THE STRUCTURE OF HANDELIN — A NEW DIGUAIANOLIDE FROM *Handelia trichophylla*

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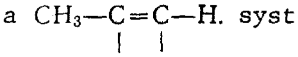
A compound of lactone nature with mp 212°C isolated from *Handelia trichophylla* Heimerl. [1], composition $C_{32}H_{40}O_8$, $[\alpha]_D^{20}$ +47.8° (c 1.3; pyridine) has been called handelin (I).

The IR spectrum of (I) (Fig. 1) has absorption bands at 1755, 1745, 1725, and 1250 cm^{-1}, which are connected with the presence of γ-lactone and ester carbonyl groups in the molecule. The lactone nature of (I) was confirmed by the formation of the saponification product — deacetylhandelin (III), the spectrum of which showed strong absorption bands at 1770 and 1740 cm^{-1}. The PMR spectrum of (II) lacked the signal of the protons of the acetyl group and, in comparison with (I), the signal of the gem-hydroxylic proton in the form of a poorly resolved multiplet appeared in a weaker field at 4.47 ppm.

The acetylation of (II) with acetic anhydride reformed handelin, showing the secondary nature of the OH (OAc) groups in these compounds.

The presence of hydroxy groups in (I) (strong absorption bands in the 3465 and 3440-cm^{-1} regions) was confirmed by the behavior of the two one-proton singlets at 5.53 and 5.96 ppm [PMR of (I), Fig. 2a]. The positions and half-widths of these signals depend on the temperature, and the addition of a very small amount of trifluoroacetic acid to the sample leads to their disappearance (Fig. 2b). Consequently, these signals are due to the protons of two hydroxy groups which show a tertiary nature: they are not acetylated by acetic anhydride and are not oxidized. With acetyl chloride, (I) gives a diacetate (III) the IR spectrum of which lacks absorption bands of hydroxy groups and the PMR spectrum of which, in comparison with that of (I), shows the signals of two additional acetyl groups — singlets at 2.08 and 2.13 ppm.

The total numbers of ester carbonyl groups in (I) and (III) correspond to the signals in the ^{13}C NMR spectra [2]: (I) (DMSO-d_6) — singlets at 177.6, 169.9, and 169.7 ppm — and for (III) (deuteropyridine) — at 184.2, 178.1, 177.8, 177.6, and 177.1 ppm. The fact that in handelin only one ester group — the acetate group — is saponified and under these conditions the other two are only opened, and reclose on acidification, giving (II), shows that its molecule contains two lactone groups. This explains the nature of all the oxygen-containing groupings of (I).

Methyl groups also participate in the formation of the structure of handelin. The signals of the protons in the 1.88-1.16 ppm region are shown in Fig. 2a. With the additional irradiation with a strong radiofrequency field of the protons of the methyl group the signal of which is located at 1.88 ppm, a considerable contraction and increase in the intensity of the one-proton singlet at 5.41 ppm is observed, from which it follows that this methyl group belongs to a  $CH_3-\underset{|}{C}=\underset{|}{C}-H.$ system.

The signals of two other methyls (singlets at 1.25 and 1.16 ppm) in (III) are shifted downfield by $\sim$0.22 ppm. This shows that each of these methyls is attached to a carbon bearing a hydroxy group. A singlet at 1.55 ppm corresponds to a methyl attached to a tertiary carbon atom.

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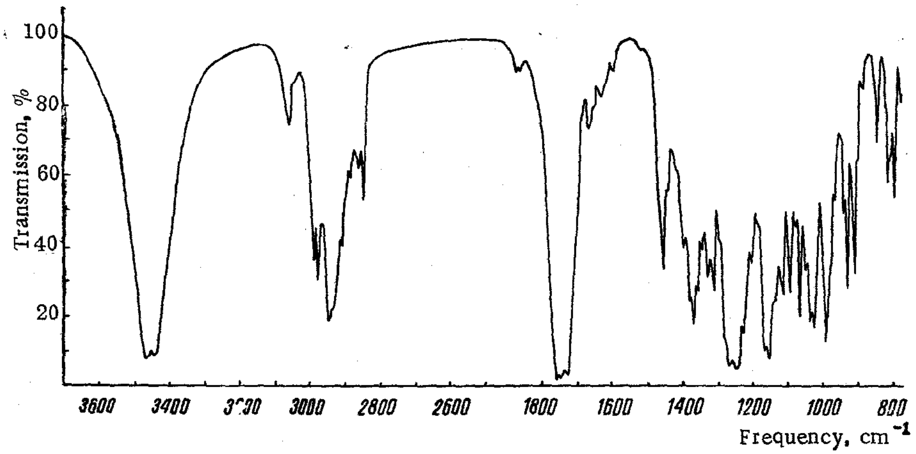


