ON TERPENES. CCXXVII.*

THE STRUCTURE OF THE SESQUITERPENIC TRIESTER LACTONE TRILOBOLIDE

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For trilobolide from the root of *Laser trilobum* (L.) BORKH. (*Umbelliferae*) the structure represented by formula *I* was deduced on the basis of chemical modification and degradation of the native substance and on the basis of spectroscopic measurements, mainly a detailed analysis of the PMR spectra of trilobolide and some of its derivatives.

In a preceding paper[1] we described the isolation of trilobolide (*I*) – a component of the root of *Laser trilobum* (L.) BORKH. (*Umbelliferae*) – and also presented some basic structural aspects following from its IR spectrum and elemental analysis. We also investigated in greater detail the structure of trilobolide and we proposed in a preliminary communication[2] a hypothetic structure, represented by formula *Ia*. In this paper we present all the facts which led to the deduction of the structure represented by formula *I*, for the trilobolide molecule.

As was mentioned earlier[1] the molecule of trilobolide (*I*) has a composition corresponding to the formula $C_{27}H_{38}O_{10}$ and according to its IR spectrum it contains a γ-lactone group, a saturated ester group, and α,β-unsaturated ester group, an acetate group, a double bond and a hydroxy group. According to active hydrogen determination[1] the molecule of trilobolide (*I*) contains two free hydroxy groups. Elemental composition of trilobolide (*I*) was confirmed by the analysis of its mass and PMR spectrum. In its mass spectrum the peak with the highest mass was at *m/e* 462, which corresponded to the molecular weight lower by 60 units than would correspond to the mentioned elemental composition. This difference may be explained by the elimination of acetic acid, which is common in similar cases[3,4] and also probable in view of the presence of an O-acetyl group, evidenced on the basis of IR and PMR spectra (singlet, 1·94 p.p.m.). The mass spectrum of trilobolide (*I*) displayed further fragments of *m/e* 362 (522−60−100) and *m/e* 100, 83 and 55, indicating the presence of an unsaturated C_5-acid residue, and fragments of *m/e* 360 (522−60−102) and *m/e* 57

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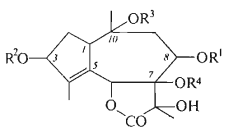
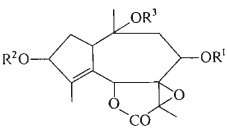


I $R^1 = CO.CH(CH_3).C_2H_5$
$R^2 = CO.C(CH_3)=CH.CH_3$
$R^3 = CO.CH_3$
$R^4 = H$

Ia, $R^1 = CO.CH(CH_3).C_2H_5$
$R^2 = CO.C(CH_3)=CH.CH_3$
$R^3 = H$
$R^4 = CO.CH_3$

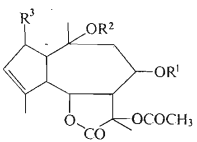


VIII, $R^1 = CO.CH(CH_3).C_2H_5$
$R^2 = CO.C(CH_3)=CH.CH_3$
$R^3 = CO.CH_3$

IX, $R^1 = CO.CH(CH_3).C_2H_5$
$R^2 = CO.C(CH_3)=CH.CH_3$
$R^3 = H$

X, $R^1, R^3 = H$
$R^2 = CO.C(CH_3)=CH.CH_3$

XI, $R^2 = CO.C(CH_3).C_2H_5$
$R^1, R^3 = H$



VI, $R^1 = CO.CH=C(CH_3)_2$
$R^2, R^3 = H$

XIII, $R^1 = CO.C(CH_3)=CH.CH_3$
$R^2, R^3 = H$

XVI, $R^1 = CO.CH(CH_3).C_2H_5$
$R^2 = CO.CH_3$
$R^3 = O.CO.C(CH_3)=CH.CH_3$

and 85 indicating the presence of a saturated C_5-acid residue and a fragment of *m/e* 260 corresponding to the elimination of all three ester residues (522 – 60 – 100 – 102). The presence of these ester groups in the molecule of trilobolide (*I*) was also corroborated by its PMR spectrum (Table I); from its detailed analysis based on decoupling experiments it further followed that the unsaturated C_5-acid is angelic acid (β-H: 6·09 p.p.m.; qq) and the saturated C_5-acid is α-methylbutyric acid (α-H: 2·50 p.p.m.; sx; α-CH_3: 1·12 p.p.m.; d; $J \approx 7$ Hz; β-CH_3: 0·89 p.p.m.; t; $J \approx 7$ Hz. The presence of two free hydroxy groups was also confirmed by PMR spectrum of trilobolide (*I*) in hexadeuteriodimethyl sulfoxide, which displayed signals of hydroxyl protons as two singlets at 5·71 and 5·81 p.p.m. (confirmed by exchange experiment). The presence of these singlets further indicated the tertiary character of both hydroxy groups, which was further corroborated by the PMR spectrum of trilobolide (*I*) measured in deuteriochloroform solution with addition of trichloroacetyl isocyanate (TAI-method, ref.[5,6]).

