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Cembrene, a Fourteen-Membered Ring Diterpene Hydrocarbon[*,1,2]

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Cembrene is a monocyclic diterpene hydrocarbon which occurs in the oleoresin of many pine trees of the subgenus *Haploxylon*. Systematic degradation of the compound established the structure as 1-isopropyl-4,8,12-trimethyl-$\Delta^{1,3,7,11}$-cyclotetradecatetraene (**8a**). Cembrene is the first 14-membered ring compound to be found in nature.

In the course of an extensive study of the composition of gum turpentine from pine trees,[4,5] Mirov isolated the crystalline diterpene cembrene (**1**) from the oleoresin of the white bark pine (*Pinus albicaulis*, subgenus *Haploxylon*, group *Cembrae*). Subsequently, this material was found in many other species of the same subgenus[1] and was also shown to be identical with thunbergene[6] (originally named *d*-thumbelene[7]) and the Wienhaus hydrocarbon.[8,9] Cembrene was optically active, possessed four double bonds (quantitative hydrogenation), two of which were conjugated [λ_{max} 245 mμ (ϵ 17,000)], and did not react with maleic anhydride.[5] These data suggested that the hydrocarbon was monocyclic and the conjugated diene was transoid. Dr. Mirov kindly supplied us with a generous supply of this hydrocarbon for our study.

The composition of $C_{20}H_{32}$ for cembrene was confirmed by its mass spectrum (parent peak 272).[10] Upon catalytic hydrogenation in ethyl acetate over palladium on carbon, 4 mole equiv. of hydrogen was absorbed and an octahydro derivative was formed. This material was shown to be a saturated hydrocarbon by its low end absorption in the ultraviolet (ϵ_{205} 200) and by the absence of vinyl proton absorption in its n.m.r. spectrum. These data along with the elemental composition establish the presence of one ring and four double bonds in the parent hydrocarbon. The mass spectrum of both cembrene and octahydrocembrene[10] yielded little structural information other than that a propyl or isopropyl group was readily lost. That this grouping was isopropyl was established by the presence in the n.m.r. spectrum of two sets of doublets centered at τ 9.13 and 9.22 (J = 2 c.p.s. for both bands, each doublet equal to three protons), indicating two nonequivalent methyl groups attached to the same carbon which, in turn, bears one hydrogen atom.

The nature of the unsaturation in cembrene was established by examination of the spectra of the material. The infrared spectrum displayed a strong band at 965 cm.$^{-1}$, characteristic of a *trans*-disubstituted double bond, and a pair of bands at 810 and 840 cm.$^{-1}$, characteristic of trisubstituted linkages. The position of the ultraviolet maximum at 245 mμ, according to Woodward rules,[11] indicated the presence of either six substituents and/or bonds exocyclic to a ring. In the n.m.r. spectrum, there were two methyl bands at τ 8.36 and 8.43, indicative of two methyl groups attached to isolated trisubstituted olefinic double bonds. In addition, there was a methyl band at τ 8.20 (triplet, J = 2 c.p.s.); this absorption was rationalized by postulating that the methyl group was attached to a conjugated double bond and was coupled with a proton on each adjacent olefinic carbon (the triplet is an overlapping quartet). At low field, between τ 3.83 and 5.37 there was absorption for five vinyl protons. A sharp doublet at τ 3.83 and 4.08 for one proton and a splitting of 16 c.p.s. is characteristic of a *trans*-disubstituted double bond. The low field absorption further suggested that this double bond was part of the conjugated diene. These spectral data indicate the presence of the following structural units in cembrene: an isopropyl group, two isolated trisubstituted double bonds carrying

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* To Professor Louis F. Fieser.

(1) This work was reported as a preliminary communication: *J. Am. Chem. Soc.*, **84**, 2015 (1962).

(2) This work was supported in part by Grant 14526 from the National Science Foundation.

(3) National Science Foundation Predoctoral Fellow, 1957–1959.

(4) N. T. Mirov, *U. S. Dept. Agr., Tech. Bull.*, **No. 1239** (1961).

(5) A. J. Haagen-Smit, T. H. Wang, and N. T. Mirov, *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 557 (1951).

(6) S. Akiyoshi, *Rept. Osaka Ind. Res. Inst.*, **17**, No. 10 (1937). We are indebted to Drs. Kobayashi and Akiyoshi for supplying us with a sample of thunbergene for direct comparison. These workers have independently established the structure of the hydrocarbon [*Bull. Chem. Soc. Japan*, **35**, 1044 (1962); **36**, 823 (1963)].

