9(11)-Fernene and Its 21-Epimer as an Epicuticular Layer on Ferns

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The glaucous epicuticular layer on the undersurface of the leaves of *Polypodium glaucinum* and the white exudate material on the lower surface of the sterile pinnae of *Plagiogyra formosana* is shown, mainly by spectroscopical methods, to consist of a mixture of 9(11)-fernene and 21-epi-9(11)-fernene, in both instances in a ratio of ca. 4:1. The ^{13}C-NMR spectrum of 9(11)-fernene has been recorded and partially assigned.

Glaucous appearance of leaf and fruit surfaces is widely encountered in higher plants. This is caused by a thin epicuticular layer of waxy material. It is known that such layers in many cases are not true waxes according to the chemical definition. They can be aliphatic compounds like alkanes, alkanols, ketones, or cyclic compounds like diterpenes, triterpenes, phytosterols [1, 2]. During an investigation of "farinose" exudate layers produced by gymnogrammoid ferns and consisting mostly of flavonoid aglycones [3] we became interested in the material causing the glaucous appearance of the under surface of the leaves of *Polypodium glaucinum* Mart. & Gal. and some other ferns, and we wanted to know what substances form the whitish layer on the lower surface of the sterile pinnae of *Plagiogyra formosana* Nakai [syn.: *Pl. glauca* (Blume) Mett. var. *philippensis* Christ.]. The results are reported in the present paper.

Materials and Methods

Fronds of *Polypodium glaucinum* were collected in Costa Rica in December 1977. A voucher is kept in the herbarium of the Museo Nacional de Costa Rica at San José (LDG 6853). The air-dried pinnae were rinsed with petrol ether to dissolve the waxy material. The major constituent was easily dissolved in boiling benzene and crystallized after addition of methanol and cooling ("PAG"). Some fronds of *Plagiogyra formosana* were kindly supplied by Miss D.-F. Rau from Taichung/Taiwan. Sterile fronds were dipped in petrol ether to dissolve the white waxy layer. Here the major constituent also could be crystallized in the same way ("P-Fo").

IR spectra were recorded on a Perkin-Elmer 257 instrument, ^{1}H-NMR spectra on a Varian A 60-A, ^{13}C-NMR spectra on a JEOL FX-100, MS on an AEI MS 902, optical rotation was measured on a Perkin-Elmer 241. GLC was performed on a Perkin-Elmer F 11 with flame ionization detector.

Results and Discussion

Compounds PAG and P-Fo both showed R_f values of 0.90 on silica (petrol ether, b.p. 100–140 °/toluene, 9:1). They were visualized by spraying with $SbCl_3$ in HOAc/chloroform, followed by heating to 120–130 °. Reddish violet spots developed rather slowly. This reaction points to a triterpenoid compound.

On GLC, the individual compounds both had a retention time of 22.1 min (column: 3% OV-17 on Chromosorb W 80/100, $5' \times 1/8''$, ca. 2200 theoretical steps; program: 5 min at 150 °, then 10 °/min to 300 °). A mixture of PAG and P-Fo showed a single, sharp peak at the same retention time.

The UV spectra (in hexane) showed no chromophore. The IR spectra (in KBr) for both substances had maxima at γ 2930 (s), 2870 (s), 1640 (w), 1455 (s), 1435 (s), 1385 (s), 1373 (s), 1208 (m), 1166 (m), 1012 (m), 973 (m), 865 (m), 814 (m) and 795 (m) cm^{-1}. Optical rotation: $[\alpha]_D^{20}$ – 16.0° (c 0.67, chloroform). Main fragments in the mass spectra: m/e (rel. int.) 410 (M^+, 85), 395 (100), 257 (14), 243 (69), 231 (14), 218 (16), 205 (17), 191 (24). m* 381 (410 → 395); M^+: found 410.3910, calc. for $C_{30}H_{50}$ 410.3913.

The ^{1}H-NMR spectra (60 MHz, $CDCl_3$) showed a one-proton multiplet at δ 5.3 and a complex pattern at δ 0.7–2.7. The ^{13}C-NMR spectra (25.3 MHz, $CDCl_3$, 75000 pulses) are given in the following. The signals in the spectrum of authentic 9(11)-fernene

