

## ON TERPENES. CCXXVI.\*

THE STRUCTURE OF LASOLIDE — A SESQUITERPENIC LACTONE  
FROM *Laser trilobum* (L.) BORKH.

M. HOLUB and Z. SAMEK

*Institute of Organic Chemistry and Biochemistry,  
Czechoslovak Academy of Sciences, Prague 6*

Received September 15th, 1972

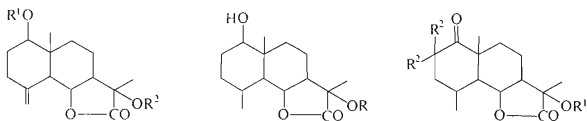
For lasolide from the root of *Laser trilobum* (L.) BORKH. the structure *I* was deduced on the basis of chemical transformations and especially a detailed analysis of the PMR spectra of the native compound and some of its derivatives.

In a preceding paper<sup>1</sup> we described the isolation of lasolide (*I*), one of the components of the root of *Laser trilobum* (L.) BORKH., m.p. 149–151°C and the composition  $C_{20}H_{28}O_5$ . In this paper we present the deduction of its structure. As mentioned before<sup>1</sup> the IR spectrum of lasolide (*I*) indicated the presence of a hydroxy group, a  $\gamma$ -lactone grouping, an  $\alpha,\beta$ -unsaturated ester group, and a double bond. The mass spectrum of the native substance *I* had the molecular peak at  $m/e$  348 and fragments  $m/e$  248 (348–100), 230 (348–100–18), 83 ( $C_4H_7CO^+$ ), 55 ( $C_4H_7^+$ ) and a metastable peak at 36.4  $m/e$ . The PMR spectrum of lasolide (*I*) (100 MHz,  $CDCl_3$ ) indicated the presence of one angelic acid residue ( $\beta$ -H: 6.15 p.p.m.; qq; 1 H), two tertiary C-methyl groups (1.59 p.p.m.; s; 3 H; 0.79 p.p.m.; s; 3 H), a non conjugated exomethylene group of the  $>C=CH_2$  type (two broadened singlets at 5.02 and 4.91 p.p.m.) and two isolated methine protons of the  $CH-O$  type of which one is of the  $CH-OH$  type (3.42 p.p.m.; dd;  $J_1 \approx 11$  and  $J_2 \approx 4.5$  Hz; confirmed by acylation *in situ* with trichloroacetyl isocyanate<sup>2,3</sup>) and the second of the type  $CH-O.CO$ . (5.02 p.p.m.; dd; multiplet superimposed by the signals of exomethylene protons).

On hydrogenation of the native compound *I* we obtained tetrahydrolasolide *II* of the composition  $C_{20}H_{32}O_5$ , which on oxidation with chromium trioxide gave ketolactone ester *III* of the composition  $C_{20}H_{30}O_5$ , the IR spectrum of which indicated the presence of a keto group in a six-membered or a larger ring ( $1700\text{ cm}^{-1}$ ). The mass spectrum of ester *III* corroborated its molecular weight and the presence of the residue of a saturated  $C_5$ -acid in the molecule. Saponification of lasolide (*I*) gave lactonediol *IV* of the composition  $C_{15}H_{22}O_4$  containing two active hydrogens.

\* Part CCXXV: Phytochemistry, in press.

Saponification of ester *III* afforded hydroxy keto-lactone *V* of the composition  $C_{15}H_{22}O_4$  the PMR spectrum of which indicated the presence of one proton of the  $\text{CH—O.CO.R}$  type (a doublet of doublets at 4.74 p.p.m.,  $J_1 \approx 6.8$  and  $J_2 \approx 9.0$  Hz).



