1775 (γ -lactone), 1715 (C=CCO₂R). MS m/z (rel. int.): 378.168 [M]⁺ (0.5) (calc. for C₂₀H₂₆O₇: 378.168), 360 [M-H₂O]⁺ (0.7), 350 [M-CO]⁺ (3), 279 [M-OAng]⁺ (4), 278 [M-AngOH]⁺ (3), 261 [279-H₂O]⁺ (3.5), 231 [261-CH₂O]⁺ (4.5), 83 [C₄H₇CO]⁺ (100), 55 [83-CO]⁺ (66); $[\alpha]_D^{24^+}$ +65 (CHCl₃; c 0.80).

$$\begin{split} & 8\alpha\text{-}Angeloyloxy\text{--}1\beta\text{,}10\alpha\text{-}epoxy\text{--}2\alpha\text{,}15\text{-}dihydroxy\text{--}11\beta\text{H}\text{-}germa-}\\ & cr\text{-}4\textbf{Z}\text{-}en\text{-}12\text{,}6\alpha\text{-}olide (9)\text{. Gum; IR } \nu_{max}^{\text{CHC1}\text{-}}\text{ cm}^{-1}\text{:} 3600 (\text{OH})\text{,} 1775 \\ & (\gamma\text{-}lactone)\text{,} 1705 (\text{C}\text{=}\text{CCO}_2\text{R})\text{. MS } m/z \text{ (rel. int.): } 380.184 \text{ [M]}^+ \\ & (1) (\text{calc. for } \text{C}_{20}\text{H}_{28}\text{O}_7\text{:} 380.184)\text{,} 362 \text{ [M}\text{-}\text{H}_2\text{O}\text{]}^+ (1.5)\text{,} 352 \text{ [M} \\ & -\text{CO}\text{]}^+ (2)\text{,} 281 \text{ [M}\text{-}\text{OAng}\text{]}^+ (4.5)\text{,} 280 \text{ [M}\text{-}\text{Ang}\text{OH}\text{]}^+ (2.5)\text{,} \\ & 252 \text{ [}280\text{-}\text{CO}\text{]}^+ (12)\text{,} 83 \text{ [}\text{C}_4\text{H}_7\text{CO}\text{]}^+ (100)\text{.} \end{split}$$

 8α -Angeloyloxy-1β,10α-epoxy-2α-hydroxy-15-oxo-germacr-11(13)-en-12,6α-olide (10). Gum; IR v_{max}^{CHCl3} cm⁻¹: 3600 (OH), 1770 (γ-lactone), 1720 (C=CCO₂R, CHO). MS m/z (rel. int.): 378.168 [M]⁺ (0.3) (calc. for C₂₀H₂₆O₇: 378.168), 350 [M -CO]⁺ (3), 279 [M-OAng]⁺ (2), 83 [C₄H₇CO]⁺ (100), 55 [83 -CO]⁺ (72).

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A RESIN ACID FROM PINUS SYLVESTRIS NEEDLES

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Key Word Index-Pinus sylvestris; Pinaceae; needles; resin acid; 12-0x0-8-hydroxy abiet-13(14)-enoic acid.

Abstract—In the course of a systematic search for resin acids in *Pinus sylvestris*, a new diterpene acid (12-oxo-8-hydroxy abiet-13(14)-enoic acid) was isolated and identified. This product, previously described from synthetic works was unknown from natural sources.

INTRODUCTION

The study of the relationships existing between the dietary acceptability of the resin acids present in the foliage of Pinus sylvestris for the sawfly Diprion pini (Hym., Diprionidae) led us [1] to the isolation of 15 diterpene acids from the needles. These products, previously reported from P. sylvestris [2] or related conifer species were: abietic acid, dehydroabietic acid, neoabietic acid, levo-pimaric acid, palustric acid, $\Delta^{7,15}$ -isopimaric acid, sandaracopimaric acid, dihydroabietic acid pinifolic acid, dehydropinifolic acid, agatholic acid and 13oxopodocarp-8(14)-enoic acid [3-14]. The isolation and structure elucidation of a new diterpene acid (1) found during the course of this study are reported. We were unable to establish any deterrent effect for 13-oxopodocarp-8(14)-enoic acid on Diprion pini [1] as previously reported [15, 16] for Neodiprion rugifrons and N. swainei.



RESULTS AND DISCUSSION

Pinus sylvestris foliage collected from 10-year-old trees from an experimental plantation of the INRA at Olivet,

France) was subjected to solvent extraction, leading, after NaOH fractionation, to the usual resin acids and their more polar congeners. Reversed-phase HPLC separated these two acid fractions which were further analysed by repeated HPLC and preparative TLC (methyl esters) according to a previously reported method [1, 13]. The identification of the already known substances resulted from direct comparisons with standards and from comparative GC-MS of their methyl esters.

