ACTA CHEMICA SCANDINAVICA 25 (1971) 607–610

The Constituents of Conifer Needles

IV.* Dehydropinifolic Acid, a Diterpene Acid from the Needles of *Pinus silvestris* L.

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A diterpene acid, dehydropinifolic acid, has been isolated from the needles of *Pinus silvestris* L. and shown to have structure (*2 a*). The occurrence of benzoic acid has also been demonstrated. Neither pinifolic acid nor dehydropinifolic acid has been detected in other parts of the same tree (bark, cambium region, sapwood, heartwood, or root). The two acids have neither been detected in the needles nor in the oleoresins of Norway spruce, *Picea abies* (L) Karst. and Common larch, *Larix decidua* Mill.

The main acetone soluble acid constituents of the needles of *Pinus silvestris* L. have previously been isolated and shown to be a diterpene acid which was named pinifolic acid (*1 a*).[1] The acid fraction has now been further investigated. Apart from pinifolic acid, a related diterpene acid, dehydropinifolic acid (*2 a*), and benzoic acid were isolated. The occurrence of pinifolic acid and dehydropinifolic acid in other parts of the tree and some other conifer species have been studied.

Fresh needles, collected in the autumn, were extracted with acetone and the concentrated extract was partitioned between water and methylene chloride. The acid fraction of the methylene chloride was filtered through a "Florisil" column and gave after recrystallisation pinifolic acid. Column chromatography of the combined mother liquors on dimethyl sulphoxide impregnated Celite gave two main fractions, of which one contained pinifolic acid. The other fraction gave after recrystallisation and sublimation a new diterpene acid ($C_{20}H_{30}O_4$; m. p. 190–192°; $[\alpha]_D$ +39.5°, *c* in EtOH 1,6), named dehydropinifolic acid.

Dehydropinifolic acid (*2 a*) exhibited spectral properties (IR, NMR, and MS) which indicated a close relationship to pinifolic acid. It gave upon reduction with sodium in butanol a dihydro product (*1 a*). Although this di-

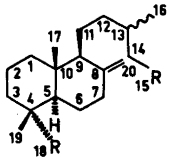
* Part III. *Acta Chem. Scand.* **19** (1965) 1792.

hydro product should be a mixture of C(13)-epimers, a direct comparison of its chromatographic (TLC and GLC) and spectroscopic (IR, NMR, and MS) properties with those of pinifolic acid (*1 a*) settled the structure of this product. The position of the C(13)–C(14)-double bond in dehydropinifolic acid (*2 a*) was shown by a comparison of the NMR-spectrum of the diol (*2 b*), prepared from dehydropinifolic acid with that of agathadiol (*3*)[2] and that of the diol (*1 b*) from pinifolic acid (see Table 1). The configuration of the C(13)–C(14)-double bond has not been determined. However, the sharp signal due to the C(16)–H_3 group indicates [6,7] a *trans* relationship between this methyl group and the C(14)-proton.

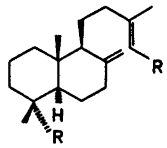
Table 1. Characteristic signals in the NMR spectra of diol (*1 b*), diol (*2 b*), and agathadiol (*3*). Abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; b = broad.

Assignment	Chemical shifts, τ, (signal pattern); Coupling constants, cps		
	Diol (*1 b*)	Diol (*2 b*)	Agathadiol (*3*)
C(14)–H (olefinic)	–	4.50 (t) *J* 7	4.62 (t) *J* 7
C(15)–H_2	6.28 (t) *J* 7	5.80 (d) *J* 7	5.86 (d) *J* 7
C(16)–H_3	9.08 (d) *J* 6	8.29 (s)	8.31 (s)
C(17)–H_3	9.26 (s)	9.26 (s)	9.34 (s)
–H_2 C(18)	AB-quartet H_A 6.56 H_B 6.84 *J* 10.5	AB-quartet H_A 6.56 H_B 6.84 *J* 10.5	–
–H_3	–	–	9.02 (s)
–H_2 C(19)	–	–	AB-quartet H_A 6.28 H_B 6.62 *J* 11.5
–H_3	9.22 (s)	9.22 (bs)	–
C(20)–H_2	5.10 (bs) 5.40 (bs)	5.10 (bs) 5.40 (bs)	5.18 (bs) 5.48 (bs)

