

STRUCTURE OF THE SESQUITERPENIC LACTONE DIPLOPHYLLOLIDE AND OTHER COMPONENTS FROM THE LIVERWORT****Diplophyllum albicans* (L.) DUM.**

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From light petroleum extracts of the liverwort *Diplophyllum albicans* (L.) DUM. two sesquiterpenic selinanolides were isolated, *i.e.* diplophyllolide A and diplophyllolide B and a sesquiterpenic alcohol of the composition $C_{15}H_{24}O$ in addition to homologous n-alkanes and cerides, a mixture of phytosterols and nonacosan-10-ol. Diplophyllolide A was assigned the structure *II* on the basis of its PMR spectrum and correlation with tetrahydroalantolactone, and its absolute configuration was also determined; according to CD spectrum it is enantiomeric with selinanolides which were isolated from higher plants earlier.

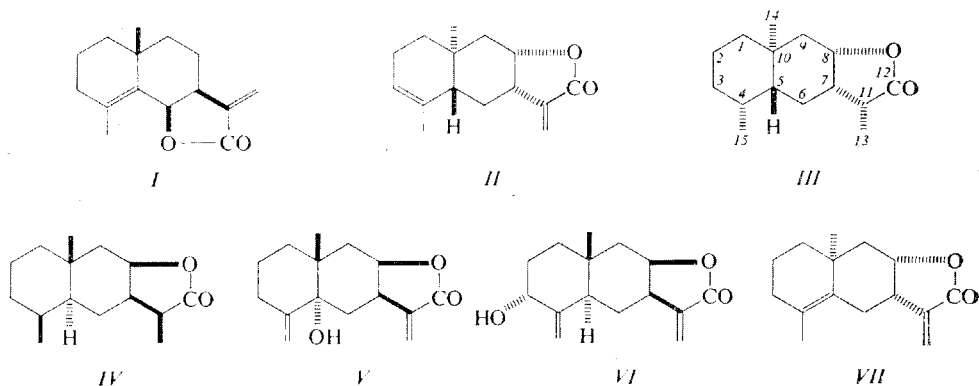
The first sesquiterpenic lactone isolated from liverworts (*Hepatica*) is the occupational allergies (contact dermatitis) provoking selinanolide *syn. eudesmanolide* from *Frullania tamarisci* (L.) DUM, to which the authors^{1,2} assigned the structure *I*. The biological activity was demonstrated for both enantiomers of which the laevorotatory one was isolated from the liverwort *F. tamarisci*, while the dextrorotatory one from *F. dilatata*. In the majority of terpenoids isolated from liverworts so far it was recently shown that they are enantiomers³⁻⁸ of the same compounds isolated earlier from higher plants.

During a complex analysis of the light petroleum extracts of the liverwort *Diplophyllum albicans* (L.) DUM. we now isolated in addition to components the occurrence of which has often been described in these plants⁹⁻¹³ a substance which displayed distinct bands of an α,β -unsaturated γ -lactone in the infrared spectrum. On repeated chromatography we found that it was a mixture of two lactones**. The less polar we named diplophyllolide A and the more polar diplophyllolide B.

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* In the course of the preparation of this paper Prof. V. Herout of this Institute obtained a private communication from Dr N. H. Andersen, University of Washington, Seattle, U.S.A. that his group had isolated from essential oils prepared by separate steam distillation of *Diplophyllum albicans* and *D. taxifolium* in both cases the same sesquiterpenic α,β -unsaturated γ -lactone; the authors gave it the name diplophyllin and assigned it the structure *VII*.

