CONSTITUENTS OF THE LIVERWORT *Bazzania trilobata* OF CZECH ORIGIN

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From *Bazzania trilobata* of Czech origin a series of sesquiterpenoids were obtained as products identical with those described in a recent paper from the same species of Japanese origin, namely α- and β-barbatene, bazzanene, cuparene, calamenene, 5-hydroxy- and 7-hydroxycalamenene, and drimenol. Furthermore we obtained ledene, gymnomitrol, gymnomitrone and viridiflorol as new components for this plant. A detailed study of the wax components was performed, too. A study of seasonal variations of the essential oil content and of its composition is presented.

The components of the liverwort *Bazzania trilobata* (L.) S. GRAY (*Jungermaniales*) have been studied quite intensively, (see, *e.g.*, the recent review of Asakawa[1]), and the latest information on the composition of a specimen of the Japanese origin was published[2] in the time when our study was in progress. Our results complete and correct in some respect the precedent papers, and add several new compounds, typical for the same botanical species grown under different geographic conditions. Toyota, Asakawa and Takemoto[2] demonstrated in the ethanolic or etherical extracts the presence of α- and β-barbatene (*I*, *II*), bazzanene (*III*), cuparene (*IV*) and 2-hydroxycuparene (*V*), (+)-calamenene (*VIa*), 5-hydroxycalamenene (*VII*), 7-hydroxycalamenene (*VIII*) and drimenol (*IX*).

In this work we have used the standard procedure[3] of isolating the volatile components from the steam-distilled essential oil of a fresh plant material, originating from a spruce forest in middle Bohemia. We isolated the waxy substances from a chloroform extract of an air-dried material. Single compounds were isolated by the combination of column and thin-layer chromatography, or by GLC in a preparative scale. All separation steps were monitored by GLC. From the material of the Czech origin, the same compounds as in the Japanese specimen were obtained, with the exception of the alcohol *V*.

Using the IR and mass spectral data, a group of three derivatives (*VI*, *VII* and *X*) of cadinane type was identified as calamenene and two its hydroxy derivatives.

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From the ^{1}H NMR spectra of these three compounds (Table I) their common structural features are clear, *i.e.*, the presence of one methyl group bound to sp^2 carbon atom, one secondary methyl on sp^3 carbon atom of an isopropyl group, and three (compound *VI*) resp. two (compounds *VII* and *X*) aromatic H. From ^{1}H NMR data and molecular formulae it follows that the compounds mentioned contain an aromatic ring substituted by a methyl group and an annelated cyclohexane ring bearing a methyl and an isopropyl group. From the multiplicity of the signals in the aromatic region of the compound *VI* it is clear that two aromatic H are mutually in position *ortho-*, the third proton lies towards them in the positions *meta-* or *para-*. Decoupling experiments enabled to find methine hydrogens on carbon atoms bearing methyl and isopropyl groups: These do not interact together, and two remaining methylene groups of the cyclohexane ring form an intensively interacting system in the upfield region of the spectrum. This is all in accord with the fact that methyl and isopropyl groups are located in the position 1,4 of a cadinane type carbon skeleton.

The hydroxy derivative *VII* shows two hydrogens forming an AB-system in the aromatic region of the spectrum, with a coupling constant $J = 8$ Hz. From this follows an *ortho*-position of aromatic H in positions 7 and 8, and of hydroxyl in position 5. The assignment of aromatic H has been done according to the comparison of observed and calculated chemical shift values (Table II). From the comparison

TABLE I

^{1}H NMR Chemical shifts of compounds *VI*, *VII*, *X*, *XI*, and TAC-derivatives of *VII* and *X* in $CDCl_3$

Proton	*VI*	*VII*	*X*	*XI*[a]	*VII* + TAI[b]	*X* + TAI[b]
$H_{(1)}$	2·86	2·87	3·06	3·07	2·90 (+0·03)	2·99 (−0·07)
$H_{(2,2')}$	1·55—	1·72—	1·49—	1·75—	1·50—[c]	1·50—[c]
$H_{(3,3')}$	—1·88	—2·15	—1·81	—2·10	—2·10[c]	—1·85[c]
$H_{(4)}$	2·59	2·73	2·69	[c]	2·54 (−0·19)	2·75 (+0·06)
$H_{(5)}$	7·03	—	6·41	6·57	—	6·79 (+0·38)
$H_{(7)}$	6·93	6·93	6·70	6·39	7·05 (+0·12)	7·04 (+0·34)
$H_{(8)}$	7·05	6·75	—	—	7·10 (+0·35)	—
$H_{(11)}$	2·25	2·03	2·38	2·46	1·93 (−0·10)	2·41 (+0·03)
$H_{(12)}$	0·77	0·92	0·60	0·83	0·88 (−0·04)	0·69 (0·00)
$H_{(13)}$	1·03	0·95	1·04	0·97	0·91 (−0·04)	1·06 (+0·02)
$H_{(14)}$	1·25	1·30	1·21	1·20	1·31 (+0·01)	1·18 (−0·03)
$H_{(15)}$	2·29	2·21	2·24	2·21	2·18(−0·03)	2·31 (+0·07)

