128. Studies Related to Biological Detoxification of Kraft Pulp Mill Effluent. V[1]). The Synthesis of 12- and 14-Chlorodehydroabietic Acids and 12,14-Dichlorodehydroabietic Acid, Fish-toxic Diterpenes from Kraft Pulp Mill Effluent

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Summary

An improved synthesis of the isomeric 12- and 14-chlorodehydroabietic acids (**3** and **2**, respectively) and 12,14-dichlorodehydroabietic acid (**4**) is described. The monochloro isomers were conveniently separated as the imidazole derivatives, and conversion of the latter to the free acids or their corresponding methyl esters could be achieved in high yield. Studies involving microbial degradation of **2**–**4** are underway.

Chlorodehydroabietic acids are important fish toxicants [2] in the effluent from the kraft mill process in the wood pulping industry. They arise from the chlorination bleaching treatment of pulp extractives containing dehydroabietic acid (**1**). Although the monochlorodehydroabietic acids have been prepared [2] and are available as mixture of isomers, their expense and incomplete characterization inspired us to investigate their synthesis, separation and identification since these compounds were required for the biodegradation studies as described in the preceding publication [1].

Chlorination of aromatic compounds is most commonly effected using chlorine in the presence of catalytic ferric trichloride [2] [3]. However, using this procedure previous authors [2] isolated the chlorinated compounds of **1** in only modest yields (30–40%). In our hands this yield was not improved using the same procedure. However, when the same catalyst was supported on silica gel (2% of $FeCl_3$) in the presence of a supported radical scavenger (0.5% of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone on silica gel), an improvement in conversion was found resulting in yields of 75–80%. It was more convenient to use chlorine dissolved in carbon tetrachloride for the chlorination with the solution being titrated (as iodine, from potassium iodide with standard thiosulfate) just prior to use. Monochlorination yielding 14- and 12-chlorodehydroabietic acid (**2** and **3**, respectively) was usually

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complete within 6–8 h at room temperature, the formation of 12,14-dichlorodehydroabietic acid (**4**) requiring 24–30 h (75–80%). Monitoring the course of chlorination was not possible using TLC., but filtered samples could be readily analyzed by ^{1}H-NMR. for disappearance of **1** or disappearance of **2** and **3**. Consumption of chlorine in all attempts was greater than the theoretical quantities. This apparent anomaly can be attributed to loss of some chlorine with the hydrogen chloride evolution. Optimal yields were obtained when highly purified **1** was employed.

In the preparation of the 14- and 12-chloro isomers **2** and **3**, it was best to avoid overchlorination since the dichloro compound **4** was difficult to remove from **3** by crystallization, while **1** was conveniently separated. The chloro isomers **2** and **3** were readily separated after formation of the imidazole derivatives as noted below.

Using the imidazole derivatives, a method developed for the analysis of isopimaric acids by HPLC. [4], the chloro acids were readily analyzed by HPLC. on reverse phase columns (*RP-8* and *RP-18*) using methanol/water mixtures (see

