	(0·08)	1·80 m	(0·11)
H-8α	2·53 ddd	(0·10)	2·70 ddd	(0·09)	2·40 ddd	(0·10)
H-8β	1·35 dt	(0·23)	1·42 dt	(0·21)	1·34 dt	(0·25)
H-9	4·23 bdd	(1·06)	4·32 bdd	(1·06)	4·00 bdd	(1·04)
H-11	2·26 dq	(−0·01)	—		2·29 dq	([a])
H-13	1·24 d	(0·01)	6·32 d	(0·02)	1·25 d	(0·02)
H-13′			5·63 d	(0·01)		
H-14	5·43 d	(−0·02)	5·46 d	(−0·02)	5·53 bs	(−0·02)
H-14′	4·83 s	(0·06)	4·85 bs	(0·06)	5·23 d	(0·04)
H-15	1·30 d	(0·01)	1·28 d	(0·00)	0·97 d	(0·06)
H_iH_j	Coupling constants[b]					
1, 2α	8·2		8·6		[c]	
1, 2β	2·6		2·0		[c]	
1, 5	7·6		7·4		11·4	
2α, 2β	19·2		19·4		[c]	
2β, 4	1·3		1·2		[c]	
3, 4	—		—		5·9	
4, 5	10·8		10·7		6·4	
4, 15	6·8		6·5		7·2	
5, 6	8·4		8·6		10·9	
6, 7	9·6		9·1		9·6	
7, 8α	2·5		2·7		3·0	
7, 8β	11·8		12·0		12·0	
7, 11	11·5		—		11·8	
7, 13	0		3·4		0	
7, 13′	0		3·0		0	
8α, 8β	12·6		12·8		12·2	
8α, 9	5·6		5·7		4·3	
8β, 9	10·6		10·8		10·8	
9, 14	1·3		1·1		$\leqq 1·0$	
11, 13	6·9		—		7·0	

The further obtained compound *VII* $(C_{15}H_{18}O_4)$ melted at 109 − 111°C and had $[\alpha]_D^{20}$ +76·8°. Its IR spectrum proved the presence of a γ-lactone $(1\ 768\ cm^{-1})$, a five-membered ring ketone $(1\ 744\ cm^{-1})$, a double bond $(1\ 647\ and\ 1\ 678\ cm^{-1})$ and a free hydroxyl $(3\ 605\ cm^{-1})$. The mass spectrum displayed a molecular peak, m/z 262, together with a characteristic fragment 244 (M − 18). CD maxima were found at 297 nm $(\Delta\varepsilon\ +3·4)$ and 220 nm $(\Delta\varepsilon\ -6·3)$. The ^1H NMR data, including the TAI-acylation shifts (Table III) are strikingly similar to those of 3-oxograndolide *(VI)*. The only difference is that, instead of one secondary methyl, the spectrum reveals another exomethylene group $(\delta\ 6·32\ d\ and\ 5·63\ d)$. Its presence in position 11(13) follows from the downfield shift of the H-7 signal $(\delta\ 2·98\ in\ VII\ vs\ 2·05\ in VI)$ and from its characteristic allylic coupling with both exomethylene protons. These coupling constants (3·4 and 3·0 Hz), together with the high value of $J(6, 7)$ 9·1 Hz, prove at the same time the *trans*-annelation of the lactone ring[15,16]. Consequently, the compound is 11,13-dehydro-3-oxograndolide and its structure is described by formula *VII*.

The absolute configuration of *VII* was derived from the positive Cotton effect at 297 nm in its CD spectra, similarly as in the case of compound *VI*. The formula *VII* thus also describes the correct absolute configuration.

