

1109. *The Isolation of Abienol from Canada Balsam, the Oleoresin of Abies balsamea (L.) Mill.*

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The diterpene alcohol abienol, previously isolated from two *Abies* oleoresins, has now been obtained from Canada Balsam, the oleoresin of *Abies balsamea* (L.) Mill. Confirmation of the previously suggested structure (I) has been obtained. A gas-chromatographic survey has shown that abienol is present in many but not all *Abies* resins. The principal acids present in Canada Balsam are abietic, neoabietic, and palustric acids.

THE genus *Abies*, or firs, is a group of some thirty-six conifers which are characterized by, among other features, the frequent occurrence in young trees of bark blisters filled with strong-smelling oleoresin. Canada Balsam, the oleoresin of *Abies balsamea* (L.) Mill. has long been an article of commerce and as such has been chemically investigated since the early nineteenth century. In the most recent study¹ the 25% of turpentine obtained by steam-distillation was shown to contain α - and β -pinenes and β -phellandrene. Ultraviolet spectrometry indicated that the acid fraction consisted largely of abietic and neoabietic acids. The remaining 20% comprised a high-boiling neutral fraction which was not examined. We have found that this neutral fraction consists largely of a single diterpene which is readily isolated by chromatography and which gives on crystallization from aqueous solvents a hydrate clearly identical with abienol hydrate isolated by Wienhaus and Mucke² from "Strasbourg Turpentine", the oleoresin of *Abies pectinata* DC. (now called *A. alba* Mill.). This in turn had been identified by them with "abietin" isolated by Cailliot³ from the same source over a hundred years previously. Abienol was isolated by Pigulevskii and Kostenko from the oleoresin of *Abies sibirica* Ledeb.^{4a-c} They corrected the earlier empirical formula and showed that abienol was a bicyclic, tertiary diterpene alcohol and a conjugated diene with one of the double bonds present as a vinyl group. The position of the ultraviolet maximum, 238 m μ , led them initially to suggest that the second double bond was exocyclic and fully substituted. Later,^{4d} when selenium dehydrogenation of the dehydration product of tetra-

¹ Lombard, Rotovic, and Criqui, *Peintures, Pigments, Vernis*, 1958, **34**, 106.

² Wienhaus and Mucke, *Ber.*, 1942, **75**, 1830.

³ Cailliot, *J. Pharm.*, 1830, **16**, 436.

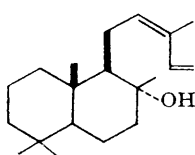
⁴ Pigulevskii and Kostenko; (a) *Vestnik Leningrad Univ.*, 1959, 154; (b) *Proc. Acad. Sci. (U.S.S.R.)*, 1959, **128**, 743; (c) *J. Gen. Chem. (U.S.S.R.)*, 1960, **30**, 1074; (d) *ibid.*, 1961, **31**, 2929.

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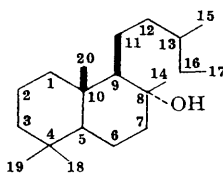
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hydroabienol had yielded 1,5,6-trimethylnaphthalene, suggesting a normal labdane skeleton, they suggested structure (I) for abienol.

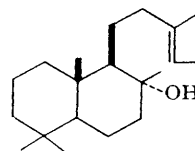
Being initially unaware of the Russian work, we repeated some of it and can supplement their spectral data. The nuclear magnetic resonance spectrum of abienol shows four vinyl protons, a vinylic methyl group, and four further methyl groups. In a labdane skeleton these functions and the vinyl group can only be accommodated by (I). Hydrogenation of abienol gives a tetrahydro-compound, the nuclear magnetic resonance spectrum of which shows no vinyl protons. Its mass spectrum supports structure (II), including peaks at m/e 294 (molecular ion), 276 (loss of water), and 191 (loss of water and saturated side-chain, C_6H_{13}) showing that the hydroxyl group is not located on the side-chain.



(I)



(II)



(III)

Confirmation of structure (I) for abienol was afforded by its reduction with sodium and propanol to isodihydroabienol with constants close to those reported⁵ for a compound with structure (III) prepared by hydrogenolysis of manoyl oxide.