The new naturally occurring substance 1 was identified as the 12-oxo-8-hydroxy abiet-13(14)-enoic acid on the basis of the physicochemical properties of its methyl ester (2) and by comparison with the reported data for this series of compounds. The high resolution mass spectrum gave a molecular formula $C_{21}H_{32}O_4$ for ester 2 and showed significant peaks in agreement with the presence of the main functions (OH, CO, methyl ester). The IR spectrum exhibited bands corresponding to the OH group and to the α,β -unsaturated carbonyl. The presence of this conjugated CO was confirmed by the UV spectra. The ¹H NMR spectrum is typical of an abietane skeleton with two tertiary methyl groups and an isopropyl linked to a double bond, the hydroxyl is tertiary judging from the lack of signal between $\delta 3$ and 4. In addition, the NMR spectrum showed a signal corresponding to the isolated olefinic proton. All the data observed for substance 2 are in agreement with those reported for a synthetic compound, in particular the ¹H NMR spectrum [17-19]. The new substance 1 could be derived from levopimaric acid via an endoperoxide (in the same way that 13-oxopodocarp-8(14)-enoic acid could be formed from neoabietic acid through an exo-epoxide).

EXPERIMENTAL

Extraction of the more polar resin acid fraction from P. sylvestris needles. Deep-frozen P. sylvestris needles were ground in a mixer and extracted with MeOH- CH_2Cl_2 (1:1) for 48 hr at room temp. leading, after filtration and concn in vacuo, to the total lipids. This fraction was dissolved in CH_2Cl_2 and the acids extracted with aq. 2% NaOH. After acidification with 2 M HCl, the aq. phase was extracted with CH_2Cl_2 . The organic phase was then dried over Na_2SO_4 and concd. The total amount of free acids varied from 8.4 g kg⁻¹ dry wt in June to 20.3 g kg⁻¹ in September.

The more polar resin acids were obtained by reversed-phase HPLC on a prep. column: Whatman Partisil M9 10/50 C8, 500 \times 9.4 mm, elution by MeOH-H₂O-*iso*-PrOH-orthophosphoric acid (3600:1500:500:1), detection by refractometry. In these conditions, the more polar resin acids are eluted first (4-8 min, flow rate 3 ml min⁻¹). The amounts recovered varied from 1.6 g kg⁻¹ dry wt in June to 19.8 g kg⁻¹ in September.

Isolation of substance 1. Semi-prep. reversed-phase HPLC of the more polar resin acids was carried out on a column of Partisil M9 10/50 ODS-2 500×9.4 mm, elution with the same solvent mixture as above, flow rate 2 ml min⁻¹, giving the known 13oxo-8(14)-podocarpenoic acid [14] together with a series of substances from the 25th to the 29th min. This fraction was submitted to analytical HPLC on a column of Beckman Altex Ultrasphere ODS 250 × 4.6 mm, elution with the previous mixture, flow rate 1 ml min⁻¹. 13-Oxo-8-podocarpenoic acid was eluted after 9.5 min, and 1 after 12 min. The amount of the latter substance varied from 6 mg kg⁻¹ dry wt in June to 100 mg kg⁻¹ in September.

Identification of substance 1. Amorphous powder. Its methyl ester (2) was prepared by brief reaction with an ethereal soln of CH₂N₂. Methyl ester 2: mp 133–136°; $[\alpha]_D^{20}$ (MeOH) 30. EIMS, *m/z* (rel. int.): 348 (77), 330 (35), 319 (15), 316 (15), 305 (27), 288 (14), 270 (27), 271 (29), 255 (16), 227 (11), 201 (10), 181 (24), 178 (12), 162 (21), 123 (32), 121 (100), 109 (30), 107 (31), 105 (25), 101 (10), 93 (30), 81 (31), 67 (26), 55 (34); MIKE MS: *m/z* 348 [M]⁺ gave the ion file 330, 320, 316, 305, 289, 271, 265, 250, 230, 219; the ion at *m/z* 330 gave the series 315, 296, 287, 271. High resolution MS: calc. for C₂₁H₃₂O₄ 348.23004, found 348.2302. IR ν_{max}^{EtOH} nm: 234, log ε 3.8. ¹H NMR of 2 (220 MHz, CDCl₃, TMS): δ 0.66 (s, 3H, Me-C-20), 1.05 and 1.08 (d, 3H, *J* = 7 Hz, Me-C-16 and C-17), 1.14 (s, 3H, Me-C-19), 3.69 (s, 3H, ester Me), 6.38 (s, 1H, C-14).

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