While diplophyllolide A was identified as an α,β -unsaturated γ -lactone on the basis of its infrared spectrum, diplophyllolide B did not produce bands for an exomethylene double bond. We devoted our main attention to diplophyllolide A in consequence of the small amount of diplophyllolide B available. From the elemental composition, IR and PMR spectra it followed that it was an isomer of alantolactone, differing in the presence of the endocyclic $\Delta^{3,4}$ double bond. This was also corroborated by hydrogenation after which we obtained tetrahydro derivative III which was identical with tetrahydroalantolactone IV on the basis of IR and PMR spectra. Tetrahydro derivative III displayed $\Delta\epsilon_{225} = -0.07$ in the CD spectrum, while tetrahydroalantolactone IV had $\Delta\epsilon_{225} = +0.21$. These facts suggested that compound III might be enantiomeric with substance IV and that diplophyllolide A has the structure II. However, the differences in $\Delta\epsilon$ -values did not permit an unambiguous conclusion. An important argument for structure II is just the CD curve of diplophyllolide A which displayed $\Delta\epsilon_{258} = +1.9$. According to empirical rules¹⁴ γ -lactones of the 7(*S*)-selin-11(13)-en-8,12-olides series should display a positive Cotton effect in the region about 255 nm, in the case of *trans*-annulation, and a negative Cotton effect in the case of *cis*-annulation of the lactone ring. As was already said¹⁵, in view of the possible enantiomerism these rules may be used for the determination of absolute configuration in those cases where the type of annulation is known. In our case the *cis*-annulation of the γ -lactone ring in II is determined both by direct correlation with alantolactone and by the PMR spectrum of II ($J_{7,8} = 5.1$ Hz, $J_{7,13} = 1.2$ Hz; $J_{7,13} = 1.1$ Hz^{16,17}). The negative Cotton effect in the CD curves of structurally close lactones, as for example telekin V ($\Delta\epsilon_{258} = -1.97$) and isotelekin VI ($\Delta\epsilon_{257} = -1.76$), indicates — in the sense of the above mentioned rules — that these substances belong to the series with the same $C_{(7)}-C_{(11)}$ bond configuration [(7*S*)-selinan-8 β ,12-olides], while the positive Cotton effect of the lactonic chromophore of diplophyllolide A indicates that they belong to the pseudoenantiomeric series with the α -configuration of the $C_{(7)}-C_{(11)}$ bond [(7*R*)-selinan-8 α ,12-olides].



In addition to sesquiterpenic lactones a homologous series of n-alkanes (clearly both odd and even numbered members of the series), beginning with C_{13} and ending with C_{33} , has been detected in the least polar chromatographic fractions of the light petroleum extract by gas chromatography. Maxima appeared in C_{27} and C_{29} , or C_{21} alkanes. n-Alkanes were followed by a homologous series of aliphatic esters comprising the homologues C_{36} — C_{50} , with distinct maxima at C_{38} and C_{48} . This mixture of esters afforded after transesterification a homologous series of methyl esters of even-numbered acids C_{16} — C_{28} (with maxima at C_{16} and C_{20}) and a homologous series of even-numbered primary alcohols C_{20} — C_{28} (with maxima at C_{22} and C_{28}). From the chromatographic fractions eluted from the column with a mixture of light petroleum with 20% of benzene a negligible amount of a substance of m.p. 67–68°C was isolated, the composition of which was $C_{15}H_{24}O$ on the basis of its high resolution mass spectrum; it is probably a sesquiterpenic alcohol, because it has a high $M - 18$ peak in its mass spectrum. From the more polar fractions a substance melting within a broader range (135–138°C, or 145–148°C) precipitated on longer standing. Mass spectrum indicated that it is a mixture of phytosterols of masses 400, 412 and 414. The last individual component isolated from the light petroleum extract was a substance of m.p. 80–81°C which on the basis of infrared data belongs among aliphatic alcohols. On comparison of its mass spectrum with the mass spectrum of authentic nonacosan-10-ol¹⁸ we proved that they are identical.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. The infrared spectra were measured in chloroform solution by means of a Zeiss UR 10 spectrophotometer. The mass spectra were measured on a MS 902 spectrometer. The PMR spectra in deuteriochloroform were recorded with a Varian HA-100 apparatus using tetramethylsilane as internal standard. Silica gel for chromatography was prepared according to Pitra and Štěrba¹⁹, and, unless stated otherwise, it was deactivated by addition of 10% of water. Light petroleum was a fraction boiling at 40–60°C. CD spectra were measured on a Roussel-Jouan, Model CD 185, Dichrograph in methanol solution.

Light petroleum extract. Dry plant material from two collections (Rudolfov, Jizera Mountains, Czechoslovakia; 1970 or 1973) was extracted at room temperature with light petroleum. The combined extracts concentrated at low temperature and *in vacuo* were chromatographed on a ten-fold amount of silica gel. The fractions which were eluted from the column with light petroleum containing 20% of benzene were concentrated, affording a poorly crystallizing residue from which diplophyllolide A and diplophyllolide B were isolated by repeated chromatography on a seven-fold amount of the same adsorbent. In an effort to obtain a larger amount of these lactones we worked up vegetal material from a locality in Hřensko (Northern Bohemia). From the lactonic fraction we isolated a third, non-crystalline lactone by the procedure described for the isolation of frullanolide^{1,2}, and we characterized it only by its IR spectrum (1163, 1164, 1762 cm^{-1}) 1762 cm^{-1}) and CD curve ($\Delta\epsilon_{200} + 0.03$; $\Delta\epsilon_{222} - 0.08$).

