Tetrahedron. Vol 33, pp. 617 to 628. Pergamon Press 1977. Printed in Great Britain

SESQUITERPENE CONSTITUENTS OF TWO LIVERWORTS OF GENUS DIPLOPHYLLUM

NOVEL EUDESMANOLIDES AND CYTOTOXICITY STUDIES FOR ENANTIOMERIC METHYLENE LACTONES

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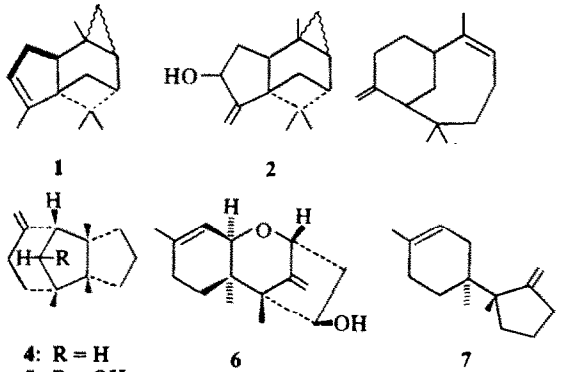
(*Received in USA* 2 *February* 1976; *Received UK for publication* 13 *July* 1976)

Abstract—The steam distillates (or hexane extracts) of the liverworts *Diplophyllum albicans* (L.) Dum. and *D. taxifolium* (Wahl.) Dum. are largely a mixture of sesquiterpenes. Both species elaborate, among the hydrocarbons, mainly *ent*-α-selinene (**8a**) and *ent*-selina-4,11-diene (**8b**) together with anastreptene (**1**) and β-elemene (**9**). The major component of each essential oil was diplophyllin (**10**), a novel *ent*-eudesmanolide. The structure of diplophyllin was established by correlation with tetrahydro-isoalantolactone (**17**). In addition, the enantiomers of diplophyllin and diplophyllolide-A (**16**) were obtained on acid isomerization of isoalantolacetone (**14a**). Diplophyllin shows significantly greater activity against human epidermoid carcinoma (KB cell culture, $ED_{50} \simeq 8\ \mu g/ml$) than its enantiomer: the first demonstration (to our knowledge) of optical selectivity for this type of cytotoxicity. Among the more polar constituents of *D. albicans* were 9α-acetoxy-diplophyllin, and several sesquiterpene alcohols including albicanol (**22**).

That the liverworts (Hepaticae) are an unusually rich source of sesquiterpenes has been convincingly demonstrated by recent work from Hüneck,[1-4] Matsuo *et al.*,[5-7] Herout's group[8-10] and our laboratories.[11-15] These findings have been reviewed regularly.[16] A number of novel skeletons have been found of which those of anastreptene (**1**)[15] and myliol (**2**),[9] bazzanene (**3**),[5,11] and β-barbatene (**4**)[11,12] and gymnomitriol (**5**)[17] remain unique to the Hepaticae. In the case of anastreptene and bazzanene related compounds neither relative nor absolute stereochemistry are established with certainty. In the case of β-barbatene the stereochemistry indicated appears firm based on CD correlation in all three degradative studies reported thus far.[6,12,17] Although the planar structure of trichodiene (**7**) is ideally suited as a biogenetic intermediate to both trichothecanes (e.g. **6**) and the barbatenes (**4**), the structures are in fact diastereomeric, with the trichodiene structure correlating with **6**. In this case the liverwort products do not correlate with either the fungal or vascular plant products. This takes on further significance since β-barbatene (**4**) is found in virtually every leafy liverwort (*Jungermanniales*) examined to date (20+).* Anastreptene (**1**) is also quite commonly encountered. The absolute stereochemistry implied by the α-CMe_2 bridge is not established, but assumed based on a biogenesis from an *ent*-germacrene.



1 **2**

4: R = H
5: R = OH

6 **7**

In the case of sesquiterpene structures presumed to be derived from 11-membered ring intermediates or germacrenes, all liverwort components, with one exception,[18] are now assigned structures enantiomeric to those of the same materials isolated from vascular plants. In this respect liverworts, although a natural phylogenetic link to the Tracheophytes, resemble the fungi[19] and marine invertibrates (or their algal symbionts).[20] The members of the genus *Scapania* examined thus far have given particularly clear spectrum of enantiomeric sesquiterpenes.[7,14] For this reason we chose to examine some species of *Diplophyllum*, the other large genus of *Scapaniaceae*. We now report the results of that investigation. Among the novel constituents were three enantiomeric eudesmanolides. These appeared to provide a unique opportunity to determine whether the cytotoxicity of sesquiterpene α-methylene lactones[21] has a chiral specificity.

RESULTS AND DISCUSSION

Essential oil characteristics. The essential oils of two species of *Diplophyllum* (*albicans* and *taxifolium*) were isolated by steam distillation after freezing and standing covered with ether, A (0.2 and 0.3% yield based on weight of partially dried plant material). In the case of *D. albicans* the oil was also isolated by bulk steam distillation, B (0.1% fresh weight) and by hexane extraction after powdering in liquid nitrogen, C (0.4% dryweight). The oils are compared in Table 1.

*Note added in proof: see p. 627.

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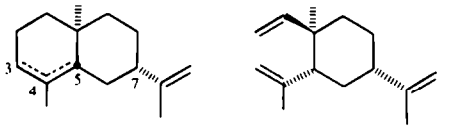
Table 1. Essential oils of *Diplophyllum* samples

Compound type	Taxifolium	Albicans		
	A	A	B	C
Sesquiterpene hydrocarbons	7%	23%	34%	26%
Lactone fraction	70	34	40	38
Polyfunctional lactones + alcohols	5	17	24	27

The major identified hydrocarbon constituents in oils from both species were *ent*-α-selinene (**8a**, identical in all respects to a sample isolated previously from *Chilos-cyphus polyanthus*[13]) and *ent*-selina-4,11-diene (**8b**).



8a: $\Delta^{3,4}$
8b: $\Delta^{4,5}$

9

In the latter case, this very likely represents the first authenticated isolation of this compound, in either enantiomeric form, from nature. The 7-epimeric compound, 10 - epi - selina - 4,11 - diene has been observed in nature, and was available for direct gc and spectral comparison.[22] The enantiomer of **8b** was available from previous studies of the isomerization of β-selinene in formic acid.[23] Figure 1, showing the CD spectra of **8b**, *ent*-**8b** (from β-selinene) and 10 - epi - selina - 4,11 - diene, leaves no doubt as to the correctness of this assignment.

Anastreptene (**1**) and β-elemene (**9**)[24] were also found in each oil. *D. Albicans* oil also contained (−)-β-bisabolene (~10% of the hydrocarbon fraction). The barbatenes were present, if at all, as trace components, only. A number of novel hydrocarbons of as yet undetermined structure were also isolated (Experimental).

The oil fraction eluted from either silica or alumina using benzene was an essentially pure α-methylene lactone (34–65% of oil depending on species and method of oil isolation), which was named diplophyllin.[25]

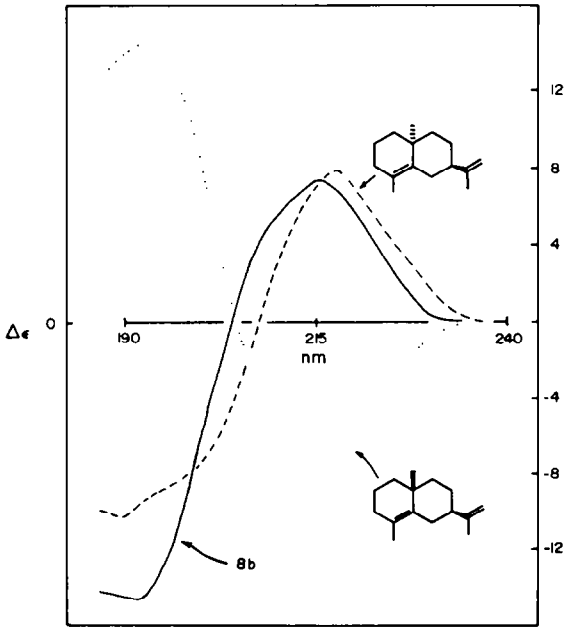


Fig. 1. CD Spectra of *ent* - seline - 4,11 - diene (**8b**), *ent*-**8b**, and 10 - epi - selina - 4,11 - diene.

The more polar fractions afforded a compound (2–4% of steam distillate of *D. albicans*, ~12% of exhaustive hexane extract of *D. albicans*) which was an acetoxy substituted α-methylene lactone, designated acetoxydiplophyllin based on NMR similarities. The TLC of *D. taxifolium* oil also shows this material as a minor constituent.

The structure elucidation of diplophyllin (**10**). Careful chromatography of the lactone fractions afforded diplophyllin as a low melting solid (**10**): m.p. 30–31.5°, $\Delta\epsilon_{267} = +2.8$, $\Delta\epsilon_{235} = -4.5$, and $\Delta\epsilon_{212} = +6.9$. The IR (1767 and 1660 cm^{-1}) and NMR spectra (5.59 and 6.19 ppm, 1 H each, doublets $J_{av} \simeq 2.3$ Hz) suggested an α - methylene - γ - butyrolactone structure; while the absence of additional vinyl-H NMR signals, the CD band at 212 nm, and the chemical shifts of the two Me signals (1.09 and 1.67 ppm) suggested a eudesmane skeleton with a $\Delta^{4,5}$-unsaturation, that is, structure **10**. To our surprise neither **10** nor its enantiomer appear to have been described previously.