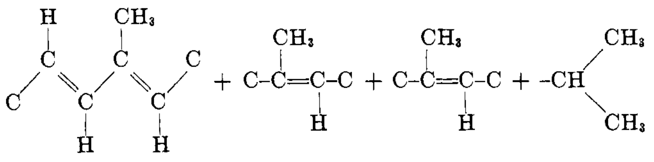
(7) Y. Sebe, *J. Chem. Soc. Japan*, **56**, 1118 (1935).

(8) H. Wienhaus, 3rd Meeting of Nordiska Kemistmotet, Finland, 1926, p. 211 (1928).

(9) The trivial name cembrene will be retained, since the material occurs widely in the subgenus *Haploxylon* of which *Cembrae* is a group.

(10) We are indebted to Dr. D. P. Stevenson of the Shell Development Co. for the mass spectral data.

(11) R. B. Woodward, *J. Am. Chem. Soc.*, **64**, 72 (1942).



methyl groups, and a conjugated diene containing a *trans*-disubstituted double bond.

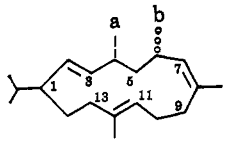
The establishment by n.m.r. spectroscopy of the above diene system with only three substituents, none of which are exocyclic to a ring, is at variance with the earlier indication obtained from the ultraviolet spectrum. The bathochromic shift of the maximum in the ultraviolet must be due either to the placement of the chromophore in a strained molecule[12] or to a transannular participation of one of the isolated double bonds.[13–15]

Reduction of cembrene with lithium in ammonia followed by rapid quenching yielded a mixture of two dihydro derivatives in the ratio of 85% dihydro **2** and 15% dihydro **3**. Slow quenching of the reaction gave a 60:40 mixture. The isomers were separated by preparative vapor phase chromatography and both products showed no maximum in the ultraviolet above 205 mμ, indicating that both isomers resulted from reduction of the conjugated diene. The major product (**2**) retained the 970-cm.$^{-1}$ band characteristic of the *trans*-disubstituted double bond, whereas the minor product (**3**) did not. This result indicates that both 1,2 and 1,4 or 3,4 reduction occurred, a result found with other dienes.[16,17] Cembrene, when hydrogenated over 5% palladium on charcoal, took up 4 mole equiv. of hydrogen, the first mole very quickly, the next two moles more slowly, and the last mole very slowly. The dihydrogenation product contained three components in the ratio of 85:10:5, the major isomer being identical with dihydro **3** prepared above.

The n.m.r. spectrum of dihydro **2** possessed low field absorption equal to four protons and high field absorption corresponding to two methyl groups on olefinic double bonds (τ 8.35), a secondary methyl group (τ 8.85), and the isopropyl group. The n.m.r. spectrum of dihydro **3** possessed absorption equivalent only to three vinyl protons, three methyl groups on olefinic double bonds, and the isopropyl group. These spectra along with the infrared spectra establish that (1) the *trans*-disubstituted olefinic bond is part of the conjugated dienic system; (2) the major product (**2**) in the chemical reduction resulted from saturation of the trisubstituted bond of the conjugated diene; and (3) the major hydrogenation product (**3**) resulted from saturation of the *trans*-disubstituted bond of the diene. The optical rotation changes from +238° for cembrene to +40° for dihydro **2** and to −28° for dihydro **3**. These large decreases in rotation upon reduction suggest that the conjugated diene is contiguous to an asymmetric center and that most likely the *trans*-disubstituted end is the nearer.

Dihydrocembrene **2** upon ozonization in methylene chloride at −70° followed by oxidation of the ozonide with hydrogen peroxide gave rise to three acids (isolated as methyl esters by v.p.c.): levulinic, 2-methylglutaric, and 2-isopropyl-5-oxohexanoic acids. These three acids contain all the 20 carbon atoms of dihydrocembrene **2**. Examination of the structure of these acids clearly show they must be derived from separate portions of the molecule. This result requires that the acids come from a ring since a side-chain fragment would be monofunctional. By counting the number of carbon atoms between the functional groups it is required that a 14-membered carbocyclic ring be present in dihydrocembrene **2** (and in cembrene **1**).