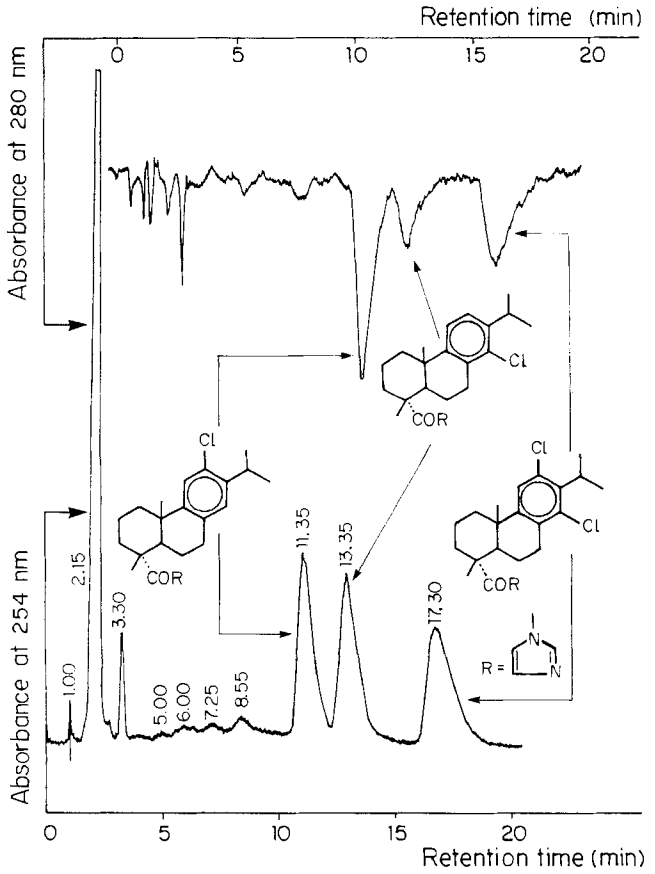


Fig. 1. *HPLC. analysis of the chlorination mixture (as imidazole derivatives) from* **1**. Conditions: reverse phase *RP-8* column; solvent: H_2O/MeOH 18:82; flow rate: 2 ml/min.

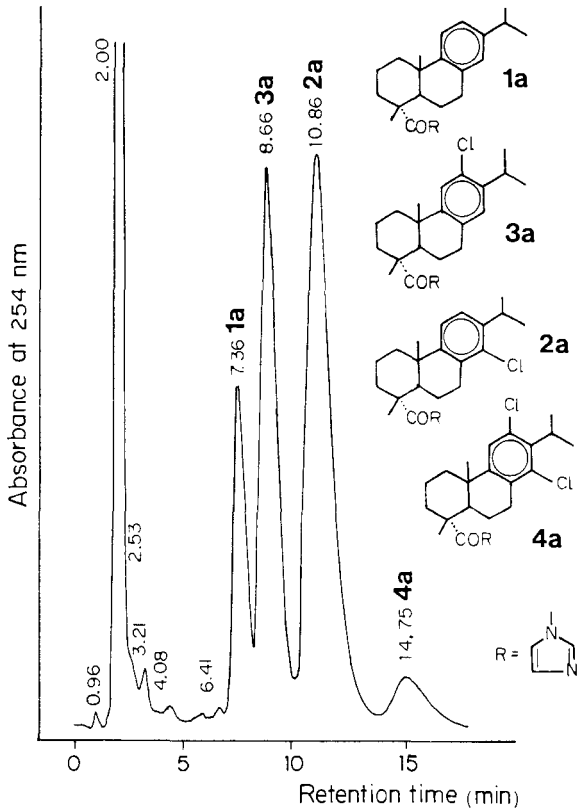
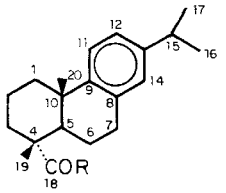


Fig. 2. *HPLC. analysis of the chlorination mixture (as imidazole derivatives) from* **1**. Conditions: reverse phase *RP-18* column; solvent: H_2O/MeOH 12:88; flow rate: 2 ml/min.

Fig. 1 and *2*). As with the other resin acids (*e.g.*, **5** and **1**), the sterically hindered imidazole derivatives can be isolated and analyzed without decomposition. Fortuitously, when the mixture of the monochlorodehydroabietic acids **2** and **3** were reacted with 1, 1'-carbonyldiimidazole [5] in acetonitrile, the isomer **2a** crystallized from solution within minutes; concentration of the mother liquors provided an almost quantitative separation of **2a**. The mother liquors containing isomer **3a** could not be induced to crystallize, although free of **2a** (by ^{1}H-NMR.) and of approximately 80% purity.

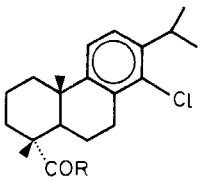
The imidazole derivative **2a** showed spectral characteristics consistent with its structure.

An accurate mass determination confirmed the molecular formula $C_{23}H_{29}ClN_2O$ with a molecular ion at *m/z* 348. High-field (400 MHz) ^{1}H-NMR. showed the characteristic imidazole protons at 8.30, 7.58 and 7.03 ppm. The important aromatic protons (H–C(11) and H–C(12)) were clearly resolved and resonated at 7.17 and 7.13 ppm as mutually coupled doublets ($J = 8.5$ Hz), thus confirming their *o*-orientation to each other. This result allowed the position of the chlorine atom to be assigned unambiguously to C(14). A septuplet at 3.43 ppm ($J = 7$ Hz) was clearly due to H–C(15), the latter being coupled to the two methyl groups at C(15) resonating in their turn as two doublets ($J = 7$ Hz) at 1.24 and 1.22 ppm. This analysis was confirmed by a double irradiation experiment.