Hydrogenation using Wilkinson's catalyst in benzene saturates only the exo-methylene affording the oily dihydro-compound (**11**), which displayed consistent spectral data. Hydrogenation using Adam's catalyst in HOAc afforded the crystalline tetrahydro-lactone (**12**, m.p. 146–146.5, $\Delta\epsilon_{225.5} = -0.227$). The enantiomeric structure (**17**) has been reported (lit.[26] m.p. 144–145°) as the hydrogenation product of alantolactone (**15**) and/or isoalantolactone (**14**). Lithium aluminum hydride reduction of lactone **12** afforded diol *ent*-**18**, m.p. 108–110° (lit.[27] m.p. 108–110° for diol **18**). Hydrogenation of authentic isoalantolactone (**14**)[28] afforded lactone **17** (m.p. 143–144°, $\Delta\epsilon_{225} = +0.21$) identical to **12** by NMR and TLC comparison.

As a confirmation of the selina-4,11-diene function we sought to prepare alcohol **13** for CD comparison with *ent* - selina - 4,11 - diene from the hydrocarbon portion of the oil. As with many α-methylene lactones, nucleophilic hydride agents gave significant amounts of conjugate reduction. This was surmounted by a two stage reduction of lactone **10**—$(iBu)_2AlH$/toluene at −78°, with a MeOH quench, followed by addition of ethanolic $NaBH_4$—which gave pure allylic alcohol **13**, m.p. 145–146°. A 0.21 ppm downfield shift of the angular methyl (relative to diene **8b**) is ascribed to a 1,3-diaxial interaction with a hydroxyl group,[29] placing this function at C-8. The CD comparison (see Fig. 2) was ample evidence for the location of the olefinic linkages.

Synthesis of ent-*diplophyllin and* ent-*diplophyllolide-A*. In order to provide an opportunity to establish whether the cytotoxicity of α-methylene lactones against carcinoma cells shows chiral specificity and to obtain some structure-activity data we set out to synthesize the enantiomers of the diplophyllum lactones (**10** and **16**) and some double bond isomers for biological evaluation. In

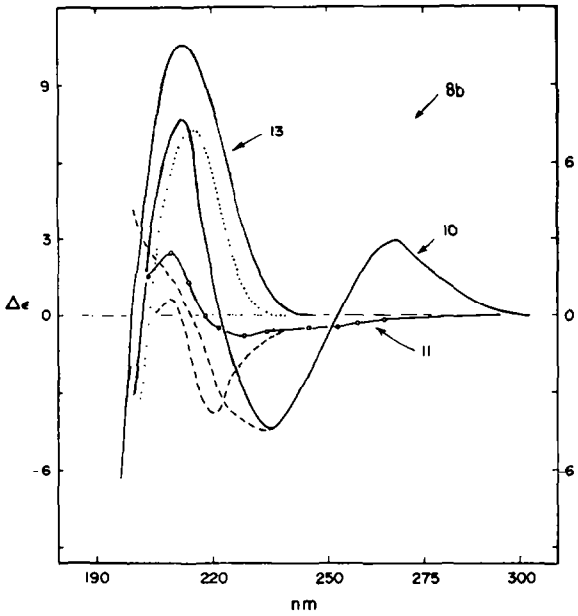


Fig. 2. The CD spectra of diplophyllin (**10**), dihydrodiplophyllin (**11**) and diol 13. That observed for diol **13** is very similar to that of diene **8b** (see Fig. 1). The broken line extensions of the spectra of **10** and **11** show the change when the CD effect for the corresponding nonoxygenated olefins are subtracted.[30]

†Lactones **10**, **16** and **14a** are readily separable by column chromatography on 15% $AgNO_3$–SiO_2 eluting in the order listed. lactone **15** cannot be separated from **10**.

addition these syntheses serve to confirm the structures assigned. In principle, acid-catalyzed rearrangement of a 4-exomethylene isomer (such as isoalantolactone, **14a**) should afford *ent*-**10** and *ent*-**16** based on the analogous transformations of β-selinene.[23] However the conjugated methylene lactone moiety responsible for cytotoxic activity is, for the same reason, subject to acid catalyzed rearrangements and additions under such isomerization conditions. Initial attempts which will not be detailed suggested that the methylene lactone would need to be protected[31] for this transformation. After some experimentation with acid media of low nucleophilicity we found that short exposures of **14a** to dilute solutions of CH_3SO_3H in trifluoroethanol (TFE)[32] effected isomerization of the 4-exomethylene giving endocyclic isomers without affecting the lactone unit. On a preparative scale, isoalantolactone on reaction for 2 hr with 10 mole % of CH_3SO_3H (as a 0.4% solution in TFE), affords a 62% yield of *ent*-**10** and 28% of *ent*-**16**.† Interestingly alantolactone (**15**) was not observed among the products and separate trials indicated that alantolactone is inert to 3% CH_3SO_3H/TFE even after 24 hr at room temperature. Similar non-reactivity of the 5,6-olefinic bond could be seen in hydrogenation studies. When crude helanin (=**14a** + **15**), rather than isoalantolactone is used for the synthesis of diol **18**, alcohol **19** is also isolated indicating that much of the alantolactone present was unaffected by hydrogenation.

The IR and NMR of *ent*-**10** corresponded in detail to those of the natural material and for *ent*-**16** with the values reported by Benešová, Samek and Vašíčková.[25]

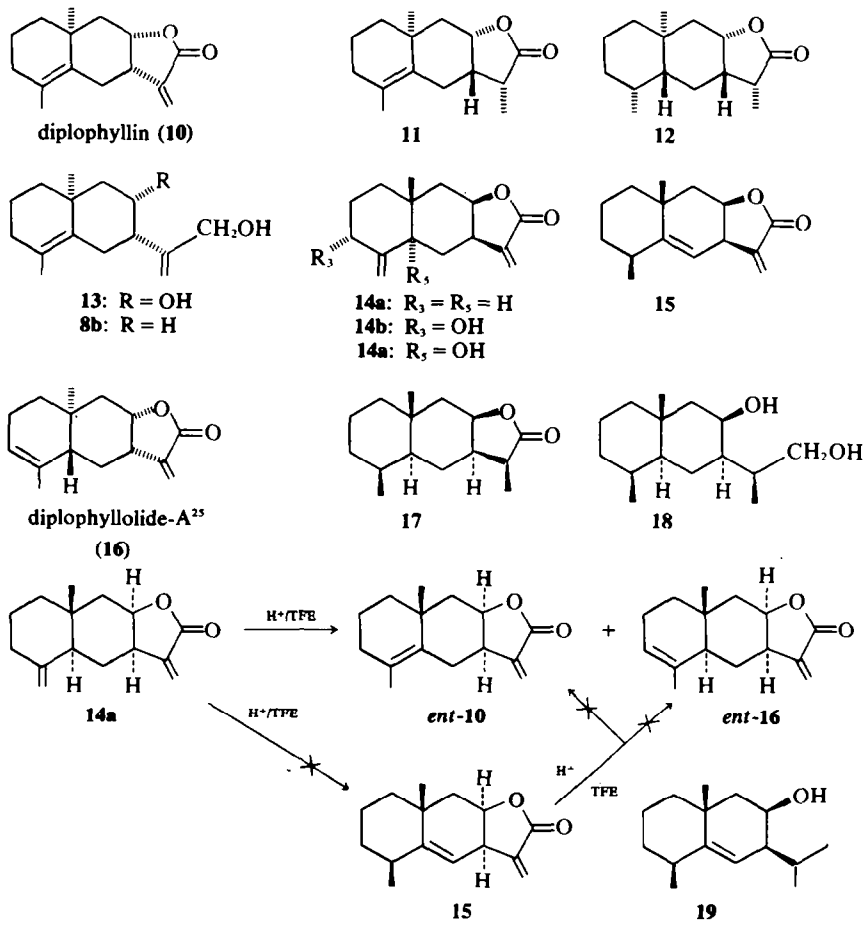


Table 2. CD Spectra of $\Delta^{12,13}$-Eudesmanolides[a]

Compound	255–267	230–240	$\Delta\epsilon$ 207–215	190–195 nm
Diplophyllin (**10**)	+2.8	−4.5	+6.9	(−ve)[b]
ent-**10**	−3.14	+4.75	−7.5	(+ve)[b]
Acetoxydiplophyllin (**20**)	+2.57	−4.2	+3.8	−8.9 ± 1.8
Diplophyllolide-A (**16**)[25]	+1.9	—	−12.3	—
ent-**16**	−2.03	~0	+13.5	(+1.5)[c]
Alantolactone (**15**)	(~+0.5)[d]	(+5)[d]	+23.8	−13!
14a	−1.68	~0	+22.4	−11!
14b[25]	−1.76	—	—	—
14c[25]	−1.97	—	—	—

[a]All data for MeOH solutions.
[b]CD crosses zero at *ca.* 201 nm (see Fig. 2).
[c]Still positive at 190 nm but extrapolation suggests a crossing to negative values by *ca.* 188 nm.
[d]The major positive band peaking at 211 nm is highly unsymmetrical suggesting an additional positive band at *ca.* 230 nm which apparently masks the weak negative band expected near 255 nm for the lactone $n \rightarrow \pi^*$ transition.

The values for acetoxydiplophyllin (**20**, vide infra), isolated from the more polar fractions of the essential oil are included since they were the basis for assigning the 4,11-diene structure. Differences in the 195–225 nm range are likely the result of the added transitions associated with the saturated ester function.

The cytotoxic activities of the synthetic and natural materials are collected in Table 3.

Diplophyllin appears significantly more active than its synthetic enantiomer. It is unlikely that this is the result of unknown impurities in the natural material since they display identical melting behavior, mirror image CD's (within experimental error) and were both subjected to identical acid conditions prior to final purification. In side-by-side assays using identical cultures diplophyllin displayed up to 10 times the activity of its enantiomer. Further exploration of cytotoxicity of ent-eudesmanolides from *Hepaticae* is planned. As expected the activity is due to the methylene lactone function (see activity of **11**). In addition the study reveals that the activity of "helanin" is associated with isoalantolactone, which had not been examined previously, not alantolactone. Previous reports of KB activity for alantolactone are likely due to incomplete separation of isoalantolactone.

