

Nor-diterpenoids from the Bark of *Pinus silvestris* L.*

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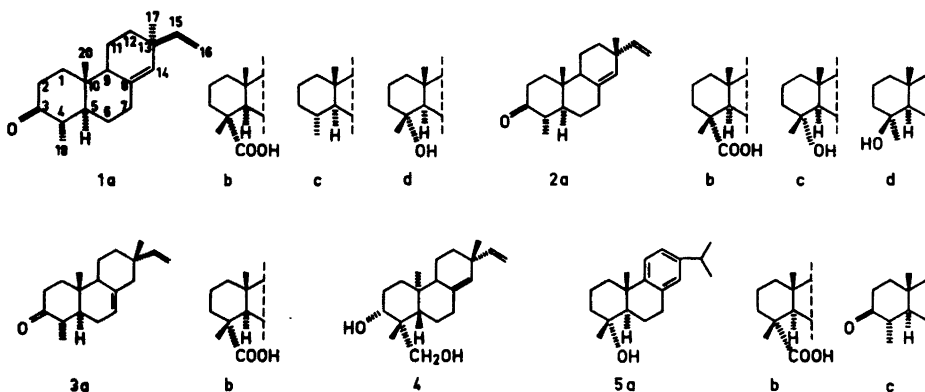
Three nor-diterpenoids have been isolated from the light petroleum soluble extractives of the bark of *Pinus silvestris* L. One of the compounds has been characterised as 19-norpimara-8(14),15-dien-3-one (1 a). Structures of the other two have been assigned to 19-norisopimara-8(14),15-dien-3-one (2 a), and 19-norisopimara-7,15-dien-3-one (3 a).

In connection with a detailed study of the lipophilic extractives of pine bark (*Pinus silvestris*)¹ a neutral light petroleum soluble fraction was obtained, which upon consecutive chromatography on silica gel and silver nitrate impregnated silica yielded the nor-diterpenoid A (C₁₉H₂₈O; MW 272, determined by mass spectrometry; $[\alpha]_D +99.6^\circ$, *c* 0.5 in chloroform; m.p. 85–86°, recrystallised from methanol) and a crystalline mixture of the nor-diterpenoids B and C. The characterisations of these compounds are described below.

A Wolff-Kishner reduction² was performed on compound A (1 a). The product was hydrogenated over platinum, and a mixture of saturated hydrocarbons were obtained. A decarboxylation³ of pimaraic acid (1 b), followed by a catalytic hydrogenation over platinum, yielded the same hydrocarbons (GLC/MS), but in different relative proportions. This experiment thus establishes the 19(or 18)-norpimarane skeleton of compound A.

The characteristic groupings of compound A (1 a) are assigned from spectral data. The NMR spectrum of the compound (see Experimental) exhibits three signals corresponding to three methyl groups. One of these signals is a doublet ($J=7$ cps) and assigned to the secondary 4-methyl group. The olefinic part of the NMR spectrum shows a significant signal pattern, which is almost identical to that of pimara-8(14),15-diene groupings, but different from those of isopimara-8(14),15-dienes and isopimara-7,15-dienes.⁴ The strong positive rotation and the shape of the ORD curve of compound A (1 a) at the low wavelength region ($[\Phi]_{222} +30500^\circ$) is characteristic of pimara-8(14),15-dienes.⁵ Isopimara-8(14),15-dienes exhibit a corresponding strong negative rotation, whereas the corresponding $\Delta^{7,15}$ -derivatives have much lower

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rotations at this wavelength region. The NMR and ORD data thus relate compound A to the normal pimara-8(14),15-diene skeleton.

The presence of a keto-group in compound A was shown by IR-data (1700 cm^{-1}). The compound exhibited a positive Cotton effect (a $+35$ in methanol). A similar positive Cotton effect is also exhibited by 19-norisopimara-8(14),15-dien-3-one (2 a), a diterpene degradation product described by Grant and Munro.⁶ These data suggest that compound A possesses a 3-keto-function, which was further confirmed by a positive Zimmermann reaction. According to Cohen and Rock⁷ the angular 19-methyl signals in steroids are shifted $0.23\ \tau$ downfield by an introduction of a 3-keto function. The angular 20-methyl signal for norpimaradiene (1 c) appears at $9.05\ \tau$, and in compound A (1 a) at $8.98\ \tau$. The difference ($0.07\ \tau$) is slightly less than the corresponding values for steroids ($0.14\ \tau$), probably due to slightly different anisotropic effects operating in the two types of compounds. However, this difference may be taken as indicative of a 3-keto group in compound A. Treatment of compound A with sulphuric acid gave no isomerisation of the 4-methyl group⁸ thus establishing the 4α -configuration. The results presented above thus establish the 19-norpimara-8(14),15-dien-3-one structure (1 a) of compound A.

Compounds B and C did not separate, using various TLC-conditions, but were easily separated by GLC. The mass spectra of the two compounds were obtained, using a combined GLC/MS technique. A comparison of the mass spectra of compounds A, B, and C with those of the methylesters of "pimarinic" (1 b) "sandaracopimarinic" (2 b) and "isopimarinic" (3 b) acids, respectively, reveals significant similarities (particularly peaks $M-15$ and $M-29$), indicating structural relationships in each pairs of compounds. Thus, compounds B and C have tentatively been formulated as 19-norisopimara-8(14),15-dien-3-one (2 a) and 19-norisopimara-7,15-dien-3-one (3 a) respectively.

