COMPONENTS OF THE EXTRACT FROM THE UNDERGROUND PARTS OF *Laserpitium siler* L. OF SLOVENIAN ORIGIN, MAINLY SESQUITERPENIC LACTONES[*]

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In the analysis of the underground parts of *Laserpitium siler* L. of Slovenian origin the authors succeeded in isolating the phenylpropane derivatives 1-(3,4-methylenedioxy-5-methoxyphenyl)propan-1-one (*IV*) and 1-(3,4-methylenedioxy-5-hydroxyphenyl)propan-1-one (*XXI*), three current phytosterols and thirteen sesquiterpenic lactones. Of these eleven are newly described, while eight have not yet been mentioned in literature at all. The structures of the following four undescribed lactones, including relative and absolute configuration, are determined: 8α-(2'-methylbutyroyloxy)-10β-hydroxy-11α-acetoxyslov-3-enolide (*XIV*), 8α-isobutyroyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*XIX*), 8α-hydroxy-10β,11α-diacetoxyslov-3-enolide (*XXIX*) and 8α,10β-dihydroxy-11α-acetoxyslov-3-enolide (*XXXII*). For the lactones derived from 1βH,5βH,6αH,7αH-guaian-6,12-olide (slovanolide) a semisystematic nomenclature is proposed.

The isolation and the structure determination of the components of *Laserpitium siler* L. (*Umbelliferae* family, *Laserpitieae* tribe) have been the subject of several communications[1-7]. In the underground part of this species of Bulgarian origin[1] silerolide was detected for which the structure was derived later, expressed by alternative formulae *I* and *II*, as well as montanolide *III* and also 1-(3,4-methylenedioxy-5-methoxyphenyl)propan-1-one (*IV*) and a mixture of phytosterols[1]. Later isomontanolide (*V*) was isolated from the fruits and acetylisomontanolide (*VI*) from the underground parts of *L. siler* L. of Slovenian origin[3]. When the light petroleum extract of the fruits of *L. siler* L. of Slovenian origin[4] was analysed, hydrocarbons of the group of paraffins, monoterpenes and sesquiterpenes were detected, further an aliphatic ketone, fats, phytosterols, aliphatic alcohols and three sesquiterpenic lactones, so far undetected in this material, *i.e.* acetylisomontanolide (*VI*), lactone *VII* (to which we gave the name gradolide[5] after the elucidation of its structure), and lactone *VIII* the structure of which has not been elucidated so far. The components from the extract of above-ground parts of *L. siler* L. of Serbian origin were investigated by Stefanović and coworkers[6] who isolated lactones *III*, *V*, *VI* and *VII*,

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the flavonoid artemetin (*IX*) and angeloyloxylatifolone (*X*). Several years ago[7] we derived for acetylisomontanolide the stereostructure *VI*. In view of the correlation of this substance with montanolide and isomontanolide the stereostructure *III* and *V* followed for these compounds. In a further paper[5] the isolation and the structure of the sesquiterpenic lactone polhovolide (*XI*) from the underground part and the structure of gradolide (*VII*) were described.

The described stereostructural type of guaianolides (1βH,5βH,6αH,7αH-guaian-6,12-olides) and the number of native substances that we showed to be derived from the mentioned sesquiterpenic base leads us to propose a more rational semisystematic nomenclature for the sesquiterpenic lactones of this type. Therefore we propose that the names should be derived from the structure represented by formula *XII* (including configuration on $C_{(1)}$, $C_{(5)}$, $C_{(6)}$ and $C_{(7)}$) for which we propose the name slovanolide. The method of use is illustrated by the names of the compounds already described.

Trivial name	Semisystematic name
acetylisomontanolide (*VI*)	8α-angeloyloxy-10β,11α-diacetoxyslov-3-enolide
montanolide (*III*)	8α-senecioyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide
isomontanolide (*V*)	8α-angeloyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide
polhovolide (*XI*)	8α-isobutyroyloxy-10β,11α-diacetoxyslov-3-enolide
gradolide (*VII*)	8α,11α-diangeloyloxy-10β-hydroxyslov-3-enolide

In this paper we compare the data concerning the components of the aerial parts of the mentioned species[6] with those of similar substances of the underground parts. Therefore we carried out a detailed analysis of the light petroleum and the chloroform extract of the roots and the rhizomes of *L. siler* of the Slovenian origin. We separated the components of the respective extract mainly by repeated column chromatography on silica gel and the separation course was monitored by thin-layer chromatography on silica gel.

The least polar substances which we isolated were 1-(3,4-methylenedioxy-5-methoxyphenyl)propan-1-one (*IV*; synonyms latifolon[8,9], crocaton[10,11]) which we identified by comparison of their IR spectra and mixture melting point with a standard. Compound *XIII* was more polar than *IV* and it had m.p. 141−143°C, $[\alpha]_D^{20}$ −138·9 and the composition $C_{22}H_{30}O_6$. It was not yet described in literature and when elucidating its structure we gave it ther name isosilerolide[12]. Further we isolated a mixture of phytosterols in which β-sitosteol, stigmasterol and campesterol were identified by gas chromatography as well as their percentual representation in the isolated mixture. We then obtained lactone *VI* and a slightly more polar compound, 8α-isobutyroyloxy-10β,11α-diacetoxyslov-3-enolide (*XI*), m.p. 125−127°C, $[\alpha]_D^{20}$ −89·2 and the composition $C_{23}H_{32}O_8$. The latter compound was identified on the basis of its IR and ^{1}H NMR spectra and mixture melting point with a standard. We found that the melting point which we found originally for compound *XI* (98−99°C) was incorrect.

A further isolated compound was lactone *VII* the identification of which was carried out on the basis of its IR and ^1H NMR spectrum and mixture melting point. We found by comparison of the IR spectrum and mixture melting point that Yugoslav authors[6] has isolated the same substance from the above-ground parts of the mentioned species and gave it the name tarolide. This substance, characterized by its m.p. 154°C, $[\alpha]_D^{20}$ $-50{\cdot}9$ and the composition $C_{25}H_{34}O_7$, was described earlier[4] without a trivial name as a component of the fruits of the investigated species.

The next substance of still higher polarity was lactone *V*.