Fig. 1. IR spectrum of handelin.

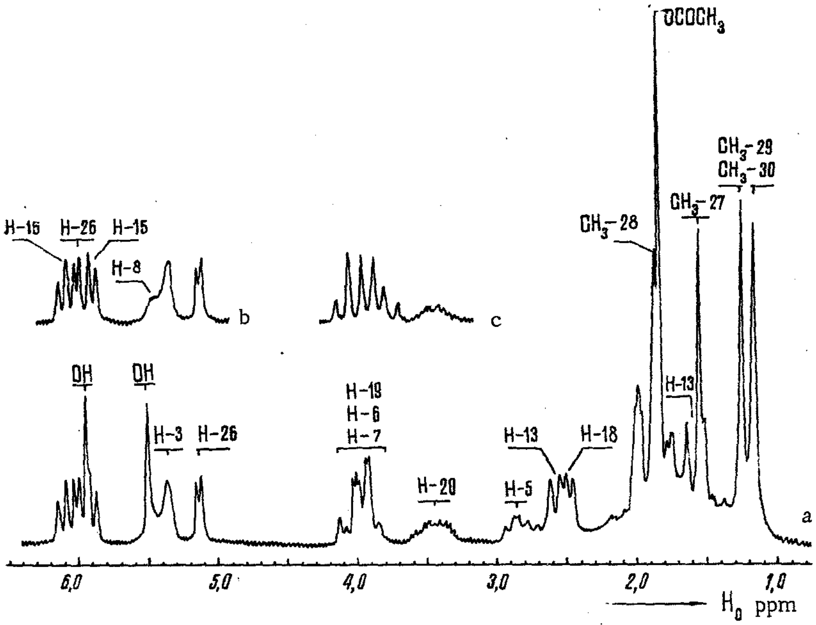


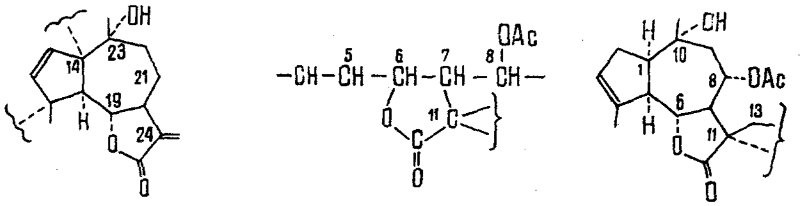
Fig. 2. PMR spectra of handelin in C_5D_5N at room temperature (a) and at +65°C (c) and in a mixture of C_5D_5N and CF_3COOH (b).

To determine the nature of the groupings adjacent to the carbons bearing methyl and hydroxy groups simultaneously, the latter were split out from (I) by means of thionyl chloride. This gave dianhydrohandelin (VI). According to its UV spectrum, its molecule contained no conjugated double bonds. The PMR spectrum, in comparison with that of (I), showed the signals of two additional olefinic protons. They can be formed only from two similar $CH_3{-}C(OH)CH_2{-}$ groupings.