[a] Data from ref.[5]; [b] TAI-induced acylation shifts are given in parentheses; [c] nondetermined value.

of the data of the compounds *VI* and *VII*, it is clear that the substitution of the aromatic ring with a hydroxyl in the position 5 influences mostly the hydrogens $H_{(4)}$, $H_{(11)}$, $H_{(12)}$ and $H_{(13)}$ of the fragment $>CH—CH(CH_3)_2$, and shows a little effect on the hydrogens $H_{(1)}$ and $H_{(14)}$, belonging to the grouping $>CH—CH_3$, once again in accord with the compound of a cadinane type. The *in situ* reaction of *VII* with trichloroacetyl isocyanate[4] (TAI) afforded the corresponding trichloroacetylcarbamoyl derivative (acylation shifts are in Table I). Their values for $H_{(7)}$ (+0·12 ppm) and $H_{(8)}$ (+0·35 ppm) are typical for acylation effects into *meta*-, resp. *para*-positions, as it follows from the comparison with TAI acylation shifts at *o*-, *m*- and *p*-cresol (Table III). Negative $\Delta\delta$ were found for hydrogens $H_{(4)}$, $H_{(11)}$, $H_{(12)}$ and $H_{(15)}$ which are sterically nearest to the acylated OH-group, obviously as a result of the lower van der Waals' effect achieved by delocalization of the free electron pair on the oxygen atom belonging to the esterified hydroxyl group (see acylation shift of 2-methylphenol (Table III). These facts are in agreement with formula *VII*.

^{1}H NMR spectrum of compound *X* demonstrates the presence of two aromatic H with a small non-zero interaction. From the form of signals broadened owing to the interaction with the methyl $C_{(15)}$, two possible structures follow having the hydroxy group in position 7 or 8. The presence of this hydroxyl in the molecule studied effectively modifies the shift of $H_{(1)}$. The TAI-acylation led to the TAC-derivative of *X* with acylation shifts of the aromatic H +0·38 ppm, resp. +0·34 ppm, thus to the values corresponding to their *ortho*- and *para*-, not to the *meta*-positions. Negative values $\Delta\delta$ were found for $H_{(1)}$ and $H_{(14)}$, and therefore, sterically they had to be near to the esterified hydroxy group. These facts document that the compound *X* is an 8-hydroxy and not a 7-hydroxy derivative. Our structure is corroborated by the

TABLE II

Comparison of calculated and observed values of chemical shifts of aromatic protons in compounds *VII*, *X* and *VIII*

Proton	*VII*		*X*		*VIII*	
	calc.[a]	obs.	calc.[a]	obs.	calc.[a]	obs.[b]
$H_{(5)}$	—	—	6·37	6·41	6·91	6·92 (6·92)
$H_{(7)}$	6·81	6·93	6·58	6·70	—	—
$H_{(8)}$	6·60	6·75	—	—	6·49	6·52 (6·62)

[a] For the calculation the data of compound *VI* and the known contribution of hydroxy group in aromatic derivatives (ref.[6]) were used. [b] Data taken from ref.[7], data for *trans*-isomer of *VIII* (ref.[8]) are given in parentheses.

comparison of the calculated and found chemical shift values for a 7-OH or an 8-OH derivative. We can assume that the minor phenolic compound found in *B. trilobata* by the Japanese authors[2] with a tentatively proposed structure of 7-hydroxycalamenene (*VIII*) was, in fact, the 8-hydroxy derivative.

Recently, Nishikawa and coworkers[5] found a stereoisomeric 8-hydroxycalamenene *XI* and proved the *trans*-configuration of the substituents on its cyclohexane cycle. In agreement with this fact, the ^{1}H NMR spectrum of their compound *XI* is not identical with our spectrum of the compound *X* (compare Table I). The substituents and their positions in both compounds are identical and the differences of spectra are therefore due to different configurations on $C_{(1)}$ and $C_{(4)}$ which confirms our presumption given above – the relative *cis*-configuration in our compounds *X*, *VI* and *VII*.