From the fractions which followed the lactone *V* we isolated compound *XIV*, m.p. 154–155°C, of the composition $C_{22}H_{32}O_7$. According to its IR spectrum it contained a hydroxyl group (3 538 cm^{-1}), a γ-lactone (1 784 cm^{-1}), an acetyl group (1 738 and 1 250 cm^{-1}), an ester group (1 719 cm^{-1}) and a double bond (1 648 cm^{-1}). The mass spectrum contained the molecular peak at 408 and characteristic signals of m/z 390 (M – 18), 348 (M – 60), 306 (M – 102), 246 (M – 102 – 60), 228 (M – 102 – 60 – 18), 85 ($C_4H_9CO^+$) and 57 ($C_4H_9^+$). CD spectrum had its maximum peak at 210 nm with $\Delta\varepsilon$ $-2{\cdot}6$. According to the mentioned data for *XIV* it followed that it has not yet been described in literature. Its ^1H NMR spectrum (Table I and II) showed the presence of two ester groups – an acetate (singlet at δ 2·09) and a 2-methylbutanoate (methyls as doublet at δ 1·17 and a triplet at δ 0·95), and three tertiary methyls (sp^2-CH_3 at δ 1·89 and two sp^3 $CH_3-\overset{|}{\underset{|}{C}}-OR$ at δ 1·22 and 1·53). At the lowest field (δ 5·56) an unresolved multiplet of the hydrogens of a trisubstituted double bond was found, with a weak interaction with the sp^2-methyl, which indicates the $-CH=\overset{|}{C}-CH_3$ grouping. Reaction with trichloroacetyl isocyanate[13,14] (TAI) gave monotrichloroacetyl carbamate (TAC-derivative) *XV* (a one-proton signal of NH at δ 8·34) with distinct acylation shifts $\Delta\delta$ of one tertiary methyl at δ 1·22 ($\Delta\delta = 0{\cdot}44$), the hydrogen multiplet at δ 2·40 ($\Delta\delta = 0{\cdot}50$) and the broad doublet at δ 1·72 ($\Delta\delta = 0{\cdot}70$). The chemical shifts of both mentioned hydrogens exclude the —CH—OR structural type and together with the distinct acylation shift of the methyl they prove the presence of a tertiary hydroxyl of the $CH_3-\overset{|}{\underset{|}{C}}-OH$ type with the mentioned hydrogens in β-positions with respect to the hydroxyl group. The acylation shifts of other hydrogens are substantially lower ($\Delta\delta < 0{\cdot}15$ ppm). The broadened doublet of doublets at δ 5·50 with $J = 11{\cdot}5$, 9·4 and 0·8 Hz was assigned to the CH—O hydrogen in the position of the bound 2-methylbutanoate group and the doublet of doublets at δ 4·76 to the CH—O hydrogen with the lactone oxygen. The acetate must be bound to a quaternary carbon together with the methyl, evidently in the γ-lactone ring. The observed splitting of further signals and the corresponding decoupling experiments led to the assignment of all hydrogens of the molecule and to the formulation of the structure of the guaianolide type with the $C_{(6)}$-lactone, represented by formula *XIV*. A comparison of the chemical shifts and the coupling constants of compound *XIV* and its TAC-derivative *XV* (Table I and II) with the ^{1}H-NMR parameters of 8α-angeloyloxy-10β,11α-diacetoxyslov-3-enolide (*VI*), 8α-senecioyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*III*), 8α-angeloyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*V*), 8α-isobutyroyloxy-10β,11α-diacetoxyslov-3-enolide (*XI*), 8α,11α-diangeloyloxy-10β-hydroxyslov-3-enolide (*VII*) and the corresponding TAC-derivatives *XVI*, *XVII* and *XVIII* (Table I and II) confirms this conclusion unambiguously and shows that the configuration on carbons 1, 5, 6, 7, 8 and 10 in compound *XIV* are also identical with the configurations on the mentioned carbon atoms in compounds *III*, *V* and *VI*. Hence, the newly isolated substance is 8α-(2'-methylbutyroyloxy)10β-hydroxy-11α-acetoxyslov-3-enolide (*XIV*). The absolute configuration of this compound was derived on the basis of a comparison of the CD spectra of this substance and of lactone *VI* the absolute configuration of which is described[7]. Since both compared substances have the same fifteen-carbon sesquiterpenic basic skeleton according to the analysis of their ^{1}H NMR spectra (Table I and II), and differ only by the nature of the ester group on $C_{(8)}$ and $C_{(10)}$, it may be assumed that the sense of the CD curve will be the same in both mentioned compounds if their absolute configuration is the same. The CD curves of both substances are practically identical (Table III) and therefore it may be concluded that 8α-(2'-methylbutyroyloxy)10β-hydroxy-11α-acetoxyslov-3-enolide has its absolute configuration analogous to 8α-angeloyloxy-10β,11α-diacetoxyslov-3-enolide (*VI*) and therefore the formula *XIV* also expresses the absolute configuration of the native substance.

The most polar compound, *XIX*, which we obtained from the light petroleum extract, had m.p. 162–164°C, $[\alpha]_D^{20}$ −86·4 and the composition $C_{21}H_{30}O_7$. As evident from the physical constants and the composition this compound has not yet been described in literature. Its IR spectrum indicates the presence of a hydroxyl group (3 540 cm^{-1}), a lactone ring (1 785 cm^{-1}), an acetate group (1 738 and 1 250 cm^{-1}), an ester group (1 719 cm^{-1}) and a double bond (1 648 cm^{-1}). The mass spectrum contained the molecular peak at 394 m.u. and characteristic signals of m/z 376 (M − 18), 334 (M − 60), 316 (M − 60 − 18), 306 (M − 88), 288 (M − 88 − 18), 246 (M − 88 − 60), 228 (M − 88 − 60 − 18), 71 ($C_3H_7CO^+$) and 43 ($C_3H_7^+$). CD spectrum had a maximum at 210 nm with Δε −2·2. The ^{1}H NMR spectra of this compound (*XIX*) and its TAC-derivative *XX* (Table I and II) are very similar to the spectra of compound *XIV* and its TAC-derivative *XV*, respectively. This compound too contains one acetate group (singlet at δ 2·09), but the second ester group is different. The methyl doublets at δ 1·19 and 1·22 with J = 7 Hz and the one-proton heptet at δ 2·55 with J = 7 Hz show unambiguously the presence of an isobutyrate. The quite analogous TAI-acylation shifts as in lactone *XIV* led to the location of the isobutyrate residue into position 8 and of the hydroxyl group into position 10. Hence, the substance isolated must be 8α-isobutyroyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*XIX*). The absolute configuration of this substance has been derived similarly as for

