

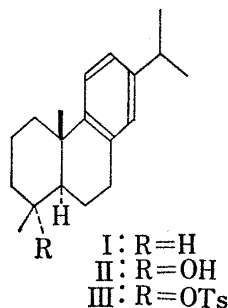
## Natural ar-Abietatriene

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While the ar-abietatriene skeleton has been observed in nature in a variety of oxidized forms, *e.g.*, *inter alia*: ar-abietatrienal (dehydroabietal),<sup>2)</sup> dehydroabietic acid,<sup>2,3)</sup> and callitric acid,<sup>4)</sup> the hydrocarbon has escaped discovery. We now wish to report the isolation of ar-abietatriene (I) from *Thujaopsis dolabrata* SIEB. et ZUCC. (M.K., A.Y. and Y.K.) and from *Podocarpus ferrugineus* D. DON (J.P.C., J.D.M., D.J.W. and E.W.).



*Thujaopsis Dolabrata* SIEB. et ZUCC.—The main diterpenic constituents of the essential oil of the leaves of Hiba (*T. dolabrata*) have been shown to be dolabradiene<sup>5)</sup> and hibaene,<sup>6)</sup> while a minor constituent could be obtained in crystalline form upon careful, fractional distillation and chromatography. Its elemental analysis and mass spectrum (Fig.

1) showed it to be a C<sub>20</sub>H<sub>30</sub> hydrocarbon, which on the basis of its ultraviolet, infrared and proton magnetic resonance spectra contained a 1,3,4-trisubstituted benzene nucleus (see Experimental). The pmr spectrum revealed the presence of five methyl groups of which two were possibly part of an isopropyl group. Comparison of the mass-spectral fragmentation pattern of the natural product with the patterns determined by

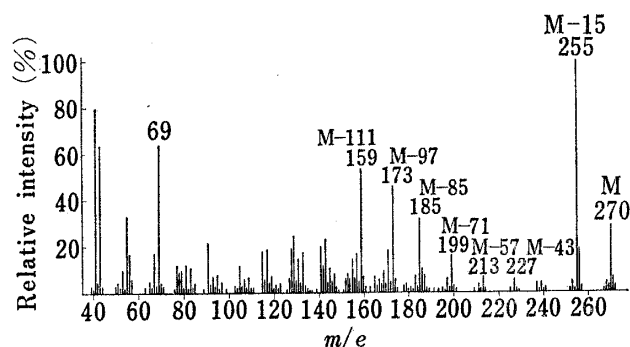


Fig. 1. Mass Spectrum of ar-Abietatriene

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Enzell<sup>7)</sup> for a variety of ring C aromatic diterpenic substances led to the assignment of structure I and to the synthesis of the hydrocarbon. Esterification of ar-abietatrienol (dehydroabietol)<sup>8)</sup> (II) with *p*-toluenesulfonyl chloride yielded a tosylate (III) whose reduction with lithium aluminum hydride produced ar-abietatriene (I), identical in all respects with the natural compound.

*Podocarpus Ferrugineus* D. Don—Earlier studies on the terpenic constituents of the New Zealand miro tree (*P. ferrugineus*) have led to the isolation of dipentene, limonene,  $\alpha$ -pinene, cineole, cadinene, rimuene, hibaene, phyllocladene, kaurene, ferruginol, sugiol and isopimaric acid.<sup>9)</sup> An investigation of the hydrocarbon constituents of the bark of the plant now has yielded the following substances: a mixture of long-chain hydrocarbons consisting predominantly of tetradecane; the sesquiterpenes longifolene,<sup>10)</sup> calamenene,<sup>11)</sup>  $\alpha$ -copaene,<sup>11,12)</sup>  $\alpha$ -muurolene,<sup>13)</sup>  $\alpha$ -curcumene,<sup>14)</sup>  $\alpha$ -bergamotene,<sup>15)</sup>  $\beta$ -selinene,<sup>16)</sup> the diterpenes isopimaradiene,<sup>17)</sup> ar-abietatriene,<sup>18)</sup> and a C<sub>19</sub>H<sub>28</sub> hydrocarbon of unknown constitution. The compounds were separated by inverted dry column chromatography<sup>19)</sup> on silica gel impregnated with silver nitrate<sup>2)</sup> or fractional distillation, by standard chromatography on silica gel impregnated with silver nitrate<sup>2)</sup> and by vapor phase chromatography and were identified by comparison with authentic samples and/or by comparison of their physical constants, especially spectral properties, with those recorded in the literature.