Concerning the conformation of compounds *VI*, *VII* and *X*, the six-membered ring bearing a methyl and an isopropyl substituent, might be in twist-chair conformations $_3T^2$, $^3T_2$ or in boat forms. $B_{1,4}$, $B^{1,4}$ (Fig. 1), owing to its anellation to the aromatic cycle. For sterical reasons, the conformation $B_{1,4}$ is very improbable, as both substituents would have to be oriented axially. The character of the spectra does not allow a direct establishment of the values of the coupling constant $J_{1,2}$, $J_{1,2'}$, $J_{4,3}$, $J_{4,3'}$. The decoupling experiment (upon irradiation at methyl $C_{(14)}$) led to approximative values only $(J_{1,2} + J_{1,2'}) \approx 11$ Hz and $(J_{4,3} + J_{4,3'}) \approx$ ≈ 12 Hz. Using approximative values $J(30°) \approx 7$ Hz, $J(60°) \approx 3$ Hz and $J(150°) \approx$ ≈ 12 Hz, it is possible to exclude the second boat conformation $B^{1,4}$, since in such a case the sum of coupling constants has to overcome the value[9] by *c.* 17 Hz. The observed value $\sum J_{H,H}$ correspond optimally with the 1 : 1 equilibrium $_3T^2 \rightleftharpoons {}^3T_2$,

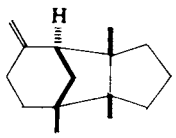
TABLE III

TAI-Induced acylation shifts in ^{1}H NMR spectra of isomeric cresols in $CDCl_3$

Proton[a]	*o*-Cresol	*m*-Cresol	*p*-Cresol
2 (*o*-)	–	+0·34	+0·32
3 (*m*-)	+0·10[b]	–	+0·17
4 (*p*-)	+0·32	+0·33	–
5 (*m*-)	+0·10[b]	+0·16	+0·17
6 (*o*-)	+0·34	+0·34	+0·32
CH_3	−0·02 (*o*-)	+0·08 (*m*-)	+0·10 (*p*-)

[a] Relative positions of protons or methyl group (in relation to hydroxy group) are given in parentheses. [b] Values are determined with lower accuracy (±0·05 ppm) due to complex character of spectra.

with the presence of approx. 55% of ${}_3T^2$ and 45% of ${}^3T_2$, being thus weakly significant for the conformation with an isopropyl substituent in the energetically more convenient equatorial orientation. The absolute configuration of our calamenene



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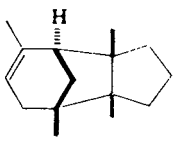

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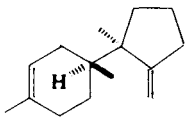

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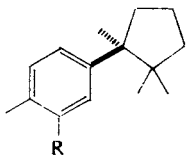
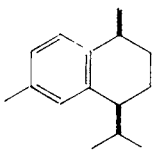
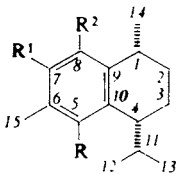


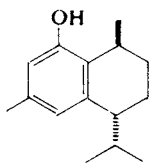
IV, R = H
V, R = OH



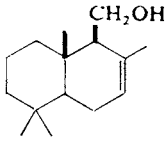
VIa



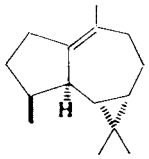
VIb, R = R^1 = R^2 = H
VII, R = OH; R^1 = R^2 = H
VIII, R^1 = OH; R = R^2 = H
X, R^2 = OH; R = R^1 = H



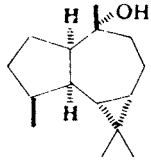
XI



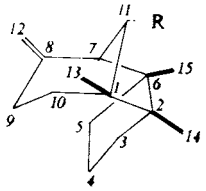
IX



XII



XIII



XIV, R = OH
XIVa, R = $OCONHCOCCl_3$
XV, R = O

XVI, $CH_3—(CH_2)_{18}—CO—(CH_3)_{11}—COOCH_3$
XVII, $CH_3—(CH_2)_{18}—CO(CH_2)_{13}—CH_2OH$
XVIII, $CH_3—(CH_2)_{18}—CO—CH_2—CH_2OH—(CH_2)_8—CO—CH_3$

as expressed by the formula *VIb*, can be deduced from its $[\alpha]_D$ value $-41{\cdot}6°$ in comparison with $[\alpha]_D$ values data summarised for stereoisomeric calamenenes by Bunko and coworkers[10]; their $[\alpha]_D$ value for [1*S*,4*S*]-calamenene (*VIb*) is $-47°$ and for [1*R*,4*R*]-calamenene (*VIa*) $+37$ up to $+41{\cdot}3°$. For biogenetical reasons, we suppose that the same absolute configuration should be ascribed to both probable congeners of calamenene in *B. trilobata*.