The carbon skeleton of trilobolide (*I*) was determined in the following manner: Hydrogenation of trilobolide (*I*) in acetic acid gave substance *II* of the composition $C_{22}H_{34}O_8$ the mass spectrum of which contained a pseudomolecular peak at *m/e* 366

(426–60). From the IR and the PMR spectrum (Table I) of compound *II* it followed that the mentioned substance is a diester lactone-diol and that therefore the hydrogenation took place under simultaneous hydrogenolysis of one of the ester residue of the C_5-acids present in the molecule of the native compound *I*. From the formation of *II* it followed that the basic carbon skeleton of trilobolide was bicyclic and that it contained one double bond. On reduction of substance *II* with lithium aluminum hydride we obtained a product which was further dehydrogenated with selenium without previous purification, and we isolated from the reaction mixture chamazulene (*III*) (characterized as trinitrobenzenate) and identified in it artemazulene (*IV*) by paper chromatography. The formation of azulenes *III* and *IV* indicated that the bicyclic skeleton of trilobolide is of the guaiane type.

The nature of the side chains of the guaiane skeleton of trilobolide (*I*) followed from its PMR spectrum which contained in addition to the mentioned methyl groups signals of the ester groups also the signals of two tertiary methyl groups and of one methyl on a double bond (Table I). The position of the double bond and the distribution of the oxygen functions was inferred from the following facts. The PMR spectrum of trilobolide (*I*) (Table I) displayed the presence of four signals of isolated methine protons which according to chemical shifts could have been of the —C<u>H</u>—OR, —C<u>H</u>—C=, or =C<u>H</u>— type. Three of these protons displayed according to detailed DR-experiments long-range couplings with the protons of the methyl group on the double bond; two of them were simultaneously coupled to the two mutually coupled protons the signals of which may be assigned on the basis of their chemical shifts only to the methylene group of the C—C<u>H</u>$_2$—C type. This topological continuity of couplings is consistent in the case of the guaiane skeleton only under the supposition of the presence of a tetrasubstituted double bond between $C_{(4)}$ and $C_{(5)}$. Three of the mentioned methine protons must therefore be of the C<u>H</u>—OR type, while one of them is bound to $C_{(3)}$, the second to $C_{(6)}$, and the third to $C_{(8)}$ or $C_{(9)}$. The fourth proton must be on $C_{(1)}$. Hence, of the total four esterified hydroxy groups in the trilobolide molecule (*I*) three are secondary and the fourth tertiary. Therefore the three tertiary oxygen functions of trilobolide (*I*) – one of the —O.CO.R type and two free hydroxy groups – are located at $C_{(7)}$, $C_{(11)}$ and $C_{(10)}$. The presence of the tertiary carbon in the position 7 is also in agreement with the absence of a typical topological continuity of vicinal couplings of protons in the fragments —$C_{(6)}$H—$C_{(7)}$H—$C_{(8)}$H—, common in the molecules of sesquiterpenic lactones.

From all these aspects, and taking into account the fact that the hydrogenolysable residue of the C_5-acid must be located at $C_{(3)}$ or $C_{(6)}$, three basic structural possibilities for the distribution of oxygen functions on this guaiane skeleton followed for the molecule of trilobolide (*I*), as represented by formulae *Ib*, *Ic*, and *Id*.

In agreement with these alternatives the PMR spectrum of the diester lactone diol *II* (100 MHz; hexadeuteriodimethyl sulfoxide) also displayed signals of only two methine protons of the —C<u>H</u>—OR type. One of them gave a triplet at 5·41 p.p.m.

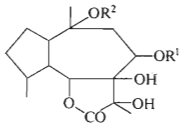
TABLE I

Characteristic Parameters of PMR Spectra of Trilobolide (*I*) and Its Derivatives