TABLE I

Proton chemical shifts in some 1βH,5βH,6αH,7αH-guaian-6,12-olides

Proton	*VI*	*III*	*XVI*	*V*	*XVII*	*XI*	*VII*	*XVIII*	*XIV*	*XV*	*XIX*	*XX*	*XXIX*	*XXX*	*XXXII*	*XXXIII*
H-1	2·77	2·41	2·95	2·43	2·95	2·75	2·43	2·95	2·40	2·90	2·40	2·91	2·93	~2·76	~2·30	2·88
H-2α	~2·16	~1·07	~2·17	2·08	2·19	~2·14	~2·07	~2·18	2·06	~2·16	2·06	2·17	[a]	[a]	[a]	~2·20
H-2β	2·30	2·19	2·33	2·22	2·34	2·28	2·21	2·34	2·18	~2·33	2·19	2·33	[a]	[a]	[a]	~2·34
H-3	5·57	5·56	5·59	5·57	5·59	5·57	5·57	5·59	5·56	5·58	5·56	5·57	5·53	5·59	5·55	5·60
H-5	2·60	2·58	2·70	2·58	2·71	2·56	2·61	2·74	2·56	2·70	2·56	2·70	2·53	2·56	2·50	2·72
H-6	4·74	4·71	4·72	4·72	4·74	4·72	4·78	4·79	4·70	4·71	4·70	4·72	4·72	4·73	4·66	4·92
H-7	3·67	3·61	3·62	3·67	3·67	3·62	3·77	3·77	3·63	3·63	3·62	3·63	3·30	3·76	3·26	3·80
H-8	5·67	5·51	5·61	5·54	5·66	5·57	5·60	5·71	5·50	5·59	5·49	5·57	4·29	5·65	4·54	5·67
H-9α	2·01	2·01	2·03	2·05	2·07	1·95	2·08	2·11	2·02	2·04	2·01	2·05	2·03	2·10	2·02	2·21
H-9β	2·67	1·81	2·43	1·85	2·48	2·58	1·83	2·48	1·72	2·42	1·73	2·43	2·59	2·79	1·79	2·73
H-13	1·54	1·54	1·52	1·55	1·53	1·53	1·61	1·59	1·53	1·51	1·53	1·50	1·66	1·64	1·69	1·61
H-14	1·60	1·22	1·66	1·23	1·67	1·55	1·23	1·67	1·22	1·66	1·22	1·66	1·41	1·55	1·26	1·70
H-15	1·90	1·88	1·91	1·89	1·91	1·89	1·91	1·93	1·89	191	1·88	1·91	1·88	1·90	1·88	1·92
$OCOCH_3$	2·02 2·13	2·09	2·06	2·06	2·04	2·06 2·10	—	—	2·09	2·06	2·09	2·07	2·06 2·10	2·04 2·12	2·11	2·05
NH	—	—	8·59	—	8·59	—	—	8·54	—	8·34	—	8·50	—	8·33	—	8·42 8·54
α-H	—	5·62	5·59	—	—	2·50	—	—	2·38	2·33	2·55	2·51	—	—	—	—
α-CH_3	1·86	—	—	1·89	1·86	1·17 1·18	1·84 1·86	1·79 1·83	1·17	1·14	1·19 1·22	1·16 1·18	—	—	—	—
β-CH_3	1·99	2·20 1·94	2·16 1·92	2·04	1·99	—	1·98 1·99	1·95 1·96	0·95	0·93	—	—	—	—	—	—
β-H	6·09	—	—	6·21	5·65	—	6·16 6·17	6·11 6·16	1·47 1·72	1·45 1·70	—	—	—	—	—	—

[a] Not determined due to the overlapping of signals.

TABLE II

Proton coupling constants in some 1βH,5βH,6αH,7αH-guaian-6,12-olides

$J_{H,H}$	*VI*	*III*	*XVI*	*V*	*XVII*	*XI*	*XI*	*VII*	*XVIII*	*XIV*	*XIX*	*XX*	*XXIX*	*XXX*	*XXXII*	*XXXIII*
^2J: $J_{2\alpha,2\beta}$	15·4	15·1	15·3	15·0	15·0	15·1	15·0	15·4	15·2	15·2	15·2	15·2	[a]	[a]	[a]	[a]
^3J: $J_{9\alpha,9\beta}$	15·1	14·8	15·2	14·5	15·2	14·9	14·8	15·1	14·8	15·2	14·8	15·2	15·2	14·5	14·8	15·0
$J_{1,2\alpha}$	~11·5	~11·5	~11·5	11·5	11·5	~11·5	~11·5	~11·5	11·4	11·6	11·6	11·6	~11·5	~11·5	~11·5	~11·5
$J_{1,2\beta}$	7·2	6·9	7·0	7·0	7·0	7·0	6·8	6·9	6·8	7·0	6·8	7·2	~7	~7	~7	~7
$J_{1,5}$	5·8	5·5	5·7	5·4	5·6	5·7	5·6	5·6	5·6	5·8	5·6	5·6	5·6	5·5	5·2	5·5
$J_{2\alpha,3}$	1·6	1·6	1·6	1·4	1·4	1·6	1·6	1·4	1·8	1·6	1·6	1·6	~1·5	~1·5	1·6	~1·5
$J_{2\beta,3}$	3·1	3·2	3·2	3·1	3·1	3·2	3·1	3·2	3·2	3·2	3·2	3·2	~3	~3	3·2	~3
$J_{5,6}$	11·6	11·6	11·6	11·5	11·6	11·6	11·5	11·5	11·6	11·6	11·5	11·6	11·6	11·6	11·6	11·6
$J_{6,7}$	9·9	9·9	9·9	10·0	9·9	10·0	10·0	10·0	9·9	10·0	9·9	10·0	10·0	10·0	10·0	10·0
$J_{7,8}$	11·1	11·5	11·4	11·4	11·4	11·0	11·5	11·4	11·5	11·3	11·5	11·4	10·6	10·8	10·7	11·2
$J_{8,9\alpha}$	10·1	9·2	9·4	9·4	9·5	10·1	9·3	9·4	9·4	9·8	9·4	9·4	9·8	10·0	9·8	10·2
$J_{8,9\beta}$	1·1	1·0	0·9	0·8	0·7	1·1	0·8	0·7	0·8	~1	0·8	0·8	1·0	1·0	1·2	~1
$J_{\alpha H,\alpha CH_3}$	—	—	—	—	—	7·0	—	—	7·0	7·0	7·0	7·0	—	—	—	—
$J_{\beta H,\beta CH_3}$	7·3	—	—	—	—	—	7·3 7·3	7·3 7·3	7·4 7·4	7·2 7·2	—	—	—	—	—	—
^4J, ^5J: $J_{1,9\beta}$	1·4	1·7	1·8	1·7	1·6	~1	1·7	1·6	~1	~1	1·8	1·8	1·7	1·7	1·6	1·6
$J_{2\alpha,15}$	2·2	2·4	2·4	2·3	2·3	2·2	2·4	2·2	2·4	2·4	2·5	2·5	~2·5	~2·5	2·4	~2·5
$J_{2\beta,15}$	1·6	1·4	1·4	1·4	1·4	1·6	1·4	1·6	1·4	1·4	1·4	1·4	~1·5	~1·5	1·5	~1·5
$J_{3,15}$	1·7	1·7	1·7	1·7	1·7	1·7	1·7	1·5	1·6	1·6	1·7	1·7	~1·5	~1·5	1·6	~1·5
$J_{\alpha CH_3,\beta H}$	1·5	—	—	1·5	1·5	—	1·6 1·6	1·5 1·5	0	0	—	—	—	—	—	—
$J_{\alpha H,\beta CH_3}$	—	1·3 1·4	1·3 1·4	—	—	—	—	—	0	0	—	—	—	—	—	—
$J_{\alpha CH_3,\beta CH_3}$	1·5	—	—	1·5	1·5	—	1·5	1·5	0	0	—	—	—	—	—	—

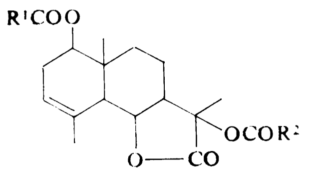
TABLE III

Specific rotation and $\Delta\varepsilon$ at 210 nm in CD spectrum of native lactones of the slov-3-enolide ($1\beta H,5\beta H,6\alpha H,7\alpha H$-guai-3-en-6,12-olide) type

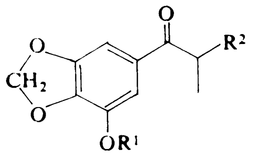
Compound	$[\alpha]_D^{20}$		Compound	$[\alpha]_D^{20}$	
III	−77·2	−4·8	*XIX*	−86·4	−2·2
V	−22·3	−3·6	*XXXII*	−55·1	−1·0
VI	−75·6	−4·1	*XIV*	—	−2·6
XI	−89·2	−3·9	*XXIX*	—	−1·4

lactone *XIV* on the basis of a comparison of CD spectra (Table III). According to this comparison formula *XIX* also represents its absolute configuration.