*II*,  $R = \text{CO.CH}(\text{CH}_3).C_2H_5$

*I*,  $R^1 = H$ ,  $R^2 = \text{CO.C}(\text{CH}_3)=\text{CH.CH}_3$

*III*,  $R^1 = \text{CO.CH}(\text{CH}_3).C_2H_5$ ,  $R^2 = H$

*IV*,  $R^1 = R^2 = H$

*V*,  $R^1 = R^2 = H$

*VII*,  $R^1 = \text{CO.NH.CO.CCl}_3$

*VI*,  $R^1 = \text{CO.NH.CO.CCl}_3$ ,  $R^2 = H$

$R^2 = \text{CO.C}(\text{CH}_3)=\text{CH.CH}_3$

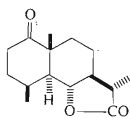
*VIII*,  $R^1 = H$ ;  $R^2 = D$

From the mentioned facts it followed that lasolide (*I*) is a sesquiterpene  $\gamma$ -lactone with a basic bicyclic carbon skeleton and one free secondary hydroxy group bound in a six-membered or higher ring, one secondary hydroxy group bound in a  $\gamma$ -lactone ring, one tertiary hydroxy group esterified with angelic acid, and one skeletal exomethylene double bond.

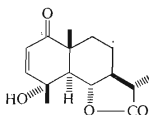
The basic topological aspects of the skeleton of lasolide (*I*) were evident from the following facts: from the weak chemical shift of one of the tertiary methyl groups in the PMR spectrum of lasolide (*I*) (0.74 p.p.m.) and its derivatives it followed that it must be an angular methyl group of the type  $\text{CH}_3\text{—C}(\text{C})(\text{C})(\text{C})$ . The structural character of the second tertiary methyl group followed from the comparison of the chemical shifts of the methyl signals in the PMR spectra of substances *III*, *V*, and trichloroacetylcarbamate *VI* (prepared by *in situ* acylation of lactone *V* with trichloroacetyl isocyanate<sup>2,3</sup>). From Table I it follows that the signal of the angular methyl (in deuteriochloroform) is practically independent of the presence of the tertiary O-acyl group, while the signal of the second methyl displays a gradual paramagnetic acylation shift  $\Delta\delta\text{CH}_3$  (*V—III*) = 0.10 p.p.m.,  $\Delta\delta\text{CH}_3$  (*III—VI*) = 0.11 p.p.m. and of  $\Delta\delta\text{CH}_3$  (*V—VI*) = 0.21 p.p.m. As mentioned before<sup>4,5</sup> this shift is typical

the  $\text{CH}_3\text{—}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{—OH}$  group situated in the  $\alpha$ -position of the  $\gamma$ -lactone carbonyl.

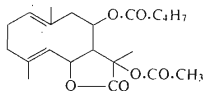
From this it follows that the bicyclic skeleton of lasolide (*I*) contains the usual lactonogenic  $C_3$ -side chain. The relative position of the  $C_1$ - and  $C_3$ -side chains of the lasolide skeleton (*I*) followed directly from a detailed analysis of the PMR spectrum of compound *III*, submitted to decoupling experiments. In the mentioned spectrum the lactonic methine proton  $\text{CH—O}$  appeared as a doublet of doublets at 4.77 p.p.m., with two couplings  $J_1 \approx 8.5$  and  $J \approx 9.5$  Hz. The first of these two couplings was to the proton forming a complex multiplet at 3.07 p.p.m., and the second to the



