Terpenoids. VI. The Structure of Thunbergene*,[1]

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Thunbergene (I) $C_{20}H_{32}$; m. p., 60°C, $[\alpha]_D$ $+233°$, is a monocyclic diterpene hydrocarbon which was first isolated from the wood turpentine oil of *Pinus thunbergii, Parl.* by one (S. A.) of the present authors[2]. The compounds which are identical with thunbergene or which are considered to be so have been found to occur in the exudates of many other *Pinus* species[3].

In their studies of the structure of this compound, the authors have arrived at the conclusion that thunbergene has the structure shown in Fig. 1. At almost the same time Professor Dauben proposed that the structure of cembrene is 1-isopropyl-4, 8, 12-trimethylcyclotetradeca-2, 4, 7, 11-tetraene[4]; direct comparison of cembrene with thunbergene proved them to be the same[5].

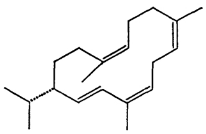

Fig. 1. Thunbergene (I)

The present paper describes the author's own work on this compound. A preliminary communication proposing the structure I (Fig. 1) has already appeared[6]. This structure was assigned to thunbergene (I) on the basis of the following evidence.

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[1] Presented before the Sixth Symposium on Organic Chemistry of Natural Products, Sapporo, Japan, July, 1962.

[2] S. Akiyoshi, *Rep. Osaka Ind. Res. Inst.*, **17**, No. 10 (1937).

[3] a) Y. Sebe, *J. Chem. Soc. Japan (Nippon Kwagaku Kwaishi)*, **56**, 1118 (1935); b) H. Wienhaus, *Nord Kemistmötet* (1926) 211; *Chem. Abstr.*, **24**, 1635 (1930); c) A. J. Haagen-Smit, T. H. Wang and N. T. Mirov, *J. Am. Pharm. Assoc.*, **40**, 557 (1951); d) N. T. Mirov and P. M. Illof, Jr., ibid., **44**, 424 (1955); e) P. M. Illof, Jr. and N. T. Mirov, ibid., **45**, 77 (1956); f) V. A. Pentegova, O. Motl and V. Herout, *Collection Czechoslov. Chem. Commun.*, **26**, 1362 (1961).

[4] W. G. Dauben, W. E. Thiessen and P. R. Resnick, *J. Am. Chem. Soc.*, **84**, 2015 (1962).

[5] The authors are indebted to Professor William G. Dauben of the Department of Chemistry, University of California, Berkeley, for identifying thunbergene with cembrene. For the systematic index of this compound, the authors suggest employing the single name, thnnbergene, which was given to the compound by its first reporter.

[6] H. Kobayashi and S. Akiyoshi, This Bulletin, **35**, 1044 (1962).

The hydrogenation of I gives an octahydro-derivative (II), $C_{20}H_{40}$, $[\alpha]_D \pm 0°$; thus I contains four double bonds and one ring. The infrared spectrum of I shows the strong band at 963 cm^{-1} characteristic of a trans-disubstituted double bond. Its ultraviolet absorption spectrum shows a band at 246 mμ (ε, 17100) indicative of a conjugated diene. The partial hydrogenation of I gives a dihydro-derivative (III) which shows no infrared band near 963 cm^{-1} and a weakened ultraviolet band at 246 mμ (ε, 355). The trans-disubstituted double bond is, therefore, a part of the conjugated diene system.

The ozonolysis of I gives levulinic acid (IV) and 2-isopropyl-5-oxohexanoic acid (V). The latter V, $[\alpha]_D$ $-30°$, is the only optically active product of the ozonolysis and affords a semicarbazone melting at 140°C[7]. The hypobromite oxidation of V furnishes an optically active 2-isopropylglutaric acid (VI), $[\alpha]_D$ $-20°$. Formaldehyde, acetaldehyde and acetone are not found to occur among the ozonolysis products, in contrast to the findings of previous reports[3a,b].