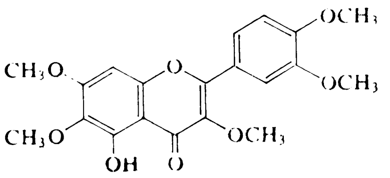
From the chloroform extract we isolated compound *XXI* as the least polar substance, m.p. 161–164°C and composition $C_{10}H_{10}O_4$. From the IR spectrum of this substance it follows that it contains an aromatic system (1 608 and 1 519 cm^{-1}), a conjugated oxo group (1 670 cm^{-1}) and a hydroxyl group (3 350 cm^{-1}). From the two last mentioned values it further follows that the hydroxyl group is not bound with the carbonyl group by an intramolecular hydrogen bond. Similar values ν(OH) and ν(CO) were described, for example in the case of (3-hydroxyphenyl)propan-1-one[15]



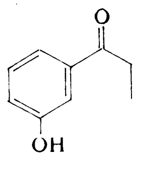
I, $R^1 = CH_3$; $R^2 = C_4H_7$
II, $R^1 = C_4H_7$; $R^2 = CH_3$



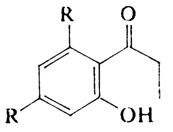
IV, $R^1 = CH_3$; $R^2 = H$
X, $R^1 = CH_3$; $R^2 = OCOC(CH_3) = CHCH_3$
XXI, $R^1, R^2 = H$
XXV, $R^1 = CONHCOCCl_3$; $R^2 = H$



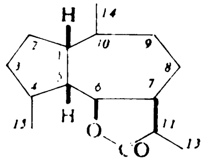
IX



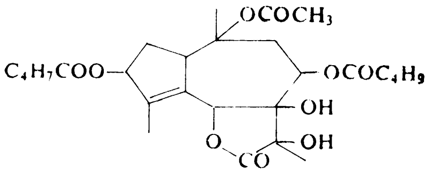
XXII



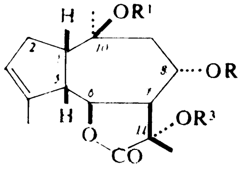
XXIII, R = H
XXIV, $R = OCH_3$



XII



XXVI



III: $R^1 = H$; $R^2 = COCH{=}C(CH_3)_2$; $R^3 = COCH_3$
V: $R^1 = H$; $R^2 = COC(CH_3){=}CHCH_3$; $R^3 = COCH_3$
VI: R^1, $R^3 = COCH_3$; $R^2 = COC(CH_3){=}CHCH_3$
VII: $R^1 = H$; R^2, $R^3 = COC(CH_3){=}CHCH_3$
XI: R^1, $R^3 = COCH_3$; $R^2 = COCH(CH_3)_2$
XIV: $R^1 = H$; $R^2 = COCH(CH_3)C_2H_5$; $R^3 = COCH_3$
XV: $R^1 = CONHCOCCl_3$; $R^2 = COCH(CH_3)C_2H_5$; $R^3 = COCH_3$
XVI: $R^1 = CONHCOCCl_3$; $R^2 = COCH{=}C(CH_3)_2$; $R^3 = COCH_3$
XVII: $R^1 = CONHCOCCl_3$; $R^2 = COC(CH_3){=}CHCH_3$; $R^3 = COCH_3$
XVIII: $R^1 = CONHCOCCl_3$; R^2, $R^3 = COC(CH_3){=}CHCH_3$
XIX: $R^1 = H$; $R^2 = COCH(CH_3)_2$; $R^3 = COCH_3$
XX: $R^1 = CONHCOCCl_3$; $R^2 = COCH(CH_3)_2$; $R^3 = COCH_3$
XXIX: R^1, $R^3 = COCH_3$; $R^2 = H$
XXX: R^1, $R^3 = COCH_3$; $R^2 = CONHCOCCl_3$
XXXII: R^1, $R^2 = H$; $R^3 = COCH_3$
XXXIII: R^1, $R^2 = CONHCOCCl_3$; $R^3 = COCH_3$

(*XXII*) (Table IV). The hydroxyl group in *o*-hydroxyacetophenone (*XXIII*; Table IV) is bound to the carbonyl group by a strong intramolecular hydrogen bond, as evident from the broad band about 3 000 cm^{-1} and a band of the carbonyl frequence at 1 643 cm^{-1} (ref.[16]). Similarly, in xanthoxylin[17] (*XXIV*; Table IV) the values ν(OH) and ν(CO) correspond to the mentioned values of *o*-hydroxyacetophenone (Table IV). From the mentioned data it is evident that compound *XXI* contains a free hydroxyl group in a *meta* position to the propan-1-one chain. The^1 H NMR spectrum of compound *XXI* contains signals of two aromatic hydrogens (doublets at δ 7·12 and 7·24), with coupling constants of 1·5 Hz, indicating their mutual *meta*-position on the ring. The two-proton singlet at δ 6·06 belongs to the methylenedioxy group and the three-proton triplet at δ 1·20 (J = 7·3 Hz), together with the quartet at δ 2·91 (J = 7·3 Hz) are evidence for the fragment $CH_3CH_2C{=}O$. The reaction with TAI confirmed the presence of the phenolic hydroxyl (in the TAC-derivative *XXV* by the singlet of the NH proton at δ 8·75). The close similarity of the ^1H NMR parameters of compound *XXI* with the known substance *IV* (Table V) practically excludes the alternative structure with the exchanged positions of the substituents OH and $COCH_2CH_3$. Hence the structure 1-(3,4-methylenedioxy-5-hydroxyphenyl)-propan-1-one (*XXI*) may be assigned to the isolated substance, and formula *XXV* to its TAC-derivative.

Further we isolated from the chloroform extract trilobolide *XXVI* with m.p. 190–192°C, $[\alpha]_D^{20}$ +69·8 and the composition $C_{27}H_{38}O_{10}$. We identified it on the basis of a comparison of its IR spectrum, ^1H NMR spectrum and mixture melting point with a standard sample which was obtained from the underground part of the species *Laser trilobum* (L.) BORKH.[8]

TABLE IV

ν(OH) and ν(CO) values of 1-(3,4-methylenedioxy-5-hydroxyphenyl)propan-1-one (*XXI*), (3-hydroxyphenyl)propan-1-one (*XXII*), *o*-hydroxyacetophenone (*XXIII*) and xanthoxyline (*XXIV*)

Compound	*XXI*	*XXII*	*XXIII*	*XXIV*
$\nu_{(OH)}$, cm^{-1}	3 350	3 260	3 000	2 800
$\nu_{(CO}$, cm^{-1}	1 670	1 663	1 643	1 622

TABLE V

^1H NMR Parameters of derivatives *IV*, *XXI* and *XXV*

Compound	CH_3	CH_2CO	O—CH_2—O	arom.H	OCH_3	NH
IV	1·21 t (J = 7·3)	2·92 q (J = 7·3)	6·07 s	7·15 d, 7·28 d (J = 1·5) (J = 1·5)	3·95 s	—
XXI	1·20 t (J = 7·3)	2·91 q (J = 7·3)	6·06 s	7·12 d, 7·24 d (J = 1·5) (J = 1·5)	—	—
XXV	1·21 t (J = 7·3)	2·91 q (J = 7·3)	6·13 s	7·41 d, 7·47 d (J = 1·5) (J = 1·5)	—	8·75 s

Two further substances, *XXVII* and *XXVIII*, were more polar than trilobolide. Compound *XXVII*, which is only slightly less polar than compound *XXVIII*, would not crystallize; $[\alpha]_D^{20}$ +12·0, composition $C_{24}H_{32}O_9$. This substance has not been described in literature so far and we propose for it the structure of 2α-hydroxy-8α-angeloyloxy-10β,11α-diacetoxyslov-3-enolide (the determination of its structure is described in the subsequent communication[18]). Compound *XXVIII* had m.p. 189–192°C, $[\alpha]_D^{20}$ +68·3 and the composition $C_{24}H_{32}O_9$. Neither has this substance been described in literature so far. The determination of its structure is also described in the subsequent communication[18].

