Acta Chemica Scandinavica B 33 (1979) 76—78

Short Communication

Hydroxylation of Dehydroabietic Acid by *Fomes annosus*

RAINER EKMAN [a] and RAINER SJÖHOLM [b]

[a] Institute of Wood Chemistry and Cellulose Technology, Åbo Akademi, SF-20500 Åbo 50, Finland and [b] Institute of Organic Chemistry, Åbo Akademi SF-20500 Åbo 50, Finland

Dehydroabietic acid is a common member of the diterpenoids occurring in coniferous plants. In Norway spruce (*Picea abies* [L.] Karst.) heartwood dehydroabietic acid is the dominating resin acid.

Oxidation and degradation of diterpenes by micro-organisms is well-known, but transformation of resin acids by fungi has received less attention.

In a series of bioassays of Norway spruce extractives we found that most common resin acids, suspended in agar cultures with growing mycelia of *Fomes annosus* (Fr.) Cke, were partially transformed to very complex mixtures of more polar constituents. However, the transformation pattern of dehydroabietic acid was more clear-cut, allowing isolation of the major products.

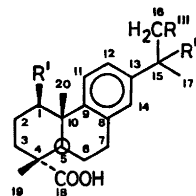
The present paper reports the isolation and identification of 1β,15-dihydroxydehydroabietic acid (*1*) and 1β,16-dihydroxydehydroabietic acid (*2*) formed in incubation experiments with *F. annosus*. Some minor transformation products were further tentatively identified.

Preliminary structural information was obtained from mass spectra of the methyl esters *1a* and *2a*.

MS of compound *1a* [IP 70 eV; *m/e* (rel.int.)]: 346 (32, M), 331 (100, M$-CH_3$), 328 (59, M$-H_2O$), 287 (10, M$-COOCH_3$), 269 (31, M$-COOCH_3-H_2O$), 253 (18, M$-CH_3-HCOOCH_3-H_2O$), 185 (74), 184 (39), 183 (44), 144 (56), 101 (59). The molecular weight and the overall characteristics of the mass spectrum were consistent with a dihydroxyderivative of dehydroabietic acid methyl ester. The high relative intensity of the fragment *m/e* 331 could be explained by a hydroxyl group located at C-15 which would strongly favour loss of the C-16 and C-17 methyls. An analogous effect has been observed for methyl 15-hydroxydehydroabietate.[1] The formation of the intense odd-electron ion at *m/e* 144 could further be rationalized by simultaneous cleavage of the C-1$-$C-10 and C-4$-$C-5 bonds with retention of the positive charge on the A-ring residue and assuming the other hydroxyl being located at C-1 and thus weakening the former bond towards electron impact.

MS of compound *2a* [IP 70 eV; *m/e* (rel.int.)]: 346 (64, M), 331 (4, M$-CH_3$), 328 (2, M$-H_2O$), 315 (100, M$-CH_2OH$), 287 (26, M$-COOCH_3$), 269 (13, M$-COOCH_3-H_2O$), 202 (22), 185 (13), 183 (23), 171 (57), 144 (84), 101 (47). The formation of the abundant fragment at *m/e* 144 also in the spectrum of *2a* was interpreted as a result of a hydroxyl group at C-1 as described for *1a* above. The other hydroxyl group in *2a* is evidently a primary one since the base peak (*m/e* 315) was formed through loss of a CH_2OH radical from the molecular ion. The primary hydroxyl could be either at C-16 or C-20.

The ^1H NMR spectrum (200 MHz, $CDCl_3$) of compound *1a* showed a six-proton singlet at δ 1.56 and two three-proton singlets at δ 1.27 and 1.29, respectively. The presence of four methyl groups, besides the ester methyl resonating at δ 3.66, excludes primary hydroxyl groups. The shift of the signal at δ 1.56, assigned to the C-16 and C-17 methyls, verifies the presence of a C-15 hydroxyl. The signals at δ 1.27 and 1.29 were assigned to the C-19 and C-20 methyls, respectively.