In their study of five different species of genus *Bazzania*, the Japanese authors gave the absolute configuration of their (+)-calamenene isolated from *B. japonica* correctly. The measured $[\alpha]_D$ value was $+54°$ and, according to the values mentioned[10], the isolated compound has prevalently been [1*R*,4*R*]-calamenene (*VIa*). However, they presume[2] the same (+)-calamenene to be present in other *Bazzania* species studied by them, including *B. trilobata*. This fact is in discordance with our findings; it should be mentioned that no optical rotation value is given throughout the paper[2] for calamenene specimens.

The presence of drimenol (*IX*) in our specimen was proved by m.p. and IR, NMR and mass spectra. Further on, we were able to identify the presence of ledene (*XII*) and of viridiflorol (*XIII*). Drimenol is often encountered in liverworts, ledene is known as a component of essential oils of liverworts in a few cases[1], but the presence of viridiflorol is new. Its identity follows unequivocally from the IR spectrum, m.p. and other data. We were not able to establish directly its stereostructure, as the value of the optical rotation of our specimen was $\approx 0°$. The $[\alpha]_D$ value given[11] for viridiflorol is very low, about $4{\cdot}0°$. In respect to the fact that without any excep-

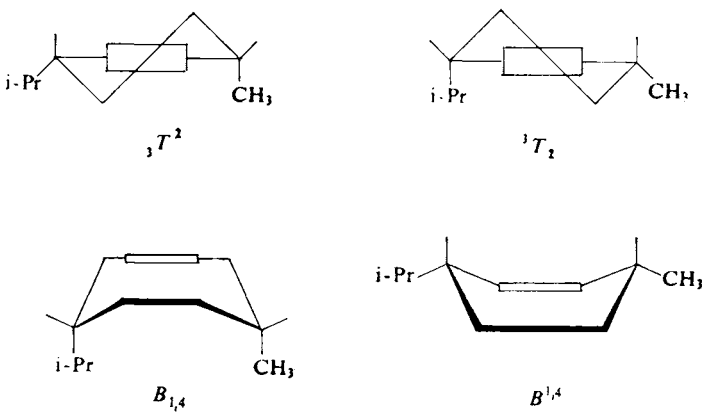


FIG. 1

Possible conformations of the six-membered ring in compounds *VIb*, *VII* and *X*

tion all aromadendrane type compounds isolated up to now from liverworts belong to the *ent*-configuration, we can suppose the same for both our ledene and viridiflorol from *B. trilobata*, and suggest the stereoformulae *XII* and *XIII* for them.

The presence of gymnomitrol (*XIV*) is new for *B. trilobata*. The compound *XIV* was identified using IR and NMR data. According to our measurement its $[\alpha]_D$ value was $-18{\cdot}6°$ (the value published by Connolly and coworkers[12] is $+7°$). A detailed ^{1}H NMR study of gymnomitrol shows the presence of three methyls bound to carbon atoms without any hydrogen atom, of one CH—O hydrogen atom and of two hydrogen atoms in the region of double-bond hydrogen shifts. Using a decoupling experiment and a detailed analysis of ^{1}H NMR spectrum of the compound *XIV* and of its TAI-derivative *XIVa*, we were able to assign signals to all hydrogen atoms belonging to the exomethylene-bearing six-membered ring (Table IV). The assignement of the signal δ 2·34 to $H_{(7)}$ is confirmed by the TAI-acylation shift of this hydrogen ($\Delta\delta = +0{\cdot}21$ ppm). This is in agreement with the values of acylation shifts of β-hydrogen atoms in secondary alcohols and the zero-value of the coupling constant with the atom $H_{(11)}$. The dihedral angle $H_{(7)}$, $H_{(11)}$ corresponds with approx. 90° to the given configuration on $C_{(11)}$.