The presence of an isopropyl group is suggested by the infrared doublet band at 1368 and 1387 cm^{-1} of I; it is confirmed by the NMR study (two doublets centered at 9.20 and 9.10 p.p.m.[8], both J, 2.3 c.p.s.) and by the occurrence of V upon the ozonolysis of I. In connection with the conformation of I, it is noted that the NMR bands associated to the methyl of the isopropyl group split to two doublets indicative of the hindered rotation of the isopropyl group.

The lack of infrared bands characteristic of terminal methylene and of vinyl group is consistent with the absence of formaldehyde among the ozonolysis products. The NMR spectrum indicates the presence of two methyl groups attached to ethylenic carbons (8.52 and 8.42 p.p.m.) and of one methyl group on a conjugated diene (8.22 p.p.m); these are concidered to be on double bonds which are in the midst of carbon chains. If this were not the case, I would give acetaldehyde upon ozonolysis.

The groupings of seventeen carbon atoms are now clarified, namely, those corresponding to two ozonolysis products, IV and V, and the conjugated diene bearing a methyl group. The three remaining carbon atoms must belong together to a single group, which cannot be trifunctional or vicinal bifunctional; therefore, the C_3-group must be a propanediylidene-type one. Thus, the groupings of all the carbon atoms are known to be bifunctional, and consequently, the ring structure of I must consist of fourteen carbon atoms and four double bonds.

Consistent with the fourteen-membered ring structure of I is its dehydrogenation with selenium to give an aromatic hydrocarbon (VII) ($C_{19}H_{20}$; m. p., 78°C[9]) which has an ultraviolet spectrum indicative of alkylphenanthrene. Its NMR spectrum can be explained by formula VII (Fig. 2); it reveals the presence of two methyls of an isopropyl group (9.0 p.p.m.), two methyl groups attached to a phenanthrene nucleus (8.0 p.p.m.) and seven hydrogens on a phenanthrene nucleus (2.5 and 1.5 p.p.m), two of them at a lower field position being at the 4- and 5-positions of the nucleus[10].

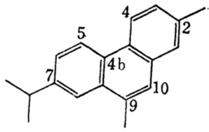

Fig. 2. (VII)

As known already, phenanthrene, if its 9- and 10- positions are both unsubstituted, is readily oxidized, giving an o-quinone which shows a characteristic visible absorption above 400 mμ[11]. The oxidation of VII brings about no change in the visible absorption of this region, indicating that VII resists the oxidation and that, consequently, either the 9- or 10-position should be substituted.

For the first maximum of the p-group[12] of the ultraviolet spectrum of VII occurring at 299 mμ, assignment of the three substituted positions can be made on the basis of the known relationship[13]. Provided that the parent cyclic system is fourteen-membered, an application of the isoprene rule indicates the unique structure of VII as 7-isopropyl-2, 9-dimethylphenanthrene (Fig. 2); this presumption has been confirmed synthetically[14]. A methyl group lost upon the dehydrogenation would have attached to the 4b-position, and the structure II is given to the octahydro-derivative (II) (Fig. 3).

Together with the preceding structural data regarding to the groupings of the carbon atoms,

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[7] O. Wallach, *Ann.*, **379**, 182 (1911).

[8] All chemical shifts are quoted in reference to the τ-scale.

[9] This aromatic hydrocarbon is presumably identical with the C_{20}-dehydrogenation product reported by Sebe, Ref. 3.

[10] J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance", McGraw-Hill Inc., New York (1959), p. 247.

[11] T. Sugasawa, *Chem. Pharm. Bull.* (*Tokyo*), **9**, 894 (1961).

[12] Clar's notation. E. Clar, *Spectrochim. Acta*, **4**, 116 (1950).

[13] a) E. Heilbronner, H. U. Däniker and Pl. A. Plattner, *Helv. Chim. Acta*, **32**, 1723 (1949); b) E. Ochiai, T. Okamoto, S. Sakai and M. Natsume, *Pharm. Bull.* (*Tokyo*), **5**, 113 (1957).

[14] H. Kobayashi, This Bulletin, **35**, 1970 (1962).