Since a sample of 19-norisopimara-8(14),15-dien-3-one, previously prepared by Grant and Munro,⁶ was not available, a direct comparison with compound B could not be made.

From the bark of *Croton oblongifolius*, Seshadri *et al.*⁹ isolated oblongifoliol (4). From this diol it should be possible to obtain the enantiomer of 19-nor-

pimara-8(14),15-dien-3-one, using a retro-aldol reaction.⁶ Such a correlation of the two compounds is planned in collaboration with Professor Seshadri.*

Nor-diterpenes have recently been isolated from the bark of some other conifer species. During the course of this work, Rowe *et al.*¹⁰ communicated the isolation of 18-nor-4 α -hydroxypimara-8(14),15-diene (*1 d*) and 18-nor-4 α -hydroxyabieta-8,11,13-triene (*5 a*) from the bark of *P. banksiana*. Recently, Quan and Swan¹¹ reported the occurrence of 18-nor-4 α -hydroxyisopimara-8(14),15-diene (*2 c*) and the corresponding 4 β -derivative (*2 d*) in the bark of *Thuja plicata* Donn.

From the soil of a *Pinus maritima* forest, Biellman *et al.*¹² recently isolated a microorganism which could metabolise dehydroabietic acid (*5 b*) to 19-norabieta-8,11,13-trien-3-one (*5 c*). In view of this finding it is an open question if the nor-diterpenoids from pine bark are products from the tree or artefacts as a result of microbial degradation of the corresponding resin acids which also occur in the bark.

EXPERIMENTAL

Light petroleum refers to a fraction with boiling range 40–60°. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian A-60 instrument operating at 60 Mc/s (solvent, CDCl₃; internal standard, TMS). Chemical shifts are given in τ units. Only characteristic spectral data are given.

The neutral light petroleum soluble part of the methylene chloride extract (21 g)¹ of the bark was chromatographed on silica. Ether (10%) in light petroleum eluted a fraction (0.84 g) which was rechromatographed on silver nitrate impregnated silica with the same eluent yielding compound A (0.32 g, R_F = 0.37) and a mixture (0.11 g, R_F = 0.33) of compounds B and C.

Compound A (19-norpimara-8(14),15-dien-3-one (1 a)) was recrystallised from methanol and had m.p. 85–86° (C₁₉H₂₈O requires C 83.8%; H 10.4%. Found: C 83.9%; H 10.1%). It gave a positive Zimmermann¹³ colour reaction for 3-keto compounds. M. W. 272 (MS). IR (KBr): 1700 (C=O); 990, 915 (–CH=CH₂); 850 (C=CH) cm⁻¹. NMR: 4.14 τ , 1H (X-part of an ABX-spectrum, J = 17.5 and 10.5 cps); 4.70 τ , 1H, broad singlet, (olefinic proton) 5.0 τ , 2H, (AB-part of an ABX-spectrum, J = 17.5, 10.5, and 2.5 cps); 7.4–8.8 τ , (methylene protons) 8.98 τ , 3H, doublet, J = 7 cps (CH₃–CH); 8.98 τ , 3H, singlet (CH₃–C); 9.04 τ , 3H, singlet (CH₃–C). ORD: $[\phi]_{400} + 525^\circ$, $[\phi]_{299} + 4380^\circ$, $[\phi]_{270} + 875^\circ$, $[\phi]_{222} + 30\ 500^\circ$; $\alpha + 35$.

Compound A (1 mg) was dissolved in ethanol (1 ml). One drop of aqueous sulphuric acid (20%) was added and the mixture refluxed for 2 h. The starting material could be recovered unchanged (GLC, TLC and mixed m.p.).

A Wolff-Kishner reduction of compound A (*1 a*) was performed according to Nagata and Itazaki² (procedure A). The product (44% yield) was a colourless oil which was hydrogenated using a platinum catalyst in acetic acid. The saturated hydrocarbon mixture thus obtained was analysed by GLC/MS (1% XE60). Three main constituents were detected (rel. ret. times 1.00, 1.32, 1.52; rel. prop. 7:29:1).

The decarboxylation of pimaric acid was carried out according to the procedure previously described for 1⁽¹⁴⁾-dihydroabietic acid by Jensen and Johnson.³ The hydrocarbon mixture thus obtained was hydrogenated using a platinum catalyst in acetic acid yielding a mixture of saturated hydrocarbons which were analysed by GLC/MS. Four main constituents were detected (rel. ret. times 1.00, 1.16, 1.32, 1.52; rel. prop. 1:2.5:5:35). The mass spectra of the constituents with relative retention times 1.00, 1.32, and 1.52 were identical to those of the corresponding products derived from compound A.

*Added in proof. Professor Seshadri has now informed us that the proposed structure (*4*) of oblongifolios has to be revised.

The proportions between compounds B and C were 1:3 (GLC). The mixture had the following properties. IR (thin film): 1700 (C=O); 3080, 1640, 995, 920, 910 ($-\text{CH}=\text{CH}_2$); 1660, 870, 830 (C=CH). NMR: 4.1–5.3 τ , signal pattern characteristic for the olefinic part of an isopimara-7,15-diene or an isopimara-8(14),15-diene, 8.92–9.1 τ ; methyl groups. The mixture gave a positive Zimmermann colour reaction, indicating the presence of a 3-keto function.

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