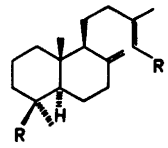
The previous evidence [1] for the C(4)-configuration of pinifolic acid rested on a comparison of pK_{MCS}-values according to the method of Simon [3] and on a comparison of cyclohexylamine salt formations and esterifications in pinifolic acid and dihydroagathic acid. Since pinifolic acid (*1 a*) and dehydropinifolic acid (*2 a*) so far should be the only resin acids of labdane type which



1a R = COOH
1b R = CH_2OH



2a R = COOH
2b R = CH_2OH



3 R = CH_2OH

possess C(4)-equatorial carboxyl groups, it was of interest to study this point in more detail using NMR data.

An investigation by Wenkert and Beak [4] has shown that the signals due to the CH_2-group of an equatorial C(4) hydroxymethylene function in diterpenoids of normal A/B configuration appear as a quartet at 0.4 ppm higher field than that of an axial C(4)-hydroxymethylene function. The axial system of agathadiol (*3*) appears at τ 6.45, whereas those of the corresponding diols (*1 b*) and (*2 b*) prepared from pinifolic acid and dehydropinifolic acid, respectively, appear at τ 6.70. Thus, there is no doubt about the equatorial configuration of the C(4)-carboxyl groups in pinifolic acid and dehydropinifolic acid.

The occurrence of pinifolic acid in other parts of *Pinus silvestris* has been studied. The acid fractions of the acetone extracts of bark, a sample from the cambial region, wood (sapwood and heartwood), and a root sample were investigated by GLC and TLC after methylation with ethereal diazomethane. Pinifolic and dehydropinifolic acids could neither be detected in any of the samples nor in the needles and oleoresins of Norway spruce, *Picea abies* (L.) Karst. and Common larch, *Larix decidua* Mill.

EXPERIMENTAL

Melting points were taken on a Kofler micro hot stage and are not corrected. NMR spectra were recorded on a Perkin Elmer R 12 instrument operating at 60 Mc/s (solvent: $CDCl_3$, *ca.* 10% v/w solutions). Chemical shifts are given in τ-units (TMS, internal standard).

Isolation of dehydropinifolic acid (2 a) and benzoic acid. Needles of *Pinus silvestris* L. were collected from all parts of the branches in the middle of September. The needles (5130 g, corresponding to 2290 g dry material) were covered with acetone and refluxed for 30 min. The solid material was filtered, milled in a Wiley-mill, and extracted twice with acetone in a Soxhlet apparatus (2 × 48 h). The combined acetone solutions were concentrated to a small volume and partitioned between water and methylene chloride. The methylene chloride phase was extracted with aqueous sodium bicarbonate (10%). The bicarbonate solution was acidified with dilute sulphuric acid (aq. 10%) and extracted with ether. The ether solution was washed with water, dried over sodium sulphate, and concentrated to yield a dark green oily acid fraction (42.8 g).

The oily acid fraction was dissolved in ether and filtered through a "Florisil" (250 g, magnesium silicate) column to separate acids of chlorophyll nature. Ether eluted a crude crystalline acid fraction (8.8 g) which according to GLC (1% E301 on Gas-chrom. P[5], temp. 205°) on a methylated sample was a mixture of pinifolic acid (*1 a*) and dehydropinifolic acid (*2 a*) in relative proportions 30:1, respectively (relative retention times: 1.0 and 1.25).

Repeated recrystallisations of this crude acid fraction from acetone/isopropyl ether mixtures gave almost pure pinifolic acid (6 g). The combined mother liquors were concentrated. The oily residue (2.8 g) was kept under vacuum (0.1 mmHg) at about 100°. Benzoic acid (0.3 g, corresponding to about 0.01% of the dry pine needles) sublimed and was collected on a cold finger. The oily residue (2.5 g) was adsorbed on a dimethyl sulphoxide impregnated Celite column (90 g, DMSO/H_2O, 85/15, on 100 g Celite). Isopropylether eluted in the first fractions pinifolic acid, followed by mixtures of pinifolic acid and dehydropinifolic acid. In the later fractions, crystalline dehydropinifolic acid (0.3 g) was eluted. Recrystallisation from an acetone/isopropyl ether mixture, followed by sublimation (0.01 mmHg, 150°), gave the pure dehydropinifolic acid (*2 a*), m.p. 190 – 192°, $[\alpha]_D + 39.5°$ (EtOH, *c* 1.6). (Found: C 71.9; H 8.9; O 19.0. $C_{20}H_{30}O_4$ requires C 71.8; H 9.0; O 19.2.)