1 R = OH

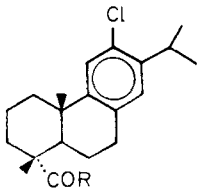
1a R = 



2 R = OH

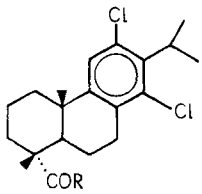
2a R = 

2b R = OCH_3



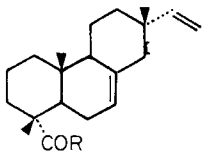
3 R = OH

3a R = 



4 R = OH

4a R = 



5 R = H

5a R = 

The imidazole derivative of the 12-chloro isomer, **3a**, obtained from the mother liquors of the crystallization, showed spectral characteristics in agreement with its structure.

The same accurate mass as for **2a** was found. In the ^{1}H-NMR. spectrum (400 MHz) of **3a** the imidazole protons resonated at 8.34, 7.61 and 7.07 ppm, but the aromatic protons ((H–C(11) and H–C(14)) appeared as two singlets at 7.22 and 6.96 ppm. The lack of any discernable coupling between the two protons confirmed their *p*-orientation to each other and allowed the assignment of the chlorine atom to the C(12) position. The proton at C(15) resonated at 3.33 ppm (*sept.*, $J = 7$ Hz) and the two H_3C–C(15) groups were seen as doublets ($J = 7$ Hz) at 1.23 and 1.22 ppm.

The above-mentioned imidazoles derivatives could be conveniently converted to either the methyl esters or the carboxylic acids. Treatment of **2a** with sodium methoxide in methanol afforded the methyl ester **2b** in a yield of over 85%. Hydrolysis of **2a** or **3a** ($KOH/H_2O/THF$) at room temperature for 24 h and acidification gave the crystalline carboxylic acids **2** and **3**, respectively, in yields over 85%.

The 12, 14-dichlorodehydroabietic acid (**4**) could be isolated as pure crystals after chlorination of **1** with an excess of reagent, followed by filtration, removal of solvent and trituration with pentane. It was shown, in separate experiments, that both **2** and **3** also could be converted to **4**, but for preparative purposes the direct chlorination of **1** was most convenient.

A further characterization of the acids **2–4** was obtained from their ^{13}C-NMR. spectra. To assign the C-atoms of each acid, a correlation with resonances of **1** was obtained, together with single-frequency off-resonance decoupling (SFORD) spectra. To further remove any ambiguity, europium-induced shifted spectra were used, and analogies with other diterpenes [6] were established to assign the resonances of **1** and therefore those of **2–4**. Although some assignments are still in doubt, a comparison of the data for **1** with those of **2–4** reveal distinct trends relating to the degree of chlorination and position of the halogen atom(s) on the aromatic nucleus. These data are summarized in *Table 1.*

Table 1. *^{13}C-NMR. data for compounds* **1–4**

Carbon Atom	Chemical shifts (multiplicity)[a]			
	1	**2**	**3**	**4**
C(18)	185.57 (*s*)	185.16 (*s*)	185.15 (*s*)	185.06 (*s*)
C(9)	146.74 (*s*)	148.84 (*s*)	148.37 (*s*)	149.60 (*s*)
C(13)	145.66 (*s*)	142.90 (*s*)	142.52 (*s*)	139.18 (*s*)
C(8)	134.66 (*s*)	133.22 (*s*)[b]	133.69 (*s*)[b]	135.39 (*s*)
C(14)	126.89 (*d*)	133.67 (*s*)[b]	127.08 (*d*)[c]	124.8 (br.)[d]
C(11)	124.06 (*d*)	123.58 (*d*)[c]	125.15 (*d*)[c]	127.74 (*d*)
C(12)	123.88 (*d*)	122.53 (*d*)[c]	130.74 (*s*)[b]	132.6 (br.)[d]
C(4)	47.44 (*s*)	47.34 (*s*)	47.39 (*s*)	47.25 (*s*)
C(5)	44.65 (*d*)	43.88 (*d*)	44.39 (*d*)	43.57 (*d*)
C(6)	37.99 (*t*)	38.22 (*t*)	37.89 (*t*)	38.03 (*t*)
C(10)	36.86 (*s*)	37.18 (*s*)	36.99 (*s*)	37.21 (*s*)
C(1)	36.81 (*t*)	36.65 (*t*)	36.77 (*t*)	36.58 (*t*)
C(15)	33.47 (*d*)	30.15 (*d*)	29.74 (*d*)	31.28 (*d*)
C(7)	30.00 (*t*)	29.15 (*t*)	29.45 (*t*)	29.15 (br.)[d]
C(20)	25.09 (*qa*)	25.04 (*qa*)	24.96 (*qa*)	24.87 (*qa*)
C(16)	23.97 (*qa*)	22.82 (*qa*)	22.80 (*qa*)	19.64 (*qa*)
C(17)	23.97 (*qa*)	22.58 (*qa*)	22.64 (*qa*)	19.51 (*qa*)
C(3)	21.79 (*t*)	21.53 (*t*)	21.63 (*t*)	21.40 (*t*)
C(2)	18.55 (*t*)	18.57 (*t*)	18.45 (*t*)	18.44 (*t*)
C(19)	16.21 (*qa*)	16.21 (*qa*)	16.22 (*qa*)	16.19 (*qa*)