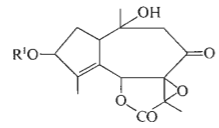
Compound[a] Solvent[b]	x $H_{(1)}$	$H_{(2)}$	$H_{(3)}$	$H_{(6)}$	$H_{(8)}$	$H_{(9)}$	$H_{(9')}$	$H_{(13)}$	$H_{(14)}$	$H_{(15)}$	Other protons[c]
	4·41	2·45[e]	5·59	5·64	5·53	3·06	2·12	1·30[f]	1·33[f]	1·88	OH: 5·71
I[d]	(4·40)		(5·55)	(5·65)	(5·65)	(3·17)		(1·47)	(1·29)		(s, 1 H), 5·83
A(B)	$J_{1,6} \neq 0$		$J_{1,15} \neq 0$	$J_{6,1} \neq 0$	$J_{8,9} = 4$	$J_{9,8} = 4$	$J_{9',8} = 4$			$J_{15,1} \neq 0$	(s, 1 H)
	$J_{1,15} \neq 0$			$J_{6,15} \neq 0$	$J_{8,9'} = 4$	$J_{9,9'} = 15$				$J_{15,6} \neq 0$	$H_{(2')}$: 1·65
										$J_{15,3} \neq 0$	OAc: 1·94
				4·94	5·41	2·86	2·17	1·25[i]	1·47[i]	0·96	OAc: 1·88
II[g,h]	(3·26)			(4·97)	(5·49)	(2·92)	(2·04)	(1·53)	(1·41)	(1·06)	$H_{(4)}$: (2·38)
A(B)				$J_{6,5} = 11$	$J_{8,9} = 4·5$	$J_{9,8} = 5$	$J_{9',8} = 4$			$J_{15,4} = 6·9$	
					$J_{8,9'} = 4·5$	$J_{9,9'} = 15·5$	$J_{9,9'} = 15·5$				
	3·95	2·45	5·55	5·32	5·01	2·79	2·45	1·57[f]	1·35[f]	1·82	OAc: 1·96
	(3·94)	(2·63)	(5·59)	(5·20)	(5·00)	(2·76)	(2·56)	(1·62)	(1·39)	(1·85)	
VIII[h]	$J_{1,6} \neq 0$	$J_{2,1} = 8·5$	$J_{3,2} = 8·5$	$J_{6,1} \neq 0$	$J_{8,9} = 3·7$	$J_{9,8} = 4$	$J_{9',8} = 4$			$J_{15,1} = 2·15$	
A(B)	$J_{1,15} \neq 0$	$J_{2,3} = 8·5$	$J_{3,2'} = 3·0$	$J_{6,15} \neq 0$	$J_{8,9'} = 3·7$	$J_{9,9'} = 14·5$	$J_{9,9'} = 14·5$			$J_{15,6} = 1·25$	
		$J_{2,2'} = 14·7$	$J_{3,15} \neq 0$							$J_{15,3} = 0·8$	
	3·10		5·48	5·40	4·95			1·52[f]	1·05[f]	1·77	
	(3·25)	(2·65)	(5·58)	(5·23)	(4·96)	(2·77)	(2·08)	(1·62)	(1·20)	(1·83)	
IX[h]		$J_{2,1} = 8·5$	$J_{3,2} = 8$		$J_{8,9} = 3·5$	$J_{9,8} = 3·5$	$J_{9',8} = 3·5$				
A(B)		$J_{2,3} = 8·5$			$J_{8,9'} = 3·5$	$J_{9,9'} = 15$	$J_{9',9} = 15$				
		$J_{2,2'} = 15$									
	3·06		5·46	5·50	3·78	2·10	1·90[e]	1·50[f]	1·08[f]	1·77	
X	$J_{1,15} \neq 0$		$J_{3,15} \neq 0$	$J_{6,15} \neq 0$	$J_{8,9} = 3·5$	$J_{9,8} = 3·5$				$J_{15,3} \neq 0$	
A					$J_{8,9'} = 3·5$	$J_{9,9'} = 14·5$				$J_{15,6} \neq 0$	
										$J_{15,1} = 2·0$	

	3·06	2·42	5·41	5·48	3·79	2·12	1·82[e]	1·50[f]	1·08[f]	1·73	$H_{(2')}$: 1·78
XI	$J_{1,15} \neq 0$		$J_{3,2} = 8{\cdot}5$	$J_{6,15} \neq 0$	$J_{8,9} = 3{\cdot}5$	$J_{9,8} = 3{\cdot}5$				$J_{15,1} = 1{\cdot}85$	$J_{2',1} = 4{\cdot}5$
A			$J_{3,2'} = 3$		$J_{8,9'} = 3{\cdot}5$	$J_{9,9'} = 14{\cdot}5$				$J_{15,3} \neq 0$	$J_{2',2} = 15$
			$J_{3,15} \neq 0$							$J_{15,6} \neq 0$	
	3·20		5·47	5·65		1·92	1·92	1·64[f]	1·10[f]	1·79	$H_{(2')}$: (2·02)
	(3·52)	(2·48)	(5·40)	(5·02)		(3·19)	(2·84)	(1·54)	(1·13)	(1·72)	$J_{2',1} = 3{\cdot}5$
XII[j]	$J_{1,14} \neq 0$	$J_{2,1} \neq 8{\cdot}5$	$J_{3,2} = 8{\cdot}5$	$J_{6,15} = 1{\cdot}6$		$J_{9,9'} = 13$	$J_{9',9} = 13$			$J_{15,1} = 1{\cdot}8$	
A(C)	$J_{1,15} \neq 0$	$J_{2,3} = 9{\cdot}0$	$J_{3,15} \neq 0$			$J_{9,14} \neq 0$				$J_{15,6} = 1{\cdot}6$	$J_{2',3} = 3{\cdot}5$
		$J_{2,2'} = 15{\cdot}5$								$J_{15,3} \neq 0$	
	2·93			4·44	4·99	2·78	1·87	1·59[i]	1·45[i]	1·02	OAc: 2·05
XIVa[k]				$J_{6,5} = 11{\cdot}5$	$J_{8,9} = 7{\cdot}2$	$J_{9,8} = 7{\cdot}5$	$J_{9',8} = 9{\cdot}5$			$J_{15,4} = 6{\cdot}8$	$H_{(4)}$: 2·37
B					$J_{8,9'} = 9{\cdot}4$	$J_{9,9'} = 15$	$J_{9',9} = 15$				$H_{(5)}$: 2·01
	2·94			4·59	4·95	2·78	1·90	1·51[i]	1·42[i]	1·10	OAc: 2·03
XIVb[l]				$J_{6,5} = 10{\cdot}8$	$J_{8,9} = 7{\cdot}5$	$J_{9,8} = 7{\cdot}5$	$J_{9',8} = 9$			$J_{15,4} = 6{\cdot}5$	$H_{(5)}$: 2·30
B					$J_{8,9'} = 9$	$J_{9,9'} = 14{\cdot}5$	$J_{9,9'} = 14{\cdot}5$				
				4·68	5·37			1·51[i]	1·52[i]	1·05	OAc: 1·92
XV				$J_{6,5} = 8{\cdot}5$	$J_{8,9} = 6$					$J_{15,4} = 6{\cdot}8$	
A					$J_{8,9'} = 6$						
V[d,m]				4·94	4·16			1·32[i]	1·22[i]	1·08	$H'_{(6)}$: 4·71
A				$J_{6,5} = 10$							$J_{6',5} = 10$
VII[d,m]				4·87				1·44[i]	1·20[i]	1·16	$H'_{(6)}$: 4·75
A				$J_{6,5} = 11$							$J_{6',5'} = 10{\cdot}5$