The non-crystalline compound *XXIX* of the composition $C_{19}H_{26}O_7$ and with the CD spectrum containing a maximum at 210 nm with $\Delta\varepsilon$ −1·4 was more polar than the preceding pair of the sesquiterpenic lactones. The IR spectrum indicated the presence of a hydroxyl group (3 500 and 3 600 cm^{-1}), a γ-lactone ring (1 780 cm^{-1}) and an acetate group (1 730 and 1 249 cm^{-1}). The mass spectrum did not contain the molecular peak but displayed characteristic peaks of fragments of *m*/*z* 306 (M−60), 246 (M−60−60) and 228 (M−60−60−18). The ^1H NMR spectrum of compound *XXIX* (Table I and II) again showed a close similarity with the spectra of lactones *XIV* and *XIX*, but in this case both ester groups were identified as acetate (singlets at δ 2·06 and 2·10). TAI-Acylation showed the presence of a hydroxyl (TAC-derivative *XXX* gives a singlet of the NH proton at δ 8·33), which, however, must be bound this time in the position 8, as evident from the characteristic acylation shift of the $C_{(8)}H$ hydrogen ($\Delta\delta$ = 1·36 ppm) and the corresponding shifts of the protons $C_{(7)}H$ ($\Delta\delta$ = 0·46) and $C_{(9)}H_2$($\Delta\delta$ = 0·20 resp. 0·07 ppm). Hence, the acetates must be bound in the positions 10 and 11. These structural modifications also explain the differing shifts of the methyl signals $C_{(13)}H_3$ and $C_{(14)}H_3$ in comparison with lactones *XIV* and *XIX*. Hence, the native compounds must have the structure *XXIX*. We determined the absolute configuration of 8α-hydroxy-10β,11α-diacetoxyslov-3-enolide *XXIX* similarly as in the case of lactone *XIV*, by comparison of CD spectra (Table III).

A further isolated substance was compound *XXXI*, m.p. 181–184°C, composition $C_{22}H_{30}O_8$. A detailed analysis of its ^1H NMR spectrum showed that it is an as yet undescribed substance and that its structure is close to that of 4β-hydroxy-8α-angeloyloxy-10β,11α-diacetoxyslov-2-enolide (*XXVIII*). We propose for it the name 4β,10β-dihydroxy-8α-angeloyloxy-11α-acetoxyslov-2-enolide; the determination of its structure is described in the following paper[18].

The most polar substance *XXXII* which we isolated from the chloroform extract was not crystalline with $[\alpha]_D^{20}$ −55·1 and the composition $C_{17}H_{24}O_6$. Its IR spectrum indicated the presence of a hydroxyl group (3 480 and 3 600 cm^{-1}), a γ-lactone ring (1 778 cm^{-1}), an acetate group (1 736 and 1 250 cm^{-1}) and a double bond (1 642 cm^{-1}). The mass spectrum contained the molecular peak at 324 m.u. and the signals of characteristic fragments of *m*/*z* 306 (M-18), 246 (M-18-60), 228 (M-18-60-18). The CD maximum was at 210 nm, with $\Delta\varepsilon$ − 1·0. The ^1H NMR spectrum of lactone

XXXII and its TAC-derivative *XXXIII* (Table I and II) again had similar characteristic features as in lactones *XIV*, *XIX* and *XXIX* or their TAC-derivatives *XV*, *XX* and *XXX*, but with only a single acetate as the ester group. According to expectation the product of TAI acylation, *XXXIII*, displayed the presence of two hydroxyls in the molecule (NH signals at δ 8·42 and 8·54), of which one must be again in position 8 (acylation shift of $C_{(8)}H$ is 1·11 ppm) and the other in position 10 (the acylation shift of the methyl protons $C_{(14)}H_3$ is 0·44 ppm), similarly as in the identified *XIV* and *XIX*. The only acetate group is again in position 11. Hence, the lactone may be assigned the structure *XXXII* with the name 8α,10β-dihydroxy-11α-acetoxyslov-3-enolide. For this substance we derived the absolute configuration, expressed by formula *XXXII*, similarly as in the case of lactone *XIX*, by comparison of CD spectra and specific rotations (Table III).

During a detailed analysis of the light petroleum and chloroform extract of the underground parts of *L. siler* L. of Slovenian origin we identified or isolated altogether 18 substances, three of them phytosterols, two phenylpropane derivatives and 13 sesquiterpenic lactones. We newly detected in the mentioned underground part 11 lactones of which two were described earlier, as components of the fruits of this species[3,4] (lactones *V* and *VII*) and one (trilobolide) was isolated earlier from the roots of *Laser trilobum* (L.) BORKH. (*Umbelliferae*, tribe *Laserpitieae*)[8]. Of the 13 sesquiterpenic lactones isolated we have now determined the complete structure for four of them, including the relative and absolute configurations. These four newly described lactones (compounds *XIV*, *XIX*, *XXIX* and *XXXII*) together with five further substances (*III*, *V*–*VII* and *XI*) which were described earlier[5,7], belong to the new, relatively recently described group of 1βH,5βH,6αH,7αH-guaian-6,12-olides[7] (slovanolides).

From a comparison of the components, especially sesquiterpenic lactones which were identified in the fruits[3,4], in the above-ground part[6] and the underground part of *Laserpitium siler* L. it follows (if disregarding the localities of the material investigated) that some substances (lactones *V*–*VII*), which occur in relatively high concentrations, are present in all the plant parts investigated, while some others, usually present in the given part in lower amounts, were so far detected only in some parts of the species investigated. For example, the above-ground part contains 1-(3,4-methylenedioxy-5-methoxyphenyl)-2-angeloyloxypropan-1-one (*X*), but not 1-(3,4-methylenedioxy-5-methoxyphenyl)propan-1-one (*IV*), which is not contained in the fruit either but is present only in the underground part, the same as, for example, isosilerolide *XIII*. The other sesquiterpenic lactones present in the underground part which are structurally related and which are also closely related structurally with the quantitatively predominant lactones, were present only in relatively small amounts

Hence, it may be concluded that from the quantitative point of view no fundamental difference exists – with the exception of isosilerolide – in the structures of the sesquiterpenic lactones occurring in the three parts of the *L. siler* L. investigated.