The three acidic fragments could have been derived from one of eight structures, **4a**–**7b**. (To reduce the number of structural formulas to be considered, a dashed (----) or a circled (oooo) line is used to represent alternate positions of methyl groups on a nucleus. When such a representation also is placed in the ring it signifies the position of a nuclear double bond to accompany the position of the methyl group.) Of these structures, **6a** and **6b** seem most unlikely since the spectroscopic evidence does not indicate two disubstituted double bonds. However, in view of the various geometrical arrangements this large ring can assume, it does not seem reasonable to rule out such structures at this time. On the basis of biogenetic concepts, **4a** is to be preferred, but in view of the unusual ring size it was essential to obtain further chemical evidence for the gross structure of cembrene.



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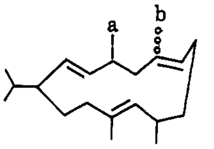

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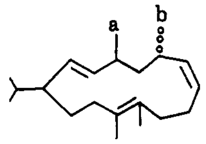

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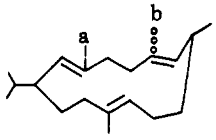

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When cembrene itself was ozonized under similar oxidative conditions, it was found that again 2-isopropyl-5-oxohexanoic acid was formed, and, in addition, there were obtained acetic, malonic, and levulinic acids (all isolated as methyl esters). The ozonide was also processed under reducing conditions using sodium iodide and the resulting mixture was steam distilled to remove malonyldialdehyde and levulinaldehyde. Addition of *p*-nitrophenylhydrazine to the residue gave the corresponding derivative of pyruvaldehyde. Since pyruvic acid is cleaved by hydrogen peroxide to carbon dioxide and acetic acid, the acetic acid isolated upon oxidative work-up must have arisen in this fashion. Thus, again all 20 carbon atoms have been isolated as bifunctional compounds, further confirming the presence of the 14-carbon ring. The finding of pyruvic and malonic acids in the place of 2-methylglutaric acid established the surroundings of the double bond lost on lithium–ammonia reduction. The pyruvic acid must have come from the central two carbon atoms and a methyl group of the conjugated diene, and thus the diene must be part of a seven-carbon unit containing

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[12] For a discussion of this effect, see G. Büchi, R. E. Erickson, and N. Wakabayashi, *J. Am. Chem. Soc.*, **83**, 927 (1961).

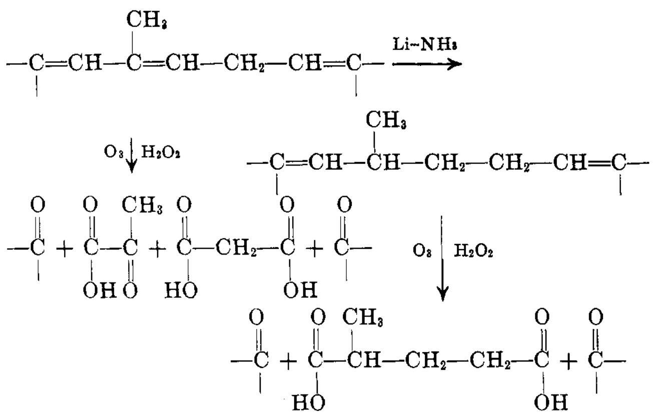
[13] F. Šorm, "Progress in the Chemistry of Natural Products," L. Zechmeister, Ed., Springer-Verlag, Vienna, **19**, 23 (1961).

[14] V. Prelog, A. M. Gold, G. Talbot, and A. Zamojski, *Helv. Chim. Acta*, **45**, 5 (1962).

[15] N. J. Leonard and F. H. Owens, *J. Am. Chem. Soc.*, **80**, 6039 (1958).

[16] A. J. Birch and H. Smith, *Quart. Rev.* (London), **12**, 17 (1958).

[17] J. Castells, E. R. H. Jones, G. M. Meakins, S. Palmer, and R. Swindell, *J. Chem. Soc.*, 2909 (1962).

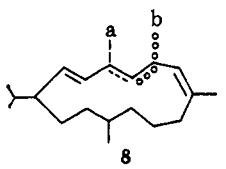


$$-C{=}CH-C(CH_3){=}CH-CH_2-CH{=}C- \xrightarrow{Li-NH_3} -C{=}CH-CH(CH_3)-CH_2-CH_2-CH{=}C-$$

$$\downarrow O_3,\ H_2O_2: \quad -C(=O)- + HO-C(=O)-C(=O)-CH_3 + HO-C(=O)-CH_2-C(=O)-OH + -C(=O)-$$

$$\downarrow O_3,\ H_2O_2: \quad -C(=O)- + HO-C(=O)-CH(CH_3)-CH_2-CH_2-C(=O)-OH + -C(=O)-$$

three double bonds. If this new unit is combined with the units which can yield levulinic and 2-isopropyl-5-oxohexanoic acid, again the same eight-carbon skeletons postulated from dihydrocembrene 2 result but now with the placement of the fourth double bond, **8a**–**11b**.