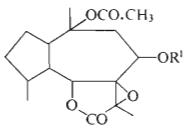
[a] All data from first-order analysis of single and multiple resonance spectra; chemical shifts are given in δ(TMS)-scale, splittings in Hz. [b] A hexadeuteriodimethyl sulfoxide, B deuteriochloroform, C hexadeuteriobenzene; in cases where two solvents are used the second solvent and the corresponding chemical shifts are given in parentheses. [c] Data for O-angelyl and O-α-methylbutyryl groups of trilobolide are: 6·09 qq (β-H); 1·12 d, ($J \cong 7$, α-CH_3), 0·89 t ($J \cong 7$, β-CH_3) and ~2·50 (α-H). In other cases these groups are always easily indicated by the corresponding signals in nearly the same positions. [d] splittings from solvent A; [e] approximate values from decoupling experiments; [f] half-line width relation $W_{14} > W_{13}$; [g] mixture of epimers; [h] splittings from solvent B; [i] tentative assignment; [j] splittings from solvent C; [k] data from the PMR spectrum of the mixture of *XIVa* and *XIVb* containing *XIVa* as the major component; prepared from *VIII*; [l] data from the PMR spectrum of the mixture of *XIVa* and *XIVb* containing *XIVb* as the major component; prepared from *II*; [m] data for major components.



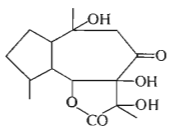
II, $R^1 = CO.CH(CH_3).C_2H_5$
$R^2 = CO.CH_3$
V, $R^1 = R^2 = H$



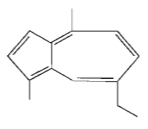
XIV, *XV*; $R^1 = CO.CH(CH_3).C_2H_5$



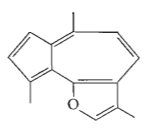
XII, $R^1 = CO.CH(CH_3)C_2H_5$



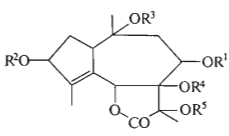
VII



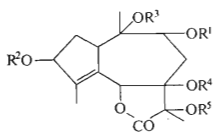
III



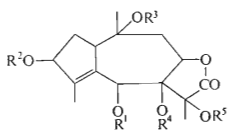
IV



Ib



Ic



Id

Ib, *Ic*, *Id*; $R^1 = CO.CH_3$ or $CO.C_4H_7$ or $CO.C_4H_9$,
$R^2 = CO.C_4H_7$ or $CO.C_4H_9$; R^3, R^4, $R^5 = CO.CH_3$ or H

($\Sigma J \approx 7$ Hz) and the second a doublet at 4·94 p.p.m. ($J = 11{\cdot}5$ Hz). The mentioned doublet can only be assigned to proton $H_{(6)}$, from which it follows that the hydrogenolysable residue of the C_5-acid is bound to $C_{(3)}$. On saponification of substance *II* (hydrolysis of trilobolide under the usual conditions did not lead to well defined

products) we obtained the saturated lactone tetrol *V* of the composition $C_{15}H_{24}O_6$ the PMR spectrum of which (Table I) displayed the presence of a methine proton signal of the —C<u>H</u>—OR type, as well as a doublet at 4·94 p.p.m. ($J \cong 10$ Hz) which can be assigned to the proton $H_{(6)}$, similarly as in the case of *II*. The invariability of the signal of proton $H_{(6)}$ in the PMR-spectra of *II* and *V* indicates that this proton is bound to the carbon carrying the closure of the lactone ring, in the sense of alternatives *Ib* and *Ic*. Oxidizing lactone tetrol *V* we obtained ketolactone triol *VII* of the composition $C_{15}H_{22}O_6$ the IR spectrum of which contained an oxo group frequency at 1 700 cm^{-1} and confirmed thus the presence of one secondary hydroxy group bound to a seven-membered ring. The location of the ester groups was further determined in the following manner. On reaction with thionyl chloride trilobolide (*I*) gave almost quantitatively substance *VIII* of the composition $C_{27}H_{36}O_9$ the IR spectrum and the PMR of which proved that this substance originated from trilobolide (*I*) by intercyclisation of both free tertiary hydroxyls, under formation of an ether cycle. On saponification of this anhydrotrilobolide *VIII* we obtained in contrast to trilobolide hydrolysis well defined products, *i.e.* the deacetyl derivative *IX* of the composition $C_{25}H_{34}O_8$ and the monoester *X* of the composition $C_{20}H_{26}O_7$. From the comparison of the PMR spectra of these substances the tertiary character of the acetyl group and the location of the α-methylbutyric acid residue at $C_{(8)}$ or $C_{(9)}$ and of the angelic acid residue at $C_{(3)}$ followed unambiguously. Hydrogenation of monoester *X* gave rise to the saturated monoester *XI* which was transformed on oxidation to esterlactone *XII* of the composition $C_{20}H_{26}O_7$. In hexadeuteriobenzene this substance afforded a suitable PMR spectrum which could be assigned on the basis of DR-experiments (Table I). These experiments demonstrated a long-range coupling between the low-field proton of the isolated methylene group of the