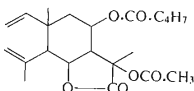
IX



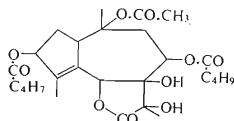
X



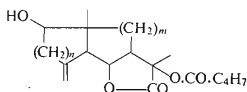
XI



XII

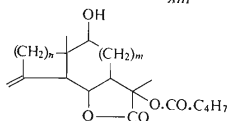


XIII



Ia, n = 2; m = 2

Ib, n = 3; m = 1



Ic, n = 1; m = 3

Id, n = 2; m = 2

Ie, n = 3; m = 1

proton which formed a triplet at 1.30 p.p.m. ( $J_1 \approx J_2 \approx 10$  Hz) and the second splitting constant of which corresponded to the coupling with the methine proton of the  $\text{CH}-\text{CH}_3$  type ( $\sim 1.10$  p.p.m.). This topological continuity of vicinal couplings is consistent with the relative 1,3-position of both  $\text{C}_1$ -side chains and with the relative 1,4-position of the  $\text{C}_1$ - and  $\text{C}_3$ -side chains. The location of the free secondary hydroxy group, relative to the side chains, was deduced from the comparison of the PMR spectra of deuteriochloroform solutions of lasolide (I) and its trichloroacetylcarbamate VII. The signal of the proton  $\text{CH}-\text{OH}$  in the spectrum of compound I appeared as a doublet of doublets at 3.42 p.p.m., with  $J_1 \approx 11$  and  $J_2 \approx 4.5$  Hz, which was shifted downfield by 1.36 p.p.m. after addition of trichloroacetyl isocyanate. Simultaneously the angular methyl group signal displayed a paramagnetic shift  $\Delta\delta\text{CH}_3(\text{TAC}) = -0.15$  p.p.m. This acylation shift is in agreement with the assumption that the hydroxy group is bound to a carbon atom which is vicinal to the tertiary carbon atom carrying the angular methyl group. This position is also in agreement with the presence of two vicinal couplings of the  $\text{CH}-\text{OH}$  proton. The presence of the corresponding  $-\text{CH}_2-\text{CO}-\text{C}-$  group was also confirmed by deuteration of lactone V, which according to the mass spectrum gave substance VIII containing two atoms of deuterium.

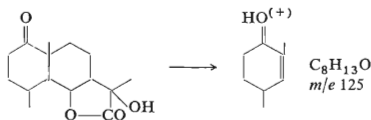
TABLE I  
Characteristic Parameters of the PMR Spectra

Compound <sup>a</sup>	H <sub>(1)</sub>	H <sub>(4)</sub>	H <sub>(5)</sub>	H <sub>(6)</sub>	H <sub>(7)</sub>	H <sub>(13)</sub>	H <sub>(14)</sub>	H <sub>(15)</sub>
<i>I</i> <sup>b</sup>	3.42 $J_{1,2} = 4.5$ $J_{1,2'} = 11$	—	1.84 <sup>c</sup>	5.02 <sup>d,e</sup> $J_{6,5} = 11$ $J_{6,7} = 9$	3.31 <sup>f</sup>	1.59	0.74	5.02 4.91
VIII <sup>g,h</sup>	4.78 $J_{1,2} = 4.5$ $J_{1,2'} = 11$	—	—	5.01 <sup>d</sup>	3.35	1.59	0.89	
III <sup>k</sup>	—	2.10 <sup>c</sup>	1.30 $J_{5,4} = 10$ $J_{5,6} = 10$	4.77 $J_{6,5} = 9.5$ $J_{6,7} = 8.5$	3.07	1.49	1.14	1.16 (6.6)
V	—	2.15 <sup>c</sup>	1.35 $J_{5,4} = 10$ $J_{5,6} = 9$	4.74 $J_{6,5} = 9$ $J_{6,7} = 6.8$	2.60	1.39	1.19	1.14 (6.4)
VI <sup>g,i</sup>	—	2.10 <sup>c</sup>	1.32 $J_{5,4} = 10$ $J_{5,6} = 10$	4.84 $J_{6,5} = 9.6$ $J_{6,7} = 8.0$	3.26	1.60	1.17	1.14 <sup>j</sup>

<sup>a</sup> All data from first-order analysis; chemical shifts given in  $\delta$  (TMS)-scale, splittings in Hz; measured on Varian HA-100 instrument in deuteriochloroform; <sup>b</sup>  $\beta$ -H 6.15 qq; <sup>c</sup> approximate position from decoupling experiments; <sup>d</sup> overlap with signals of H<sub>(15)</sub> and H<sub>(15')</sub>; <sup>e</sup> splittings from spectrum of hexadeuteriobenzene solution; <sup>f</sup> overlap with signal of H<sub>(1)</sub>; <sup>g</sup> prepared by *in situ* acylation with trichloroacetyl isocyanate; <sup>h</sup>  $\beta$ -H 6.16 qq, NH 8.41; <sup>i</sup> NH 8.48; <sup>j</sup> splitting uncertain due to overlap with signals of H<sub>(14)</sub>; <sup>k</sup> mixture of epimeric esters containing *R*- and *S*- $\alpha$ -methylbutyryl residues;  $\alpha$ -H:  $\sim$ 2.35,  $\alpha$ -CH<sub>3</sub>: 1.13 dd,  $J_1 = 7.1$  and  $J_2 = 6.9$  ( $A(R,S) \cong \cong 0.5$  Hz),  $\beta$ -CH<sub>3</sub>: 0.91 td,  $J = 7$ , ( $A(R,S) \cong 1.5$  Hz).