Constituents of the more polar fractions. Fractions from column chromatography eluting after diplophyllin contained mixtures of sesquiterpene alcohols and a new lactone. On standing the lactone crystallizes, m.p. 92–95°. Based on NMR and CD similarities (Table 2) this new substance was designated acetoxydiplophyllin. The acetoxy methine signal appeared as a doublet (5.04 ppm, 9.7 Hz) while the lactone methine was a doubled doublet (4.35 ppm, J = 8.4 and *ca.* 10 Hz) suggesting that the acetoxy group was located at C-9. Although hydroxylation at C-9 is common in eremophilanes[36] it has not been noted in intact eudesmanes. Although a direct NMDR confirmation was not possible due to the small chemical shift difference between H-8 and H-9 an indirect one tends to confirm the assignment: irradiation at H-7 (*ca.* 3.0 ppm) produced a simple AB pattern ($J_{AB} \simeq 10$ Hz) for the two downfield methines.

Table 3. Activity against human epidermoid carcinoma (KB cell culture)

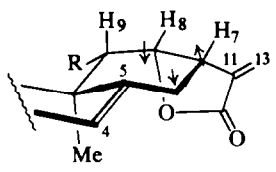
Compound	Name	ln ED_{50} (μg/ml)[a]
ent-**10**		3.3 ± 0.3
10	Diplophyllin	2.1 ± 0.7
11	Dihydrodiplophyllin	5[+]
"helanin"[b]		1.6 ± 0.6
14	Isoalantolactone	0.9 ± 0.8
15	Alantolactone	4[+]
ent-**16**		*ca.* 3.0

[a]Log effective doses are given in order to express the accuracy of the data. The given value is the average of, typically, three separately submitted samples.
[b]Commercial helanin is a crude mixture of alantolactone and isoalantolactone from natural sources.

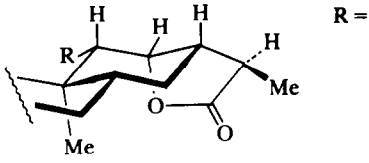
The remaining question is the stereochemistry at C-9. We tentatively prefer the 9α-OAc (i.e. acetoxydiplophyllin is structure **20**) based on an analysis of the δ-values and coupling constants for H-9 in **20** and the sequential hydrogenation products (**21a** and **21b**), and a comparison with the comparable data for diplophyllin and related lactones.

The combined effect of the C-5 sp^2 center and the essentially flat conjugated lactone ring result in a flattened B ring making $J_{8,9\alpha} \simeq J_{8,9\beta}$ in diplophyllin suggesting dihedral angles on the order of 30 and 150°. As the conjugation in the lactone ring is removed, the values approach more typical values for J_{ec} and J_{ea}: a trend which continues on saturating the 4,5-olefinic linkage. This corresponds to increased puckering of the B ring as shown by the arrows in the illustration. The changes for $J_{8,9}$ in acetoxydiplophyllin are best approximated by the C-9 proton signal designated as H-9 (not H-9′). This resonance is usually observed as an A of ABX (J_{AB} = 15–15.5 Hz) downfield from the methylene envelope in the lactones studied: for **10**, 2.00 ($J_{AX} \simeq 6$); for **11**, 2.13 ($J_{AX} \simeq 2.6$); for **14a**, 2.20 ($J_{AX} \simeq 2$); for **15**, 2.12 ($J_{AX} \simeq 3$); for **16**, 2.16 ($J_{AX} \simeq 1.8$); and for **17**, 1.99 ppm (J_{AX} = 2.3 Hz). The other C-9 proton (H-9′) is less well resolved but can generally be located at 1.35–1.65 ppm. The upfield position of H-9′ is consistent with the shielding expected from the C-10→C-1 and C-10→Me bonds[33] leading to a 9α assignment for H-9′. The changes in δ(H-9) are consistent with π-bond anisotropy effects for the series of lactones studied with a 9β assignment. However we view a 9α-OAc assignment for acetoxydiplophyllin as tentative only until confirmed by synthesis. Attempts at such a confirmation are in progress.

The mother liquors from the isolation of acetoxydiplophyllin (**20**) and chromatography fractions eluting just



	$J_{8,7}$	$J_{8,9}$	$J_{8,9'}$	sat'rn at
R = H	6.7	6.7	6.7	none
	~3.4	2.8	~3.8	11,13
	4.1	2.3	4.1	4,5; 11,13
R = OAc	8.4	9.7	—	none
	~3.3	3.7	—	11,13
	3.7	2.7	—	4,5; 11,13



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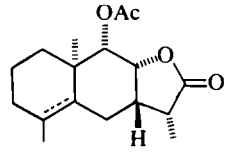
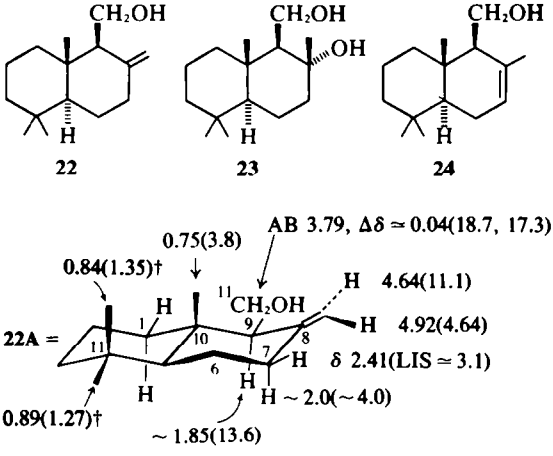


21a: $\Delta^{4,5}$
21b: sat'd

before **20** contained a mixture of alcohols. The major component, albicanol, was isolated by rechromatography or preparative GC and has been assigned structure **22**, not previously observed in nature. The *endo* isomer, (−)-drimenol (**24**), is well represented in nature, including an occurrence in a liverwort, *Bezzania trilobata*.[2] Our structure assignment (tentative) was based on the LIS-values observed on addition of $Eu(FOD)_3$:[38] these are shown on projection formula **22A**. The LIS of the angular-Me was inconsistent with a *trans*-diaxial relationship between CH_2OH and Me. The one literature report of structure **22** was as a by-product in a synthesis of **24** via dehydration of diol **23**.[34] The literature NMR data however did not correspond with ours with respect to δ-values. An examination of the actual NMR trace[35] however revealed an identical AB of ABX for the $\underline{CH_2OH}$ grouping and correspondence in every detail except for a linear error in δ-values, suggesting a machine calibration error in one or the other determination. The stereochemical assignment was confirmed by comparing the hydrogenation products of **22** and authentic (−)-drimenol (**24**),[37] and other chemical correlations (Experimental).



22 23 24

Preparative GC of acetoxydiplophyllin (**20**) mother liquors and fractions eluting (SiO_2 column) immediately after **20** afforded yet another sesquiterpene alcohol, diploalbicanol, m.p. 47–48°, of unknown structure. The NMR spectrum indicates a tertiary methyl carbinol (MeCO at 1.10) with two additional singlet Me resonances (1.00 and 1.02) and a doublet Me (0.92 ppm, J = 7.5 Hz). The NMR also suggests a cyclopropyl ring (~2H at 0.39–0.71 ppm). Careful studies of the $Eu(FOD)_3$ shifted spectra revealed five protons shifting at rates comparable to the carbinol-Me and gave well resolved spectra which gave integrals suggesting the formula $C_{15}H_{26}O$.‡ This alcohol has been named diploalbicanol based on NMR and fingerprint IR similarities to β-diploalbicene and an endocyclic isomer (D.a.-10-K, Experimental).§

From the most polar fractions of the exhaustive hexane extract of *D. albicans*, we isolated another methylene lactone in impure form. NMR comparison and TLC data before and after acetylation suggest assignment as the desacetyl derivative of **20**. Further characterization will have to await isolation in pure form from a larger collection of this or related liverworts.

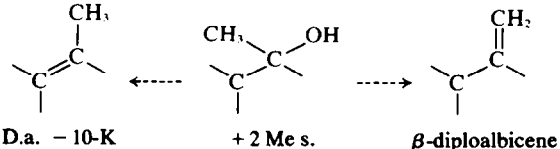
CONCLUSIONS

The liverworts continue to be the source of many intriguing sesquiterpene structures. The present study confirms the occurrence of *ent*-selinenes in liverworts and extends the list of *ent*-sesquiterpenes from liverworts to include β-elemene and (−)-β-bisabolene. The isolation of *ent* - selina - 4,11 - diene may represent the first fully documented natural occurrence of the substance which should be termed γ-selinene based on analogy with the

†The assignments of the C-4 Me signals may be reversed.

‡Unfortunately this could not be confirmed by mass spectroscopy due to equipment failures.

§In particular, all three substances have shown the appropriate Me signal to have the relationship below:



Each compound shows a similar pattern for cyclopropyl hydrogens at *ca.* 0.35–0.80 ppm and many corresponding bands in the IR fingerprint region.

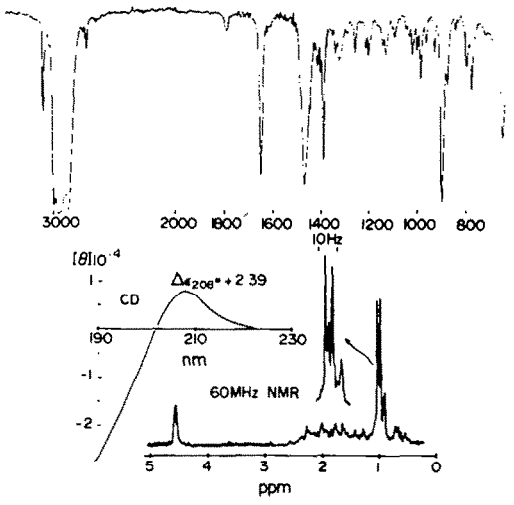


Fig. 3. Spectral data for β-diploalbicene.

well known α-, β-, γ-eudesmols,† in this case supported by a comparison with the *nat*-selinene derived from β-selinene and with 10 - epi - γ - selinene.[22]

The presence of albicanol (**22**) an obvious, but previously unknown in nature, isomer of drimenol, indicates that drimanes are widespread in liverworts.[2,37,50] It is worth noting that with the drimanes the absolute stereochemistry found in the liverworts is the same as that in the majority of vascular plants. The drimane (bicyclofarnesane) biogenesis bears a closer relationship to that of sterols and triterpenes rather than sesquiterpenes, and these higher terpenoids, when found in liverworts,[10,16,51] are identical to vascular plant products, not enantiomeric.