—C—C<u>H</u>$_2$—CO— type and the protons of the tertiary methyl group located on $C_{(10)}$

(the assignment of the signals of these methyl protons was simultaneously confirmed by long-range coupling with the proton $H_{(1)}$). From these experiments the position of the keto group in the molecule of substance *XII* followed, and hence also the position of the α-methylbutyric acid residue in the molecule of trilobolide on $C_{(8)}$, in the sense of the alternative *Ib*.

The position of the tertiary acetyl group, and hence also the position of the two free tertiary hydroxyl groups in the molecule of trilobolide (*I*) has also been determined by PMR spectroscopy. We studied the problem of the structural assignment of tertiary methyl groups in sesquiterpenic lactones of the type $CH_3—C_{(i)}R_1R_2—OR$ ("i" indicates the position in the conventional numbering of sesquiterpenic skeletons; $R_k(i)$ are the vicinal groups). We used this method recently in connection with the structure study of montanolide (*VI*) and isomontanolide[3] (*XIII*). We found that the signals of the tertiary methyl groups bound to homocyclic systems display to a certain extent a cha-

racteristic dependence of the paramagnetic acetylation shift $\Delta^{i}\delta CH_3(R) = \delta CH_3-C_{(i)}-OH - \delta CH_3-C_{(i)}-OR < 0$ ($R = CO.CH_3$) on the structural character of the vicinal groups R_k of the $C_{(i)}$ atom. For methyl groups of type *A* with i(A) = 4,10 and $R_k(A) = CH, CH_2$ (homocyclic system) these shifts are relatively large (in $CDCl_3$ $\Delta^{A}\delta CH_3(Ac) = 0{\cdot}2-0{\cdot}4$ p.p.m.), while for methyls of type *B* with i(B) = 11 and $R_1 = CH$, $R_2 = -CO-O$ (γ-lactone ring) they are smaller (in $CDCl_3$ $\Delta^{B}\delta CH_3(Ac) = 0{\cdot}05-0{\cdot}2$ p.p.m.). Acetylation in the position i(B) does not practically affect the chemical shift of the methyl group in i(A) and *vice versa*. For practical purposes the measurement of paramagnetic acylation shifts by means of in situ acylation with trichloroacetyl isocyanate (TAI) in deuteriochloroform solutions was found to be very advantageous (TAI-method[6]; $R = -CO.NH.COCCl_3$). We found[7,8] that $\Delta\delta CH_3(TAC)$ are for both types, *A* and *B*, larger (by approximately 0·10 p.p.m.) than the corresponding $\Delta\delta CH_3(Ac)$. In some cases we also observed that both types of hydroxyls, *A* and *B*, also differ in their reactivity toward trichloroacetyl isocyanate. In the case of types *A* with $R_k(A) = CH, CH_2$ the reaction took place very rapidly, practically during the time necessary for the manipulation (2 to 3 min), while in the case of types *B* we observed in a number of cases a slow course lasting up to several hours[8], in dependence on the excess of TAI. We also studied these effects with trilobolide (*I*) and deacetylanhydrotrilobolide *IX*. With trilobolide we observed the formation of monotrichloroacetyl carbamate only after several days standing, while in the case of substance *IX* the trichloroacetyl carbamate (TAC) was formed immediately. The obtained values of $\Delta\delta CH_3(TAC)$ are listed in Table II. The low value of $\Delta\delta(TAC) = -0{\cdot}26$ of the variable signal, as well as the low reactivity of the corresponding hydroxyl indicate the type *B*, while $\Delta\delta CH_3(TAC) = -0{\cdot}33$ and $\Delta\delta CH_3(Ac) = -0{\cdot}19$ of the variable signal in the case of deacetylanhydrotrilobolide *IX* and the high reactivity of the corresponding hydroxyl indicate the type *A*. On the basis of the mentioned effects it may be supposed with great probability that the O-acetyl group is bound in trilobolide to $C_{(10)}$ and hence, that the free tertiary hydroxyl groups in this compound are located at $C_{(7)}$ and $C_{(11)}$. Therefore, in anhydrotrilobolide (*VIII*) the epoxide ring bound to atoms $C_{(7)}$ and $C_{(11)}$ is present. In agreement with these conclusions are also the acylation shifts of the $H_{(1)}$ proton which are also listed in Table II. These effects on the chemical shift of proton $H_{(1)}$ correspond well to the known β-acylation shifts[9] and their interpretation from the point of view of transannular effects in alternative cases of the positions of the O-acetyl group at $C_{(7)}$ or $C_{(11)}$ in trilobolide and especially in compound *VIII* is not evident. The interpretation of the solvent effects which they have on the chemical shift of the signals of tertiary methyl groups of the $CH_3-\overset{|}{\underset{|}{C}}-OH$ type in substances *I* and *IX* also lead to the same conclusions. From the evaluation of the available experimental material[8] the relation $\delta CH_3(A) < \delta CH_3(B)$ (with the ranges $\delta CH_3(A) = 0{\cdot}9-1{\cdot}3$ p.p.m. and $CH_3(B) = 1{\cdot}4-1{\cdot}6$ p.p.m.) follows for the signals of the protons of the