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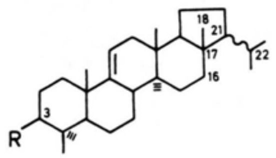
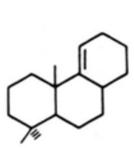
have been assigned tentatively by comparison with the completely assigned spectra of cylindrin (**2**) [4] and podocarp-9(11)-ene (**3**) [5]. δ 151.4 (C-9), 115.4 (C-11), 59.5 (C-21), 51.9 (C-18), 44.7, 42.9 (C-17), 42.3 (C-3), 41.4, 39.9 (C-10), 38.0 (C-14), 37.6 (C-1), 36.7, 36.1 (C-16), 33.6, 32.7, 30.7 (C-22), 29.2, 28.2 (C-20), 25.0, 23.0 (C-30), 22.1 (C-29), 21.6 (C-24), 20.1, 19.5, 17.9, 15.8, 15.4 (C-27), 14.0 (C-28). The samples PAG and P-Fo show the same signals and some additional signals which are given here: δ 56.6, 52.1, 41.9, 40.4, 34.3, 33.2, 29.6, 27.5, 26.6, 26.4, 21.5, 18.7, 16.7.

Both samples formed white needles, m. p. (capillary) 148−150° (PAG), 144−146° (P-Fo), no depression on admixture. However, in a m. p. microscope, softening was observed for both samples from ca. 130°, and the samples did not melt completely until a temperature of 168−170° was reached.

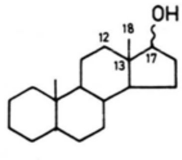
The IR spectrum indicates a trisubstituted double bond. The MS is indicative of a triterpene hydrocarbon. Weak but informative fragments are observed at *m/e* 367 (M-43, loss of C_3H_7) and *m/e* 325 (characteristic of a double bond in 9(11)-position [6]. Peaks at *m/e* 257, 243, 231 and 205 are common in pentacyclic triterpenes [6].



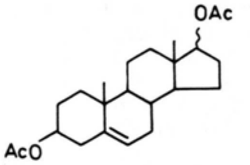
1: R=H , 21β-isopropyl
2: R=OCH$_3$, 21β- 〃
4: R=H , 21α- 〃



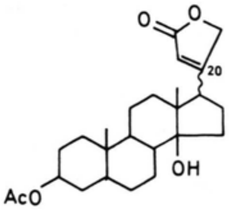
3



5: 17α-OH
6: 17β-OH



7: 17α-acetoxy
8: 17β- 〃



9: 17α-(2',5'-dihydro-5'-oxo-3'-furyl)
10: 17β-(2',5'-dihydro-5'-oxo-3'-furyl)

All the spectroscopical data discussed above are in good agreement with the ones published for 9(11)-fernene (**1**) [7, 8]. This substance, however, has a m. p. of 170−172°. Thus it would seem that samples PAG and P-Fo consist of more than one substance. The ^{13}C-NMR spectra of these substances show, in addition to all of the signals from 9(11)-fernene, some small additional peaks.

From this, it can be concluded that PAG and P-Fo consist mainly of 9(11)-fernene, but that one or more additional substances are present. By fractional crystallization of PAG and P-Fo from heptane, small amounts of substances melting fairly sharply at 169−170° and at 149−150° were obtained.

Since only one spot is visible on TLC, one peak is observed on GLC, and the UV- IR, MS and ^{1}H-NMR spectra of PAG, P-Fo and authentical 9(11)-fernene are nearly identical, it is supposed that the other substance is closely related to 9(11)-fernene (**1**). Such a substance has been described by Barton *et al.* [8]. Tentatively identified as 21-epi-9(11)-fernene (**4**), it was obtained from the rhizomes of *Polypodium vulgare* along with 9(11)-fernene. The epimeric compound could not be separated from **1** by TLC, and its spectral data were nearly the same as those observed from **1**. ^{13}C-NMR data, however, were not recorded. Barton *et al.* [8] reported a m. p. of 151−153° for 21-epi-9(11)-fernene.

Model studies of 9(11)-fernene, in which the isopropyl group on C-21 is in the β-position, and of the epimeric compound (α-position) shows that steric interactions between the isopropyl group and the methyl group on C-17 are stronger for the β-bound isopropyl group. This should lead to deshielding of C-21 in the β-form relative to the α-form. A similar phenomenon should be expected in 17 β-versus 17α-substituted steroids. Although 17 α- and 17 β-isopropyl substituted pairs of steroids are known [9], their ^{13}C-NMR data seem to be unrecorded. However, ^{13}C-NMR spectra have been recorded for 17 α- and 17β-hydroxy-5α-androstane (**5**, **6**) [10], for 17α- and 17β-diacetoxy-androst-5-ene (**7**, **8**) [11], and for 17α- and 17β-(2′,5′-dihydro-5′-oxo-3′-furyl)-3β-acetoxy-5β-androstane-14β-ol (**9**, **10**) [12].