Discussion of the NMR *Results*

In our previous paper[7] we determined on the basis of spectral data and chemical correlations the configurations 1βH,5βH,6αH,7αH,8αH,10βOR,11αOR for 8α-senecioyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*III*), 8α-angeloyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*V*) and 8α-angeloyloxy-10β,11α-diacetoxyslov-3-enolide (*VI*). The 100 MHz ^1H NMR spectra used at that time did not permit the assignment of some hydrogens (H-2α and H-2β not at all, and in the case of *III* not even H-1, H-5, H-9α and H-9β either) and the determination of a number of coupling constants. The structural analysis of newly isolated lactones described in this paper was carried out on a 200 MHz FT-NMR spectrometer. The spectra displayed a distinct similarity with the formerly published data[7] and they also permitted us to assign all signals and coupling constants. In order to confirm the identity of the skeletons of the newly described lactones with the derivatives of 8α-senecioyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*III*), we carried out a new measurement of compounds *III*, *V* and *VI* on a 200 MHz spectrometer and also a complete assignment of the protons in these substances as well (for data see Tables I and II). All hydroxy derivatives were converted to corresponding TAC-derivatives *XVI* and *XVII* by *in situ* acylation with TAI and we measured their ^1H NMR spectra. The shifts of hydrogens, induced by TAI-acylation, are surveyed in Table VI. A comparison of NMR parameters shows unambiguously that compounds *III*, *V*–*VII*, *XI*, *XVI*–*XXII*, *XXIX*, *XXX*, *XXXII* and *XXXIII* possess the same guaian-6,12-olide skeleton with the configurations 1βH,5βH,6αH,7αH and that they differ only in the number and the character of the ester groups in positions 8α, 10β and 11α. A complete analysis of the vicinal coupling constants, together with the long-range interactions provides sufficient argument for the proposal of the probable preferred conformations (Fig. 1), common for the whole set of substances. For greater clarity the projections representing approximate orientations of the atoms around individual C—C bonds are represented in Fig. 1, which are in agreement with the observed values of the coupling constants. The hydrogens of the methylene groups in positions 2 and 9 were assigned configurationally as α and β on the basis of the $J_{1,2}$, $J_{2,3}$ or $J_{8,9}$ values. The assignment was confirmed by differing homoallylic interactions $J_{2,15}$, $J_{2',15}$, the observation of ^4J interaction (σ-type) of hydrogen H-9β with H-1 (requiring a *W*-coupling pathway) and a distinct TAI-acylation shift of H-9β, comparable to the shift of H-1 which has an equivalent position with respect to $C_{(10)}$-OR as H-9β in the conformation presented.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. For column chromatography silica gel according to Pitra and Štěrba (30–60 mμ, deactivated by addition of 11% of water) was used, while silica gel G Merck according to Stahl was used for thin-layer

TABLE VI

TAI-Induced acylation shifts of protons in some hydroxy-1βH,5βH,6αH,7αH-guaian-6,12-olides

Proton	*XIV*—*III*[a] ($C_{(10)}$)	*XVII*—*V*[a] ($C_{(10)}$)	*XVIII*—*VII*[a] ($C_{(10)}$)	*XV*—*XIV*[a] ($C_{(10)}$)	*XX*—*XIX*[a] ($C_{(10)}$)	*XXX*—*XXIX*[a] ($C_{(8)}$)	*XXXIII*—*XXXII*[a] ($C_{(8)}$, $C_{(10)}$)
H-1	0·54	0·52	0·52	0·50	0·51	—0·17	0·58
H-2α	0·10	0·11	0·11	0·10	0·11	*b*	*b*
H-2β	0·14	0·12	0·13	0·15	0·14	*b*	*b*
H-3	0·03	0·02	0·02	0·02	0·01	0·06	0·05
H-5	0·12	0·13	0·13	0·14	0·14	0·03	0·22
H-6	0·01	0·02	0·01	0·01	0·02	0·01	0·26
H-7	0·01	0·00	0·00	0·00	0·01	0·46	0·54
H-8	0·10	0·12	0·11	0·09	0·08	1·36	1·23
H-9α	0·02	0·02	0·03	0·02	0·04	0·07	0·19
H-9β	0·62	0·63	0·65	0·70	0·70	0·20	0·94
H-13	—0·02	—0·02	—0·02	—0·02	—0·03	—0·02	—0·08
H-14	0·44	0·44	0·44	0·44	0·44	0·14	0·44
H-15	0·03	0·02	0·02	0·02	0·03	0·02	0·04
$OCOCH_3$	—0·03	—0·02	—	—0·03	—0·02	—0·02 0·02	—0·06
α-H	—0·03	—	—	—0·05	—0·04	—	—
α-CH_3	—	—0·03	—0·05 —0·03	—0·03	—0·03 —0·04	—	—
β-CH_3	—0·04 —0·02	—0·05	—0·03 —0·03	—0·02	—	—	—
β-H	—	—0·56	—0·05 —0·01	—0·02 —0·02	—	—	—

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[a] Acylation shift ($\Delta\delta_{H_i} = \delta_{H_i}$ (R-OTAC)- δ_{H_i} (R-OH)) of the hydroxylactone and its TAC-derivative with the localisation of the OH or the OTAC group, respectively; [b] not determined due to the overlapping of signals.

chromatography. The IR spectra were measured in chloroform on Specord 71 IR and Perkin Elmer PE 580 instruments. The ^1H NMR spectra were measured on a Varian XL-200 instrument in deutericchloroform, using tetramethylsilane as internal reference. The *in situ* acylations were carried out using a standard procedure[13,14], on addition of trichloroacetyl isocyanate (TAI) into a solution of the substance in the NMR cell. Chemical shifts and coupling constants were obtained by first order analysis from expanded spectra, using the exponential multiplication with gaussian apodization function for resolution enhancement. The mass spectra were measured on an AEI MS 902 spectrometer. Optical rotations were measured in methanol with an objective Perkin Elmer 141 polarimeter. The circular dichroism was measured on a Roussel Jouan CD 185 dichrographe, in methanol.

Isolation of Light Petroleum Extract Components

Dried, ground rhizomes and roots (14·9 kg) of *L. siler* L. of Slovenian origin were extracted with light petroleum at room temperature to completion. After evaporation of the solvent 700 g o