[a]) Chemical shifts in ppm relative to tetramethylsilane (= 0 ppm); multiplicities from SFORD spectra. [b])[c]) Assignments may be interchanged. [d]) Signal broadened by chlorine quadrapole.

Figures 3 and *4* show a plot of ^{13}C-NMR. chemical shifts of **1** as a function of the mol ratio Eu(fod)$_3$:substrate. As expected, most resonances experienced downfield shifts (except C(4) which is not shown for clarity) consistent with the *McConnell-Robertson* equation [6] applied in a purely qualitative manner. From *Dreiding* models and using the *McConnell-Robertson* equation, the assignments of C-atoms in the ^{13}C-NMR. spectra of **1** were made based on the relative slope of the lines in *Figures 3* and *4*. In general, the proximity effect dominates and clearly differentiates all the methyl and some of the aromatic C-atom resonances.

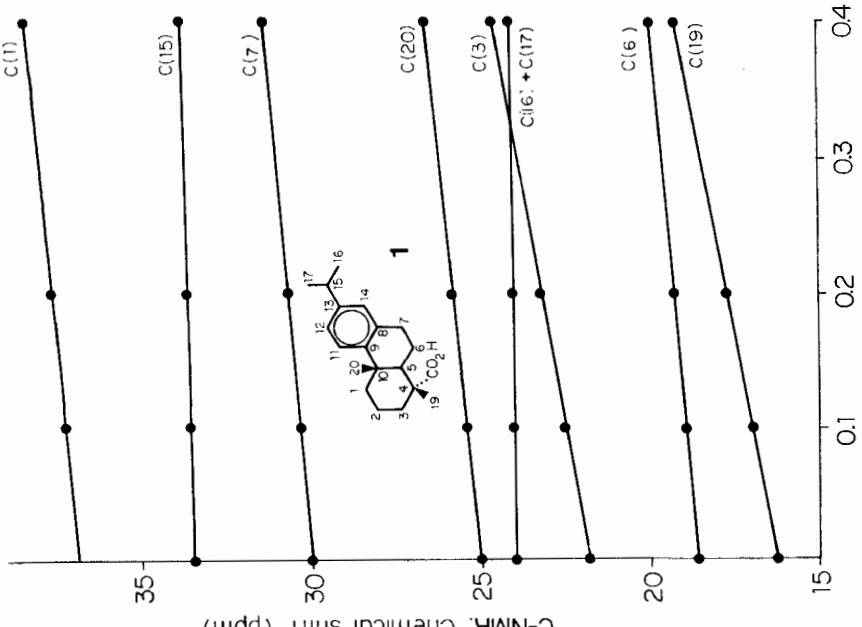


Fig. 3. *Influence of Eu (fod)$_3$ concentration on the ^{13}C-NMR. chemical shifts of* **1**