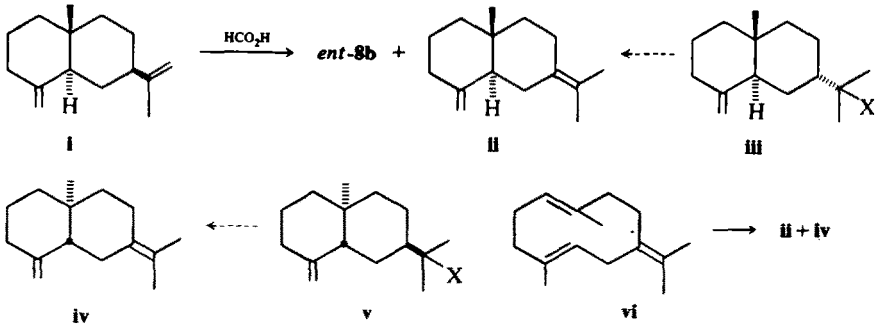
The major components of the oils of these two *Diplophyllum* species proved to be *ent*-eudesman-8,12-olides with a 4(5)-olefinic linkage and in some cases further hydroxylation at C-9.‡ Both feature previously unobserved in eudesmanolides. While these studies were in progress, Benešová *et al.* reported the presence of a Δ^3 - eudesman - 8,12 - olide in one of these species (*D. albicans*) of European origin. This suggests that distinct chemical races exist.

As a part of the structure proof of diplohyllin (**10**), isoalantolactone was converted to *ent*-diplophyllin and *ent*-diplophyllolide-A and the synthetic enantiomers were compared with the natural products as antitumor agents (KB cell culture screen). Diplophyllin shows significantly greater activity ($ED_{50} \simeq 1.2$–12 μg/ml) than its enantiomer: the first demonstration (to our knowledge) of optical selectivity for this type of cytotoxity. These observations led us to a preliminary examination of twenty species of American liverworts and we have found that over half of these species display (by TLC) spots giving characteristics indicative of sesquiterpene α-methylene lactones.[52] These new extracts will be tested for antitumor activity and their constituents will be the subject of structure elucidations. These studies will be reported in due course.

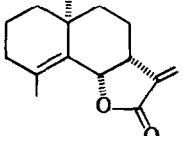
EXPERIMENTAL

General methods. The apparatus described by W. S. Johnson and W. P. Schneider (*Organic Synthesis*, Coll. Vol. 4, p. 132. Wiley, New York, 1963, was used to maintain a N_2 atmosphere over sensitive reactions. When argon was employed as the inert gas, a positive pressure was maintained on a thoroughly flushed, serum-capped, flask by an argon filled balloon. The general isolation procedure for chemical reactions consisted of addition of water, through extraction with the specified solvent, washing the combined extracts with saturated brine soln, and drying the extracts over $MgSO_4$ or Na_2SO_4. The solvent was removed from the filtered extracts under reduced pressure on a rotary evaporator affording the crude product which is subject to GC analysis and any required purification methods. Micro-analyses were per-

†Structure *ent*-**8b** has been suggested for a constituent of a *Chamecyparis* species[44] together with compatible NMR data and α_D +32° (MeOH). However the NMR data does not allow the 10 - epi - selina - 4,11 - diene structure (α_D −109°)[22] to be eliminated with full confidence. Synthetic *ent*-**8b** (from (+)-β-selinene, **i**) displays α_D +23° (pentane).[23] The name γ-selinene had earlier been applied to a substance (α_D +3°) assigned structure **ii**.[45] A compound for which structure **ii** (α_D +106°) is established beyond a doubt has since been prepared from β-selinene[23] and isolated from *Streptomycetes fradiae*.[46] In the latter case an origin from structures such as **iii** seems plausible. The enantiomeric structure (**iv**, α_D − 112°) occurs in vetiver oil in association with structures of type **v**.[47] The first authenticated occurrence of selina - 4(14),7(11) - diene, and its 3(4)-isomer, was in hops[48] and a subsequent study[49] reporting $\alpha_{270} = +10°$ for the 4(14),7(11)-diene from hops can, in retrospect be taken as proof of a nonenzymatic origin from germacrene-B (**vi**). Structures **ii** and **iv** show rotation of +980° and −1000° respectively at that wavelength.



‡A Δ^4 - eudesman - 6,12 - olide has been reported to occur in liverworts of the genus *Frullania*[53] and appears to be associated with a dermatitis of woodcutters. *Frullania* liverworts grow on the bark of living trees. The lactone from *F. dilatata* was shown to have structure **vii**, an *ent*-santonin. But that from *F. tamarisci* is the optically pure enantiomer of **vii**. The latter would be the first established case of a germacrene-derived sesquiterpene from a liverwort with a β-isopropyl group. In light of the habitat of these liverworts it is possible that in the latter case the liverwort utilizes a precursor produced by its vascular plant host.



vii

formed by Chemalytics, Inc., Tempe, Arizona. High resolution mass spectral data (AEI-MS-9) was obtained by a computer controlled scan of masses for compound and a perfluoro standard. The computer reports masses for non-standard peaks to 0.0001 amu together with the best fitting formula, with error (in mmass) by comparison with five neighboring standard peaks. Thereore our mass spectral data is reported as follows: mass (% of base peak, formula or other designation of peak identity, error in mmass). The PDP-12 computer and software for this were obtained by NSF funding (No. GP-18433). Gas chromatographic analyses were performed on a Hewlett-Packard Model 700 instrument equipped with 24 ft × 0.125 in. columns and a dual thermal conductivity detector. The stationary phases are: A, apiezon-L; C, Carbowax-20 M; D, DEGS. The columns were used only so long as tests reveal 250–800 theoretical plates per foot. Preparative GC was accomplished on the same instrument using 10–24 ft × 0.25–0.50 in. columns without the use of stream splitting. The samples were condensed in *ca.* 1 mm i.d. tubes attached directly to the heated exit port. To minimize losses due to aerosols the collecting tube (8–12 in. long) is appressed onto a hot plate surface (~120°) with a cloth bag full of crushed dry ice. Unless otherwise specified hydrocarbons were collected from columns maintained at 150–180°, oxygenated compounds from columns at 180–210°.

Procedures

(a) Column chromatography on $AgNO_3/SiO_2$ and $AgNO_3/Al_2O_3$ use supports prepared by the methods described in the thesis of Syrdal.[39] For TLC commercial precoated glass plates (250 μ layer, SiO_2, incorporating a fluorescent indicator) were used throughout. The plates were stored in equilibrium with ambient air moisture. Silver nitrate was incorporated by dipping the plates for 5 sec in 5% $AgNO_3$ in 1:1 ethanol-acetonitrile. Evaporation of solvent (5 min in a drafty hood) gives plates which should be used immediately. Spots were visualized by spraying with 2% $Cu(OAc)_2$-15% H_3PO_4 (for SiO_2 plates) or isopropanolic phosphomolybdic acid (for $AgNO_3/SiO_2$ plates) followed by heating at *ca.* 120° on a hot plate.

(b) Hydrogenations were performed on 1–50 mg of compound by dissolving the compound in the stated solvent (HOAc, EtOH, ϕH-EtOH) in a 10–250 ml round-bottomed flask; flushing with nitrogen; adding 5–10 mg of catalyst; flushing with hydrogen; and stirring for 1–16 hr after sealing with a serum cap.

(c) Compounds requiring purification by preparative GC for spectroscopic characterization were examined by NMR (Varian EM-360, T-60, or HA-100) and IR PE-257, Beckmann Acculab-4) directly as obtained from the GC exit port. In the case of hydrocarbons, the collected material was dissolved in pentane and eluted through Woelm basic Alumina (Activity I) prior to determination of rotatory, CD, or UV data. Rotation data is obtained for pentane soln (*c*, 0.05–0.5 g/100 ml) unless otherwise indicated, CD spectra were recorded for *ca.* 0.2–10 mM solution in pentane, acetonitrile, or methanol (l = 0.1–10 mm) on a Cary 6001 recording spectropolarimeter.

(d) Heterogeneous acid-catalyzed rearrangements of sesquiterpenes were performed on an analytical scale by adding the polar acid media to an equal or greater (up to 3 times) volume of a stirred 1–10% solution of sesquiterpene in n-decane under nitrogen. The extent of reaction is monitored by stopping the stirring, allowing the phases to separate, and removal of an aliquot (0.2–2 μl) of the upper n-decane phase for direct GC analysis. The polar acid phases were made up from 98+% HCO_2H (Reagent grade), CF_3CO_2H (distilled from BaO), CH_3SO_3H (freshly distilled), TFE (PCR, redistilled), ethylene glycol (freshly distilled at aspirator pressure), and THF (distilled from benzophenone + sodium) in the proportion indicated in each experiment. The work-up consists of the addition of 10–50 volumes of petroleum ether (30–60°) and washing with water, aq. $NaHCO_3$ and brine.