#### Experimental<sup>19)</sup>

***Thujopsis Dolabrata* SIEB. et ZUCC.**—Repeated fractional distillation of the essential leaf oil of Hiba on a spinning band column of 60 theoretical plates led in *ca.* 1% yield to a fraction boiling at 166–170°/0.5–1.0 mmHg. Chromatography of 10.9 g thereof on 65 g of silica gel and elution with petroleum ether while monitoring the fractions by gas chromatography<sup>20)</sup> yielded a solid in later fractions whose crystallization from ethanol gave 0.65 g of colorless needles of ar-abietatriene, mp 41–43°;  $[\alpha]_D^{25} +0.49^\circ$  ( $c=1.0$ , MeOH); molecular ion,  $m/e$  270;  $\lambda_{\max}$  (MeOH) 275  $\mu$  ( $\log \epsilon$  2.91);  $\nu_{\max}$  (KBr) 3050, 1615, 1498, 888, 828  $\text{cm}^{-1}$ ;  $\delta$  (CCl<sub>4</sub>) 0.96 (s, 3H), 1.19 (d,  $J=6\text{Hz}$ , 6H), 6.75 (broad s, 1H), 6.82 (q,  $J=8$ , 2Hz, 1H), 7.06 (q,  $J=8$ , 1Hz, 1H). *Anal.* Calcd. for C<sub>20</sub>H<sub>30</sub>: C, 88.82; H, 11.18%. Found: C, 88.59; H, 11.16%.

**ar-Abietatrienyl *p*-Toluenesulfonate (III)**—A mixture of 2.65 g of ar-abietatrienol<sup>8)</sup> (II) and 3.4 g of *p*-toluenesulfonyl chloride in 25 ml of dry pyridine was allowed to stand at room temperature for 15 hr

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- 20) Melting points are uncorrected. Proton magnetic resonance spectra were recorded on a Varian Associates A-60 spectrometer.

and then heated on a steam-bath for 6 hr. It was diluted with water and extracted with ether. The extract was washed with 1N hydrochloric acid and water and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave an oily product which solidified on standing. Crystallization thereof from 1:1 benzene-petroleum ether afforded colorless prisms, mp 92–93°; IR  $\nu_{\max}$  (KBr): 3050, 1602, 1499, 1332, 1172, 955, 928, 848, 812  $\text{cm}^{-1}$ . *Anal.* Calcd. for  $\text{C}_{27}\text{H}_{36}\text{O}_3\text{S}$ : C, 73.60; H, 8.24%. Found: C, 73.56; H, 8.22%.

**ar-Abietatriene (I)**—A mixture of 2.7 g of the tosylate and 0.75 g of lithium aluminum hydride in 70 ml of dry dioxane was refluxed for 20 hr. It then was treated with dilute hydrochloric acid and extracted with ether. The extract was washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave an oil whose petroleum ether solution was passed through an alumina column yielding 0.75 g of a crystalline solid. Crystallization of the latter from ethanol gave colorless needles of ar-abietatriene, mp 42–44° (lit.<sup>17</sup>) mp 38–42°;  $[\alpha]_D^{25} + 0.63^\circ$  ( $c=1.0$ , MeOH); identical in all respects with the natural hydrocarbon (*vide supra*). *Anal.* Calcd. for  $\text{C}_{20}\text{H}_{30}$ : C, 88.82; H, 11.18%. Found: C, 88.97; H, 11.32%.