TABLE IV
Comparison of X-ray and ^1H NMR conformational data of compound *VI*

H_i, H_j	$\Phi_{i,j}$ (X-ray)	$J(H_i, H_j)$, Hz	$\Phi_{i,j}$ (NMR)[a]
1, 2α	32·8	8·2	21
1, 2β	−88·3	2·6	−115
1, 5	−36·5	7·6	−23
4, 5	143·9	10·8	161
5, 6	−166·6	8·4	−152
6, 7	−139·0	9·6	−161
7, 8α	−65·5	2·5	−63
7, 8β	176·3	11·8	165
7, 11	137·2	11·5	167
8α, 9	52·5	5·6	43
8β, 9	170·8	10·6	165

[a] The $\Phi_{i,j}$ values calculated using a Karplus-like equation from ref.[14]. In the cases where *J* value corresponds to two possible angles only one, closer to $\Phi_{i,j}$ (X-ray), is given.

[a] The parameter could not be determined; [b] absolute values of coupling constants are given only; [c] chemical shift equivalence of H-2α and H-2β allows to estimate only the sums $J(2\alpha, 1) + J(2\alpha, 3) \approx J(2\beta, 1) + J(2\beta, 3) \approx 18$ Hz.

Compound *VIII*, m.p. 186−190°C, $[\alpha]_D^{20}$ −11·5°, composition $C_{15}H_{22}O_4$, was the most polar grandolide isolated by us. Its IR spectrum showed the presence of a γ-lactone (1 768 and 1 177 cm^{-1}), a double bond (1 644 cm^{-1}) and a hydroxyl (3 610 and 3 490 cm^{-1}). The mass spectrum contained characteristic fragments m/z 248 (M − 18) and 230 (M − 18 − 18) but no molecular peak. One CD maximum at 218 nm (Δε +0·9) was observed. The ^1H NMR spectrum (Table III) exhibited signals of two secondary methyl groups (doublets at δ 1·25 and 0·97; $J \approx$ 7 Hz) one exomethylene group (δ 5·53 bs and 5·23 d) (similarly to 3-oxograndolide (*VI*)) and three CH—O protons at δ 4·06 dd, 4·00 bdd and 4·26 dt. TAI-acylation afforded a di-TAC-derivative (a two-proton NH signal at δ 8·43), the TAI-acylation shifts of the CH—O protons being 0·05, 1·04 and 0·92 ppm. It was thus possible to assign the signal at δ 4·06 to a lactone CH—O—CO grouping whereas the other two at δ 4·00 and 4·16 (with large TAI-acylation shifts) indicate the presence of two CH—OH groups. A detailed analysis of ^1H NMR data, particularly $^3J(H, H)$ values, led to the proposed structure *VIII*. A conformational analysis of the five-membered ring and determination of configuration of the hydroxyl at C-3 are complicated by the very similar chemical shifts of the H-2 and H-2′, and also H-4 and H-5, proton signals. Structure *VIII* (without absolute configuration) has been ascribed to a compound obtained in 1983 by Halim and coworkers[8]. Their ^1H NMR data (400 MHz) differ somewhat from ours just in the region of the mentioned protons. Comparison of our values of $\sum J(1, 2) + J(1, 2')$, $J(1, 5)$ and $J(4, 5)$ with those for oxograndolides *VI* and *VII* indicates that the five-membered ring in the 3-hydroxy derivative *VIII* exists in a different conformation.

The absolute configuration at C-11 in *VIII* was determined from the positive CD-maximum at 218 nm using the sector rule[6,7], similarly as in the case of *VI*. The absolute configuration of the discussed lactone, 3β-hydroxygrandolide, is thus depicted by formula *VIII*.

The compound *VIII* was isolated, along with 3-oxograndolide (*VI*), also from *Vernonia angusticeps* species.