The exhaustive hydrogenation of (I) in the presence of platinum oxide saturated the three double bonds with the formation of hexahydrohandelin (V). On comparing the weak-field regions of the spectra of (I) and (V), the signals of five olefinic protons were detected in the former. Doublets at 6.06 and 5.18 ppm with ^4J = 3.3 and 2.6 Hz (Fig. 2a, b) are the signals of the protons of an exocyclic methylene group attached to a lactone ring. The H-20 proton (fragment A) experiences allyl coupling with it and its signal at 3.48 ppm is strongly broadened because of vicinal interaction with another three protons — the lactone proton (H-19) with ^3J = 9.8 Hz and the methylene protons attached to the C-21 carbon. The fine structure of the multiplet with its center at 4.0 ppm appears more distinct with a rise in the temperature of a solution of (I) at 65°C (Fig. 2c). It is caused by the superposition of the signals of three protons one of which is the triplet signal of the H-19 lactone proton at 4.04 ppm. The presence of a second large SSCC (^3J = 9.8 Hz) shows that the H-18 methine proton is adjacent to it. It was shown by the INDOR method that the latter is represented by a doublet with broadened components at 2.53 ppm having ${}^3J = 9.8$ Hz. Consequently, the H-18 proton has only the H-19 lactone proton in the vicinal position.

Using the INDOR method, coupling was brought about between the H-15 and H-16 protons with ${}^3J = 5.7$ Hz to which (Fig. 2b) two doublet signals at 5.94 and 6.17 ppm correspond. The values of SSCC was characteristic for the vinyl group —HC=CH— in a five-membered ring [3]. Because of the absence of other vicinal interactions, there can be no protons attached to the carbons in the α positions to this group. We detected only allyl spin—spin coupling (∿0.7 Hz) between the H-18 and H-16 protons, which was confirmed by the double resonance spectra of deacetyldehydrohandelin (IV) where a doublet signal with broadened components from the H-18 proton (${}^3J = 9.7$ Hz) is in a region of the field free from other signals at 3.37 ppm, while its irradiation does not affect the resonance transitions of the other nuclei. Thus, the functions of the three double bonds in (I) and the system in which they are present have been elucidated.

The formation of chamazulene (VII) by the dehydrogenation of (I) and (V) with selenium shows the presence in handelin of at least one guaiane fragment. The mutual positions of a number of protons that have been found in (I) makes it possible to trace the link between the lactone ring with the exomethylene group and the five-membered ring with the isolated double bond, i.e., the elements characteristic of lactones of the guaiane type. This enables us to put forward a partial structure (fragment A) of the handelin molecule:



A B C

Scheme 1

In this fragment, as shown above, two bonds remain free at the C-14 and C-17 carbons. The lactone ring is trans-linked with the main skeleton, and the H-18 has the α orientation (${}^3J_{19.20} = {}^3J_{18.19} = 9.8$ Hz).

The acetyl group of (I) is not present in this fragment, since the only possible position at C-21 is excluded because there is no sign of spin—spin coupling between H-20 and the gem-acetyl proton (multiplet at 5.45 ppm) in the double resonance spectra. Furthermore, in the spectrum of (II) the difference in the chemical shifts of the signals of the protons of the exocyclic methylene amounts to 0.76 ppm, which shows the absence of a hydroxy group in the γ position to it [4]. Thus, fragment A of the handelin molecule has the composition $C_{15}H_{18}O_3$ and a structure similar to that of a γ-lactone of the guaiane type. To determine the structure of the second fragment, the composition of which, judging from the total formula of (I), is $C_{17}H_{22}O_5$, (II) was oxidized with chromium trioxide to deacetyldehydrohandelin. Its carbonyl group is not conjugated with double bonds (UV spectrum), and in the IR spectrum there is an absorption band at 1705 cm^{-1} which is characteristic for cyclic ketones with a ring larger than six-membered [5]. Then, taking into account the structure of the groupings not present in fragment A, the main skeleton of the second half of (I) must consist of ortho-conjugated five- and seven-membered carbon rings, i.e., it is a derivative of bicyclo [5.3.0] decane.

It follows from the PMR spectrum of (III) that the H-6 lactone proton (fragment B), the signal of which has the form of a triplet at 4.25 ppm interacts vicinally with the protons on the neighboring carbon atoms with the same value of ${}^3J$ of 10.0 Hz.