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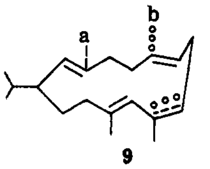

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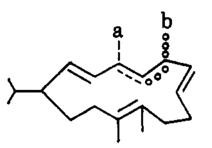

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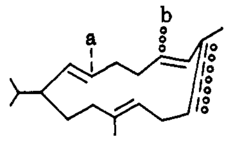

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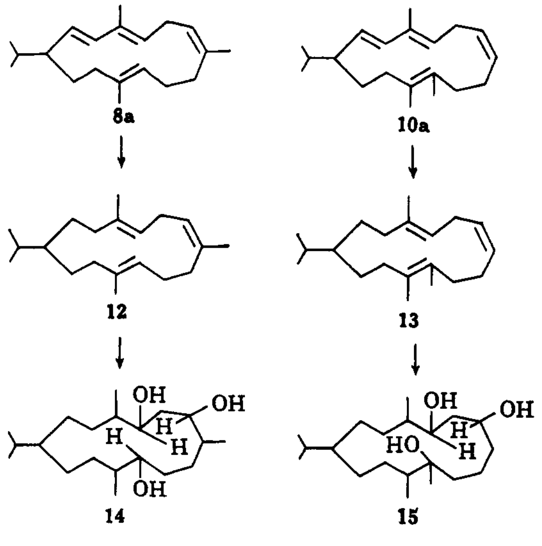
The n.m.r. spectrum of cembrene and the hydrogenation and reduction data demand that a *trans*-disubstituted double bond be part of the conjugated diene system. This feature is only met by structures **8a**, **10a**, **10b**, and **11b**. To further differentiate between these four structures, dihydrocembrene **3**, the isomer containing three trisubstituted olefinic bonds, was ozonized under oxidation conditions. For the first time, a neutral product was obtained (~50% yield), but it could not be identified. The only acids produced in this degradation were malonic and levulinic. There are eight possible dihydrocembrenes containing three methyl groups on double bonds which can result from 1,2 or 1,4 hydrogenation of cembrene. However, only those derivatives derived from **8a** and **10a** possess structures which can yield malonic and levulinic acids upon ozonization. Thus, dihydrocembrene **3** must possess either structure **12** or **13**.

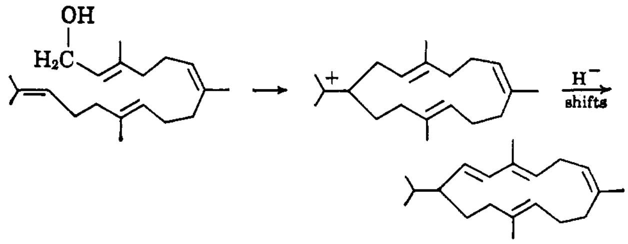


The two remaining structures for cembrene, **8a** and **10a**, differ only in the manner in which a single five-carbon unit is attached, **8a** being biogenetically favored. These two structures can be differentiated by study of their related dihydro derivatives, **12** and **13**, respectively. These olefins were differentiated by examination of the triols obtained from them by hydroboration and oxidation.[18] The n.m.r. spectrum of the triols possessed a broad band at τ 6.28, characteristic of a hydrogen on a carbon holding a hydroxyl group,[19] which corresponded to the absorption of three protons. Acetylation of the triols with acetic anhydride and sodium acetate at 145° gave the triacetate whose n.m.r. spectrum showed a broad band at τ 5.20, corresponding to three-proton absorption. The downfield shift of 1.08 p.p.m. is characteristic of an acetate of a secondary alcohol.[19] These spectra are only possible for triol **14** containing three secondary hydroxyl groups. Thus, the triol precursor, dihydrocembrene **3**, is 1-isopropyl-4,8,12-trimethyl-$\Delta^{4,7,11}$-cyclotetradecatriene (**12**). It follows that dihydrocembrene **2** is the isomeric $\Delta^{2,7,11}$-triene (**4a**) and that cembrene is 1-isopropyl-4,8,12-trimethyl-$\Delta^{2,4,7,11}$-cyclotetradecatetraene (**8a**).