As the most polar of the native lactones we isolated deacetylarctolide (*IX*), m.p. 150−152°C, $[\alpha]_D^{20}$ +93·5°, composition $C_{15}H_{18}O_5$. Its IR spectrum indicated the presence of a γ-lactone (1 769 cm^{-1}), a double bond (1 668 cm^{-1}) and a hydroxyl (3 605 cm^{-1}). No molecular peak was observed in the mass spectrum, only characteristic fragments m/z 260 (M − 18) and 242 (M − 18 − 18). The CD spectrum displayed a maximum at 205 nm (Δε +11·8). The ^1H NMR spectrum (Table III) was again very similar to that of arctolide (*I*). The only marked difference was the absence of the acetate signal and an upfield shift of the H-3 signal (δ 4·71 for *IX*, compared with 5·65 for *I*). The TAI-acylation proved two hydroxyl groups (NH signals at δ 8·49 and 8·44) and was accompanied by marked acylation shifts of the H-1 (1·26 ppm), H-6α (0·58 ppm) and H-3 (1·11 ppm) protons, proving the presence of a tertiary hydroxyl on C-5 and a secondary hydroxyl on C-3. The $^3J(H, H)$

coupling constants, practically identical with those of arctolide (I), confirmed the deacetylarctolide structure IX.

Neither deacetylarctolide (IX) nor the above-discussed 3-deacetyl-3-propionyl-arctolide (IV) could be assigned absolute configuration in the manner described for I or V because in the CD spectra of IV and IX the Cotton effect due to the $n \to \pi^*$ transition of the α-methylene-γ-lactone chromophore was not discernible. However, the structural relationship, common appearance and the similarity of the CD curves of all the four lactones I, V, IV and IX (Table V) suggest that the absolute configuration of the two latter lactones is very likely expressed by formulae IV and IX.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. Silica gel for column chromatography was prepared according to Pitra and Štěrba (30—60 mµ, deactivated with 11% of water). Thin-layer chromatography was carried out on silica gel G according to Stahl (Merck). HPLC was performed on a Waters HPLC apparatus with an RI-detector. The IR spectra were measured in chloroform, unless stated otherwise, on a spectrophotometer UR 20 (Carl Zeiss, Jena). The mass spectra were measured on an AEI MS 802 spectrometer, optical rotation was determined in methanol on a Perkin–Elmer 141 objective polarimeter. The CD spectra were measured on a Roussel Jouan CD 185 dichrographe, the ORD curves on a Jasco UV-5 spectropolarimeter. The ^{1}H and ^{13}C NMR spectra (at 200 MHz or 50·3 MHz, respectively) were measured on a Varian XL-200 FT-NMR instrument in deuterochloroform, using tetramethylsilane as internal reference. The TAI-acylation shifts were measured on TAC-derivatives prepared by in situ acylation — addition of small excess of TAI to a CDCl$_3$ solution of corresponding hydroxy compound in an NMR sample tube[3,4].

Isolation of Mixture of Sesquiterpenic Lactones

Aerial parts of *Arctotis grandis* THUNB. species (*Compositae, Arctotae*) afforded the so-called lactone fraction from which the greatest part of arctolide (I) was separated by crystallization[1]. The concentrated mother liquors (10·4 g) were chromatographed on a column of silica gel (500 g) and the compounds were eluted with light petroleum containing increasing amount of ethyl acetate. The course of the chromatography is summarized in Table VI.

10,14-Deoxoarctolide (II)

Fraction 2 (Table VI; 0·1 g) was chromatographed on silica gel (10 g) in ethyl acetate–light petroleum (1 : 4) to give noncrystalline II, $[\alpha]_D^{20} +17·9°$ (c 0·016). IR spectrum (cm^{-1}): 1 771 (γ-lactone), 1 745 and 1 246 (acetate) and 3 480 (hydroxyl). Mass spectrum, m/z: 244 (M — 60), 226 (M — 60 — 18). CD spectrum (nm, $\Delta\varepsilon$): 236, +0·8; 211, +2·8. ^{1}H NMR spectrum: see Table I. For C$_{17}$H$_{20}$O$_5$ (304·3) calculated: 67·09% C, 6·62% H, 0·33% H-act.; found: 67·17% C, 6·85% H, 0·43% H-act.