From the point of view of all aspects mentioned so far the alternatives *Ia* to *Ie* follow in principle for the topology of the lasolide (*I*) skeleton, of which only alternatives *Ia* and *Ie* correspond to the biogenetically normal and usual eudesmane type. This assumption was supported directly by the mass spectrum of lactone *V* and its dideuterio derivative VIII, which contained characteristic corresponding fragments with —CO—CH<sub>2</sub>— and —COCD<sub>2</sub>—groups, of masses  $m/e$  125 and 127 respectively. The composition C<sub>8</sub>H<sub>13</sub>O corresponds to the mass  $m/e$ , and it may be assumed that it is the usual fragment of the angular splitting of keto derivatives containing a carbonyl group vicinal to the angular methyl (*cf.* 4-ketopseudoguaianolides<sup>6</sup>). The formation of this fragment and its structure may be explained rationally and satisfactorily only in the case of the structure of the hydroxy keto-lactone represented by formula

V, which corresponds with the alternative *Ia* for lasolide, in the sense of the splitting represented in Scheme 1. The presence of the fragment of *m/e* 125 was also visible in the mass spectrum of 1-oxosantanolide IX, which was prepared from tauremisin<sup>7</sup> (X). On the basis of all the facts mentioned we propose for lasolide the structure expressed by formula I.



SCHEME 1

Lasolide *I* is another sesquiterpene lactone isolated from *Umbelliferae* which belongs to the group of eudesmanolides. Several sesquiterpene lactones of this type, which are rather common in *Compositae*, were isolated from the species of *Umbelliferae* family quite recently, and their structure was determined<sup>8-10</sup>. In the roots of *L. trilobum* we have proved up to now five sesquiterpene lactones<sup>1</sup> and we have elucidated the structure of four of them. From the biogenetic point of view it is interesting that each of the four mentioned lactones belongs to a different basic carbon skeleton type: laserolide (XI) belongs to germacranolides<sup>11</sup>, isolaserolide (XII) to elemanolides<sup>12</sup>, trilobolide (XIII) to guaianolides, and the lasolide (*I*) described in this paper belongs to eudesmanolides.

## EXPERIMENTAL

Melting points were determined on a Kofler block and were not corrected. IR spectra were measured on a Unicam SP 200 spectrophotometer in chloroform, and mass spectra on a AEI MS 902 spectrograph.

**Tetrahydrolasolide (II):** A solution of lasolide (*I*; 161 mg) in 8 ml of acetic acid was added with 24.5 mg of PtO<sub>2</sub> and hydrogenated. The consumption of hydrogen at 22°C and 726 Torr (31.5 ml) corresponded to 2.2 mol of hydrogen. After the conventional working up tetrahydrolasolide *II* (150 mg) was obtained, m.p. 152–154°C (diisopropyl ether). For C<sub>20</sub>H<sub>32</sub>O<sub>5</sub> (352.5) calculated: 68.15% C, 9.15% H, 0.28% H act.; found: 68.38% C, 8.99% H, 0.33% H act. IR spectrum: 3600 (OH), 1770 (γ-lactone), 1723 (saturated ester), 1470, 1385, 1140, 1105, 1080, 1005 and 980 cm<sup>-1</sup>.