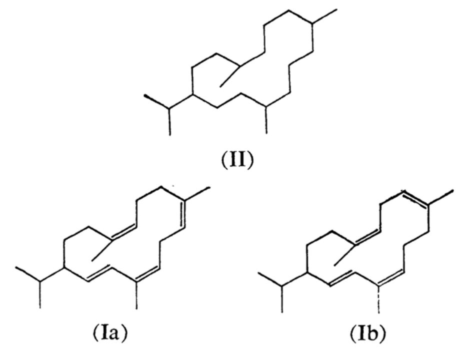

Fig. 3.

structure II gives two equally probable structures, Ia and Ib, for I (Fig. 3). From the biogenetic point of view, structure Ia is rather·preferable since this diterpene hydrocarbon I is considered to be derived from geranylgeraniol in a manner analogous to the formation of the ten-membered monocyclic sesquiterpenoids from farnesol[15] (Fig. 4).

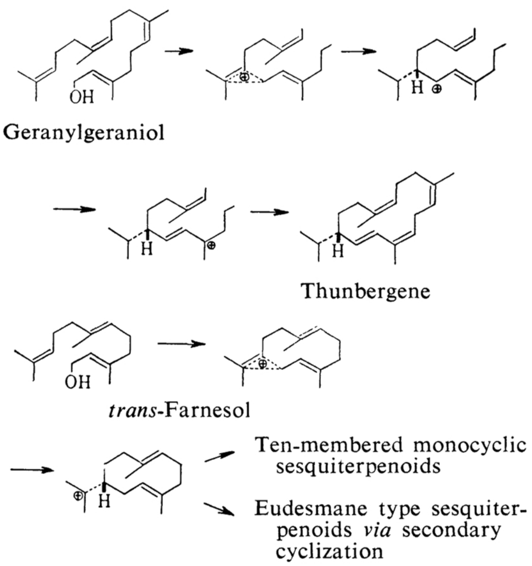

Fig. 4. Hypothetic biogenesis of thunbergene. Comparison with that of sesquiterpenoids proposed by Hendrickson[15].

Structure Ia coincides with that proposed by Dauben[4].

The absolute configuration of the only asymmetric carbon can be directly related to that of (−)-VI, which was confirmed by Fredga[16]. The configuration should be retained throughout the degradation sequence from I to (−)-VI since all the degradation reaction would not affect the asymmetric center.

Thus, Fig. 1 represents the structue of thunbergene (I). This compound is a novel type of diterpene and is the first characterized naturally- occurring fourteen-membered carbon ring compound. It is interesting in connection with the hypothetic biogenesis of I that the absolute configuration of the isopropyl group of I is quite analogous to these found in ten-membered monocyclic sesquiterpenoids or related compounds (Fig. 4).

The stereochemistry of the three trisubstituted double bonds remains to be elucidated. The results of the NMR studies of the stereochemistry of ethylenic double bonds[17] are not considered to be applicable to the elucidation of this problem, because the long-range shielding effect of the double bonds on the methyl groups is difficult to ascertain. Probably the only final solution to the problem will be obtained by the X-ray analysis of a suitable derivative of I.

Experimental[18]

Isolation of Thunbergene (I). — A neutral turpentine oil from the root of *Pinus thunbergii, Parl.* was fractionated by distillation under reduced pressure. Thunbergene (I) was separated as a crystalline mass from the fraction boiling at 135∼155°C/3 mmHg. The minimum content of I found in the wood turpentine oil was 1% by weight. Repeated recrystallizations from ethanol gave colorless needles melting at 59∼60°C, $[\alpha]_D$ +233° (*c* 0.70 in chloroform); molecular weight, 272 (from a parent peak of the mass spectrum of I[19]).

Found: C, 88.14; H, 11.77. Calcd. for $C_{20}H_{32}$: C, 88.16; H, 11.84%.

C-Methyl number: 2.61, 2.64 and 2.54. IR $\tilde{\nu}_{max}$ 1667(w), 1387(s), 1368(s), 963(s), 938, 907 and 837 cm^{-1} (in carbon tetrachloride): UV λ_{max}: 246 mμ (ε, 17100) (in hexane). NMR spectrum[20]: 9.20(3) (doublet, *J* 2.3 c.p.s.) 9.10(3) (doublet, *J* 2.3 c.p.s.), 8.52(3), 8.42(3), 8.22(3), 8.15∼7.80 (broad band), 5.30∼4.30 (broad band), and 3.95 p.p.m.(1) (doublet, *J* 15 c.p.s.).