TABLE II

Comparison of Chemical Shifts of $H_{(1)}$, $H_{(13)}$ and $H_{(14)}$ in Trilobolide (*I*) and Deacetylanhydrotrilobolide *IX* and in Their Adducts with Trichloroacetyl Isocyanate (prepared *in situ* in deuteriochloroform; chemical shifts in δ (TMS)-scale)

Compound	$H_{(1)}$	$H_{(13)}$	$H_{(14)}$	$\Delta\delta H_{(1)}$[a]	$\Delta\delta H_{(13)}$[a]	$\Delta\delta H_{(14)}$[a]
I	4·40	1·47	1·29			
I-TAC[b,c]	4·42	1·73	1·26	0·02	0·26	−0·03
IX	3·25	1·62	1·20	0·66[e]	0·00[e]	0·33[e]
IX-TAC[b,d]	3·91	1·62	1·53	(0·69)	(0·00)	(0·19)

[a] Acylation shifts are defined as $\Delta\delta H(Acyl) = \delta H(Acyl) - \delta H(OH)$; [b] monotrichloracetyl carbamate derivative; [c] NH: 8·68 p.p.m. (1 H); [d] NH: 8·26 p.p.m. (1 H); [e] values in parentheses are the corresponding acylation shifts of the acetyl group (Table I).

tertiary methyl groups $\delta(CH_3—\overset{|}{\underset{|}{C}}—OH)$ of sesquiterpenic lactones in deuteriochloroform solutions. In solutions of sesquiterpenic lactones in hexadeuteriodimethyl sulfoxide the signals of types *A* and *B* were shifted upfield, usually by 0·15–0·20 p.p.m.[8]. From the comparison of the PMR spectra of trilobolide (*I*) and deacetylanhydrotrilobolide (*IX*) in deuteriochloroform and hexadeuteriodimethyl sulfoxide (Table I; *cf.* substance *VIII*) it follows that only one of the two signals of tertiary methyl groups present displays a significant solvent effect. This fact alone excludes the position of the O-acetyl group at $C_{(7)}$. In the case of trilobolide (*I*) the variable signal $\delta CH_3(CDCl_3) = 1{\cdot}47$ p.p.m. may therefore be assigned to the type *B*, while in the PMR spectrum of deacetylanhydrotrilobolide *IX* the variable signal δCH_3 $(CDCl_3) = 1{\cdot}20$ p.p.m. corresponds to the type *A*; this is in agreement with the preceding interpretation of the acylation shifts.

The probability of the formation of the epoxide ring followed from the correlation of substances *II* and *VIII*: the PMR spectrum of diester lactonediol *II* indicated that this substance is a mixture of stereoisomers. We also carried out the cyclisation of substance *II* under the same conditions as in the preparation of anhydrotrilobolide *VIII* from trilobolide (*I*), and we succeeded in the isolation of two products, *XIV* and *XV*, which had according to IR, mass, and PMR spectra equal functional groups and did not contain free hydroxy groups. The product *XIV* was according to PMR spectrometry clearly a mixture of two stereoisomers (*XIVa* and *XIVb*, resp.) which differed probably only in the configuration of the center at $C_{(4)}$. We also obtained the mixture of stereoisomers *XIVa* and *XIVb* by hydrogenolysis of anhydrotrilobolide *VIII*, but with opposite quantitative ratio of both components (*XIVa*, *XIVb*)

than in the preceding experiment. In view of the fact that the stereoisomers *XIVa*, *XIVb*, and *XV* must differ only in one of the asymmetric carbons, namely $C_{(1)}$, $C_{(5)}$ or $C_{(7)}$, their formation from substance *II* indicates rather the sterical independence of the relative arrangement of both hydroxy groups, and makes the transannular cyclisation improbable.

Hence, from the sum of all the above-mentioned facts the structure represented by formula *I* follows for the molecule of trilobolide.

The structure of trilobolide (*I*) indicates that this native compound has analogous structural characteristics to a series of sesquiterpenic lactones isolated from the species of the *Laserpitieae* tribe (*Umbelliferae*)[3,4,7,10] the molecules of which have a relatively high number of oxygen functions, containing mainly several different ester-bound organic acids and the oxygen function on $C_{(11)}$.

Some aspects of the structural relationship of trilobolide (*I*) with archangelolide (*XVI*) were mentioned in one of the preceding papers[4]. Trilobolide (*I*) differs from all described native sesquiterpenic lactones, regardless of whether they were isolated from the species of *Daucaceae* or *Compositae* or other families, by the oxygen substituent at $C_{(7)}$, which was not yet described in any of the native sesquiterpenic lactones.