**Podocarpus Ferrugineus D. DON**—Dry, coarsely ground bark of the New Zealand tree *P. ferrugineus*, 13.5 kg, was extracted in 1 kg portions with methylene chloride in a Soxhlet apparatus for 48 hr/portion. Evaporation of the solvent from the combined extracts yielded 320.8 g (2.38% of dry weight) of red-brown gum whose solution in 4 liter of petroleum ether was washed with four 500 ml portions of 5% aqueous potassium hydroxide solution and four 500 ml portions of water. These washings were combined and designated the “acid fraction”; their investigation will be reported later. Drying of the petroleum ether solution over anhydrous magnesium sulfate and evaporation of the solvent gave 200 g (1.48% of dry weight) of brown gum, designated the “neutral fraction”. After preadsorption on 400 g of neutral alumina (activity I) it was chromatographed on a 4 kg column of the same support. The petroleum ether and 9:1 petroleum ether-ether eluates were combined and designated the “hydrocarbon fraction.” The investigation of the more polar eluates will be reported later. Inverted dry column chromatography<sup>18</sup> of the “hydrocarbon fraction” on 450 g of 200–325 mesh silica gel impregnated with 10% silver nitrate and elution with 7:3 petroleum ether-methylene chloride produced four hydrocarbon mixtures: A 1.78 g, B 1.71 g, C 0.80 g and D 1.91 g.

Chromatography of mixture A on 50 g of 100–200 mesh silica gel impregnated with 10% silver nitrate and elution with petroleum ether yielded 0.41 g of hydrocarbons, shown by pmr analysis to be of the straight-chain type, and 0.92 g of hydrocarbons of other type. Gas chromatography<sup>21</sup> of the former showed it to be 77% tetradecane by comparison with an authentic specimen (retention time 7 min, SE-30 column, 130°). Preparative chromatography of the second mixture yielded 10 mg of (+)-longifolene,  $[\alpha]_D^{20} + 9.5^\circ$  ( $c=1.6$ , hexane) retention time 22 min at 140°, identical in all respects with an authentic sample<sup>10</sup>; 8 mg of (–)-calamenene,  $[\alpha]_D^{20} - 13^\circ$  ( $c=0.8$ , hexane) retention time 27 min at 160°, identical in all respects with an authentic sample<sup>10</sup>; and 263 mg of (+)-ar-abietatriene, mp 42–43°, retention time 55 min at 200°, identical in all respects with an authentic specimen.<sup>17</sup>

Chromatography of mixture B on 50 g of the above treated silica gel yielded 916 mg from the petroleum ether eluates, 186 mg from petroleum ether-ether elution and 283 mg from the ether eluates. Preparative gas chromatography of the first mixture yielded 501 mg of (+)-longifolene, that of the second mixture led to 5 mg of (+)-copaene,  $[\alpha]_D^{20} + 2.2^\circ$  ( $c=0.5$ , hexane), retention time 20 min at 120°, identical in all respects with an authentic specimen,<sup>11,12</sup> 4 mg of a hydrocarbon of unknown constitution and 16 mg of (+)-longifolene, and, finally, chromatography of the third mixture gave 12 mg of unidentified hydrocarbons (retention time 87 min at 110°) and 13 mg of (–)- $\alpha$ -muurolene,  $[\alpha]_D^{20} - 24^\circ$  ( $c=0.75$ , hexane), retention time 102 min at 110°, infrared bands and proton magnetic resonance signals identical with those recorded for this hydrocarbon.<sup>13</sup>

Chromatography of mixture C on 50 g of activity I alumina impregnated with 10% silver nitrate and elution with petroleum ether yielded 398 mg of an oil whose preparative gas chromatography gave 6 mg of (–)- $\alpha$ -muurolene, 41 mg of (+)- $\alpha$ -bergamotene,  $[\alpha]_D^{20} + 24^\circ$  ( $c=4.1$ , hexane), retention time 9 min at 150°, identical in all respects with an authentic sample, and 10 mg of (+)- $\alpha$ -curcumene,  $[\alpha]_D^{20} + 30^\circ$  ( $c=0.1$ , hexane), retention time 14 min at 150°, identical in all respects with an authentic specimen.<sup>14</sup>

Chromatography of mixture D on 50 g of 100–200 mesh silica gel impregnated with 10% silver nitrate and elution with petroleum ether and with 1–10% ether in petroleum ether yielded 356 mg and 1.28 g of oil, respectively. Preparative gas chromatography of the first eluate gave 11 mg of (+)- $\alpha$ -bergamotene and 2 mg of an unidentified hydrocarbon. The second fraction afforded 20 mg of (–)- $\gamma$ -muurolene (with slight  $\beta$ -selinene contamination),  $[\alpha]_D^{20} - 244^\circ$  ( $c=0.15$ , hexane), retention time 18 min at 150°, identical in

21) A standard, analytical 0.4 × 200 cm column packed with 10% silicone grease on 40–60 mesh Diasolid M was employed at 188°, hydrogen flowing as carrier gas at 45 ml/min. Hibaene, dolabradene and ar-abietatriene showed retention times of 8.6, 9.4 and 11.6 min, respectively.