3-Deacetyl-3-(2′-methyl)butyrylarctolide (V)

Chromatography of fraction 3 (Table VI; 200 mg) on a silica gel column (20 g) in light petroleum––ethyl acetate (75 : 25) afforded a mixture of lactones II and V (80 mg); R_F 0·4 (acetone–hexane 1 : 1). This mixture was subjected to semi-preparative HPLC using a pack of 5 columns (8 mm

internal diameter, packed with 10μ Lichrosorb Si 60) in series in triple solvent system (hexane––chloroform–propanol 48 : 48 : 4), flow rate 5 ml/min., pressure 2 560 psi. The chromatography furnished noncrystalline 3-deacetyl-3-(2′-methyl)butyrylarctolide (*V*), composition $C_{20}H_{26}O_6$. Mass spectrum, m/z: 260 (M − 102), 242 (M − 102 − 18), 85 ($C_4H_9CO^+$), 57 ($C_4H_9^+$). IR spectrum (cm^{-1}): la l 770 γ-ctone), 1 731 (ester), 1 626, 1 670 (double bond), 3 525 (hydroxyl). CD spectrum (nm, Δε): 260, +0·1; 205, +6·4. ^{1}H and ^{13}C NMR spectrum: see Tables I and II.

TABLE V
Circular dichroism (CD) and specific optical rotation ($[α]_D^{20}$) data of sesquiterpene lactones *I*, *IV*, *VIII* and *IX*

Compound	CD				$[α]_D^{20}$
	nm	Δε	nm	Δε	
I	206	+6·6	270	+0·1	+64·1°
IV	209	+4·9	—	—	+46·8°
VIII	205	+6·4	260	+0·1	*a*
IX	205	+11·8	—	—	+93·5°

a Not determined (too small amount of sample isolated).

TABLE VI
Chromatography of sesquiterpene lactones from *Arctotis grandis*

Fraction	Eluant	Residue weight, g	Main constituent
	ethylacetate––light petroleum		
1	1 : 4	0·6	
2	1 : 3	0·1	10,14-deoxoarctolide
3	1 : 3	0·2	3-deacetyl-3-(2′-methyl)butyrylarctolide
4	1 : 3	0·7	3-deacetyl-3-propionylarctolide
5	1 : 3	0·3	
6	1 : 3	2·3	arctolide
7	1 : 1	0·5	
8	3 : 2	1·1	3-oxograndolide + 11,13-dehydrograndolide
9	3 : 2	0·1	
10	3 : 2	1·4	3α-hydroxygrandolide + deacetylarctolide
11	3 : 2	0·1	
12	acetone	1·5	

For $C_{20}H_{26}O_6$ (362·4) calculated: 66·28% C, 9·17% H, 0·42% H-act.; found: 66·09% C, 9·33% H, 0·61% H-act.

3-Deacetyl-3-propionylarctolide (IV)

Repeated chromatography of fraction 4 (Table VI; 0·7 g) on silica gel (70 g) in ethyl acetate–light petroleum (3 : 7) afforded 350 mg of noncrystalline 3-deacetyl-3-propionylarctolide (IV), $[\alpha]_D^{20}$ +48·6° (c 0·34). Mass spectrum, m/z: 260 (M − 74), 242 (M − 74 − 18), 57 ($C_2H_5CO^+$), 29 ($C_2H_5^+$). IR spectrum (cm^{-1}): 1 770 (γ-lactone), 1 730 (saturated ester), 1 670, 1 630 inflex (double bond), 3 525 (hydroxyl). CD spectrum (nm, Δε): 209, +4·9. ^{1}H and ^{13}C NMR spectrum: see Tables I and II. For $C_{18}H_{22}O_6^?$(334·4) calculated: 64·64% C, 6·63% H, 0·30% H-act.; found: 64·50% C, 6·79% H, 0·32% H-act.