EXPERIMENTAL

Melting points were determined on a Kofler block and they were not corrected. For column chromatography silica gel according to Pitra and Štěrba[11] (30—60 μ, deactivated by addition of 11% of water) was used, while for thin-layer chromatography silica gel Merck (according to Stahl) was used. IR spectra were measured in chloroform on a Unicam SP 200 and Zeiss UR-10 (Jena) spectrometer. PMR spectra were measured on a Varian HA-100 apparatus. Mass spectra were measured on an AEI MS 902 and MCh 1303 (USSR) spectrometer. Optical rotation was determined with a Jasco ORD/UV 5 spectropolarimeter, in methanol.

Saturated diester lactone II: Trilobolide (*I*; 932 mg) was dissolved in 30 ml of acetic acid, PtO_2 (92·0 mg) was added to it and the mixture was saturated with hydrogen. Consumption, 184 ml of H_2 (23°C, 738 Torr), corresponded to 3 mol of hydrogen. After the conventional work-up saturated diester lactone *II* (865 mg) was prepared, m.p. 159—160°C (benzene–light petroleum), $[\alpha]_D^{20}$ −18·1° (*c* 1·1). For $C_{22}H_{34}O_8$ (426·5) calculated: 61·95% C, 8·02% H, 0·47% H act. (2); found: 62·24% C, 8·13% H, 0·48% H act. IR spectrum: 1778 cm^{-1} (γ-lactone), 1724 cm^{-1} (acetate), 1713 cm^{-1} (saturated ester), 3570 and 3450 cm^{-1} (hydroxyl). Mass spectrum: *m/e* 366 (426—60), 322 (426—18—86), 262 (426—18—86—60), 246 (426—60—102—18), 228 (426—60—102—18—18), 85 ($C_4H_9.CO^+$), 57 ($C_4H_9^+$).

Dehydrogenation of trilobolide derivatives: A solution of diester lactone *II* (2·0 g) in 200 ml of ether was added in portions and under stirring to a suspension of 500 mg of lithium aluminum hydride in 200 ml of ether. The mixture was then refluxed for 4 h. The product was isolated in the usual manner (1·2 g) and mixed, without further purification, with 2·2 g of metallic selenium. After 8 minutes heating at 300°C the mixture was cooled and extracted with light petroleum. The extract was chromatographed on 10 g alumina (alkaline, act. II) and a part of the azulenic fraction was chromatographed on paper[12] Whatman No 1 (20% paraffin oil solution as stationary phase and 20% HCl as the mobile phase, ascending arrangement). The R_F values of spots, 0·66 and 0·82, were practically identical with the R_F values of parallelly chromatographed chamazulene (*III*; R_F 0·67) and artemazulene (*IV*; R_F 0·81). A further part of the azulenic fraction was re-

chromatographed on 5 g of alumina of the same quality; elution with light petroleum afforded chamazulene (*III*) the trinitrobenzenate of which had m.p. 128—129°C, undepressed on admixture of authentic trinitrobenzenate of chamazulene (m.p. 129—131°C).

Lactone tetrol V: A solution of lactone *II* (1·7 g) in 30 ml of methanol was mixed with 2 g of NaOH in 10 ml of water and 40 ml of methanol and the mixture was refluxed for 30 min. After cooling water was added and the solution extracted with ether. The extract was washed with aqueous sodium hydrogen carbonate and further worked up in the conventional manner. Lactone tetrol *V* (900 mg) obtained was crystallised from benzene, m.p. 222—224°C $[\alpha]_D^{22}$ —55·5° (*c* 0·22). For $C_{15}H_{24}O_6$ (300·3) calculated: 59·99% C, 8·05% H, 1·34% H act. (4); found: 60·23% C, 8·08% H, 1·36% H act.. IR spectrum: 1 760 cm^{-1} (γ-lactone), 3 400 cm^{-1} (hydroxyl).

Ketolactone triol VII: To a solution of lactone tetrol *V* (200 mg) in 3 ml acetone 30 ml of a solution obtained by mixing 6·7 g of chromium trioxide, 25 ml of water, and 5·3 ml of conc. H_2SO_4 was added dropwise at 0°C under stirring and cooling. After the addition the mixture was allowed to stand at room temperature for 20 min, then diluted with water, filtered, extracted with ether, and further processed in the usual manner. The obtained ketolactone triol *VII* (120 mg) had m.p. 177—179°C (diisopropyl ether). For $C_{15}H_{22}O_6$ (298·3) calculated: 60·39% C, 7·43% H, 1·02% H act. (3); found: 60·57% C, 7·51% H, 1·19% H act. IR spectrum: 1 770 cm^{-1} (γ-lactone), 1 770 cm^{-1} (ketone), 3 400 cm^{-1} (hydroxyl).

Triester lactone VIII: To a solution of trilobolide (*I*, 3·0 g) in 120 ml of pyridine, 15 ml of thionyl chloride were added dropwise and under stirring at 0°C. The mixture was allowed to stand for 20 min at room temperature and then poured in several portions onto ice and worked up in the conventional manner. Triester lactone *VIII* (2·5 g) of m.p. 109°C (diisopropyl ether) was thus obtained, $[\alpha]_D^{20}$ +13·6° (*c* 0·20). For $C_{27}H_{36}O_9$ (504·5) calculated: 64·27% C, 7·20% H; found: 64·53% C, 7·20% H. IR spectrum: 1 789 cm^{-1} (γ-lactone), 1 738 cm (saturated ester), 1 715 cm^{-1} (unsaturated ester), 1 651 cm^{-1} (double bond), 1 250 cm^{-1} (acetate). Mass spectrum: *m/e* 444 (504—60), 344 (504—60—100), 342 (504—60—102), 242 (504—60—100—102), 85 ($C_4H_9.CO^+$), 83 ($C_4H_7.CO^+$), 57 ($C_4H_9^+$), 55 ($C_4H_7^+$).