The H-7 proton (quartet at 3.09 ppm with ${}^3J = 10.0$ and 7.8 Hz) also interacts with the gem-acetyl H-8 proton (broadened signal at 5.50 ppm) with ${}^3J = 7.8$ Hz. The signal of the H-5 proton is masked in this spectrum but it appears at 2.86 ppm in the form of a quartet with ${}^3J = 10.0$ and 7.0 Hz in the spectrum of (I), i.e., it interacts not only with H-6 but also with another proton with ${}^3J = 7.0$ Hz. The bond found between the methines forming a chain to which the lactone ring and acetyl group are attached explains the presence in the second part of the molecule of (I) of the system B, where the C-11 carbon is quaternary. Then, possibly, the second fragment (C) of the handelin molecule is also a sesquiterpene γ-lactone with free bonds at C-11 and C-13. In this part of the molecule the double bond is between C-3 and C-4, since in the PMR spectrum of (II) no signs of spin–spin coupling between the proton of the $CH_3-\underset{|}{C}=\underset{|}{C}-H$ grouping (signal at 5.39 ppm) and the gem-hydroxylic proton (signal at 4.46 ppm) are observed.

According to the value of $^3J_{6,7}$ = 10.0 Hz [spectrum of (III)], the lactone ring is trans-linked with the carbon skeleton, and the H-1 and H-5 protons are present in the cis position relative to one another and are α-oriented ($^3J_{1,5}$ = 7.0 and $^3J_{5,6}$ = 10.0 Hz). The acetyl group also has the α-orientation ($^3J_{7,8}$ = 7.8 Hz).

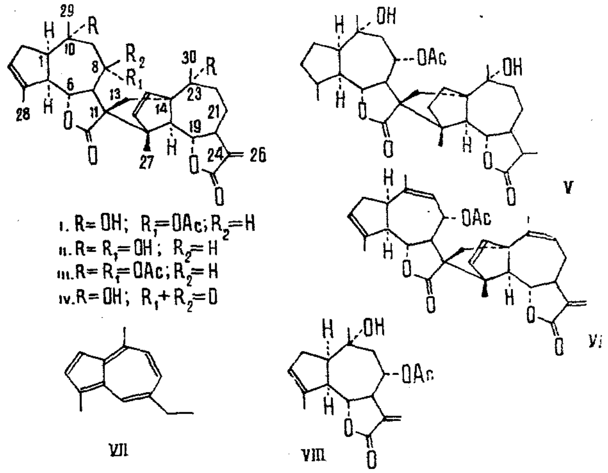
A compound with a structure similar to that of fragment C is cumambrin A (VIII) [6], which has also been isolated from the plant under investigation [7]. They differ only by the nature of the bonds between the C-11 and C-13 carbons.

In the spectrum of (I) by the INDOR method we detected doublet signals at 2.58 and 1.58 ppm of two protons interacting with one another with ^2J = 12 Hz. These signals, by their chemical shifts and SSCC value are characteristic of nonequivalent protons of an isolated group. In the handelin molecule only the protons at C-13 correspond to this condition if the bond with fragment A is between C-11 and C-13.

On comparing the PMR spectra of (I) and (IV), it was found that in (IV) the H-18 proton is descreened by 0.84 ppm. This effect is undoubtedly connected with the spatial propinquity of H-18 and a carbonyl group [8]. It follows from an analysis of their Dreiding models that the linkage of fragments A and C is realized by carbon–carbon bonds between C-11–C-17 and C-13–C-14. Spatially, the lactone ring of fragment C and the five-membered ring spiro-linked to it are arranged in such a way that in the case of (IV) a NOE (20%) is observed between the H-6 lactone proton and one of the methylene protons at C-13. A comparative study of the chemical shifts of the protons of (I) and its derivatives (II-IV) showed the β-orientation of the methyl groups at C-10 and C-23. Thus, the most probable structure of handelin corresponds to formula (I), where the orientation of the substituents and of the asymmetric carbon atoms is given as for the individual fragments A and C.

In view of the fact that the bond between fragments A and C must possess reduced stability because of the participation of one of them in the spiro linkage and because of their α-position with respect to the rings, a cleavage reaction was performed by boiling (I) in decalin [10] with the addition of dioxane. Under these conditions, in fragment C as a result of the splitting out of the second half, A, an ethylenic bond was formed between C-11 and C-13 and it was isolated as cumambrin A, which completely confirmed its structure.