The methylene hydrogens on $C_{(9)}$ and $C_{(10)}$ were assigned on the basis of the analysis of multiplets with chemical shifts δ 2·43, 2·13, 1·75 and 1·40. The assignment of the multiplet at δ 2·43 of the axial hydrogen $H_{(9ax)}$ agrees with the values of vicinal coupling constant $J_{9ax,10ax} = 12{\cdot}2$ Hz, $J_{9ax,10eq} = 8{\cdot}7$ Hz and with allylic interactions of exomethylene hydrogen atoms $J_{9ax,12} \approx J_{9ax,12'} = 2{\cdot}7$ Hz (the angle between H_{9ax} and the plain of the double bond *c.* 110°). The second hydrogen atom on $C_{(9)}$ of the methylene double bond with the chemical shift δ 2·13 shows a vicinal coupling $J_{9eq,10ax} = 8{\cdot}3$, $J_{9eq,10eq} = 0{\cdot}7$ Hz and allylic interactions $J_{9eq,12} = J_{9eq,12'} = 0{\cdot}7$ Hz (in agreement with an angle of *c* 10° between $H_{(9eq)}$ and the plain of the double bond). The second pair of CH_2-hydrogens corresponds to the signals with the chemical shift δ 1·75 and 1·40. To the axial hydrogen $H_{(10ax)}$ there belongs the multiplet at δ 1·40 with coupling constants $J_{10ax,9ax} = 12{\cdot}2$ Hz and $J_{10ax,9eq} = 8{\cdot}3$ Hz; the atom $H_{(10eq)}$ at 1·75 has the corresponding coupling constants $J_{10eq,9ax} = 8{\cdot}4$ Hz and $J_{10eq,9eq} = 0{\cdot}7$ Hz. The observed TAI-acylation shifts of the methyl groups confirm the *cis*-orientation of methyls $C_{(14)}$ and $C_{(5)}$ to $C_{(11)}$—OH group and also the configuration on carbons $C_{(2)}$ and $C_{(6)}$; these shifts were used to their speculative assignment. The methyl $C_{(15)}$ was assigned to the signal δ 0·96 and its acylation shift was only $+0{\cdot}02$ ppm; therefore, it is minimally influenced by the acylation of the hydroxyl group. Methyl $C_{(15)}$ was assigned to the signal with a chemical shift δ 1·24 according to its upfield shift after the TAI-acylation, $\Delta\delta = -0{\cdot}06$ ppm. The last methyl signal (δ 1·09) has to belong to the methyl $C_{(13)}$ and that is in accord with the value of its TAI-acylation shifts $\Delta\delta = +0{\cdot}05$ ppm, in which the inductive effect of the more electro-negative substituent is probably compensated by the upfield effect of the delocalized electron-pair of the oxygen atom.

TABLE IV

^{1}H NMR Parameters of the compounds *XIV* and *XIVa*

Compound	Chemical shifts										
	$H_{(7)}$	$H_{(9ax)}$	$H_{(eq)}$	$H_{(10ax)}$	$H_{(10eq)}$	$H_{(11)}$	$H_{(12)}$	$H_{(12')}$	$H_{(13)}$	$H_{(14)}$	$H_{(15)}$
XIV	2·34 s	2·43 dddt	2·13 ddp	1·40 ddd	1·75 ddd	3·72 bs	4·64 dd		1·09 s	0·96 s	1·24 s
XIVa[a]	2·55 s	2·48 dddt	2·21 ddq	1·58 ddd	[b]	4·86 d	4·74 dd	4·77 dd	1·14 s	0·98 s	1·18 s
	(+0·21)	(+0·05)	(+0·08)	(+0·18)	—	(+1·14)	(+0·10)	(+0·13)	(+0·05)	(+0·02)	(−0·06)

Compound	Interaction constants							
	$J_{9ax,10ax}$	$J_{9ax,10eq}$	$J_{9eq,10ax}$	$J_{9eq,10eq}$	$J_{9ax,9eq}$	$J_{10ax,10eq}$	$J_{9ex,12}$	$J_{9eq,12}$
XIV	12·2	8·4	8·3	0·7	16·8	14·2	2·7	0·7
XIVa	12·2	8·8	8·2	0·7	16·8	14·2	2·6	0·7

[a] TAI induced acylation shifts are given in parentheses. [b] The signal is superimposed.

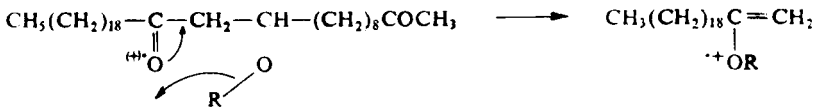
In the fraction accompanying gymnomitrol, we suppose the presence of a little amount of a carbonyl derivative, which is – according to its IR and NMR spectral data – very probably gymnomitrone (*XV*).

From another plant specimen the waxy components of *B. trilobata* were studied in a chloroform extract. A mixture of compounds insoluble in hexane was present which after transesterification[13] yielded, in a methyl ester portion, a series of methyl esters of fatty acids and an individual oxo-acid methyl ester. This derivative has $M^{+\cdot}$ 508 (1·9%) and the molecular formula $C_{33}H_{64}O_3$, and hence it is twice unsaturated. The elimination of a neutral fragment $\cdot OCH_3$ (*m/z* 477, 4·0%) from the molecular ion and the presence of peaks *m/z* 74 (16%) corresponding to $CH_2 = \overset{+\cdot}{C}(OH)OCH_3$ and *m/z* 87 (14%) corresponding to $CH_2CH_2\overset{+\cdot}{C}OOCH_3$, prove the fact that the compound *XVI* is a methyl ester of carboxylic acid. The third oxygen atom is bounded in a carbonyl group and its presence and position confirm the α-fission [*m/z* 295 (15·7%) $CH_3(CH_2)_{18}\overset{+}{C}O$; *m/z* 241 (11·3%) $OC(CH_2)_{11}COOCH_3$] and Mc Lafferty's shifts [*m/z*] 310 (31·60) $CH_3(CH_2)_{18}—\overset{+\cdot}{C}(OH) = CH_2$, *m/z* 256 (45·9%) $CH_2 = \overset{+\cdot}{C}(OH)(CH_2)_{11}COOCH_3$]. The ions *m/z* 310 eliminate a molecule of water (*m/z* 292, 6·5%) and from the ions *m/z* 256 methanol is eliminated (*m/z* 224, 11·9%). Hence the structure of compound *XVI* corresponds to the methyl ester 13-oxodotriacontanoic acid.