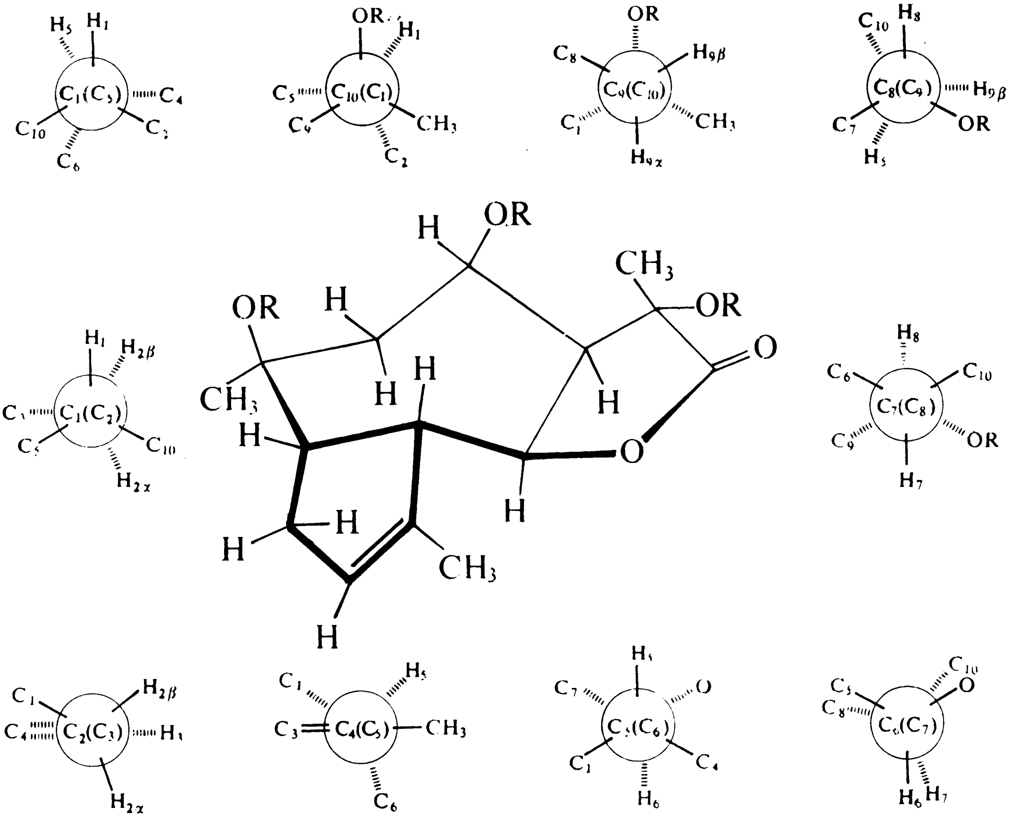

FIG. 1

Schematic representation of the probable conformation of 1βH,5βH,6αH,8βH,10βOR, 11αOR-guaian-6,12-olides with the Newman projections of some C—C bonds extract were obtained a part of which (60 g) was chromatographed on a silica gel column (1 200 g) using toluene and ether–toluene mixtures of various ratios; 15 main fractions were thus obtained (Table VII).

1-(3,4-Methylenedioxy-5-methoxyphenyl)propan-1-one (*IV*)

From fraction 2 (Table VII) ketone *IV* (130 mg) was obtained by repeated column chromatography on silica gel, m.p. 87—88°C (light petroleum), composition $C_{11}H_{12}O_4$. The identity with authentic sample was confirmed by comparison of their mass spectra, IR spectra and ^1H NMR spectra and mixture melting point which was undepressed.

Isosilerolide (*XIII*)

From fraction 4 (Table VII) isosilerolide (*XIII*; 1·79 g) was obtained by crystallization; m.p. 141—143°C (diisopropyl ether) and $[\alpha]_D^{20}$ $-138{\cdot}9$ (*c* 0·51). For $C_{22}H_{30}O_6$ (390·6 calculated: 67·66% C, 7·74% H; found: 67·55% C, 7·52% H.

Phytosterols

From the mother liquor (5·0 g) after the crystallization of isosilerolide a mixture of phytosterols (30 mg) was obtained by repeated silica gel column chromatography, in which β-sitosterol (77%), stigmasterol (22%) and campesterol (1%) were identified by gas chromatography (on PYE 10/64 chromatograph with FI detection, glass column 0·4 × 150 cm packed with 3% SE-30 G.C. Grade

TABLE VII

Chromatography of the light petroleum extract

Fraction	Solvent	Weight of residue, g	The main component
1	toluene	8·3	—
2	toluene	7·2	*IV*
3	toluene	3·7	phytosterols
4	toluene	8·3	*XIII*, phytosterols
5	toluene	5·7	—
6	toluene	6·4	*VI*
7	toluene with 5% of ether	7·0	—
8	toluene with 5% of ether	1·3	*XI*
9	toluene with 5% of ether	1·0	—
10	toluene with 5% of ether	3·8	*VII*
11	toluene with 5% of ether	4·3	—
12	toluene with 5% of ether	4·5	*V*
13	toluene with 5% of ether	1·5	—
14	toluene with 10% of ether	3·0	*XIV*, *XIX*
15	toluene with 20% of ether	1·9	—

on Gas-Chrom Q (80—100 mesh), temperature of the column 260°C, temperature of the injector and detector 280°C).

8α-Angeloyloxy-10β,11α-diacetoxyslov-3-enolide (*VI*)

Fraction 6 (Table VII) was crystallized, affording lactone *VI* (2·26 g), m.p. 132—134°C (diisopropyl ether), $[\alpha]_D^{20}$ −75·6 (*c* 0·47) and composition $C_{24}H_{38}O_8$. The identity with a standard[3] was confirmed by comparison of their mass, IR and ^1H NMR spectra and mixture melting point, which was undepressed.

8α-Isobutyroyloxy-10β,11α-diacetoxyslov-3-enolide (*XI*)

From fraction 8 (Table VII) lactone *XI* (360 mg) was isolated by crystallization, m.p. 125—127°C (diisopropyl ether), $[\alpha]_D^{20}$ −89·2 (*c* 0·39), composition $C_{23}H_{32}O_8$. The identity with an authentic sample[5] was shown by comparison of their mass, IR and ^1H NMR spectra and mixture melting point (without depression).

8α,11α-Diangeloyloxy-10β-hydroxyslov-2-enolide (*VII*)

From fraction 10 (Table VII) compound *VII* (1·72 g) was obtained by crystallization, m.p. 149 to 151°C (diisopropyl ether), $[\alpha]_D^{20}$ −49·8 (*c* 0·52), composition $C_{25}H_{34}O_7$. The identity with a standard compound[4,5] was confirmed by comparison of their mass, IR and ^1H NMR spectra and mixture melting point which was undepressed.

8α-Angeloyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*V*)

From fraction 12 (Table V) lactone *V* (450 mg) was obtained by crystallization, m.p. 174—176°C (diisopropyl ether), $[\alpha]_D^{20}$ −22·3 (*c* 0·31), composition $C_{22}H_{30}O_7$. Identity with the standard[3] was proved by comparison of their mass, IR and ^1H NMR spectra and mixture melting point (without depression).

8α-(2′-Methylbutyroyloxy)-10β-hydroxy-11α-acetoxyslov-3-enolide (*XIV*)

Repeated chromatography of fraction 14 (Table V) on a silica gel column (with toluene–ether 9 : 1) afforded lactone *XIV* (14 mg), m.p. 154—155°C (diisopropyl ether). IR spectrum (in cm^{-1}): 3 538 (hydroxyl), 1 784 (γ-lactone), 1 738, 1 250 (acetate), 1 719 (ester), 1 648 (double bond). Mass spectrum (*m*/*z*): 408 (M), 390 (M−18), 348 (M−60), 306 (M−102), 246 (M−102−60), 228 (M−102−60−18), 85 ($C_4H_9CO^+$), 57 ($C_4H_9^+$). CD spectrum (nm, Δε): 210 (last reading), −2·6. For $C_{22}H_{32}O_7$ (408·5) calculated: 64·68% C, 7·90% H, 0·25% H act.; found: 64·94% C, 7·83% H, 0·30% H act.