The absence of the molecular ion from the mass spectrum of (I) and the decomposition of the fragment with m/e 306 (the ion with the highest mass number) in a similar manner to cumambrin A confirms the ease of cleavage of the bonds between the main parts of the molecule. The second part of the molecule (246 amu) was eliminated in the form of a neutral particle.



Scheme 2

In the spectrum of deacetyldehydrohandelin, the molecular weight of which is 44 less than that of (I), peaks of ions with m/e 262 and 246 as the highest values are present. They are probably those fragments into which the molecule of (IV) decomposes initially under the action of electron impact. They then each eliminate a molecule of water, forming intense ions with m/e 244 and 228. The latter decomposes with the loss of 15 mass units (CH_3) and the formation of an ion with m/e 213.

Handelin diacetate is the most volatile derivative of (I), and its spectrum shows the peak of the molecular ion with m/e 636, the decomposition of which is accompanied by the successive elimination of three molecules of acetic acid (ions with m/e 576, 516, 456), which shows the spatial propinquity of the two parts of the molecule. The strongest peaks are those of ions with m/e 228 (100%) and 288 (16%), which possibly arise by the decomposition of fragments with m/e 456 and 576.

The absence of double bonds in hexahydrohandelin is responsible for the fragmentation of its molecular ion with m/e 558 in two main directions: 1) the successive loss of two molecules of water, acetic acid, and a methyl group (ions with m/e 540, 522, 462, and 447, respectively); and 2) the loss of water, acetic acid, water, and a methyl group (ions with m/e 540, 480, 462, and 447, respectively). In this spectrum, the strongest peaks are those in the region of small mass numbers, the ion with m/e 43 being the 100% ion.

The compositions of the ions from (I) were established by high-resolution mass spectrometry: with m/e 306, 1522 ($C_{17}H_{22}O_5$); m/e 246, 1316 ($C_{15}H_{18}O_3$); m/e 228, 1105 ($C_{15}H_{16}O_2$); m/e 213, 0941 ($C_{14}H_{13}O_2$). The information obtained in a consideration of the mass spectra of handelin and its derivatives agrees well with the structure (I) proposed for it, confirming its composition, the sesquiterpene nature of its main fragments, and the nature of their linkage.

EXPERIMENTAL

The IR spectra were taken on a UR-20 instrument (tablets with KBr), the mass spectra on Varian CH-5 and on a MKh-1303 instruments, the UV spectra on a Hitachi EPS-3T spectrometer, and the NMR spectra on Varian JNM-4H-100, XL-100, and CFT-20 spectrometers. All the PMR spectra considered were taken in deuteropyridine solution; 0 — HMDS.

The value of R_f were obtained on "Silufol-R" plates in the chloroform—methanol (9:1) system with a 0.5% solution of vanillin in concentrated sulfuric acid as the revealing agent. The elementary analyses corresponded to the calculated figures.

The ^{13}C NMR spectra were taken and interpreted by O. A. Subbotin.

Handelin (I). The leaves and small stems of *Handelia trichophylla* Heimerl., collected in May, 1974, (22 kg) were extracted with chloroform. The concentrated extract was dissolved in ethanol and the solution was diluted with water to give a 60% ethanolic solution. After a day, the precipitate that had deposited was separated off and the combined lactones were extracted from the solution with chloroform. Elimination of the solvent gave 237 g of a crystallizing resin. Treatment of this with ethyl acetate yielded 7 g of crystalline (I). An additional 4 g of (I) was obtained by chromatographing 230 g of the resin on KSK silica gel (fractions 51-65, eluent benzene). Its mp was 212°C (decomp; dioxane—ethanol), $C_{32}H_{40}O_8$, R_f 0.57 (bright crimson spot).