The alcoholic portion of the reesterification products yielded two oxo-alcohols *XVII* and *XVIII*. The compound *XVII* has $M^{+\cdot}$ 508 (5·3%) and the molecular formula $C_{34}H_{68}O_2$; molecular ions eliminate water and carbon monoxide *m/z* 490 (1·5%), resp. *m/z* 480 (1·6%). The composition of the molecular formula corresponds to one unsaturation which, supposably, is a carbonyl. The second oxygen is a hydroxyl as follows from an intensive peak *m/z* 31 (14·7%) $CH_2=\overset{+}{O}H$. The presence of a carbonyl group and its position too, follow from α-fissions [*m/z* 295 (31·7%) $CH_3(CH_2)_{18}.\overset{+}{C}\equiv O$; *m/z* 241 (10·0%) $HO(CH_2)_{14}—\overset{+}{C}\equiv O$] and from Mc Lafferty's shifts [*m/z* 256 (100%) $HO(CH_2)_{14}—\overset{+\cdot}{C}(OH)=CH_2$; *m/z* 310 (41·1%) $CH_3(CH_2)_{18}—\overset{+\cdot}{C}(OH)=CH_2$]. Both ions eliminate water after the shift. Hence, the compound is 1-hydroxytetratriacontan-15-one (*XVII*).

The compound *XVIII* has $M^{+\cdot}$ 494 (1·2%) and the molecular formula $C_{32}H_{62}O_3$ with two unsaturations. The molecular ions eliminate successively two molecules of water (*m/z* 476 = M−18; 1·1% and *m/z* 458 = M−18−18; 0·2%) demonstrating the presence of hydroxyl and/or carbonyl groupings. The basic peak of the spectrum (*m/z* 43; $CH_3\overset{+}{C}O$) and the peak *m/z* 58 [42·4%; $CH_2 = \overset{+}{C}(OH)CH_3$ originating by a Mc Lafferty's shift], both indicate an oxo group in the position 2.

The position of the second carbonyl group is defined by α-fissions [*m/z* 295 (10%) $CH_3(CH_2)_{18}C{\equiv}\overset{+}{O}$; *m/z* 227 (12·2%) $\overset{+}{O}{\equiv}(CH_2CH(OH)\ (CH_2)_8COCH_3]$ and by Mc Lafferty's shifts [*m/z* 242 (22·8%) $CH_2 = C(\overset{+\bullet}{O}H)CH_2CH(OH)(CH_2)_8COCH_3$, *m/z* 310 (21·1%) $CH_3(CH_2)_{18}{-}C(\overset{+\bullet}{O}H){=}CH_2$]; both ions arising by the shift eliminate water [*m/z* 224 (12·2%) and *m/z* 292 (3%)]. From the presented fragmentation there follows the presence of two carbonyl groups in position 2 and 13; between them a further oxygen atom is present (as hydroxy or in ether group). After labelling of the compound *XVIII* using $C_2H_5O^2H$, the mass of the molecular peak was shifted by one unit to $M^{+\bullet}$ 495 and similarly, there were shifted the peaks of ions *m/z* 227 resp. 242 to *m/z* 228 resp. 243. From this fact it follows that the third oxygen atom is present in the form of a hydroxy group. Remarkably, also the mass of the ion *m/z* 310 [from McLafferty's shift of an oxo-group] was shifted by one unit. This fact may be explained by the circumstance that the hydroxy group is located on $C_{(11)}$ and during the shift deuterium is transposed on the carbonyl atom. This explanation is supported by the fact that the trimethylsilylated compound *XVIII* behaves analogically: This grouping was transferred from the hydroxyl to the carbonyl oxygen atom, as follows from the presence of ions *m/z* 382 (13%) and of the molecular formula $C_{24}H_{50}OSi$ (Scheme 1).