Gas chromatographic retention data. Gas chromatographic data for sesquiterpenes and their derivatives were obtained on 24–50 ft × 0.125 in. o.d. columns of stationary phases coated on silanized supports. For oxygenated compounds the retention data are reported as relative retentions (RR) with the stationary phase designator as a subscript and the temperature as a superscript. The standard for all oxygenated compounds is cedrol (RR = 1.00).† For sesquiterpene hydrocarbons we continue to use a variant of Kovats' indices in which sesquiterpenes are employed as standards.[40] Our data is based on coinjection with a mixture of α-copaene and γ-cadinene, using interpolation on a log scale.†

$$I_x = I(\alpha\text{-copaene}) + \frac{\ln t_R(X) - \ln t_R(\alpha\text{-copaene})}{\ln t_R(\gamma\text{-cadinene}) - \ln t_R(\alpha\text{-copaene})} \quad (\Delta I)$$

Retention data for standard sesquiterpene hydrocarbons are collected in Table 4.

Cytotoxicity assays were performed at A. D. Little as part of the screening program of the Drug Development Branch, Division of Cancer Treatment, NCI, using the usual methods for the KB cell culture screen. All compounds were submitted at least on two seaprate occasions.

Sources of sesquiterpene standards. α-Copaene, γ-cadinene, (+)-longifolene and calamenane for GC standardization were obtained from Alaska Cedar oil as previously described.[41] β-Selinene was obtained by fractional distillation of celery seed oil. Acid-catalyzed rearrangement of β-selinene afforded α-selinene, selina - 4,11 - diene and other isomers.[23] Semihydrogenation of selina -, 4,11 - diene afforded 4-eudesmene.[23] *Ent*-α-selinene,[13] β-barbatene,[11,12] and β-bisabolene[41] were available from previous work. Authentic elemenes were the gift of B. Lawrence (Stang Canada Ltd.). Gift samples of drimenol,[37] drimanol[37] and isoalantolactone[28] were used.

Commercial helanin (Fluka) was the source for alantolactone and isoalantolactone. TLC on silica (3% EtOAc/ϕH) shows only a single spot at R_f 0.49 which is resolved on $AgNO_3$–SiO_2 plates in the same system: R_f = 0.23 (isoalantolactone) and R_f = 0.59 (alantolactone). A 2-g portion was chromatographed on 100 g of 15% $AgNO_3$–SiO_2 eluting with 4% EtOAc in benzene. Early fraction afforded 0.71 g (35%) of alantolactone (**15**): m.p. 82–82.5° (lit. m.p. 79,[43] 78.5–80°[28]), $[\alpha]_D$ = +197 ± 6 (*c* 0.2, $CHCl_3$) (lit.[43] α_D +175, $CHCl_3$); $[\alpha]_D$ = +243°, $\Delta\epsilon_{211}$ = +23.8, $\Delta\epsilon_{192}$ = −13! (*c* 0.13, MeOH). Later fractions afforded 0.99 g (50%) of isoalantolactone (**14a**): m.p. 113.5–114° (lit.[28] m.p. 112–113°); $[\alpha]_D$ = +169 ± 8 (*c* 0.2, $CHCl_3$) (lit.[43] α_D +172°, $CHCl_3$); $[\alpha]_D$ = +189, $\Delta\epsilon_{255}$ = −1.68, $\Delta\epsilon_{208}$ = +22.4, $\Delta\epsilon_{190}$ = −11! (*c* 0.14, MeOH). The NMR spectra of

†All retentions are corrected for void volume by measuring from the air peak rather than the point of injection.

Table 4. GC retention indices for sesquiterpene standards

	I_A^{190}	I_A^{155}	I_C^{150}	I_C^{165}	I_D^{160}
α-longipinene	1423	1395	1524.5	1541	1652
α-copaene	1433	1410	1537	1551	1665
longifolene	1494	1464	1619	1640	1802.5
β-barbatene	1536	1503	1690	1712	1902.5
β-selinene	1556	1530	1749	1765.5	1958
calamenene	1568	1548	1838.5		2087
γ-cadinene	1573	1555	1778	1792	1978.5
10-epi-α-selinene	1581		1788	1803.5	1995

both substances corresponded to the literature report.[28] Apparently the $AgNO_3/SiO_2$ column produces material of greater purity than fractional crystallization.[28,43] This is also borne out by the cytotoxicity assays (Discussion).

Essential oil of Diplophyllum albicans

(A) *Sample No.* 21. The liverwort was collected on 10/14/73 at Granite Falls, WA and carefully freed of soil and other species of liverworts and mosses. Partially dried plant material (330 g) was steam distilled after mashing in liquid N_2 and standing overnight covered with ether. Ether extraction of the steam distillate afforded 710 mg (0.22%) of essential oil. A crude polarity separation was effected by chromatography on neutral Al_2O_3 eluting sequentially with n-hexane, benzene, and ethyl acetate. The n-hexane fraction (D.a.-A-I, 160 mg, 23%) contained sesquiterpene hydrocarbons. Benzene eluted 120 mg (D.a.-A-II, 17%) of an essentially pure lactone, diplophyllin (**10**): R_f ($CHCl_3$, SiO_2; longiborneol = 0.51) = 0.63; R_f (ϕH, SiO_2; longiborneol = 0.20) = 0.30. The ethyl acetate fraction (D.a.-A-III, 230 mg) contained diplophyllin and more polar components.

(B) *Large scale isolation, sample No.* 50. Crudely cleaned sample, 4.1 kg (fresh weight), was steam distilled in the same manner as A above affording 4.0 g (0.1%) of essential oil. Chromatography on silica gave 7 fractions:

Fraction No.	Weight (g)	TLC (4% EtOAc/ϕH, SiO_2)
D.a.-B-Ia	1.0	0.95*•
D.a.-B-Ib	0.35	0.95* (major), 0.81 and 0.75* (minor)
D.a.-B-II	0.08	0.74* and 0.61*
D.a.-B-III	1.57	0.39* (diphophyllin) and 0.61 (v. minor)
D.a.-B-IVa	0.10	0.32*, 0.23, and 0.06
D.a.-B-IVb	0.69	0.23, 0.17* (acetoxydiphophyllin), 0.07
D.a.-B-V	0.16	0.02*

*Spots indicated by * were visualized by quenching of a UV fluor prior to treatment with the visualizing agent (2% $Cu(OAc)_2$ in 15% aq H_3PO_4, 120°).

Combined fractions D.a.-B-I (34%) was used as the source of hydrocarbons. Fraction D.a.-B-III was chromatographed to afford pure diplophyllin. Further chromatography of D.a.-B-IV afforded three new constituents.

(C) *Extractive isolation, sample No.* 72. Liverwort sample No. 72 (~2 kg fresh wt, collected 3/75 at Granite Falls, WA) was exhaustively extracted with hexane after freezing with liquid nitrogen. The liquid nitrogen fragmented sample was mashed affording 620 g of a dry free-flowing powder in order to facilitate solvent extraction. Three liters of hexane were used in three portions. The thoroughly dried ($MgSO_4$) extracted was concentrated to afford 1.8 g of essential oil (~0.4%, dry basis). TLC (5% EtOAc/ϕH, SiO_2) revealed three major, one moderate, and a number of minor components: ~0.76 (major, hydrocarbons), 0.36 (major, diplophyllin), 0.24 (moderate, diplophyllin alcohols), and 0.20 (major, acetoxydiplophyllin).

The hexane extract, 1.5 g, was chromatographed on a 100-g column of silica using a hexane to benzene to EtOAc gradient with the following results. The fractions were bulked to give samples comparable to those in B. (above) with a similar fraction notation.

Fraction	Eluted with	Wt	Components
D.a.-C-Ia	hexane	0.38 g	hydrocarbons
D.a.-C-II	1:1 hexane-ϕH	0.081 g	
D.a.-C-III	ϕH-2% EtOAc	0.57 g	diplophyllin
D.a.-C-IVa	4–5% EtOAc	0.08 g	alcohol mixture
D.a.-C-IVb	5–6% EtOAc	0.26 g	acetoxydiplophyllin + very slightly more polar alcohol
D.a.-C-V	8% EtOAc	0.06 g	more polar alcohols
D.a.-C-VI	10–15% EtOAc	0.08 g	new methylene lactone

D.a.-C-III and D.a.-C-IVb corresponded by NMR as well to the comparable fractions from part B.

D.a.-C-IVa was examined by GC, several columns indicated a mixture of alcohols predominantly two substances: 25% novel alcohol ($RR_A^{200} = 0.50$) and 38% albicanol ($RR_A^{200} = 1.74$).

Rechromatography of D.a.-C-IVb (SiO_2, 4% EtOAc/ϕH) afforded pure acetoxydiplophyllin.

Fraction D.a.-C-VI was examined by NMR due to the presence of a major UV visualized spot. The NMR spectrum showed among other resonances the usual patterns for the exomethylene and a doublet (δ_{CCl_4} 3.58 ppm, J = 10 Hz) suggesting a CH(OH)–C– grouping as would be found in the desacetyl derivative of **20**. Attempts to selectively remove the acetyl grouping of **20** to confirm this assignment failed. Treatment of fraction D.a.-C-VI with acetic anhydride and pyridine in CH_2Cl_2 led to a material retaining the methylene lactone freatures (UV, NMR) but with the 10 Hz doublet shifted from 3.58 → 5.00 ppm. By TLC the polar UV visualized spot of D.a.-C-VI disappeared to be replaced by a spot running precisely with acetoxydiplophyllin.

Essential oil of Diplophyllum taxifolium. Liverwort sample No. 53, 120 g fresh weight, was treated as in A, above, affording 400 mg (0.3%) of essential oil. TLC comparison with the oil from sample No. 50 (*D. albicans*) and fractions D.a.-B-I → V. The major component was diplophyllin ($R_f \sim 0.4$) with minor spots for hydrocarbons ($R_f \sim 0.95$), alcohols (($R_f \sim 0.24$, 0.07), and a very small spot at $R_f \sim 0.19$ (acetoxydiplophyllin), in all cases corresponding to components of *D. albicans.*

A 45-mg portion of the oil was chromatographed on alumina: hexane eluted 3 mg of hydrocarbon (D.t.-I); benzene eluted 32 mg (70%) of oily diplophyllin, identical by NMR, TLC and IR to material from D.a.-A-II; and ethyl acetate eluted 2 mg of more polar compounds.