This establishment of the structure of cembrene as **8a** with a 14-carbon ring best accounts for the anomolous ultraviolet spectrum as being due to a transannular effect. No definitive information is available with regard to the stereochemistry of the trisubstituted double bonds but from a study of Dreiding models the structure written containing one *cis* bond appears more favored than an all *trans* structure.

The absolute configuration of the asymmetric carbon was shown to be *S* in that the 2-isopropyl-5-oxohexanoic acid obtained upon ozonization possessed the same sign of optical rotation as the same acid derived from (+)-piperitone. This latter ketone has been related to (*R*)-isopropylsuccinic acid *via* (+)-α-phellandrene.[20] After completion of this work, Minato[21] transformed the enantiomeric keto acid to (*S*)-α-isopropylglutaric acid *via* the haloform reaction.

Cembrene, the first naturally occurring 14-carbon ring compound to be identified, is specially significant from the biogenetic standpoint since it is the monocyclic diterpene derived from geranylgeraniol in a manner analogous to the postulated formation of the germacrane sesquiterpenes from farnesol.[22] The occurence



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[18] H. C. Brown and G. Zweifel, *J. Am. Chem. Soc.*, **83**, 2544 (1961).

[19] L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Ltd., London, 1959, p. 55.

[20] (a) A. Fredga and J. K. Miettinen, *Acta Chem. Scand.*, **1**, 371 (1947); (b) A. J. Birch, *Ann. Rept. Progr. Chem.* (Chem. Soc. London), **47**, 192 (1950).

[21] H. Minato, *Tetrahedron*, **18**, 365 (1962).

[22] J. B. Hendrickson, *ibid.*, **9**, 82 (1959).

of cembrene in the oleoresin of all pines belonging to the *Haploxylon* subgenus and its absence in pines of the *Diploxylon* subgenus suggests that the presence of the hydrocarbon in pine oleoresin may be of phytogenetic significance. Concurrent with the establishment of the structure of cembrene, hydroxylated derivatives of it were shown to be present in tobacco leaves and cigarette smoke.[23–25]

Experimental[26]

Cembrene (1).—Oleoresin collected from *Pinus armandi* was supplied by the Mutual Security Agency, Taipei, Formosa.[27] The oleoresin was processed as described by Mirov and Illoff[28] and the cembrene was obtained as white crystals: m.p. 59–60°; $[\alpha]^{23}\text{D}$ +238° (c 1.13, $CHCl_3$); λ_{max}^{EtOH} 245 mμ (ε 17,000); $\nu_{max}^{CS_2}$ 2920 (s), 1660 (w), 1640 (w), 1380 (m), 1360 (m), 967 (s), 942 (m), 911 (m), 842 (m), 792 (m), and 749 (w) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{32}$: C, 88.16; H, 11.84; mol. wt., 272.46. Found: C, 88.03; H, 11.71; mol. wt., 272 (mass spectrum).

Dihydrocembrene 2.—A solution of 4.2 g. (15.4 mmoles) of cembrene in 300 ml. of absolute ether was added to a 1-l. three-necked flask having a stopcock at the bottom and fitted with a sealed stirrer and a Dry Ice condenser topped with a soda lime tube. Liquid ammonia (400 ml., directly from cylinder) was condensed into the flask, and lithium wire was added with stirring, a few small pieces at first, then as rapidly as possible until 5.1 g. (0.735 g.-atom) had been used. Stirring was continued for 15 min. and the reaction mixture was drained rapidly into a 2-l. beaker containing 48 ml. of absolute ethanol. The resulting mixture was stirred until the blue color had disappeared and then allowed to stand until the ammonia had evaporated. Water was added to the residue and the aqueous layer was separated and extracted three times with ether. The combined ethereal extracts were washed with water until the washes were neutral, and with saturated sodium chloride solution, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure, the residual colorless oil was dissolved in petroleum ether, and the solution was filtered through alumina (activity I). The solvent was evaporated and the colorless oil (4.5 g.) showed no maximum in the ultraviolet above 205 mμ (ϵ_{205}^{EtOH} 11,700). The material was analyzed by v.p.c. using a 5 ft. × 0.25 in. silicone SF-96 column at 215°; two completely separated peaks were obtained and their area ratio was 85:15. The two components were separated using a preparative column.

The major component had $[\alpha]^{22}\text{D}$ +40° (c 0.91, $CHCl_3$); $\nu_{max}^{CS_2}$ 2930 (s), 1660 (w), 1380 (m), 973 (s), and 837 (w) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{34}$ (274.47): C, 87.51; H, 12.49. Found: C, 87.47; H, 12.29.