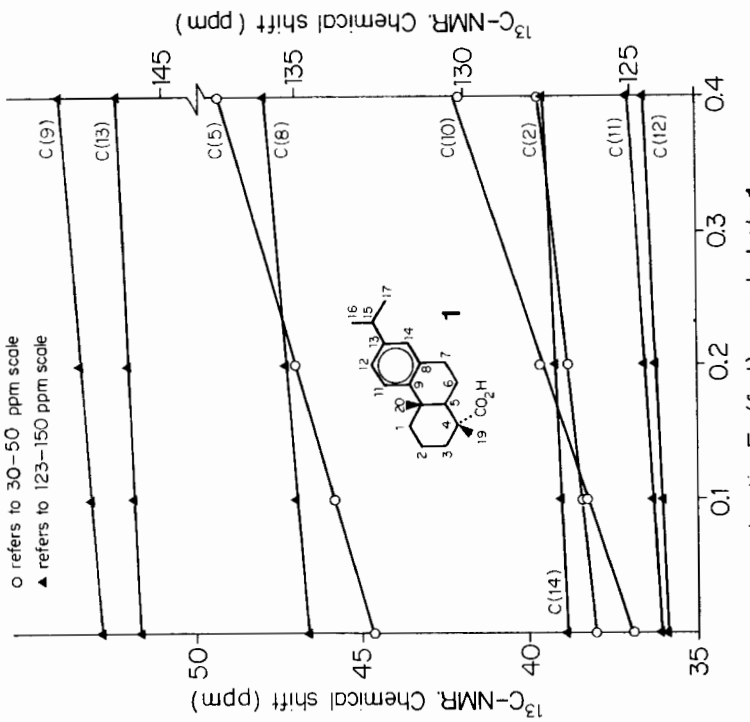


Fig. 4. *Influence of Eu (fod)$_3$ concentration on the ^{13}C-NMR. chemical shifts of* **1**

With the chlorinated acids in hand and their characterization complete, the biotransformation studies of these toxicants has now begun, and one such study is reported in [1].

Experimental Part

General Remarks. S. [1]. In addition: ^{13}C-NMR. spectra were obtained on the *Bruker WH-400* at 100.6 MHz using $CDCl_3$ as solvent. HPLC.: C_8 or C_{18} reverse phase analytical columns and the solvent system methanol/water at compositions and flow rates as shown in *Figure 1* were used.

Preparation of the chlorine solution. A Cl_2-solution in CCl_4 was readily and conveniently prepared by the addition of conc. hydrochloric acid to solid $KMnO_4$, with the Cl_2 dried ($CaCl_2$) and passed through a sinter into ice-cooled CCl_4. In this way a solution of approximately 2–2.5 M can be readily obtained. The Cl_2-concentration was determined by titration of liberated I_2 from KI (aqueous) and standardized thiosulfate just prior to use. Storage of the solution below 0° showed little change within 2 months.

Preparation of supported catalyst. $FeCl_3$ on SiO_2 was prepared by dissolving anhydrous $FeCl_3$ in dry CH_3CN followed by the addition of sufficient silica gel (dry, 0.04–0.06 mm particle size) to give a 2% loading. The solvent was removed in the rotary evaporator and the yellow powder dried on the pump overnight.

The supported radical scavenger 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was prepared (0.5% DDQ) in exactly the same way.

Synthesis of 14- and 12-chlorodehydroabietic acids (**2** *and* **3**, *respectively*). To a solution of **1** (pure, 6.5 g, 21.7 mmol) in CCl_4 (50 ml) was added the supported catalyst (1 g, 2% $FeCl_3$ on SiO_2) and radical scavenger (0.1 g, 1% DDQ on SiO_2). While the stirred suspension was cooled in ice, a solution of Cl_2 (26 mmol) was added at once in the dark. Small samples were taken, filtered and examined by ^{1}H-NMR. After 4 h approximately, 25% of **1** remained, and after 8 h less than 10%. A further addition of chlorine (2 mmol) and reexamination after 3 h showed no **1** and only traces of **4** (< 5%) by ^{1}H-NMR. Filtration and evaporation of the solvent gave the crude **2** and **3** (approx. 3:2 by ^{1}H-NMR. integration; 8 g) as a solid foam.