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[15] J. B. Hendrickson, *Tetrahedron*, **7**, 82 (1959).

[16] A. Fredga and J. Miettinen, *Acta Chem. Scand.*, **1**, 137 (1947).

[17] a) R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.*, **82**, 5749 (1960); b) L. M. Jackman and R. H. Wiley, *J. Am. Chem. Soc.*, **1960**, 2881; c) R. B. Bates, R. H. Carnighan, R. O. Rakutis and J. H. Schauble, *Chem. Ind. (London)*, **1962**, 1020.

[18] All melting points were uncorrected and were measured on a Koffer micro hot stage. Elementary analyses were performed by the Service Center of Elementary Analysis of Organic Compounds, Department of Chemistry, Kyushu University. All spectra in the ultraviolet and visible regions were recorded on a Hitachi model EPS-2 spectrophotometer, and in infrared region on a Nippon Koken model DS-301 double-beam spectrophotometer. The authors wish to thank Miss Yasuko Ikejiri for measuring the ultraviolet and visible spectra, and Miss Yuko Tomita for measuring the infrared spectra.

[19] The authors are indebted to Dr. Curt Enzell of the Royal Institute of Technology, Stockholm, for the mass spectral datum.

[20] The authors are indebted to the staff of the Japan Electron Optics Laboratory, Tokyo, for the NMR spectrum of I. The spectrum was measured in deutrochloroform (an about 20% solution) on a JNM C-60 instrument.

Hydrogenation of I. — Upon catalytic hydrogenation in an ethyl acetate solution (25 ml.) over platinum black, I (1.15 g.) absorbed 502 ml. of hydrogen in the standard state (the calculated amount for 4 mol. of hydrogen, 497 ml.), giving an oily octahydro-derivative (II), n_4^{29} 0.8632, n_D^{29} 1.4730, $[\alpha]_D \pm 0°$ (*c* 7.84 in chloroform), C-methyl number: 2.62.

Found: C, 85.48, 85.35; H, 14.10, 14.30. Calcd. for $C_{20}H_{40}$: C, 85.63; H, 14.37%.

Thunbergene (I) (500 mg.) in ethyl acetate (15 ml.) was hydrogenated with palladium black (100 mg.) until one mole of hydrogen (41 ml. in the standard state) was absorbed. Dihydro-derivative (III) was an oil with the following properties: d_4^{22} 0.890, n_D^{22} 1.5020, $[\alpha]_D$ $-2.2°$ (*c* 8.13 chloroform). UV, λ_{max}: 246 mμ (ε, 355) (in hexane).

Ozonolysis of I. — Ozone was passed through a solution of I (9.8 g.) in methylene chloride (200 ml.) at $-70°$C until the solution turned purple. The resulting ozonide in methylene chloride was added drop by drop into hot 30% hydrogen peroxide (15 ml.). The solvent was taken off by distillation. After the ozonide in hydrogen peroxide had been refluxed for an hour, manganese dioxide was added to decompose the excess hydrogen peroxide. The resulting mixture was steam-distilled. The residue was acidified and extracted with ether, the ethereal extract giving a non-volatile acid (4.85 g.). The aqueous layer was further extracted with ether for 3 hr. by means of an Asahina continuous extraction apparatus[21]. From the second ethereal extract, levulinic acid (IV) (0.91 g.) was obtained; this gave succinic acid upon being treated with sodium hypobromite at 0°C for 3 hr. The semicarbazone of levulinic acid (IV) melting at 184°C was identified by the comparison of the infrared spectra and the mixed melting point determination with an authentic sample.

The non-volatile acid (2.60 g.) was partition-chromatographed on silica-gel (60 g.) holding 0.5 N sulfuric acid (30 ml.). Eluant was wet ether saturated with 0.5 N sulfuric acid. A keto-acid was isolated from the middle fractions and was purified via semicarbazone (m. p., 138~140°C), which was prepared according to Asahina and Kuwata[22] and was recrystallized from water.