22) Two standard, analytical 1/8 in × 5 ft column packed with 5% SE-30 on Chromosorb W or with 10% QF-1 on Chromosorb W were employed at various temperatures, nitrogen flowing as carrier gas at 25 ml/min. A preparative 3/8 in × 5 ft column packed with 30% XE-60 on Chromosorb P was used in an Aerograph model A-700 gas chromatograph, helium flowing as carrier gas at 132 ml/min.

all regards with an authentic sample<sup>13)</sup> and 15 mg of (+)- $\beta$ -slinene (with slight  $\beta$ -muurolene contamination),  $[\alpha]_D^{20} +25.0^\circ$ , retention time 22 min at 150°, identical in all respects with an authentic specimen.<sup>16)</sup>

Distillation was used as an alternate mode of separation of the "hydrocarbon fraction." A 9.30 g portion was distilled at 30—77°/0.2 mmHg and the pot residue, 1.20 g, chromatographed on 40 g of 100—200 mesh silica gel impregnated with 10% silver nitrate. Elution with petroleum ether led to 196 mg of a long-chain hydrocarbon mixture, 549 mg of ar-abietatriene, 169 mg of isopimaradiene,  $[\alpha]_D^{20} -30.4^\circ$  ( $c=1.7$ ,  $\text{CHCl}_3$ ), identical in all particulars with an authentic specimen<sup>17)</sup> and a hydrocarbon of unknown constitution. Preparative thin-layer chromatography of the latter on 750  $m\mu$  layers of silica gel G impregnated with 10% silver nitrate and elution with chloroform afforded 22 mg of colorless prisms, mp 54—55°, mass  $m/e$  256.2192+0.0005 (Calcd for  $\text{C}_{16}\text{H}_{28}$ : 256.2191),  $\lambda_{\text{max}}$  (hexane) 233  $m\mu$  ( $\log \epsilon$  2.43),  $\nu_{\text{max}}$  (KBr) 3115, 2940, 2859, 1641, 1440, 1372, 1180, 1139, 999, 909, 889, 860, 825 and 734  $\text{cm}^{-1}$ ,  $\delta$  ( $\text{CDCl}_3$ ) 0.70 (s, 3H), 0.89 (s, 3H), 1.95 (broad s, 5H), 4.65 (d,  $J=13\text{Hz}$ , 2H), 5.35 (broad m, 1H), 12-line signal 4.7—6.1 (3H, vinyl group), ord ( $c=0.08$ , hexane), plain positive curve,  $[\alpha]_{700} 0^\circ$ ,  $[\alpha]_{600} +12^\circ$ ,  $[\alpha]_{589} +15^\circ$ ,  $[\alpha]_{500} +75^\circ$ ,  $[\alpha]_{450} +100^\circ$ ,  $[\alpha]_{400} +150^\circ$ ,  $[\alpha]_{350} +220^\circ$ ,  $[\alpha]_{300} +300^\circ$ ,  $[\alpha]_{250} +720^\circ$ .

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### Kinetics of Diphenylhydantoin Disposition in Man

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Diphenylhydantoin (DPH) is one of the most useful drugs for the treatment of convulsive disorders. It has been, however, proved that there was only a narrow range between the optimum therapeutic and minimally toxic plasma level of DPH.<sup>2)</sup> It was demonstrated that no DPH or only small amounts, approximately 1%, of DPH in unmetabolized form could be recovered in the urine of human subjects<sup>3)</sup> or rats.<sup>4)</sup> Most ingested DPH is hydroxylated in the liver and the product, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH) is finally eliminated through the kidney after being conjugated mainly with glucuronic acid. In order to use DPH effectively, it is necessary to know the rate of its metabolism and excretion and the variable factors which influence the rate.

The rate of drop of plasma DPH levels in human subjects received a single 400 mg oral dose of diphenylhydantoin sodium (DPH-Na) was 50% in 18 to 24 hours.<sup>4)</sup> Solomon and Schrogie<sup>5)</sup> reported that healthy female volunteers were orally given 100 mg of DPH 3 times

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