Arctolide (I)

Crystallization of fraction 6 (Table VI; 2·3 g) afforded arctolide (I; 1·6 g), m.p. 142−144°C (methanol–ether); $[\alpha]_D^{20}$ +55·6° (c 0·33). Its identity with an authentic sample[1] was proven by IR, ^{1}H NMR and mass spectra and by mixture melting point.

3-Oxograndolide (VI) and 11,13-Dehydro-3-oxograndolide (VII)

Chromatography of fraction 8 (Table VI; 1·1 g) on a silica gel column (100 g) afforded a mixture of lactones VI and VII which was separated by repeated column chromatography on silica gel. Elution with ethyl acetate–light petroleum (3 : 7) afforded 3-oxograndolide (65 mg), m.p. 134 to 136°C (ethyl acetate–light petroleum); $[\alpha]_D^{20}$ +90·6° (c 0·37). Mass spectrum, m/z: 264 (M), 246 (M − 18). IR spectrum (cm^{-1}): 1 777 (γ-lactone), 1 742 (ketone in a 5-membered ring), 1 648 (double bond), 3 490, 3 605 (hydroxyl). CD spectrum (nm, Δε): 297, +3·1; 220, +1·0. ORD (nm, Φ): 311, +5 030; 296, ±0; 275, −6 810; a = +134. ^{1}H and ^{13}C NMR spectrum: see Tables II and III. For $C_{15}H_{20}O_4$ (264·3) calculated: 68·16% C, 7·63% H, 0·38% H-act.; found: 68·34% C, 7·91% H, 0·48% H-act. Elution with ethyl acetate–light petroleum (2 : 3) afforded 11,13-dehydro-3-oxograndolide (216 mg), m.p. 109−111°C (ethyl acetate–light petroleum); $[\alpha]_D^{20}$ +76·8° (c 0·38). Mass spectrum, m/z: 262 (M). IR spectrum (cm^{-1}): 1 768 (γ-lactone), 1 744 (ketone in a 5-membered ring), 1 647, 1 678 (double bond), 3 496, 3 605 (hydroxyl). CD spectrum (nm, Δε): 297, +3·3; 220, −6·3. ORD (nm, Φ): 311, +6 570; 298, ±0; 275, −9 110; a = +157. ^{1}H NMR spectrum: see Table III. For $C_{15}H_{18}O_4$ (262·3) calculated: 68·68% C, 6·92% H, 0·38% H-act.; found: 68·32% C, 6·91% H, 0·35% H-act.

3α-Hydroxygrandolide (VIII) and Deacetylarctolide (IX)

Fraction 10 (Table VI; 1·36 g) was chromatographed on a silica gel column (130 g) in ethyl acetate–light petroleum (3 : 2) to give two fractions. Repeated chromatography of the first fraction on a silica gel column afforded 3α-hydroxygrandolide (VIII; 78 mg), m.p. 186−190°C, $[\alpha]_D^{20}$ −11·5° (c 0·39). Mass spectrum, m/z: 266 (M), 248 (M − 18), 230 (M − 18 − 18). IR spectrum (cm^{-1}): 1 768, 1 177 (γ-lactone), 1 644 (double bond), 3 610, 3 490 (hydroxyl). CD spectrum (nm, Δε): 218 +0·9. ^{1}H and ^{13}C NMR spectrum: see Tables II and III. For $C_{15}H_{22}O_4$ (266·3) calculated: 67·64% C, 8·33% H, 0·76% H-act.; found: 67·61% C, 8·34% H, 0·66% H-act. The second fraction on crystallization afforded deacetylarctolide (IX; 371 mg), m.p. 150−152°C (ethyl acetate); $[\alpha]_D^{20}$ +93·5° (c 0·39). Mass spectrum, m/z: 260 (M − 18), 242 (M − 18 − 18). IR spectrum (cm^{-1}): 1 769 (γ-lactone), 1 668 (double bond), 3 605 (hydroxyl). CD spectrum (nm, Δε): 205, +11·8. ^{1}H NMR spectrum: see Table I. For $C_{15}H_{18}O_5$ (278·3) calculated: 64·73% C, 6·52% H, 0·72% H-act.; found: 64·45% C, 6·46% H, 0·63% H-act.