Sesquiterpene hydrocarbons of Diplophyllum taxifolium

Gc analysis of D.t.-I. Sample D.t.-I was analyzed by GC on two phases with the results below:

Peak No.	$I_A^{190°}$	$I_C^{165°}$	~%	Correspondences
1	1403.6	1577	16.9	anastreptene
2	1424.8		1.9	(β-elemene)
3	1469.2	1642	25.2	novel (=D.t.-3)
4	1500.0	(~1660?)	4.2	(D.a.-7-G)
5	1518.6	1718	27.6	selina-4,11-diene
6	1539	(~1751?)	5.4	(D.a.-11-J?)
7	1554.7	1767	14.6	*ent*-α-selinene

All correspondences are tentative since the amount of hydrocarbon fraction was insufficient for GC isolation of components. The assignments are based on the similarity with *D. albicans* hydrocarbon fractions. The component giving peak No. 3 appears to be unique for *D. taxifolium* and has been designated diplotaxifolene.

Sesquiterpene hydrocarbons of Diplophyllum albicans

(A) *Further analysis of D.a.-A-I.* Fraction D.a.-A-I was analyzed by GC on two phases without further purification. The results are given below:

Peak desig.	$I_A^{190°}$	~%	Peak desig.	$I_C^{165°}$	~%
A	1327	0.5	1	1464.3	0.4
B	1363	0.6	2	1523.6	0.6
C	1403	6.0	3	1546	0.6
C'	1411	~0.5	4	1576.2	2.0
D	1426.5	8.5	5	1602.3	0.6
F	1479	4.6	6	1626.7	7.6
F'	1490	tr?	7	1660.8	11.8
F''	1497	tr?	8	1696.3	2.1
G	1501	14.2	9	1717	25.6
H	1519	20.9	10	~1733	5.1
I	1525	15.8	11	1747.6	14.2
J	1539	6.2	12	1765	17
K	~1545	3.5	12'	1771	9.6
L	1555	15.0	13	~1788	2.0

Due to the complexity of oil it was not possible to correlate between the peaks observed on the two phases. Chromatography of D.a.-A-I on 15% $AgNO_3$–Al_2O_3 using a hexane to 3% EtOAc in hexane gradient and analysis of sequential fractions on both GC phases allowed a correlation of peaks on the two phases and indicated at least 22 components rather than 14 as indicated by either GC analysis. These correlations are given below.

Designation	$I_A^{190°}$	$I_C^{165°}$	%	Correspondence
1-A	1327	1464	0.4	
2-B	1363	1523.6	0.6	
3'-C'	1411	~1550	0.4	
4-C	1403	1579	2–3	anastreptene
5-X		1602		
6-D	1426.5	1627	8	(β-elemene)
6'-X		~1640		
7-F	1478.7	1660.8	2	
7-X		~1660	tr.	
7'-G	1498.5	1671.5	~2	
7-Fg	(1480) 1501	1663	~8	isolated
7-G	1501	1659.8	~4	isolated
x-G'	1490.3		tr.	
8-X		1696	~1	isolated
8-X'		1699	tr.	
9-G	1499	1715	~2	
9-H	1519.6	1717.5	20	*ent*-selina-4,11-diene
9'-I	1524.1	1728.8	<2	
11-I	1524	1746	~10	(β-bisabolene)
10-K	1546.5	1737	3	isolated
11-L	1555	1741	~2	
11-J	(1539)	1750	2?	
12-L	1560	1765.4	15	*ent*-α-selinene

The components were eluted in the following order: 5-X, 6'-X, x-G', 7-G, 10-K, 1-A, ~2-B, ~7-X, 9'-I, 4-C, 8-X, 11-L, 9-G, 7-F, 7'-G, 9-H, 7-Fg, 8-X', and then 12-L. Components designated 11-J, 6-D, and 11-I were not eluted which is in accord with the later assignments (from D.a.-B-I) 11-I = β-bisabolene and 6-D = β-elemene.

Twenty-one fractions were eluted from the $AgNO_3$–Al_2O_3 chromatography of D.a.-A-I (130 mg). The first eight fractions contained very small amounts of oil. Fraction No. D.a.-A-I-Ag-9 showed a nearly pure compound on both columns (I_C^{165} = 1659.4, I_A^{190} = 1501.2); and preparative GC (phase A) afforded a pure sample (D.a.-7-G): δ (CCl_4) 0.83, 0.92 and 1.05 (3 Me s), 1.63 ppm (vinyl-Me)-vinyl-H signals either absent or not discernible due to noise.

Fraction No. D.a.-A-I-Ag-11 was largely one compound (I_C^{165} = 1734.9), which was isolated by preparative GC (D.a.-10-K): IR (film) no vinyl-H, 1386 (v. sharp singlet, no CMe_2), 1322, 1255, 1140, 1098, 985 and 965 cm^{-1}; δ (CCl_4) no vinyl-H, 1.52 (vinyl-Me), 1.00 and 1.07 (2 Me, s) and 0.93 (Me, d, 7 Hz).

Fraction No. D.a.-A-I-Ag-14 afforded crystalline anastreptene (**1**) on preparative GC: δ (CCl_4) 0.77 (3 H, Me, s), 1.02 (6 H, Me, s), 1.70 (vinyl-Me, m) and 5.12 ppm (1 H, vinyl-H, m).

Fraction Ag-18 was essentially pure *ent* - selina - 4,11 - diene (**8b**): $\Delta\epsilon_{215.5}$ = +7.3, $\Delta\epsilon_{204}$ = 0, $\Delta\epsilon_{192}$ = −15.1; δ (CCl_4) 1.04 (Me s), 1.59 and 1.73 (2 vinyl-Me), and 4.68 ppm ($C{=}CH_2$). The NMR and IR spectra matched with authentic sample of the enantiomer[23] ($\Delta\epsilon_{216}$ ~ −8, $\Delta\epsilon_{193}$ ~ +14) derived from β-selinene (Fig. 1).

Fraction Ag-19 contained among other materials a novel sesquiterpene, $C_{15}H_{24}$ (*m/e* = 204), designated diploalbicene (I_C^{165} = 1663.9): IR (film) 3080, 3030 (sh), 1790, 1642, 894 ($C{=}CH_2$), 1385 (very sharp, no CMe_2?), 1251, 1210, 1196, 1135, 1125, 1015, 995, 978, 958, 923, and 768 cm^{-1}; δ (CCl_4) 0.95 (Me, d, 6.5 Hz), 0.97 (Me, s), 1.03 (Me, s), and 4.55 ppm ($C{=}CH_2$) (Fig. 3).

Fraction Ag-21 contained *ent*-α-selinene (**8a**: $\Delta\epsilon_{213}$ ~ +3, $\Delta\epsilon_{204}$ ~ −3.4!; δ (CCl_4) 0.80 (Me, s), 1.59, 1.74 (2 vinyl-Me), 4.67, 5.28 ppm) identical by NMR, GC and CD to material derived from *Chiloscyphus polyanthus*.[13]

(B) *Further chromatography of D.a.-B-I for the isolation of anastreptene* (**1**), (−)-β-*bisabolene*, β-*elemene* (**9**), *hydrocarbon D.a.-7-F, and selina - 4,11 - diene* (**8b**). Combined fractions D.a.-B-I (~1.25 g) were chromatographed on 150 g of dry-packed SiO_2 using n-hexane for elution. The first portions contained 0.64 g of a gross mixture D.a.-B-I-1. The next portion (0.17 g) contained only three components: 15% β-elemene (I_A^{190} = 1433), 62% β-bisabolene (I_A^{190} = 1525.3), and 18% of an unknown (I_A^{190} = 1556, D.a.-11-L?). The final 0.12 g portion was: β-elemene, diploalbicene (I_A^{190} = 1501.6), and *ent*-α-selinene (I_A^{190} = 1557.3) together with seliene **8b** and unknown D.a.-10-K.

Preparative GC of D.a.-B-I-2 afforded β-elemene (identified by NMR and IR,[42] and by GC coinjections), and (−)-β-bisabolene: $[\alpha]_D$ = −83.4 (n-C_5H_{12}, lit. α_D = +75 for *ent.*), $\Delta\epsilon_{225}$ = +0.23 ± 0.17, $\Delta\epsilon_{205}$ = −2.97 ± 0.52; NMR checks with previous sample.[41] Diploalbicene showed the NMR recorded in A (above) including resonances at 0.3–0.8 suggesting a cyclopropyl unit and was

optically active: $\Delta\epsilon_{208} = +2.39$, $\Delta\epsilon_{202} = 0$ and $\Delta\epsilon_{190} = -8.2$!

Fraction D.a.-B-I-1 was rechromatographed over $AgNO_3$–SiO_2 using 1% ethyl ether in n-hexane giving in order of elution: Anastreptene (**1**) – m.p. 91–93°, $[\alpha]_D = +32°$(pentane)-65 mg not requiring further purification; 160 mg of *ent* - α - selinene (NMR, CD), and 60 mg of a mixture of diploalbicene, *ent* - α - selinene, and *ent* - selina - 4,11 - diene.

Further fractionation of oxygenated sesquiterpenes from D. Albicans

(A) *D.a. - A - II, D.a. - B - III, and D.a. - C - III.* Rechromatography (100 g SiO_2/g.) using 1% EtOAc in benzene for elution afforded pure diplophyllin (**10**): m.p. 30–31.5°; $[\alpha]_D = -108°$, $\Delta\epsilon_{267} = +2.8$, $\Delta\epsilon_{235} = -4.5$ (lactone); $\Delta\epsilon_{212} = +6.9$ (olefin $\pi \rightarrow \pi^*$) (MeOH, 9 mM); δ_{CDCl_3} (100 MHz) 1.09 (3 H, Me, s), 1.67 (3 H, vinyl-Me), 2.65–3.2 (2 H, m), 4.48 (1 H, H-8, q, 6.7), 5.59 and 6.19 ppm (1 H each, $C=CH_2$, d, 2.3 Hz);† IR (film) 1767, 1660 (α-methylene butyrolactone), 1260, 1115, 1002 and 810 cm^{-1}; ms 232.1452, (59, $C_{15}H_{20}O_2$ −1.0), 217.1226 (100, $C_{14}H_{17}O_2$ +0.0), 171.1176 (29, $C_{13}H_{15}$ +0.4), 161.0598 (12, $C_{10}H_9O_2$ −0.4) and 121.1018 amu (32%, C_9H_{13} +0.2 mmass).