The minor component possessed an identical infrared spectrum and showed the same v.p.c. retention time on a 5 ft. × 0.25 in. 5% neopentyl glycol succinate column at 148° as the major product obtained by catalytic dehydrogenation (next experiment).

Anal. Found for $C_{20}H_{34}$: C, 87.43; H, 12.44.

Dihydrocembrene 3.—A mixture of 174 mg. of 5% palladium on carbon and 40 ml. of ethyl acetate was prehydrogenated until no further uptake of hydrogen occurred. A solution of 2.56 g. (9.48 mmoles) of cembrene in 40 ml. of ethyl acetate was added and the hydrogenation was continued at atmospheric pressure and room temperature until 1 mole equiv. of hydrogen was absorbed (∼15 min.). The hydrogenation proceeded rapidly to this point and slowed markedly thereafter. The catalyst was filtered and the solvent was evaporated to yield 2.55 g. of a colorless oil. The material was analyzed by v.p.c. using a 5 ft. × 0.25 in. 5% neopentyl glycol succinate column at 180°; one major (85%) and two minor peaks were obtained. The major peak was collected and had $[\alpha]^{24}\text{D}$ −28° (c 1.65, $CHCl_3$); ϵ_{200}^{EtOH} 14,500; $\nu_{max}^{CS_3}$ 2945 (s), 1440 (m), 1380 (m), and 1365 (m) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{34}$ (274.47): C, 87.51; H, 12.49. Found: C, 87.18; H, 12.39.

Hexahydrocembrene.—A mixture of 0.290 g. of 5% palladium on carbon and 40 ml. of ethyl acetate was prehydrogenated, a solution of 1.085 g. (3.98 mmoles) of cembrene in 40 ml. of ethyl acetate was added, and the hydrogenation was continued at atmospheric pressure and room temperature until 3 mole equiv. of hydrogen was absorbed (168 min.). The catalyst was filtered, the solvent was evaporated, and the residue was distilled to yield 0.617 g. (56%) of a colorless oil, b.p. 135° (0.05 mm.). The material was analyzed by v.p.c. using a 5 ft. × 0.25 in. 5% neopentyl glycol succinate column at 150°; there were three major peaks and three to five smaller peaks. The mass spectrum[29] showed 82% hexahydro, 17% tetrahydro, and 1% octahydro. The n.m.r. spectrum showed broad absorption centered at τ 5.13, corresponding to 0.93 vinyl proton.

Anal. Calcd. for $C_{20}H_{38}$ (278.50): C, 96.25; H, 13.75. Found: C, 86.15; H, 13.56.

Octahydrocembrene.—Following the above hydrogenation procedure, a solution of 1.00 g. (3.68 mmoles) of cembrene in 40 ml. of ethyl acetate containing 100 mg. of 5% palladium on carbon was hydrogenated until absorption of hydrogen ceased (∼4 hr.) and 3.95 mole equiv. had been absorbed. The catalyst was filtered, the solvent was evaporated, the residual oil was dissolved in petroleum ether (b.p. 30–60°), and the solution was filtered through a column of alumina (activity I). The solvent was removed under reduced pressure to yield 950 mg. of a colorless oil: $n^{25}\text{D}$ 1.4744; $[\alpha]^{22}\text{D}$ +1.6° (0.5-dm. tube, neat); $\nu_{max}^{CS_2}$ 2925 (s), 1430 (m), 1365 (m), 1355 (m), and 730 (w) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{40}$: C, 85.63; H, 14.37; mol. wt., 280.52. Found: C, 85.47; H, 14.21; mol. wt., 280 (mass spectrum); Kuhn–Roth methyl no., 2.0, 2.71.

Ozonolysis of Dihydrocembrene 2.—Ozone was passed through a solution of 2.74 g. (10.1 mmoles) of crude dihydrocembrene **2** (the 85:15 mixture obtained in the lithium–ammonia reduction) in 70 ml. of methylene chloride cooled in a Dry Ice–methanol bath until the solution turned blue. The ozone uptake was approximately 30 mmoles. The solution was allowed to warm to room temperature and the methylene chloride was removed under reduced pressure at 25°. To the residue there was added 10 ml. of 30% hydrogen peroxide and 20 ml. of water and the mixture was warmed on a steam bath for 4 hr. at which time the oily phase had disappeared. The aqueous solution was extracted with four 40-ml. portions of ether and then extracted continuously with ether for 24 hr.