Diplophyllolide A. The combined chromatographic fractions which were eluted from the column with a mixture of light petroleum and benzene (1 : 1) afforded after evaporation of the

solvent a substance of m.p. 60–62°C; MS (*m/e*): 232 ($C_{15}H_{20}O_2$) CD: $\Delta\epsilon_{258} + 1.9$; $\Delta\epsilon_{215} - 12.3$; IR: 1153, 1408, 1640, 1668, 1757 cm^{-1} ; PMR: 5.38 m (H_3), 3.00 m (H_7); 4.52 m (H_8); ($J_{7,8} = J_{8,9} = 5.1$ Hz; $J_{8,9} = 1.5$ Hz); 6.12 dd (H_{13} , $J_{7,13} = 1.2$, $^2J \cong 0.4$); 5.58 dd ($H_{13}f$; $J_{7,13} = 1.1$ Hz, $^2J \cong 0.4$ Hz); 2.15 dd (H_9 , $J_{9,8} \cong 1.5$, $^2J = 15.5$), 1.43 dd (H'_9 , $J'_{9,8} \cong 5$, $^2J = 15.5$), 0.89 t ($J \cong 0.7$, H_{14}); 1.62 m (H_{15} , $J_{3,15} \cong 1.2$).

Diplophyllolide B. Combined chromatographic fractions eluted from the column with light petroleum and benzene (1 : 2) afforded after evaporation of solvent a substance of m.p. 92 to 100°C; MS (*m/e*): 234 ($C_{15}H_{22}O_2$); CD: $\Delta\epsilon_{240} - 0.98$; IR 1165, 1760 cm^{-1} .

Tetrahydro derivative III. Diplophyllolide A was hydrogenated in ethanolic solution in the presence of fresh Adams catalyst until saturation was complete. After evaporation of the solvent a substance remained which after two crystallizations from light petroleum had m.p. 144°C. MS (*m/e*): 236 ($C_{15}H_{24}O_2$); CD: $\Delta\epsilon_{225} - 0.07$; IR: identical with authentic tetrahydroalantolactone *IV*. PMR: 4.42 m (H_8), 2.75 q (H_{11}), 2.37 m (H_7), 1.98 dd (H_9 , $J_{9,8} \cong 2$, $^2J = 15$), ~ 1.34 (H_9), ~ 1.77 (H_4), 1.19 d (H_{13} , $J_{13,11} = 7.2$), 0.98 (H_{14}), 0.88 d (H_{15} , $J_{4,15} = 7.3$). Tetrahydro derivative *III* when mixed with authentic tetrahydroalantolactone *IV* (m.p. 145°C) displayed melting point depression.

Tetrahydroalantolactone IV. An authentic sample of tetrahydroalantolactone^{20,12} was purified chromatographically on a small column of silica gel before CD measurement. The melting point remained unchanged, CD: $\Delta\epsilon_{225} + 0.21$.

n-Alkanes and wax esters. *n*-Alkanes were eluted from the column with light petroleum in the first fractions, while esters could be eluted with light petroleum containing 2–10% of benzene. Gas chromatographic analyses were carried out on a Pye Series 104 Chromatograph, Model 24, provided with a dual column and FI detector system and the possibility of programmed heating. Columns of 0.4 × 150 cm size were filled with 2.5–3% of SE-30 G.C. Grade on Gas Chrom P (100–120 mesh). Chromatographic peaks were identified under the conditions described earlier¹³. Transesterifications of esters were carried out with methanol and gaseous hydrogen chloride in sealed ampoules in tetrachloromethane. The homologous series of methyl esters of monocarboxylic acids and free alcohols were identified together by gas chromatography¹³.

Sesquiterpenic alcohol. On concentration of fractions eluted from the column with a mixture of light petroleum and 20% of benzene a substance precipitated, m.p. 68–72°C. Its amount was sufficient for a measurement of MS (*m/e*) only: 220 ($C_{15}H_{24}O$).

Phytosterols. Benzene eluted from the column fractions which after concentration and longer standing afforded a substance melting unsharply even after several crystallizations, in the 135 to 138°C or 145–148°C intervals. Its mass spectrum indicated that it is a mixture of phytosterols with the following molecular masses: 400, 412 and 414.

Aliphatic alcohol. The fractions containing the mixture of phytosterols were followed by a series of fractions which were eluted from the column with a mixture of benzene with 30% of ether. After concentration crystals melting at 80–81°C crystallized out from these fractions. MS (*m/e*) 423 ($M - 1$), 422, 406 ($M - 18$), 297 ($C_{19}H_{39}CH = ^+OH$), 157 ($C_9H_{19}CH = ^+OH$). From the IR bands of the spectrum it followed that it is an aliphatic alcohol. The identity of this alcohol with an authentic sample of nonacosan-10-ol¹⁸ was demonstrated by comparison of the mass spectra of both substances, which were identical.

For the discovery of the locality with the liverwort Diplophyllum albicans in Rudolfov (Jizera Mountains), for help during its collection, and for his continuous interest in this work the authors

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