1 R'=R''=OH, R'''=H
2 R'=R'''=OH, R''=H
3 R'=R'''=H, R''=OH
4 R'=R''=H, R'''=OH
1a–4a: methyl esters

The assignment of the other hydroxyl group to C-1 is based on the following argument. Based on steroid models, the downfield shift ($\Delta = 0.09$ ppm) of the C-20 methyl signal as compared to methyl dehydroabietate, could be due to a C-1 or a C-3 hydroxyl.[2] Moreover, the shift is also indicative of a β-configuration

0302-4369/79/010076-03$02.50
© Acta Chemica Scandinavica

of the hydroxyl group.[2] The benzylic protons at C-7 gave a two-proton multiplet at δ 2.89 which eliminates the possibility of a hydroxyl group located at C-7. The ring-junction proton at C-5 showed a set of two doublets, consistent with the X part of an ABX system, at δ 2.20 with $J_{AX}+J_{BX}=15.6$ Hz, confirming the axial character of this proton and excluding a C-5 or C-6 hydroxyl. The proton geminal to the hydroxyl group showed a non-resolved multiplet at δ 3.95. The shift and the half-height width (W/2 = 18 Hz) of the signal are both characteristic of an axial proton.[2] The strongest evidence for the exact location and orientation of the secondary hydroxyl group is to be found in the aromatic part of the spectrum which shows three signals: δ 8.19 (H-11, d, J 8.6 Hz), 7.23 (H-12, dd, J 8.6 and 2.4 Hz), 7.16 (H-14, d, J 2.4 Hz). The extremely large downfield shift of the C-11 proton can only be explained by a non-bonded interaction between the proton at C-11 and an equatorial C-1 hydroxyl group. Inspection of a molecular model of compound *1a* shows that the distance between the C-1 oxygen and the C-11 hydrogen atom is considerably smaller than the sum of their van de Waals radii. This condition is known to cause unusually large deshielding of the proton.[3] The same effect has been observed in the spectrum of *trans*-7-methoxy-4a-methyl-1,2,3,4,4a,9,10,10a-octahydro-4β-phenanthrol[4] having an equatorial β-hydroxyl at the position corresponding to C-1 in dehydroabietanes. No corresponding downfield shift has been observed in the spectrum of teideiadiol possessing an axial C-1 α-hydroxyl group.[5] These facts show that only an equatorial C-1 β-hydroxyl can account for the dramatic downfield shift of H-11 in the spectrum of compound *1a*.

The ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of compound *2a* showed a three-proton doublet at δ 1.24 (J 6.8 Hz) and two three-proton singlets at δ 1.26 and 1.29, respectively. The presence of only three methyl groups besides the ester methyl resonating at δ 3.66, shows that one of the hydroxyl groups is primary. This is further verified by the presence of two methylene protons, exhibiting a doublet at δ 3.67 (J 6.8 Hz) and hence attached to a carbon bearing a hydroxyl group. Thus the methyl and methylene doublets at δ 1.24 and 3.67 were assigned to the C-17 and C-16 protons, respectively. Irradiation of the C-15 methine proton, displaying a multiplet at δ 2.86 (J 6.8 Hz), resulted in a singlet at δ 3.67 and collapsing of the doublet at δ 1.24. This verified that the primary hydroxyl group is located at C-16. The singlets at δ 1.26 and 1.29 were assigned to the C-19 and C-20 methyls, respectively.

The presence of a C-1 β-hydroxyl group was established by an argumentation analogous to that for compound *1a* above, based on the following ^{1}H NMR data ($CDCl_3$): δ 3.96 (H-1, m, W/2 = 18 Hz), 2.18 (H-5, dd, J 12.0 and 2.9 Hz), 2.87 (H-7, m), 8.14 (H-11, d, J 8.2 Hz), 7.00 (H-12, dd, J 8.2 and 1.9 Hz), 6.88 (H-14, d, J 1.9 Hz).