The combined extracts from the first four extractions were dried over magnesium sulfate, and the ether was removed under reduced pressure. The oily residue (1.0 g.) was esterified with diazomethane and the resulting esters were separated by v.p.c. using a 5 ft. × 0.25 in. silicone SF-96 column at 180°.

The first component had the same infrared spectrum and retention time as authentic methyl levulinate. The 2,4-dinitrophenylhydrazone, m.p. 144–145°, gave no melting point depression on admixture with authentic methyl levulinate 2,4-dinitrophenylhydrazone.

The second component had the same infrared spectrum and retention time as authentic dimethyl 2-methylglutarate. A 70-mg. portion of the ester was boiled with a solution of 400 mg. of potassium hydroxide, 46 ml. of methanol, and 4 ml. of water for 1 hr. The reaction mixture was diluted with water, acidified, and extracted continuously with ether. The ethereal extract was dried, and the solvent was removed under reduced pressure to give 50 mg. of a pale yellow oil which crystallized on standing. The solid was recrystallized from water to yield colorless prisms, m.p. 75–76°. No melting point depression was obtained upon admixture with an authentic sample, m.p. 75–76°.[30]

The third component had the same infrared spectrum and retention time as authentic methyl 2-isopropyl-5-oxohexanoic

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(23) D. L. Roberts and R. L. Rowland, *J. Org. Chem.*, **27**, 3989 (1962).

(24) R. L. Rowland and D. L. Roberts, *ibid.*, **28**, 1165 (1963).

(25) R. L. Rowland, A. Rodgman, J. N. Schmacher, D. L. Roberts, L. C. Cook, and W. E. Walker, Jr., *ibid.*, **29**, 16 (1964).

(26) Analyses were performed by the Microanalytical Laboratory, College of Chemistry, University of California, Berkeley. All chemical shifts are quoted in reference to external tetramethylsilane.

(27) We are indebted to Dr. H. G. Schenk and Mr. P. Zengraff of the Mutual Security Agency and to Dr. N. T. Mirov for their assistance in procuring the oleoresin sample. We are also indebted to Mr. V. Alhadeff for the earlier isolation and chemical studies.

(28) N. T. Mirov and P. M. Iloff, Jr., *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 424 (1955).

(29) The mass spectrum was kindly determined by Dr. Richard Teeter of the California Research Corp., Richmond, Calif.

(30) F. H. Howles, J. F. Thorpe, and W. Udall, *J. Chem. Soc.*, **77**, 942 (1900).

acid. It possessed $[\alpha]^{26}\text{D}$ −12.2° (*c* 1.12, $CHCl_3$) and its semicarbazone melted at 124–126°.

The ethereal solution obtained from the continuous extraction of the original aqueous solution was dried with magnesium sulfate, the solvent was evaporated, and the residue was esterified with diazomethane to yield 1.2 g. V.p.c. analysis showed the presence of methyl levulinate and dimethyl 2-methylglutarate.

Ozonolysis of Dihydrocembrene 3.—A solution of 2.66 g. (9.70 mmoles) of dihydrocembrene **3** in 80 ml. of methylene chloride was ozonized treated with hydrogen peroxide as described above. The hydrogen peroxide solution was cooled, made basic with potassium carbonate, and extracted with two portions of methylene chloride. The aqueous layer was acidified with sulfuric acid and the resulting aqueous solution was continuously extracted with ether for 50 hr. The ether was dried, and the solvent was evaporated to yield 2.0 g. of crude acids which were esterified by heating with methanol and sulfuric acid. The esters were separated, preparatively, using a 5 ft. × 0.25 in. silicone SF-96 column at 180° and were identified as dimethyl malonate and methyl levulinate by identity of retention times and infrared spectra with those of authentic samples.

The neutral organic layer was dried and the solvent was removed to give 1.55 g. of an oily residue. The infrared spectrum of the oil showed the presence of hydroxyl and carbonyl absorption but no crystalline derivatives could be prepared.

Ozonolysis of Cembrene (1). A. Oxidative Procedure.—Ozone was passed through a solution of 2.72 g. (10.0 mmoles) of cembrene in 70 ml. of methylene chloride cooled in a Dry Ice–methanol bath until a blue color persisted. The ozone uptake was about 40 mmoles. The solution was allowed to warm to room temperature and the solvent was removed under reduced pressure at 25°. The residual oil was treated with 20 ml. of water and 10 ml. of 30% hydrogen peroxide, and the mixture was heated on a steam bath until the oily layer was no longer visible. The aqueous solution was extracted with four 40-ml. portions of ether.