For these substances, it has been found that the C-17 signals in β-substituted substances are shifted 1−3 δ-units to lower field relative to α-substituted ones. Thus, the C-21 signal in 21-epi-9(11)-fernene should be found at 56.5−58.5 [9(11)-fernene: C-21 at δ 59.5]. In the spectra of PAG and P-Fo [but not in the 9(11)-fernene spectrum], a signal is found at δ 56.6. For C-20 in substances **9** and **10**, deshielding of the β-form relative to the α-form leads to a difference in shift position of 3.5 δ-units. Since the C-22 signal in 9(11)-fernene is found at δ 30.7, it might be expected that the C-22 signal in the epi-form is found at ca. δ 27. Peaks which occur in PAG and P-Fo spectra, but not in the 9(11)-fernene spectrum are found at δ 26.4, 26.6 and 27.5.

In the steroids, 17β-substitution leads to shielding of the C-18 signal (2.5−6 δ-units) relative to 17α-substitution. The corresponding signal in 9(11)-fernene, C-28, is found at δ 14.0. Additional peaks in PAG and P-Fo are found at δ 16.7 and 18.7.

Finally, C-12 in the steroids is deshielded 4.5−9 δ-units for 17β-substitution relative to 17α-substitution. In 9(11)-fernene, the corresponding carbon atom, C-16, is found at δ 36.1, and peaks at δ 27.5 and 29.6 in the PAG/P-Fo spectra might thus be assigned to the 21-epimer.

In summarizing these results, it can be concluded that the PAG and P-Fo spectra show signals which are to be expected from 21-9(11)-fernene. Since the C-21 signal occurs in a part of the spectrum where few other signals are found, this signal would seem to be of particular importance. As PAG and P-Fo both contain substances with m.p.s in good accordance with those reported for 9(11)-fernene and 21-epi-9(11)-fernen, it seems reasonable to assume that the samples contain a mixture of the two 21-epimeric forms of 9(11)-fernene. The relative signal intensity of the signals discussed (59.6 *vs.* 56.6, 30.7 *vs.* 26.4, 14.0 *vs.* 16.7 and 36.1 *vs.* 27.5) is 80%:20% for PAG and 78%:22% for P-Fo, in both instances with a standard deviation of 1.7%. If the assumption is made that the relaxation rate of the signals from the two epimers is the same, this should show their distribution in the mixture.

9(11)-fernene was first described by Ageta *et al.* [7] as a constituent of the ferns *Polypodium vulgare, Dryopteris crassirhizoma, Adiantum pedatum,* and *A. monochlamys.* It has since then been described in a large number of ferns [8, 13−16], in two mosses [17, 18] and in the coniferous plant *Podocarpus saligna* [19]. This enumeration is not intended to be complete.

21-Epi-9(11)-fernen has, to our knowledge, only been reported from the rhizome of *Polypodium vulgare* [8], where it co-occurs with 9(11)-fernene. Since the chromatographic and spectroscopic behaviour of these two substances are nearly identical (with the probable exception of the ^{13}C-NMR spectrum), 21-epi-9(11)-fernene might well be more wide-spread in nature than has been believed hitherto.

In all cases cited above, the plant material was subjected to extraction, usually with petrol ether, sometimes with diethyl ether or with ethanol. No attention was paid to the fact that the hydrocarbons could be present as a constituent of the epicuticular layer and not of leaf and rhizome tissue. This is now demonstrated unambiguously for the thin glaucous layer on *Polypodium glaucinum* and for the whitish layer on *Plagiogyra formosana.* We want to emphasize that whenever lipophilic compounds are isolated from plant material, the possibility of their epicuticular origin should be kept in mind [20].

In a greenhouse at the Botanical Garden at Heidelberg a fern is grown under the name "*Polypodium aureum* var. *mexicana*". On the young rhizome of this plant a rather thick, chalky covering is observed, which chromatographically is identical with PAG and P-Fo. Spectral analysis has not been performed on this material. It may be assumed, however, that it consists of the same pentacyclic triterpene hydrocarbons. In all three cases the epicuticular material forms very thin and long filaments as can be observed by scanning electron microscopy. SEM pictures have been published recently (as *Polypodium aureum*) by Barthlott and Wollenweber [21].

Note added in proof: There exists some confusion about the use of the names *Polypodium aureum, Phlebodium aureum* (L.) J. Sm. (*cf.* [22]), or *Polypodium glaucinum* Mart. & Gal. At any rate all varieties and forms of this fern produce epicuticular material in various amounts and this has led horticulturists to select for the "chalky" strains which have gone into commerce under such names as "blue fern", "glazed polypody" etc. Taxonomists have in the past given varietal names to such forms. Presumably the chemical composition of the epicuticular layers is the same for all, varying only in quantitative aspects.

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