Separation of **2** *and* **3** *as 1′-(14- and 12-chlorodehydroabietoyl)imidazoles* (**2a** *and* **3a**, *respectively*). To the crude **2** and **3** (8.0 g) dissolved in dry CH_3CN (80 ml) was added a solution of 1,1′-carbonyldiimidazole (1.1 equiv.) in CH_3CN (20 ml) and the mixture allowed to stand at 0° for 4 h. The isomer **2a** began to crystallize within 10 min affording 3.4 g as a microcrystalline white solid. Concentration of the mother liquors to 20 ml and cooling provided a second crop (0.3 g), totalling 3.7 g of **2a** (45% based on **1**), m.p. 213.5–215° (CH_3CN). – IR. (nujol): 1707, 1270, 1240, 1220, 945, 832. – ^{1}H-NMR. ($CDCl_3$, 400 MHz): 8.30 (*d*, J = 1, 1H, H–C(2′)); 7.58 (*t*, J = 1, 1H, H–C(5′)); 7.17 (*d*, J = 8.5, 1H, H–C(11) or H–C(12)); 7.13 (*d*, J = 8.5, 1H, H–C(11) or H–C(12)); 7.03 (*d*, J = 1, 1H, H–C(4′)); 3.43 (*sept.*, J = 7, 1H, H–C(15)); 3.00 (*d*×*d*, J = 6.5 and 18, 1H, H_β–C(7)); 2.78 (*d*×*d*×*d*, J = 8, 11 and 18, 1H, H_α–C(7)); 2.40 (*m*, 2H); 1.53 (*s*, 3H, H_3C–C(4)); 1.52 (*m*, 1H); 1.30 (*s*, 3H, H_3C–C(10)); 1.24, 1.22 (2*d*, J = 7, 6H, 2 H_3C–C(15)). – MS.: 384 (M^+), 289, 273, 207 (100). High-resolution molecular-weight determination: Calc. (^{35}Cl) 384.1968, found 384.1966.

$C_{23}H_{29}ClN_2O$	Calc.	C 71.76	H 7.59	Cl 9.21	N 7.28%
	Found	„ 71.60	„ 7.60	„ 9.25	„ 7.16%

The mother liquors from the crystallization were evaporated to dryness, dissolved in CH_2Cl_2 (50 ml), washed with water (2 times 20 ml), dried (Na_2SO_4) and evaporated to give a foam of crude **3a** (3.9 g) free of **2a** (^{1}H-NMR.) which could not be induced to crystallize. – IR. (nujol): 1710, 1270, 1230, 1200, 1110, 1080, 1050, 930, 890, 850, 830. – ^{1}H-NMR. ($CDCl_3$, 400 MHz): 8.34 (*s*, 1H, H–C(2′)); 7.61 (*m*, 1H, H–C(5′)); 7.22 (*s*, 1H, H–C(11)); 7.07 (*m*, 1H, H–C(4′)); 6.96 (*s*, 1H, H–C(14)); 3.33 (*sept.*, J = 7, 1H, H–C(15)); 2.88 (*m*, 2H, 2H–C(7)); 2.39 (*m*, 2H); 1.53 (*s*, 3H, H_3C–C(4)); 1.31 (*s*, 3H, H_3C–C(10)); 1.23, 1.22 (2*d*, J = 7, 6H, 2 H_3C–C(15)). – MS.: 384 (M^+), 368, 289, 273, 207 (100). High-resolution molecular-weight determination: Calc. (^{35}Cl) 384.1968, found 384.1967.

Formation of 14-chlorodehydroabietic acid (**2**) *from* **2a**. A solution of **2a** (3.65 g) in THF (50 ml) was stirred with 5% aq. NaOH-solution (100 ml) for 48 h. The volatile solvent was removed in the rotary evaporator and the aq. residue was acidified, cooled and extracted with CH_2Cl_2. Removal of the

solvent gave a colorless foam (3.44 g). Crystallization from pentane give colourless needles of **2** (3.2 g, quantitative), m.p. 159–160°. – IR. (nujol): 3000–3400 br., 1700, 1290, 1210, 1160, 960, 840. – ^{1}H-NMR. ($CDCl_3$, 400 MHz): 7.16 (*d*, J=8, 1H, H–C(11) or H–C(12)); 7.10 (*d*, J=8, 1H, H–C(11) or H–C(12)); 3.42 (*sept.*, J=7, 1H, H–C(15)); 3.00 ($d\times d$, J=6 and 18, 1H, H_β–C(7)); 2.82 ($d\times d\times d$, J=8, 12 and 18, 1H, H_α–C(7)); 2.31 (br. *d*, J=13, 1H); 2.18 ($d\times d$, J=2 and 13, 1H); 1.29 (*s*, 3H, H_3C–C(4)); 1.22 (*s*, 3H, H_3C–C(10)); 1.24, 1.20 (2 *d*, J=7, 6H, 2 H_3C–C(15)). – MS.: 334 (M^+), 319 (100), 273, 231. High-resolution molecular-weight determination: Calc. (^{35}Cl) 334.1709, found 334.1704.