Diol (*1 b*). Pinifolic acid was treated with ethereal diazomethane, and the dimethyl ester thus obtained was reduced with lithium aluminium hydride in ether. The product was worked up in the usual way to yield the diol (*1 b*) which after recrystallisation from an ether/light petroleum (b.p. 40 – 60°) mixture had m.p. 79 – 80.5°, $[\alpha]_D + 39.0°$ ($CHCl_3$, *c* 0.9). (Found: C 77.9; H 11.7. $C_{20}H_{36}O_2$ requires C 77.9; H 11.8.)

Diol (*2 b*). The dimethyl ester of dehydropinifolic acid, prepared by treatment of the acid in ether with ethereal diazomethane, was reduced with lithium aluminium hydride in ether. The product thus obtained was recrystallised from a mixture of ether/light petroleum (b.p. 40 – 60°) to yield the pure diol (*2 b*), m.p. 107.5 – 109°, $[\alpha]_D + 41.5°$ ($CHCl_3$, *c* 0.9). (Found: C 78.4; H 11.3; O 10.4. $C_{20}H_{34}O_2$ requires C 78.4; H 11.2; O 10.4.)

The diols (*1 b*) and (*2 b*) could also be obtained by a similar reduction of a mixture of the dimethyl esters of pinifolic acid and dehydropinifolic acid. The diol mixture thus obtained was separated by preparative TLC (Al_2O_3) using a mixture of methanol/ether (3:100) for the development of the plates (R_F-values; diol (*1 b*) 0.55, diol (*2 b*) 0.45).

Sodium in butanol reduction of dehydropinifolic acid (*2 a*). The acid (*2 a*) was reduced according to the method previously [1] described for the preparation of dihydroagathic acid. The product thus obtained showed identical chromatographic properties (TLC and GLC; for conditions, see Ref. 5) to those of pinifolic acid (*1 a*). The spectral properties (IR, NMR, and MS) of the product were almost identical to those of pinifolic acid. The minor differences must be due to the fact that the product is a mixture of C(13)-epimers, whereas natural pinifolic acid is homogeneous. The product could not be induced to crystallise, which also may be due to the fact that it was an epimeric mixture.

Samples from other parts of P. silvestris and from other species. Bark, wood (a sample containing both sapwood and heartwood), a sample from the cambial region, and a root sample from *P. silvestris* were extracted with acetone. The acid fractions of the ether soluble parts of these acetone extracts were methylated with ethereal diazomethane and analysed for the presence of pinifolic and dehydropinifolic acids by GLC and TLC (for conditions, see Ref.5). The acids could not be detected in any of the samples.

A similar investigation of needles and oleoresins from Norway spruce, *Picea abies* (L.) Karst. and from Common larch, *Larix decidua* Mill, did not reveal the presence of pinifolic and dehydropinifolic acids.

Acknowledgement. A part of this investigation has been financially supported by the *Swedish Forest Products Research Laboratory* (project No. 1011).

REFERENCES

1. Enzell, C. and Theander, O. *Acta Chem. Scand.* **16** (1962) 607.
2. Carman, R. M. *Australian J. Chem.* **17** (1964) **393**, 395.
3. Sommer, P. F., Arya, V. P. and Simon, W. *Tetrahedron Letters* **1960**, No. 20, 18.
4. Wenkert, E. and Beak, P. *Tetrahedron Letters* **1961** 358: *cf.* also Gaudemer, A., Polonsky, J. and Wenkert, E. *Bull. Soc. Chim. France* **1964** 407.
5. Norin, T. and Westfelt, L. *Acta Chem. Scand.* **17** (1963) 1828.
6. Bates, R. B., Carnighan, R. H., Rakutis, R. O. and Schauble, J. H. *Chem. Ind. (London)* **1962** 1020.
7. Rowe, J. W. and Scroggins, J. H. *J. Org. Chem.* **29** (1964) 1554.

Received June 27, 1970.