Deacetylhandelin (II). A solution of 0.7 g of (I) in 0.6 liter of ethanol was treated with 0.9 g of KOH. The mixture was heated in the water bath at 60-70°C for 0.5 h and was then concentrated to 50 ml, diluted with water, and treated with chloroform. The alkaline solution was acidified with hydrochloric acid, and the reaction product was extracted with chloroform and purified on a column of alumina. The yield of (II) was 0.3 g, mp 203-204°C (decomp, acetone),$C_{30}H_{38}O_7$, R_f 0.31 (crimson spot). IR spectrum, cm^{-1}: 3490, 3420 (OH), 1770, 1740 (lactone carbonyls), 1660, 1630 (C=C).

Compound (II) (0.1 g) was acetylated with acetic anhydride in pyridine, the reaction mixture being heated in the water bath for 3 h. After the usual working up, (I) was isolated.

Handelin Diacetate (III). A solution of 0.7 g of (I) in 135 ml of freshly distilled acetyl chloride was kept at 35°C for 3.5 h. The residue after the elimination of acetyl chloride was purified on a column of alumina. The yield of (II) was 0.5 g, mp 233-234°C (decomp., ethyl acetate—ethanol), composition $C_{36}H_{44}O_{10}$, mol. wt. 636 (mass spectrometry), R_f 0.83 (crimson spot). IR spectrum, cm^{-1}: 1760, 1737, 1250 (lactone and acetyl carbonyls), 1670, 1680 (C=C).

Deacetyldehydrohandelin (IV). In drops, 0.15 g of chromium trioxide in 3 ml of 90% acetic acid was added to a cooled (0°C) solution of 0.15 g of (II) in 9 ml of 90% acetic acid. The reaction mixture was kept at 0°C for 3.5 h and was then diluted with ice water, and the (IV) was extracted with chloroform; yield 0.7 g, mp 213°C (decomp; acetone—ethanol), $C_{30}H_{36}O_7$, R_f 0.53 (violet spot). IR spectrum, cm^{-1}: 3515 (OH), 1760, 1740 (lactone C=O's), 1705 (ketone C=O), 1670, 1650, 1635 (C=C).

Hexahydrohandelin (V). Adams platinum oxide (20 mg) was added to a solution of 0.1 g of (I) in 20 ml of chloroform and hydrogenation was carried out for 3.5 h. This gave 0.09 g of (V) with mp 282-283°C (acetone—ethanol), $C_{32}H_{46}O_8$, mol. wt. 558 (mass spectrometry). IR spectrum, cm^{-1}: 3475, 3385 (OH), 1765, 1730, 1250 (lactone and acetate C=O's).

Dianhydrohandelin (VI). In drops, 0.6 ml of thionyl chloride in 3.5 ml of pyridine was added to a solution of 0.3 g of (I) in 7 ml of pyridine cooled to 0°C. After 10 min the mixture was poured onto ice. The reaction product was extracted with chloroform and purified by chromatography on alumina. The yield of (VI) was 0.1 g, mp 178°C (decomp., acetone—ethanol), $C_{32}H_{36}O_6$. IR spectrum, cm^{-1}: 1760, 1730, 1240 (lactone and acetate C=O's), 1670, 1640. C=C (cm^{-1}): R_f (dark green spot).

Chamazulene (VII). Heating 0.2 g of (I) or (V) with an equal quantity of selenium at the boiling point for 5 min gave a dark blue liquid hydrocarbon which, after purification on a column on alumina was identified from its IR spectrum and its picrate (mp 110-112°C from ethanol) as chamazulene [9].

Cumambrin A (VIII). In a mixture of dioxane and decalin, 0.07 g of handelin was heated to 154-158°C for 45 min. The decomposition products were chromatographed on neutral alumina. Benzene—hexane (9:1) extracted 0.01 g of a substance with mp 186-187°C, $C_{17}H_{22}O_5$, identical according to its IR spectrum and a mixed melting point with cumambrin A.

SUMMARY

1. A new lactone, handelin, with the composition $C_{32}H_{40}O_8$ has been isolated from the epigeal part of *Handelia trichophylla* Heimerl.

2. It has been established by chemical and spectral investigations that handelin is a diguaianolide, and the nature of the linkage of its guaiane fragments and the stereochemistry of the molecule as a whole have been determined.

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