The acid fraction was examined by gas chromatography. The separation of the methyl esters of the common abietane and pimarane resin acids has been reported by several groups^{6a-d} though no single stationary phase has been found to effect a complete separation. We confirmed the presence, as major constituents, of abietic and neoabietic acids together with small quantities of isopimaric, pimaric, and dehydroabietic acids. A further major peak could have been due either to methyl laevopimarate or palustrate. By chromatographing the mixed methyl esters on silver nitrate-silica gel^{6e} we obtained fractions in which the material responsible for this peak was concentrated. Its ultraviolet spectrum showed a maximum at 265 $m\mu$ with no appreciable shoulder at 272 $m\mu$ and we conclude that it consists largely of methyl palustrate.⁷

The presence of abienol in all three of the *Abies* species so far examined suggested that its occurrence might be general in the genus and have taxonomic significance. Through the courtesy of Mr. A. Westall, Supervisor of The National Pinetum, Bedgebury, Kent, we were able to collect fresh samples of oleoresin from nineteen *Abies* species and these, after treatment with diazomethane, were examined by gas chromatography. In four species,⁸ *A. amabilis* Forbes, *A. concolor* (Gordon) Hildebr., *A. mariesii* Mast., and *A. sachalinensis* (Schmidt) Mast., abienol was found to be by far the major diterpenoid constituent. It was also an important constituent in the following species, listed roughly in order of diminishing concentration: *A. firma* Sieb. and Zucc., *A. veitchii* Lindley, *A. koreana* E. H. Wilson, *A. nephrolepis* (Trautv.) Maxim., *A. holophylla* Maxim., *A. homolepis* Sieb. and Zucc., *A. sutchuenensis* (Franchet) Rehd. and Wils., *A. delavayi* Franchet, and *A. squamata* Mast. It was present only in traces in *A. grandis* Lindley and *A. pinsapo* Boiss., and appeared to be absent from *A. cephalonica* Loudon, *A. bracteata* (D. Don) Nutt., *A. procera* Rehder, and *A. magnifica* A. Murray. The remaining species were not examined. Several other neutral constituents

⁵ Hodges and Reed, *Tetrahedron*, 1960, **10**, 71.

⁶ (a) Hudy, *Analyt. Chem.*, 1959, **31**, 1754; (b) von Rudloff and Sato, *Canad. J. Chem.*, 1963, **41**, 2165; (c) Norin and Westfeld, *Acta Chem. Scand.*, 1963, **17**, 1828; (d) Nestler and Zinkel, *Analyt. Chem.*, 1963, **35**, 1747.

⁷ Loeblich, Baldwin, and Lawrence, *J. Amer. Chem. Soc.*, 1955, **77**, 2823.

⁸ In use of names we follow Dallimore and Jackson, "A Handbook of Coniferae," 3rd edn., Edward Arnold, London, 1961. In some cases the names have been amended in accordance with "A Hand-List of Coniferae," H.M. Stationery Office, London, 1961.

were observed in these resins. In particular, a major peak with the same retention time as manool was noted in *A. magnifica*, *A. pinsapo*, and *A. delavayi*.

EXPERIMENTAL

Rotations are in chloroform at room temperature. Light petroleum refers to the fraction b. p. 40–60°. Alumina for chromatography was neutralized by standing in ethyl acetate for 24 hr., filtering, washing with methanol, and reactivating at 180° for 15 hr.

Isolation of Abienol.—In the first experiments the oleoresin was steam-distilled to remove volatile terpenes. This resulted in the formation of considerable amounts of abienol polymer and it was found best to proceed as follows. Canada Balsam (173 g.) was dissolved in ether (1 l.) and divided into acid and neutral fractions in the usual way by extraction with aqueous 2% sodium hydroxide solution. Acidification of the aqueous alkaline extract with dilute acetic acid and isolation with ether gave the acids (96 g., 55%). The neutral fraction (78 g., 45%) was dissolved in light petroleum and chromatographed on alumina (1 kg.). Elution with light petroleum (1 l. fractions) gave, in the first fraction, 45 g. of terpene hydrocarbons (26%). The next three fractions gave 4.5 g. of oil containing terpenes and three minor diterpenoids [gas-liquid chromatography (g.l.c.)] which were not examined. Further elution with light petroleum and with light petroleum-ether gave a series of fractions each showing a single peak (by g.l.c.). Combined, they gave 22 g. (12.7%) of colourless abienol which set to a crystalline mass in the refrigerator. It could readily be crystallized from aqueous acetone to give long needles of abienol hydrate, m. p. 65°, with prior softening, $[\alpha]_D + 22^\circ$ (*c* 1.6), λ_{\max} . (in EtOH) 238 m μ (ϵ 19,800), ν_{\max} . (Nujol) 3100, 1820, 1650, 1600, 995, and 905 cm.⁻¹ (Found: C, 77.6; H, 11.9. Calc. for C₂₀H₃₄O, H₂O: C, 77.85; H, 11.75%). Crystallization from acetonitrile gave stout prisms of abienol, m. p. 41–42°, $[\alpha]_D + 24^\circ$. Pigulevskii and Kostenko^{4b} report m. p. 40–42° for crystals obtained from the melt. The n.m.r. spectrum of abienol (in CDCl₃), $\tau = 2.85$ –5.05 (four vinyl protons), 8.27 (C₍₁₅₎ methyl), 8.91 (C₍₁₄₎ methyl), and 9.2, 9.23, and 9.27 (C₍₁₈₎, C₍₁₉₎, and C₍₂₀₎ methyls).