**Keto ester III:** A solution of tetrahydrolasolide (*II*; 150 mg) in 5 ml of pyridine was mixed with a suspension of 200 mg of chromium trioxide in 2 ml of pyridine. The mixture was allowed to stand at room temperature for 16 hours, then worked up, affording keto ester *III* (100 mg) of m.p. 138–139°C (diisopropyl ether). For C<sub>20</sub>H<sub>30</sub>O<sub>5</sub> (350.4) calculated: 68.75% C, 8.63% H; found: 68.58% C, 8.41% H. IR spectrum: 1775 (γ-lactone), 1725 (saturated ester), 1700 (C=O), 1470, 1385, 1105, 1015, and 985 cm<sup>-1</sup>. Mass spectrum: *m/e* 248 (350–102), 230 (350–102–18), 85 (C<sub>4</sub>H<sub>9</sub>.CO<sup>+</sup>), 57 (C<sub>4</sub>H<sub>9</sub><sup>+</sup>).

*Lactonediol IV*: A mixture of lasolide (*I*; 80 mg) and 5 ml of a 5% methanolic KOH solution was allowed to stand at room temperature for 18 h. Working up of the mixture afforded diol *IV* (30 mg), m.p. 175°C (ethyl acetate–light petroleum). For  $C_{15}H_{22}O_4$  (266.3) calculated: 67.65% C, 8.33% H, 0.77% H act.; found: 67.51% C, 8.27% H, 0.67% H act. IR spectrum: 3580 (OH), 3400 (OH), 1760 ( $\gamma$ -lactone), 1610, 1450, 1385, 1350, 1120, 1090, 1040, 980  $cm^{-1}$ .

*Hydroxy keto-lactone V*: A mixture of lactone *III* (45 mg) in 0.5 ml of methanol and 0.5 ml of 20% methanolic KOH solution was stirred at room temperature for 6 h. After working up, lactone *V* (20 mg) of m.p. 205–207°C (benzene) was obtained. For  $C_{15}H_{22}O_4$  (266.3) calculated: 67.65% C, 8.33% H, 0.38% H act.; found: 67.76% C, 8.22% H, 0.45% H act. IR spectrum: 3550, 3430 (OH), 1770 ( $\gamma$ -lactone), 1705 (C=O), 1470, 1390, 1135, 1105, 1020, 1005, 975, 930  $cm^{-1}$ . Mass spectrum: *m/e* 266 and 248 (266–18).

*Deuteration*: Lactone *III* (22 mg) was mixed with 2 ml of deuterium oxide, 2 ml of deuterioethanol and 12 mg of potassium hydroxide and the mixture was worked up as described<sup>13</sup>. After filtration through silica gel the obtained product *VIII* (15 mg) had *m/e* 268.

*Elemental analyses were carried out by Mrs V. Rusová and Mr V. Štěřba of the Analytical Department of our Institute (head Dr J. Horáček). The IR spectra were measured by Mrs S. Holubová and the mass spectra by Dr A. Trka (head Dr L. Dolejš). We express our thanks to all those mentioned as well as to Miss H. Němcová for technical assistance.*

#### REFERENCES

1. Holub M., De Groote R., Herout V., Šorm F.: This Journal 33, 2911 (1968).
2. Trehan I. R., Monder C., Bose A. K.: Tetrahedron Letters 1968, 67.
3. Goodlet V. W.: Anal. Chem. 37, 431 (1965).
4. Holub M., Samek Z., De Groote R., Herout V., Šorm F.: This Journal, in press.
5. Samek Z., Holub M., Vokáč K.: Unpublished results.
6. Tsai L., Highet R. J., Herz W.: J. Org. Chem. 34, 945 (1969).
7. Rybalko K. S., Dolejš L.: This Journal 26, 2909 (1961).
8. Serkerov S. V.: Chim. Prir. Sojed. 1972, 63.
9. Serkerov S. V.: Chim. Prir. Sojed. 1971, 590.
10. Serkerov S. V.: Chim. Prir. Sojed. 1969, 245; 1971, 667.
11. Holub M., Samek Z., Popa D. P., Herout V., Šorm F.: This Journal 35, 284 (1970).
12. Holub M., Samek Z.: Phytochemistry 11, 3053 (1972).
13. Büchi G., Schach M., Wittenau V., White D. M.: J. Am. Chem. Soc. 81, 1968 (1959).

Translated by Ž. Procházka.