Found: C, 52.30; H, 8.55; N, 18.14. Calcd. for $C_{10}H_{19}N_3O_3$: C, 52.38; H, 8.35; N, 18.33%.

The keto-acid (V) (96 mg.) ($[\alpha]_D$ $-30°$ (*c* 0.56 in chloroform)) was regenerated from the semicarbazone (200 mg.) by heating it together with 25% sulfuric acid (10 ml.) for an hour.

The keto-acid (V) (123 mg.) was oxidized to a dicarboxylic acid (VI) (117 mg.) by treating it for 3 hr. at 0°C with a sodium hypobromite solution. Recrystallization from dilute hydrochloric acid gave a sample (VI) (27 mg.) melting at 92~95°C. Repeated recrystallizations from benzene raised the melting point to 94~95°C, $[\alpha]_D$ $-20°$ (*c* 1.64 in chloroform).

The dicarboxylic acid (VI) was dehydrated by heating it together with acetic anhydride for one and a half hour. The anhydride was separated by distillation under reduced pressure and recrystallized from petroleum ether (b. p. 50~70°C); m. p., 72°C. IR $\tilde{\nu}_{max}$ 1810 and 1770 cm^{-1} in carbon tetrachloride.

The degradation products, V, VI and their derivatives, were identified by infrared spectral comparisons in solution with corresponding racemic authentic samples derived from diosphenol[23].

Dehydrogenation of I.—A mixture of I (2.00 g.) and selenium (3.00 g.) was heated at about 300°C for 3 hr. The resulting mixture was washed with 20% aqueous potassium hydroxide and was fractionated by distillation under reduced pressure. Fractions boiling above 180°C/1.5 mmHg (n_D above 1.6025) were combined and chromatographed on alumina. Elution with petroleum ether (b. p. 30~50°C) afforded a fluorescent oil, which gave a picrate (350 mg.) upon treatment with a 6% ethanol solution of picric acid. Repeated recrystallizations from ethanol gave a picrate as orange needles melting at 133.5~134.7°C. From the lower boiling fraction (n_D 1.5832), 50 mg. of the same picrate was obtained.

The aromatic hydrocarbon (VII) was regenerated from the picrate by eluting it from an alumina column using petroleum ether (b. p., 30~50°C)-ethyl ether (1 : 1) as an eluant. The evaporation residue crystallized upon treatment with a small amount of ethanol to give the hydrocarbon (VII) (190 mg.), which was then recrystallized from ethanol; m. p., 77.0~77.8°C. Infrared and ultraviolet spectra were superimposable on these of the authentic sample[14], and the mixed melting point was undepressed.

Found: C, 91.90; H, 8.19. Calcd. for $C_{19}H_{20}$: C, 91.88; H, 8.12%.

UV λ_{max} mμ (log ε): 224 (4.44), 249 (4.48), 257 (5.00), 274 (4.41), 281 (4.37), 288 (4.19), 299 (4.08), 319 (2.46), 328 (2.40), 335 (2.45) (in hexane). NMR spectrum[24]: 1.5, 2.5, 8.0 and 9.0 p.p.m., relative intensities: 2, 5, 6 and 6 respectively.

The chromic acid oxidation of VII and the visible absorption spectroscopy of the oxidation product were performed according to Sugasawa[11].

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[21] Y. Asahina, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **No. 533**, 531 (1926).

[22] Y. Asahina and S. Kuwata, ibid., **No. 491**, 3 (1923).

[23] F. W. Semmler and Mc. Kenzie, *Ber.*, **39**, 1164 (1906).

[24] The proton chemical shifts were measured in a carbon tetrachloride solution in reference to water as an external standard on a Nippon Electron Optics Laboratory model JNM-1 NMR spectroscope, and were converted to the τ-value above quoted (±0.2 p. p. m. accurate), assuming the proton chemical shift of 5.0 p. p. m. for the water reference. The authors wish to thank Mr. Ganzo Kawano for his technical assistance with the NMR spectroscopy.