$C_{20}H_{27}ClO_2$ Calc. C 71.73 H 8.13 Cl 10.59% Found C 72.00 H 8.07 Cl 10.33%

Conversion of **2a** *to methyl 14-chlorodehydroabietate* (**2b**). The derivative **2a** (100 mg) was dissolved in methanol (10 ml) containing CH_3ONa (approx. 50 mg). After 2 h, the solvent was removed, the residue extracted with hexanes (3 times 10 ml), the extract washed with water and evaporated to give **2b** (80 mg, 88%), m.p. 166–167° (pentane). – IR. (nujol): 1715, 1240, 1195, 1130, 1110, 1050, 840. – ^{1}H-NMR. ($CDCl_3$, 80 MHz): 7.15 (*AB*, J=8, 2H, H–C(11) and H–C(12)); 3.7 (*s*, 3H, CO_2CH_3); 3.45 (*sept.*, J=7, 1H, H–C(15)); 2.9 (*m*, 2H); 1.25 (*s*, 3H, H_3C–C(4)); 1.18 (*s*, 3H, H_3C–C(10)); 1.19, 1.17 (2 *d*, J=7, 6H, 2 H_3C–C(15)). – MS.: 348 (M^+), 333, 273 (100). High-resolution molecular-weight determination: Calc. (^{35}Cl) 348.1855, found 348.1860.

$C_{21}H_{29}ClO_2$ Calc. C 72.29 H 8.33 Cl 10.16% Found C 71.97 H 8.42 Cl 10.01%

Formation of 12-chlorodehydroabietic acid (**3**) *from* **3a**. The crude **3a** (3 g) was hydrolyzed with aq. NaOH-solution/THF as described for **2a**. Isolation gave crude **3** (2.5 g) which was crystallized from hexanes (2.21 g, 31% based on **1**), m.p. 178–180°. – IR. (nujol): 3400–3000 br., 1690, 1160, 1140, 980. – ^{1}H-NMR. ($CDCl_3$, 400 MHz): 7.20 (*s*, 1H, H–C(11)); 6.94 (*s*, 1H, H–C(14)); 3.32 (*sept.*, J=7, 1H, H–C(15)); 2.85 (*m*, 2H, 2H–C(7)); 2.27 (*d*, J=12, 1H); 2.20 ($d\times d$, J=2 and 12, 1H); 1.26 (*s*, 3H, H_3C–C(4)); 1.20, 1.19 (2 *d*, J=7, 6H, 2 H_3C–C(15)); 1.19 (*s*, 3H, H_3C–C(10)). – MS.: 334 (M^+), 319, 273 (100), 231. High-resolution molecular-weight determination: Calc. (^{35}Cl) 334.1709, found 334.1704.

$C_{20}H_{27}ClO_2$ Calc. C 71.73 H 8.13 Cl 10.59% Found C 71.59 H 8.27 Cl 10.45%

Synthesis of 12,14-dichlorodehydroabietic acid (**4**). Chlorination of **1** (2.7 g) was conducted similarly as for the preparation of **2** and **3**, except using an excess of chlorine (3–3.5 equiv.) for 24 h. Filtration and evaporation of the solvent gave a foam which was induced to crystallize by addition of hexanes to provide colorless needles of **4** (2.5 g, 76%), m.p. 219–220°([2]: 218–220°). – IR. (nujol): 3300–2900 br., 1690, 1600*w*, 1550*w*, 1290, 1200, 1000, 980, 800, 780. – ^{1}H-NMR. ($CDCl_3$, 400 MHz): 7.19 (br. *s*, 1H, H–C(11)); 3.93 (br. *s*, 1H, H–C(15); but *sept.* at 80 MHz, J=7); 2.96 ($d\times d$, J=6 and 18, 1H); 2.75 ($d\times d\times d$, J=8, 11.5 and 18, 1H); 2.27 (br. *d*, J=13, 1H); 2.14 ($d\times d$, J=2.5 and 13, 1H); 1.42 (*d*, J=7, 6H, 2 H_3C–C(15)); 1.30 (*s*, 3H, H_3C–C(4)); 1.22 (*s*, 3H, H_3C–C(10)). – MS.: 368 (M^+), 353, 307 (100), 265, 227, 117. High-resolution molecular-weight determination: Calc. (^{35}Cl) 368.1310, found 368.1308.

$C_{20}H_{27}Cl_2O_2$ Calc. C 65.04 H 7.10 Cl 19.20% Found C 64.97 H 7.05 Cl 19.15%

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