Tetrahydroabienol.—Hydrogenation of abienol over pre-reduced platinum oxide as already described^{4b} gave tetrahydroabienol, m. p. 47–49°, $[\alpha]_D - 3^\circ$ (*c* 1.1) (Found: C, 81.7; H, 13.05. Calc. for C₂₀H₃₈O: C, 81.6; H, 13.0%). The n.m.r. spectrum showed no vinyl proton. The mass spectrum included major peaks at *m/e* = 41 (79), 43 (73), 55 (68), 57 (65), 69 (100), 71 (70), 81 (68), 82 (55), 83 (70), 95 (93), 97 (48), 109 (70), 123 (54), 157 (20), 177 (33), 191 (59; loss of H₂O and C₆H₁₃ side-chain), 261 (32; loss of H₂O and CH₃), 276 (40; loss of H₂O), and 294 (14; molecular ion). The figures in parentheses give peak intensities as percentages of the strongest peak.

Isodihydroabienol.—Abienol (3 g.) in propanol (150 ml.) was treated with sodium (9 g.), the mixture being heated on a steam-bath when reaction slowed. After 1.5 hr., part of the propanol was removed *in vacuo*, water was added, and the product isolated with ether. Crystallization from aqueous methanol gave isodihydroabienol (2.05 g.), m. p. 90–96°. Recrystallization from the same solvent, from light petroleum, and from acetonitrile raised the m. p. to 97–99°, $[\alpha]_D + 1.5^\circ$ (*c* 1.1), [reported for (III),⁵ m. p. 99–100.5°, $[\alpha]_D - 1^\circ$]; ν_{\max} . (Nujol) 827 cm.⁻¹ (trisubstituted double bond) (Found: C, 82.45; H, 12.8. Calc. for C₂₀H₃₆O: C, 82.15; H, 12.4%).

Acid Fraction and Examination of Oleoresins.—Gas chromatography was carried out with a Pye "Panchromatograph" using either a hydrogen-flame or argon-ionization detector. Glass columns 5 ft. by 0.25 in. were used packed with 3% E301 silicone or XE-60 cyanoethylsilicone on 100–120-mesh silanized Celite, at 200°, with argon at 25 lb./sq. in. inlet pressure. With the XE-60 column some decomposition of abienol occurred, with formation of extra peaks, and the E301 column was preferred generally. The acid fraction or whole Canada Balsam, after methylation by the convenient procedure of Schlenk and Gellerman,⁹ showed major peaks due to methyl abietate and methyl neoabietate, smaller amounts of methyl isopimarate and methyl dehydroabietate, and a trace of methyl pimarate. Another major peak was due either to methyl laevopimarate or methyl palustrate. 1 g. of the acid fraction was methylated with diazomethane and chromatographed on 100 g. of silver nitrate-silica gel,^{6c} eluting with increasing concentrations of ether in light petroleum. No homogeneous fraction was obtained, but later fractions showed mainly the laevopimarate-palustrate peak with only traces of abietate and neoabietate. They had λ_{\max} . (EtOH) 265 m μ and consist therefore largely of methyl palustrate.

Retention times, using the argon-ionization detector, were as follows (XE-60 values in parentheses); manool 3.3 (5.3), abienol 4.3 (7.3), methyl pimarate 5.6 (8.2), methyl laevopimarate-

⁹ Schlenk and Gellerman, *Analyt. Chem.*, 1960, **32**, 1412.

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palustrate 6·7 (10·0), methyl isopimarate 6·7 (10·4), methyl dehydroabietate 7·3 (12·4), methyl abietate 8·2 (13·8), methyl neoabietate 8·8 (15·7) min.

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