3-Oxograndolide (*VI*) and 3α-Hydroxygrandolide (*VIII*) from *Vernonia angusticeps*

Dried ground aerial parts of *Vernonia angusticeps* EKM species (900 g; collected in April 1980 at Gran Piedra, Santiago de Cuba, Cuba; voucher HAJB 41 577 deposited in the herbarium of National Botanical Garden in Havana, Cuba) were extracted successively with light petroleum and chloroform at ambient temperature. The chloroform extract (15 g) was chromatographed on a column of silica gel (400 g) in toluene, containing increasing amount of ether and then with ether with increasing amount of ethyl acetate. Toluene with 40% of ether eluted 3-oxograndolide (*VI*; 1·5 g), m.p. 139—143°C (ethyl acetate); $[\alpha]_D^{20}$ +93·0° (*c* 0·35). CD spectrum (nm, $\Delta\varepsilon$): 297, +3·7; 245, +0·1; 219, +1·2; 209, ±0. ^{1}H NMR, mass and IR spectra were identical with those of 3-oxograndolide (*VI*), isolated from *Arctotis grandis*; no mixture melting point depression. 3α-Hydroxygrandolide (*VIII*; 2·1 g) was eluted with ether–ethyl acetate (1 : 1); m.p. 162—165°C (acetone–diisopropyl ether), $[\alpha]_D^{20}$ +6·5° (*c* 0·42). ^{1}H NMR, mass, IR and CD spectra were identical with those of 3α-hydroxygrandolide (*VIII*), isolated from *Arctotis grandis*, no mixture melting point depression.

REFERENCES

1. Samek Z., Holub M., Drożdż B., Grabarczyk H.: Collect. Czech. Chem. Commun. *42*, 2117 (1977).
2. Buděšínský M., Šaman D., Pelnař J., Vašíčková S., Drożdż B., Grabarczyk H., Le Viet Ngoc-Phuong, Holub M.: Collect. Czech. Chem. Commun. *54*, 166 (1989).
3. Goodlett V. W.: Anal. Chem. *37*, 431 (1965).
4. Samek Z., Buděšínský M.: Collect. Czech. Chem. Commun. *44*, 558 (1979).
5. Stöcklin W., Waddell T. G., Geissman T. A.: Tetrahedron *26*, 2397 (1970).
6. Halim A. F., Zaghloul A. M.: Planta Med. *38*, 183 (1980).
7. Buděšínský M., Šaman D.: Collect. Czech. Chem. Commun. *52*, 453 (1987).
8. Halim A. F., Zaghloul A. M., Zdero Ch., Bohlmann F.: Phytochemistry *22*, 1510 (1983).
9. Jennings J. P., Klyne W., Scopes P. M.: J. Chem. Soc. *1965*, 7211, 8229.
10. Snatzke G., Ripperger H., Horstmann C., Schreiber K.: Tetrahedron *22*, 3103 (1966).
11. Samek Z., Holub M., Vokáč K., Drożdż B., Jommi G., Gariboldi P., Corbella A.: Collect. Czech. Chem. Commun. *37*, 2611 (1972).
12. Halim A. F., Zaghloul A. M., Bohlmann F.: Phytochemistry *19*, 2767 (1980).
13. Rychlewska U.: Acta Crystallogr., C *41*, 540 (1985).
14. Buděšínský M., Trnka T., Černý M.: Collect. Czech. Chem. Commun. *44*, 1949 (1979).
15. Samek Z.: Collect. Czech. Chem. Commun. *43*, 3210 (1978).
16. Samek Z., Harmatha J.: Collect. Czech. Chem. Commun. *43*, 2779 (1978).

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