For comparison the ^{1}H NMR (200 MHz, $CDCl_3$) data of methyl dehydroabietate are given: δ 2.28 (H-1β, broad doublet, $J_{1\alpha,1\beta}$ 12.2 Hz), 2.23 (H-5, dd, J 12.4 and 2.1 Hz), 2.85 (H-7, m), 7.17 (H-11, d, J 8.0 Hz), 7.00 (H-12, dd, J 8.0 and 1.5 Hz), 6.89 (H-14, d, J 1.5 Hz), 2.79 (H-15, m, J 6.8 Hz), 1.22 (H-16, 17, d, J 6.8 Hz), 1.27 (H-19, s), 1.20 (H-20, s), 3.66 (COOCH_3, s).

GLC-MS analysis of total incubation mixture resulted further in identification of 15-hydroxydehydroabietic acid (*3*) using authentic reference material. A minor transformation product was tentatively characterized as 16-hydroxydehydroabietic acid (*4*) (MS: intense peak *m/e* 299 formed by loss of a CH_2OH group from the parent ion *m/e* 330). However, no indication of the presence of a C-1-substituted monohydroxy derivative was found. Thus, hydroxylation of dehydroabietic acid by *F. annosus* appears to occur first in the C-15 and C-16 positions, then in the C-1β position.

Incubation, isolation and yield. An acetone solution of dehydroabietic acid was suspended by shaking in warm (60 °C), sterilized 0.5 % malt extract agar to obtain a 0.1 % concentration of the acid. Five ml of the agar mixture was poured into two sterilized Petri dishes (40 mm diam.). One of the portions was inoculated with a 2×2 mm piece of *F. annosus* mycelium from an agar culture and the other served as control. The mycelium was allowed to grow in darkness for four weeks at 24 °C and the control was kept in the same place. The agar mixtures were boiled under reflux together with EtOAc for 4 h. The two-phase mixtures were allowed to cool and the EtOAc layers, containing the inoculation products and the dehydroabietic acid control were evaporated to dryness and methylated. Gas chromatographic analysis of the incubated sample gave following composition: 64 % dehydroabietic acid, 11 % *1*, 15 % *2*, 7 % *3* and 3 % *4*, as determined by area normalization of the peaks. Compounds *1*–*4* were not detected in the control.

The incubation experiment was repeated in a similar manner but on a bigger scale to allow isolation of the individual components.

Experimental. The crude mixture of the acids was chromatographed on preparative TLC-plates of silica gel using benzene:acetone (60:40 v/v) as solvent. The main fraction of the transformation products at R_F 0.30–0.45 was isolated and methylated in methanolic Et_2O with freshly prepared diazomethane. Resulting esters *1a* and *2a* were then chromatographed on analytical TLC-plates using benzene:EtOAc (60:40 v/v) as solvent. Because of partial overlapping of *1a* (R_F 0.45) and *2a* (R_F 0.40) the procedure had to be repeated twice to obtain pure fractions.

The gas chromatographic analyses of the methyl esters were performed with a Varian 2400 gas chromatograph equipped with a

packed glass column (2 m × 1.8 mm i.d., 1 % XE-60, 160 – 230 °C, 4 °C/min). Mass spectra were recorded with an LKB-9000 instrument using a column similar to that mentioned above. ^{1}H NMR spectra were obtained with a Bruker WP-200 Spectrometer operating at 200 MHz.

Acknowledgements. The authors are greatly indebted to Professor Torbjörn Norin for discussions, gifts of reference samples and access to the NMR equipment.

1. Norin, T. and Winell, B. *Acta Chem. Scand. 6* (1972) 2289.
2. Bhacca, N. S. and Williams, D. H. *Applications of NMR Spectroscopy in Organic Chemistry,* Holden-Day, San Fransisco 1964.
3. Jackman, L. M. and Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon, Oxford 1969.
4. Whitlock, Jr., H. W., Reichardt, P. B. and Silver, F. M. *J. Am. Chem. Soc. 93* (1971) 485.
5. Bretón, J. L., González, A. G. and de León, G. *An. Quim. 66* (1970) 293.

Received November 20, 1978.