8α-Isobutyroyloxy-10β-hydroxy-11α-acetoxyslov-3-enolide (*XIX*)

From fraction 14 (Table VII) lactone *XIX* (32 mg) was obtained by repeated chromatography on a silica gel column using 10% ether in toluene; m.p. 162—164°C (diisopropyl ether) and $[\alpha]_D^{20}$ −86·4 (*c* 0·34). IR spectrum (cm^{-1}); 3 540 (hydroxyl), 1 785 (γ-lactone), 1 738, 1 250 (acetate), 1 719 (ester), 1 648 (double gond). Mass spectrum (*m*/*z*): 394 (M), 376 (M−18), 334 (M−60), 316 (M−60−18), 306 (M−88), 288 (M−88−18), 246 (M−88−60), 228 (M−88−60−18), 71 ($C_3H_7CO^+$), 43 ($C_3H_7^+$). CD spectrum (nm, Δε): 210 (last reading), −2·2. For $C_{21}H_{30}O_7$ (394·5) calculated: 63·93% C, 7·67% H, 0·26% H act.; found: 63·74% C, 7·68% H, 0·28% H act.

Isolation of the Components in the Chloroform Extract

After exhaustive extraction of the underground parts with petroleum ether the material was further extracted exhaustively with chloroform at room temperature. After evaporation of the solvent a residue was obtained (190 mg) from which a part (60 g) was chromatographed on a silica gel column (1 200 g) using toluene, toluene–ether mixtures of various ratios, ether and ether–methanol mixtures of various ratios and methanol for elution; 17 basic fractions were obtained (Table VIII).

1-(3,4-Methylenedioxy-5-hydroxyphenyl)propan-1-one (*XXI*)

From fraction 3 (Table VIII) ketone *XXI* (20 mg) was obtained by repeated chromatography on a silica gel column using toluene with 10% ether for elution; m.p. 161—164°C (ethyl acetate). IR spectrum (cm^{-1}): 3 350 (hydroxyl), 1 670 (conjugated ketone), 1 608, 1 519 (aromatic system). Mass spectrum (m/z): 194 (M). For $C_{10}H_{10}O_4$ (194·3) calculated: 61·81% C, 5·19% H, 0·52% H act.; found: 61·96% C, 5·17% H, 0·43% H act.

Trilobolide (*XXVI*)

From fraction 4 (Table VIII) trilobolide (*XXVI*; 340 mg) with m.p. 190—192°C, $[\alpha]_D^{20}$ +68·9 (c 0·76) and composition $C_{27}H_{38}O_{10}$ was obtained by repeated chromatography on a silica gel column, using 15% ether in toluene for elution. The identity with an authentic sample of triloboli-

Table VIII

Chromatography of chloroform extract

Fraction	Solvent	Weight of residue, g	Main component
1	toluene	4·7	—
2	toluene with 5% of ether	7·7	—
3	toluene with 10% of ether	3·1	*XXI*
4	toluene with 15% of ether	2·0	*XXVI*
5	toluene with 20% of ether	1·1	—
6	toluene with 30% of ether	2·9	*XXVII*, *XXVIII*
7	toluene with 40% of ether	1·6	—
8	toluene with 50% of ether	1·3	*XXIX*
9	ether	3·3	—
10	ether with 1% of methanol	2·4	—
11	ether with 2% of methanol	5·9	*XXXI*
12	ether with 5% of methanol	1·5	*XXXII*
13	ether with 10% of methanol	0·9	—
14	ether with 15% of methanol	4·9	—
15	ether with 20% of methanol	0·9	—
16	ether with 30% of methanol	1·6	—
17	methanol	0·6	—

de[8] was demonstrated by comparison of mass, IR and ^1H NMR spectra and mixture melting point which remained underpressed.

2α-Hydroxy-8α-angeloyloxy-10β,11α-diacetoxyslov-3-enolide (*XXVII*)

From the mother liquor after crystallization of compound *XXVIII* non-crystalline lactone *XXVII* (68 mg) was obtained by repeated chromatography on a silica gel column and preparative thin-layer chromatography on the same adsorbent, using toluene with 30% ether for elution. It had $[\alpha]_D^{20}$ +12·0 (*c* 0·32). For $C_{24}H_{32}O_9$ (464·5) calculated: 62·05% C, 6·95% H, 0·22% H act.; found: 61·99% C, 7·12% H, 0·34% H act.

4β-Hydroxy-8α-angeloyloxy-10β,11α-diacetoxyslov-2-enolide (*XXVIII*)

Fraction 6 (Table VIII) afforded lactone *XXVIII* (42 mg) on repeated chromatography on silica gel with 30% of ether in toluene as eluent. M.p. 189—192°C (ethyl acetate), $[\alpha]_D^{20}$ +68·3 (*c* 0·37). For $C_{24}H_{32}O_9$ (464·5) calculated: 62·05% C, 6·95% H, 0·22% H act.; found: 62·25% C, 7·18% H, 0·30% H act.

8α-Hydroxy-10β,11α-diacetoxyslov-3-enolide (*XXIX*)

From fraction 8 (Table VIII) non-crystalline lactone *XXIX* (26 mg) was isolated by repeated column chromatograpy on silica gel using 50% of ether in toluene and preparative thin-layer chromatography on the same sorbent. IR spectrum (cm^{-1}): 3 500, 3 600 (hydroxyl), 1 780 (γ-lactone), 1 730, 1 249 (acetate). Mass spectrum (*m*/*z*): 306 (M—60), 246 (M—60—60), 228 (M—60—60—18). CD spectrum (nm, Δε): 210 (last reading), −1·4. For $C_{19}H_{26}O_7$ (366·4) calculated: 62·28% C, 7·15% H, 0·28% H act.; found: 62·04% C, 7·32% H, 0·35% H act.

4β,10β-Dihydroxy-8α-angeloyloxy-11α-acetoxyslov-2-enolide (*XXXI*)

From fraction 11 (Table VIII) lactone *XXXI* (17 mg) was obtained by repeated column chromatography on silica gel (with 2% methanol in ether) and preparative thin-layer chromatography, m.p. 181—184°C (ethyl acetate, diisopropyl ether). For $C_{22}H_{30}O_8$ (422·5) calculated: 62·54% C, 7·16% H, 0·48% H act.; found: 62·76% C, 6·95% H, 0·36% H act.

8α,10β-Dihydroxy-11α-acetoxyslov-3-enolide (*XXXII*)

From fraction 12 (Table VIII) non-crystalline *XXXII* (30 mg) was isolated by repeated column chromatography on silica gel, using 5% methanol in ether for elution, and preparative thin-layer chromatography on the same adsorbent: $[\alpha]_D^{20}$ −55·1 (*c* 0·13). IR spectrum (cm^{-1}): 3 480, 3 600 (hydroxyl), 1 778 (γ-lactone), 1 736, 1 250 (acetate), 1 642 (double bond). Mass spectrum (*m*/*z*): 324 (M), 306 (M—18), 246 (M—18—60), 228 (M—18—60—18). CD spectrum (nm, Δε): 210 (last reading), −1·0). For $C_{17}H_{24}O_6$ (324·4) calculated: 62·94% C, 7·46% H, 0·62% H act.; found: 63·13% C, 7·44% H, 0·71% H act.

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