R = —H *m/z* 310
R = —D *m/z* 311
R = —$Si(CH_3)_3$ *m/z* 282

SCHEME 1

Originally, the silylated compound *XVIII* had in its mass spectrum $M^{+\bullet}$ 566 (5·2%) and, in addition to the mentioned peak *m/z* 382, further characteristic peaks *m/z* 43 (100%) $\overset{+}{C}H_3CO$; *m/z* 257 (37·0%) $(CH_3)_3Si{-}\overset{+}{O}{=}CH(CH_2)_8COCH_3$ (*i.e.*, ions arising by an α-fission), and further *m/z* 314 (27·8%) $CH_2{=}C(\overset{+\bullet}{O}H)CH_2CHOSi.(CH_3)_3(CH_2)_8COCH_3$ (as a product of McLafferty's shift). Hence the compound *XVIII* is 11-hydroxytriacontan-2,13-dione. The structure of compounds *XVI*, *XVII* and *XVIII*, isolated after reesterification, demonstrate the presence of a waxy compound (probably of estolide type) in *B. trilobata*. The homologous oxo-acid and the same primary oxo-alcohol of this unusual type were found by saponification of the wax from cochineal inset *Dactylopius confusus* and the same C_{66} keto ester was produced by the wooly alder aphid *Prociphilus tessalatus*, and given by Meinwald and coworkers[14].

The hexane soluble components of *B. trilobata* contained in their hydrocarbon portion the homologous series of n-alkanes in the range C_{15}—C_{35}; the odd homologues showed predominancy. The soluble wax esters proved – after transesterification and a systematic GLC and mass spectroscopic analysis – the presence of primary aliphatic alcohols with a predominant C_{18} member. The normal fatty acid series (analyzed in the form of methyl esters) contained C_{10}—C_{26} members with three predominant acids (C_{14}, C_{16} and C_{20}). The fraction of the free fatty alcohols contained a series of members very similar to the data given by Huneck and Klein[15], a few years ago.

As a summary, *B. trilobata* represents a liverwort species rich in sesquiterpenoids, as it is characteristic for many plants belonging to *Jungermanniales*. It grows prevalently in spruce forests [*Picea excelsa* (LAM.) LINK] in large quantities forming extense mats. We tried to investigate (analogically as published by Huneck and coworkers[16]) the variations in the content of the essential oil and its components during the season of the year. We followed the data during the years 1980–1981, collecting the necessary plant material in the same place (middle Bohemia near Bernartice) on the elevation of *c.* 600 m). Unlike Huneck's experimental plant the water liverwort *Scapania undulata* (L.) DUM. – our *B. trilobata* is a typical terrestric plant and, hence, exposed to a large amount of seasonal stresses by wet and dry seasons, cold winter (plant covered with snow) and summer times. Specimens of the plant were collected once a month, a fresh sample of *c.* 50–80 g material distilled by a standard procedure and the obtained essential oil analysed for quantitative composition by GLC combined with an integrator. In the essential oil content unexpectedly great variations were found, min. 0·06% in March, max. 0·68% in January. The ratio of single compounds in the prepared essential oils varied highly: The extreme data for the six main components are given in Table V.

TABLE V

Seasonal variations of the main components in the essential oil of *B. trilobata*

Compound	Maximally		Minimally	
	%	month	%	month
5-Calamenenol (*VII*)	29·2	I	12·0	II
Bazzanene (*III*)	28·5	V	12·0	I
β-Barbatene (*II*)	27·8	IX	9·5	I
Drimenol (*IX*)	18·9	I	8·2	VI
Viridiflorol (*XIII*)	10·2	V	2·4	VIII
cis-Calamenene (*VIb*)	7·1	X	3·2	I

EXPERIMENTAL

The specimens of *B. trilobata* were collected in middle Bohemia (near Bernartice, about 600 m above sea level).

All the instrumentation and/or methods used were the same as in the precedent paper[3]. ^{1}H NMR spectra were measured on VARIAN XL-200 (at 200 MH_2) in deuteriochloroform (Aldrich Chemicals); TAI acylations were performed using trichloracetyl isocyanate (Merck) by an *in situ* reaction directly in the NMR tube; the spectra were referenced to tetramethylsilane; the values are given in ppm.

Essential Oil

was obtained from fresh plant material which was hand sorted and washed of soil. In a typical run, 820 g of liverwort (200 g dry weight) were distilled using overheated steam (110°C) and the yield was 2·8 g (0·34% of fresh plant) of light green oil. The separation by column chromatography on silica gel (100 g) afforded 0·80 g of the hydrocarbon portion and 1·46 g of the oxygen containing substances. All the individual substances were isolated from both fractions using column chromatography and preparative, GLC, and were identified by comparison of their retention data, further on by IR, mass (or GLC-MS) and ^{1}H NMR spectra with those of adequate standards. The structures of compounds not yet known were established using mainly ^{1}H NMR spectroscopy.