Diester lactone IX *and monoester lactone* X: Triester lactone *VIII* (1·0 g) in 30 ml of methanol was mixed with a solution of 2·5 g KOH in 45 ml methanol and 5 ml water, and allowed to stand at room temperature for 4 h. After dilution with water the mixture was acidified with 5% H_2SO_4 and extracted with ether. The combined ethereal fractions were worked up in the usual manner, affording 700 mg of a product which gave after crystallisation from methanol–diisopropyl ether 250 mg of lactone *X*, m.p. 90°C, $[\alpha]_D^{20}$ —55·8° (*c* 0·59). For $C_{20}H_{26}O_7.CH_3OH$ (410·5) calculated: 61·43% C, 7·37% H; found: 61·19% C, 7·40% H. IR spectrum: 1 782 cm^{-1} (γ-lactone), 1 706 cm^{-1} (unsaturated ester), 1 649 cm^{-1} (double bond), 3 610 cm^{-1} (hydroxyl). The mother liquors of lactone *X* were chromatographed on 15 g of silica gel with benzene containing 10% of ether as eluent. From the first fractions liquid diester lactone *IX* (50 mg) was isolated. For $C_{25}H_{34}O_8$ (462·5) calculated: 64·92% C, 7·41% H, 0·22% H act.; found: 65·17% C, 7·67% H, 0·27% H act. IR spectrum: 1 780 cm^{-1} (γ-lactone), 1 730 cm^{-1} (saturated ester), 1 700 cm^{-1} (unsaturated ester), 1 645 cm^{-1} (double bond), 3 600 cm^{-1} (hydroxyl).

Monoester lactone XI: A mixture of unsaturated monoester lactone *X* (190 mg) in 5 ml of acetic acid and 43 mg of PtO_2 was hydrogenated until the consumption of hydrogen ceased. After the usual work-up lactone *XI* (160 mg) was obtained, m.p. 85—87°C (methanol–diisopropyl ether), $[\alpha]_D^{20}$ —45·3° (*c* 0·35). For $C_{20}H_{28}O_7.CH_3OH$ (412·5) calculated: 61·14% C, 7·82% H; found: 60·96% C, 7·87% H. IR spectrum: 1 775 cm^{-1} (γ-lactone), 1 720 cm^{-1} (saturated ester), 3 450 cm^{-1} and 3 600 cm^{-1} (hydroxyl).

Ketoester lactone XII: Monoester lactone *XI* (120 mg) in 3 ml of acetone was oxidized under the same conditions as in the case of the preparation of *VII*, using 20 ml of a solution prepared from 2·2 g of chromium trioxide, 17 ml of water, and 3·5 ml of conc. H_2SO_4. After the usual working up lactone *XII* (60 mg) was obtained, m.p. 146—148°C (benzene). For $C_{20}H_{26}O_7$ (378·4) calculated: 63·48% C, 6·93% H, 0·27% H act.; found: 63·57% C, 6·95% H, 0·47% H act.. IR spectrum: 1 785 cm^{-1} (γ-lactone), 1 720 cm^{-1} (double intensity; saturated ester and ketone). 3 560 and 3 450 cm^{-1} (hydroxyl).

Diester lactone XIV: *a*) A mixture of triester lactone *VIII* (520 mg) in 20 ml of acetic acid, 132 mg of 30% Pd/C catalyst, and several crystals of *p*-toluenesulfonic acid was saturated with hydrogen for 2 days. Applying the conventional procedure a product was obtained (432 mg) which was chromatographed on 30 g of silica gel with benzene. From the first fractions lactone *XIV* (150 mf) was obtained, m .p. 86—87°C (light petroleum), $[\alpha]_D^{20}$ +49·3° (*c* 0·38). For $C_{22}H_{32}O_7$ (408·5) calculated: 64·68% C, 7·90% H; found: 64·99% C, 8·14% H. IR spectrum: 1 775 cm^{-1} (γ-lactone), 1 730 cm^{-1} (saturated ester and acetate; double intensity), 1 250 cm^{-1} (acetate). Mass spectrum: *m/e* 366 (408—42), 264 (408—42—102), 246 (408—102—60), 85 ($C_4H_9.CO^+$), 57 ($C_4H_9^+$).

b) To a cooled solution (—10°C) of lactone *II* (720 mg) in 30 ml of pyridine thionyl chloride (5 ml) was added dropwise and the mixture allowed to stand at room temperature for 15 min. It was then poured in several portions onto ice and worked up in the conventional manner. The product (620 mg) was chromatographed on 40 g of silica gel with benzene. From the fore-fractions lactone *XIV* (100 mg) was obtained, m.p. 84—86°C (light petroleum), which according to its mixture melting point, IR, PMR and mass spectrum was identical with the substance obtained in the preceding section.

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