(B) *D.a.-B-IVb and D.a.-C-IVb.* These fractions contained 40–80% acetoxydiplophyllin (**20**) by NMR and TLC analysis. Rechromatography using 3% EtOAc in benzene afforded fractions which crystallized. Trituration with cold pentane afforded pure lactone **20**: m.p. 92–95°; $[\alpha]_D = -132 \pm 6°$, $\Delta\epsilon_{266} = +2.57$, $\Delta\epsilon_{250} = 0$, $\Delta\epsilon_{231} = -4.23$, $\Delta\epsilon_{211} = +3.76$, $\Delta\epsilon_{195} = -8.9 \pm 1.8$ (6 mM, MeOH); δ_{CDCl_3} 1.10 (Me, s), 1.70 (Me, s), 2.11 (MeCO, s), 2.8–3.4 (2 H, m)‡ 4.35 (H-8, $J_9 = 10$, $J_7 = 8.4$), 5.04 (H-9, d, 9.7), 5.65 (d, 2.2) and 6.27 ppm (d, 2.5 Hz);‡ IR ($CHCl_3$) 1770, 1750, 1663, 1380, 1373, 1258, 1211, 1123, 1065, 965, 921, 821 cm^{-1}; MS 290.1492 (18, $C_{17}H_{22}O_4$ −2.2), 230.1294 (57, P-HOAc −1.2), 215.1074 (48, P-HOAc-Me +0.4), 123 (42), 105 (25), 91.0554 (32, C_7H_7, +0.6), and 43.0168 amu (100%, C_2H_3O −1.6 mmass). Hydrogenation—$(\phi_3P)_3RhCl/H_2/\phi H$ - EtOH—of acetoxydiplophyllin afforded the 11,13-dihydro derivative: δ_{CDCl_3} 1.15 (Me, s), 1.21 (Me, d, 7.5), 1.69 (vinyl-Me), 2.08 (MeCO, s), 2.2–2.8 (2 H, m), 4.21 (H-8, ~trip., 3.5), and 5.10 ppm (H-9, d, 3.7 Hz). Further hydrogenation—PtO_2/H_2/EtOH—gave the tetrahydro derivative: δ_{CDCl_3} 4.94 (H-9, d, 2.7), 4.21 (H-8, dd, 2.7, 3.7), 2.2–2.9 (~3 H, m), 2.10 (MeCO, s), 1.20 (Me, d, 7.6), 1.03 (Me, s) and 0.90 ppm (Me, d, 7.4 Hz).

(C) *Albicanol* (**22**). D.a. - B - IVa was combined with early fractions from the chromatography in *B* (just above). The resulting oily residue showed one major component by GC ($RR_A^{190} = 1.84$, $RR_C^{190} = 2.37$). Column chromatography (basic alumina, 5% EtOAc in benzene) afforded albicanol (**22**) as an oil: IR (film) 3340 (OH), 3085, 1645, 890 ($C=CH_2$), 1465, 1395, 1372, 1314, 1205, 1168, 1142, 1120, 1025 and 970 cm^{-1}; δ_{CDCl_3} (60 MHz) 0.75, 0.84, and 0.89 (Me, s), 2.41 (1 H, allyl-H, m), 2.6 (OH), 3.79 (2 H, AB of ABX, CH_2OH, $J_{AX} = 5.5$, $J_{BX} = 9.0$, $\Delta\nu_{AB} = 7$, $J_{AB} = 11$),§ 4.64 and 4.92 ppm ($C=CH_2$, ~d, 1.5 Hz); see discussion for LIS data (**22A**) and discussion of apparent lack of agreement with literature NMR data.[34]

On acid treatment (CH_3CO_3H, TFE) albicanol and authentic drimenol[37] gave nearly identical mixtures by GC (phases A and C). Hydrogenation (PtO_2, HOAc) afforded after column chromatography drimanol,[37] m.p. and mixed m.p. 109–111°.

(D) *Diploalbicanol.* The mother liquors from acetoxydiplophyllin fractions and those fractions eluting immediately after acetoxydiplophyllin (including D.a.-C-V displayed a major component by GC ($RR_C^{200} = 1.07$, $RR_A^{200} = 1.29$). Collection of the material eluted during this GC peak gave a new crystalline alcohol:¶ m.p. 47–48°; IR (film) 3380 (OH), 3020 (OH), 3020 sh?, 1460, 1455, 1382, 1253, 1120, 1095, 987, 940 and 892 cm^{-1}; δ_{CDCl_3} 0.39–0.71 (~2 H, cyclopropyl-H), 0.92 (Me, d, 7.5 Hz), 1.00 and 1.02 (Me, s), and 1.10 ppm (MeCO, s). The Eu(FOD)$_3$ induced shifts for these signals were: cyclopropyl, ~2–2.9; Me d, 1.70; Me s, 1.74 and 0.91; and carbinol-Me, 9.00 ppm. The spectrum observed on addition of 0.23 eq. of Eu(FOD)$_3$ was integrated: 4.0–5.0 (3 H, LIS = 12–13), 3.3–4.0 (2 H, apparent quartet, LIS ≃ 8 ppm), 3.17 (3 H, carbinol-Me), 1.6–2.8 (~6 H), 0.82–1.6 ppm (~11 H, including Me singlets at 1.25 and 1.40, Me d at 1.31 ppm). This data indicates that the methyl-carbinol unit is well separated from the other Me groups and the cyclopropyl and that there are at least 3 β-H. No further refinement of the structure was possible.

Dihydrodiplophyllin (**11**). Diplophyllin (22 mg) and 10 mg of tris - (triphenylphosphosine) - rhodium chloride in 2 ml of benzene were stirred in an atmosphere of H_2 for 15 hr. The mixture was eluted through a column of alumina to afford 10 mg of oily product (**11**): $\Delta\epsilon_{250} = -0.44$ (inflec.), $\Delta\epsilon_{227} = -0.70$, $\Delta\epsilon_{209} = +2.48$ (olefin $\pi \rightarrow \pi^*$?) (MeOH, 12 mM): IR (film) 1775, 1460, 1450, 1285, 1163, 954, 890, 870 and 750 cm^{-1}; δ_{CDCl_3} 4.43 (H-8, m), 2.2-3.0 (3 H, m), 1.67 (vinyl-Me), 1.22 (3 H, Me, d, 7.2 Hz) and 1.13 ppm (3 H, Me, s); Ms confirms the molecular formula: 234.1627 amu (=$C_{15}H_{22}O_2$ +0.8 mmass).

Tetrahydrodiplophyllin (**12**). Diplophyllin (40 mg) was hydrogenated at 1 atm using Adams' catalyst in 2 ml of HOAc. The reduction product crystallized; recrystallization from ethanol afforded **12**: m.p. 146–146.5°, $\Delta\epsilon_{225.5} = -0.227$ (MeOH, 14.6 mM); δ_{CDCl_3} 0.79 (Me, d, 7), 0.99 (Me, s), 1.19 (Me, d, 7), 1.97 (1 H, A of ABX, $J_{AB} = 15$, $J_{AX} = 2.3$, H-9β?), 2.50 (1 H, m), 2.79 (H-11, quintet, 7), and 4.46 ppm (H-8, t of doublets, $J_d = 2.3$, $J_t = 4.1$ Hz). Hydrogenation of authentic isoalantolactone (**14**)[28] in like manner produced tetrahydroalantolactone (**17**) – m.p. 143–144°, $\Delta\epsilon_{225} = +0.21$, lit.[26] m.p. 144–145° – which was identical by TLC and NMR.

Lithium aluminum hydride reduction of lactone **12** afforded alcohol *ent*-**18**: m.p. 108–110° (recrys. from n-hexane); lit. m.p. 108–110°, reported for the enantiomer (**18**).[27]

Ent - *Selina* - 4,11 - *diene* - 8,13 - *diol* (**13**). To a soln of 23 mg of diplophyllin (0.1 mmole) in dry toluene was added 0.15 ml (0.2 mmole) of 1.45 M toluene soln of Al(i-Bu)$_2$H dropwise at −78° under stirring. After the mixture was stirred at −78° for 45 min MeOH was added to decompose the excess of hydride. About 3 equivs of $NaBH_4$ (saturated soln in EtOH) was added to the mixture at 0° and stirred for 2.5 hr at 0 ~ 5°. Usual work up gave 14 mg of crystalline product which was recrystallized from benzene, (**13**): m.p. 145–146°. Repetition of this sequence on 130 mg of diplophyllin afforded 78 mg (60%) of diol **13**: m.p. 145–146°; R_f (40% EtOAc/ϕH, SiO_2) = 0.29; $\Delta\epsilon_{212.5} = +10.8$, $\Delta\epsilon_{201} = 0$, $\Delta\epsilon_{195} = -9$! ($CH_3CN$, 9 mM); δ_{CDCl_3} 1.26 (3 H, Me, s), 1.62 (vinyl-Me), ~2.3 (2 H, OH), 3.9–4.4 (3 H, m, CH—O, $-CH_2$–O), 5.06 and 5.24 ppm ($C=CH_2$); MS 236.1780 (34, $C_{15}H_{24}O_2$ +0.6) 218.1676 (100, $C_{15}H_{22}O$ +0.6), 203.1432 (83, $C_{14}H_{19}O$ −0.4), 185.1356 amu (63%, $C_{14}H_{17}$ +2.8 mmass). Found (236.1783, ms): C, 76.10; H, 10.38. Calc. for $C_{15}H_{24}O_2$: C, 76.23; H, 10.24%).