By the preparative GLC of the hydrocarbon portion on a packed column (3% SE-30, 120°C), the following series of sesquiterpenes were obtained successively: α- and β-Barbatenes, identified using IR, mass and ^{1}H NMR spectra and comparison with those previously[3] obtained in our Laboratories and with the published data of Andersen and coworkers.[17].

Ledene (XIII) was isolated as a compound forming a minor peak admixed to calamenene, by chromatography on silica gel. Its IR spectrum agreed with the spectrum obtained by dehydratation of ledol (*cf.*[18]). ^{1}H NMR spectrum showed the following signals: δ 1·09 d (7·0), CH_3—CH; δ 1·13 s, 1·20 s, 2 × CH_2—C< and δ 1·68 bs, CH_3—C=C<.

(1*S*,4*S*)-*Calamenene* (VIb) was identified using the comparison of IR and mass spectra with those of the calamenene from *Lophocolea heterophylla* (*cf.*[3]) and the data of Andersen and Huneck[19]; its $[\alpha]_D^{20} = -41{\cdot}6°C$ (*c* 0·8, $CHCl_3$).

Cuparene (IV) was identified by IR and mass spectra[20]; $[\alpha]_D^{20} = -28{\cdot}0°C$ (*c* 0·8, $CHCl_3$).

Bazzanene (III) showed IR and mass spectra identical with those reported by Matsuo[21].

The portion of oxygen-containing compounds yielded — using preparative GLC (3% SE-30; 130°C) — sesquiterpenols, mostly purified by further chromatography on silica gel. The following compounds were obtained:

Viridifiorol (XII) was identified by IR spectrum, identical with a specimen prepared in our Laboratories (*cf.*[11,22]); m.p. 73·5—74·5°C, $[\alpha]_D^{20}$ ±0°C.

Gymnomitrol (XIV) was identified by the comparison of the data given by Connolly and coworkers[23]. Its m.p. was 114·5—116°C, $[\alpha]_D^{20}$ −18·6°C (*c* 0·67, $CHCl_3$). The interpretation of the IR and ^{1}H NMR spectra of a less pure specimen led to a conclusion that a structurally related carbonyl derivative is admixed [i.e., probably gymnomitrone (*XV*)].

5-*Hydroxycalamenene* (VII) represented the main component of sesquiterpenols. Its spectra were identical with the data given in the literature[2,24]; the detailed data on its ^{1}H NMR see Table IV.

Drimenol (IX) was the second main sesquiterpenol; it was identified by comparison of its spectral data with the spectra given by Huneck[25]; m.p. 95—96°C, $[\alpha]_D^{20}$ −18·8°C (c = 2·1, $CHCl_3$).

8-*Hydroxycalamenene* (X) represented the last identified peak in GLC. Its IR spectrum showed peaks 3 612, 3 500 (OH group), 1 621 and 1 580 cm^{-1} (aromatic ring); $[\alpha]_D^{20}$ +31·5°C (c 4·2, $CHCl_3$); for detailed ^1H NMR data see Table I.

Chloroform Extract

Air dried liverwort (160 g) was percolated four times with chloroform (2 000 ml in total) and the extract (4·82 g) was dissolved in n-hexane; the portion insoluble in hexane (0·81 g) was reesterified using methanol and dry HCl (*cf.*[13]). After column chromatography (300 g of silicagel) the product yielded a fraction of fatty acid methyl esters (according to GLC analysis formed by a homologous series of even numbers C_{12}—C_{18}, max. at $C_{16(0)}$ and $C_{18(1)}$); further components were the methyl ester of the oxo-acid *XVI* (mass spectrum M^+ 508; $C_{33}H_{64}O\alpha$), the oxo-alcohol *XVII* (M^+ 494, $C_{32}H_{62}O_3$: this compound yielded a trimethylsilyl derivative M^+ 566, $C_{35}H_{70}O_3Si$).

The portion soluble in hexane yielded after a column chromatography (500 g of silicagel) following fractions: n-Alkanes (84 mg), sesquiterpene hydrocarbons (0·80 g), wax esters (0·28 g), free primary aliphatic alcohols (0·70 g) and sesquiterpene alcohols (1·02 g); further obtained fractions have not been studied.

The study of all wax components was performed using the same standard procedure as given in a precedent paper[3]; the wax esters were reesterified and separated into fatty acid methyl esters and aliphatic alcohols; both fractions were analysed by GLC.

Seasonal Composition

The content of essential oil in the specimens of fresh plant material collected monthly throughout the whole years 1980—1981, was established in a classical way using the Clevenger apparatus[26]. The approx. weight of dry plant specimen was found by weighing the plant residue after distillation in dried specimen to a constant weight. The amount of essential oil varied in the following manner: January 0·63%, February 0·38%, March 0·06%, April 0·17%, May 0·07%. ...

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