Ent-*Diplophyllin* (ent-**10**) + *isomerization of isoalantolactone* (**14**)

(A) *Preliminary experiments.* Isoalantolactone (7 mg) in 100 μl of trifluoroethanol (TFE) was treated with 1 μl of CH_3SO_3H with stirring at room temp. TLC showed complete isomerization after 24 hr, R_f (8% EtOAc/ϕH, $AgNO_3$–SiO_2): 0.35 (absent , authentic **14**), 0.70 (minor, lactone **16**) and 0.80 (major, corresponds to diplophyllin).

Similar treatment of helanin (Fluka, =**14** + **15**) gave a comparable TLC result, R_f (3% EtOAc/ϕH, $AgNO_3$–SiO_2): 0.50 (minor, lactone **16**) and ~0.61 (major). But NMR spectroscopy revealed residual alantolactone (R_f ~0.61). In a separate trial, pure alantolactone was subjected to the isomerization conditions without rearrangement. Prolonged treatment and more concentrated acid (3% CH_3SO_3H in TFE) were also without effect on alantolactone.

†In CCl_4 (60 MHz): 1.09, 1.67, 2.5–3.2, 4.38, 5.48 and 6.08 ppm. The solvent shifts implied have been confirmed by the 60 MHz (CCl_4 & $CDCl_3$) spectra of synthetic *ent*-diplophyllin.

‡NMDR experiments establish that H-7 is one of these two resonances, centered at *ca.* 3.0 ppm. These experiments indicate $J_{7,8} = 8$–8.5 Hz and that the vinyl hydrogen doublets are due to $J_{7,13}$ not a two bond coupling.

§The downfield member of this AB pattern ($J_{AX} = 5.5$) shifts more rapidly and the coupling constants approach $J_{AX} \rightarrow 3.5$, $J_{BX} \rightarrow 11$ Hz as Eu(FOD)$_3$ is added. This indicates changes in rotamer population in the free and complexed alcohol.

¶The NMR pattern at 0.3–0.7 ppm is the same as that for diploalbicene and D.a.-10-K. In addition all three substances show very sharp IR bands at 1382–1390, 1250–1256 and 982–987 cm^{-1} as well as many subtler similarities.

(B) *Preparative reaction.* Isoalantolactone (500 mg) in 5 ml of TFE was treated with 20 μl of CH_3SO_3H for 2 hr with stirring. Dilution with water and ether extraction afforded 480 mg of oily product, *ent*-**10** and -**16** by TLC. The product was chromatographed on 15% $AgNO_3$–SiO_2 using 2% EtOAc in benzene affording 310 mg (62%) of *ent*-**10** [IR, NMR identical to natural diplophyllin; $\Delta\epsilon_{267} = -3.14$, $\Delta\epsilon_{235} = +4.75$, $\Delta\epsilon_{212} = -7.5$ (MeOH); m.p. 31–32°] and 140 mg (28%) of lactone *ent*-**16**: m.p. 81–82° (from EtOH–H_2O);† δ_{CDCl_3} 0.88 (Me, s), 1.61 (vinyl-Me), ~3.05 (H-7, m), 4.50 (H-8, m), 5.36 (H-3, m), 5.58 and 6.09 ppm (C=CH_2, 2 doublets, J ~ 1.5 Hz); $[\alpha]_D = +132$, $\Delta\epsilon_{258} = -2.03$, $\Delta\epsilon_{243} = 0$, $\Delta\epsilon_{215} = +13.5$, $\Delta\epsilon_{190\to} 0$ (c, 0.18 MeOH); ms 232.1464 (78, $C_{15}H_{20}O_2$ +0.2), 217.1236 (100, $C_{14}H_{17}O_2$ +1.0), 203.1072 (7, $C_{13}H_{15}O_2$ +0.2), 178.1008 (9, $C_{11}H_{14}O_2$ +1.6), 171.1182 (25, $C_{13}H_{15}$ +1.0) and 131.0854 amu (25%, $C_{10}H_{11}$ −0.6 mmass).

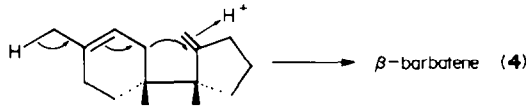
Synthesis of Diol **18**

(A) *Hydrogenation of* ent-*diplophyllin. Ent*-**10** (250 mg) in 5 ml HOAc was hydrogenated at 1 atm using Adams' catalyst affording 250 mg of crystalline **17**: $\Delta\epsilon_{225} = +0.22$. The entire sample, in 10 ml ether, was added to a suspension of 300 mg of LAH in 20 ml of ether. After 4 hr, excess LAH was decomposed by dropwise addition of water. Diol **18** (m.p. 108–110°). 245 mg, was isolated with ether after dissolving the aluminates in dilute aqueous H_2SO_4.

(B) *From helanin.* Helanin (Fluka), 2.0 g, was hydrogenated as above affording 1.8 g of crystalline product indistinguishable from the previous sample by TLC. LAH reduction afforded 1.5 g of a mixture, R_f (20% EtOAc/ϕH, SiO_2) 0.79 (trace = starting material), 0.35 (spot A), and 0.22 (spot B = diol **18** by TLC comparison). Chromatography over 50 g of SiO_2 using 20% EtOAc in benzene afforded 400 mg (~18%) of spot A material, assigned structure **19** based on the NMR and CD spectra observed: δ_{CDCl_3} 5.1 (vinyl-H, d, 5.5 Hz), 4.27 (1 H, m), 1.95–2.6 (2 H, allyl-H, m), 1.13, 1.04, 0.96 and 0.84 ppm (sharp lines integrating to 12 H, 4 Me groups); $\Delta\epsilon_{207} = -0.24$, $\Delta\epsilon_{192} = -0.8$! (pentane 16 mM).

Later fractions afforded 710 mg (32%) of diol **18**, m.p. 108–110°, identical to material obtained from pure *ent*-**10** or **10** by NMR, TLC and IR.

Note added in proof: Additional chemical evidence on the structure of bazzanene now excludes structure **3**, and is consistent with structure **viii**, the diastereomer of trichodiene, a structure which can serve as the biogenetic precursor of the barbatenes.



viii

Y. Asakawa (Dept. of Chem., FAculty of Science, Hiroshima) has independently concluded that bazzanene has a trichodiene-related structure.

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[15]Anastreptene, first encountered in large amounts in *Anastrepta orcadensis* has now been found in representatives of the genera: *Scapania*, *Diplophyllum*, *Barbilophozia*, and *Orthocaulis* as well. Anastreptene has been chemically correlated with a myliol degradation product establishing the common skeleton, see N. H. Andersen, P. Bissonette, C.-B. Liu, B. Shunk, Y. Ohta, C.-L. W. Tseng and A. Moore, *Phytochemistry*, in press. Experimental studies to confirm the skeleton and stereochemistry of anastreptene and myliol are in progress.

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[19]Fungal sesquiterpenes are enantiomeric to those of higher plants where a direct comparison can be made (see for example culmorin [D. H. R. Barton and N. H. Werstiuk, *J. Chem. Soc.* (C), 148 (1968)], (+) - epi - cubenol [N. N. Gerber, *Phytochemistry* **10**, 185 (1971)], (−)-sativene [P. de Mayo and R. E. Williams, *J. Am. Chem. Soc.* **87**, 3275 (1965)], and the extensive recent work of Arigoni presented at the recent I.U.P.A.C. symposium (9th Conference on Natural Products, Ottawa, Canada, 24–28 June 1974). The one exception that comes to mind is 4(14),7(11) - selinadiene and related compounds from *Streptomyces fradiae* [N. N. Gerber, *Phytochemistry* **11**, 385 (1972)] where a CD comparison in these laboratories indicates a β - angular - Me. This does not preclude an origin from a 7α-isopropyl precursor.

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[23]This acid-catalyzed isomerization of β-selinene provides essentially all of the selinadienes, unpublished work with D. P. Svedberg.

[24]The absolute stereochemistry of β-elemene depicted is assumed based on its co-occurrence with *ent*-selines.

[25]On hearing of our work, V. Herout let us know of their isolation of two different *ent*-eudesmanolides, diplophyllolide-A and B, from the same species of liverwort. An account of this work has just appeared: V. Benešová, Z. Samek and S. Vašíčková, *Coll. Czech. Chem. Commun.* **40**, 1966 (1975). Our present work included the synthesis of *ent* - diplophyllolide - A (*ent*-**16**) and thus we were able to ascertain that our samples of *D. Albicans*

†The reported values for naturally-derived lactone **16** are: m.p. 60–62°, $\Delta\epsilon_{258} = +1.9$, $\Delta\epsilon_{215} = -12.3$.[25] On reading this report we repeated a m.p. determination on our sample (obs. m.p. 79–81°). This would appear to be a case of dimorphism.

contains no detectable **16** (less than 1%). Both our and the Czechoslovakian specimens were growing on granitic rocks. This would appear to be a case of distinct chemical races.

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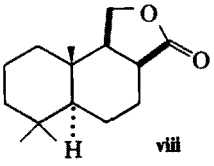

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[51]See Ref. 16, see also A. Matsuo, M. Nakayama, S. Hayashi and S. Yasuda, *Agr. Biol. Chem. Tokyo* **36**, 2241 (1972); A. Matsuo, M. Nakayama, S. Hayashi and S. Yasuda, *Phytochemistry* **12**, 2413 (1973).

[52]Unpublished work with C. B. Liu and M. Chan.

[53]The report of *F. tamarisci* constituents appears as H. Knocke, G. Ourisson, G. W. Perold, J. Fousseraeu and J. Maleville, *Science* **166**, 239 (1969). The report of *ent*-santanolide **vii** as a note in proof (with J. C. Muller) in this same article.