The combined ethereal extracts were re-extracted with two 20-ml. portions of 5% sodium bicarbonate solution. The bicarbonate extract was acidified with 20% hydrochloric acid and the acidified mixture was extracted with four 40-ml. portions of ether. The ethereal extracts were evaporated under reduced pressure to yield 1.1 g. of a liquid which had a strong odor of acetic acid. The residue was distilled and acetic acid, b.p. 118°, was obtained; the infrared spectrum of the product was identical with that of an authentic sample. The pot residue which remained after partial removal of the acetic acid was dissolved in ether and esterified with diazomethane to give 0.7 g. of a pale yellow oil. V.p.c. analysis using a 5 ft. × 0.25 in. silicone SF-96 column at 110° showed the presence of three major constituents.

The first component was methyl acetate. The second component was separated and possessed the identical retention time and infrared spectrum as authentic methyl levulinate. The 2,4-dinitrophenylhydrazone, m.p. 144–145° (crystallized from ethanol), gave no melting point depression on admixture of authentic methyl levulinate 2,4-dinitrophenylhydrazone. A mixture of 45 mg. of the second component, 300 mg. of potassium hydroxide, 37 ml. of methanol, and 3 ml. of water was heated under reflux for 30 min. The reaction mixture was diluted with 50 ml. of water, acidified with dilute hydrochloric acid, and exhaustively extracted with ether. Evaporation of the ether yielded an oil which was allowed to react with 2,4-dinitrophenylhydrazine in the usual manner. The solid derivative formed, m.p. 199–201°, gave no melting point depression on admixture with an authentic sample of levulinic acid 2,4-dinitrophenylhydrazone. The oil also yielded a *p*-bromophenacyl ester, m.p. 83–85° (crystallized from ethanol), which gave no melting point depression on addition of authentic *p*-bromophenacyl levulinate.

The third component was separated and possessed $[\alpha]^{22}\text{D}$ −13° (*c* 0.50, $CHCl_3$). The material also possessed retention time and infrared spectrum identical with those of authentic methyl 2-isopropyl-5-oxohexanoate. The ester (100 mg.) was saponified and 70 mg. of an acidic oil was obtained which gave an infrared spectrum identical with that of 2-isopropyl-5-oxohexanoic acid and which yielded a semicarbazone, m.p. 135–137° (crystallized from water), which gave no melting point depression on admixture with authentic 2-isopropyl-5-oxohexanoic acid semicarbazone.

The original aqueous layer was continuously extracted with ether for 24 hr. The ethereal solution was dried, concentrated to a 30-ml. volume, and added to a solution of diazomethane. The ether was removed by distillation through a glass-packed column. V.p.c. analysis of the residue with a 5 ft. × 0.25 in. silicone SF-96 column at 80° showed the presence of two major components. The higher boiling component had the same retention time as authentic methyl levulinate. The lower boiling component had the same retention time and infrared spectrum as authentic dimethyl malonate.

B. Reductive Procedure.—Ozone was passed through a solution of 1.37 g. (5.0 mmoles) of cembrene in 20 ml. of methylene chloride and 50 ml. of methanol cooled in a Dry Ice–methanol bath until a blue color persisted. The solution was allowed to warm to room temperature, diluted with a solution of 5 g. of sodium iodide and 2 ml. of glacial acetic acid in 20 ml. of water, and allowed to stand at room temperature for 1 hr. Sufficient sodium thiosulfate solution was added to remove the iodine color, the reaction solution was diluted with 300 ml. of water, and the mixture was steam distilled until 300 ml. of distillate has been collected. The distillation residue was filtered and treated with a solution of 5.7 g. of *p*-nitrophenylhydrazine in 3 ml. of glacial acetic acid and 125 ml. of 95% ethanol. The precipitate which formed was filtered and recrystallized twice from dimethylaniline to give red crystals, m.p. 283–285°. A mixture melting point with authentic pyruvaldehyde *p*-nitrophenylosazone showed no depression. The melting points of the osazones were strongly dependent on the rate of heating.

Hydroboration of Dihydrocembrene 3.—To a mixture of 2.77 g. (10.0 mmoles) of dihydrocembrene **3**, 1.25 g. (33.0 mmoles) of sodium borohydride, and 60 ml. of diglyme cooled to 5° and under nitrogen atmosphere there was added with stirring over a 30-min. period 7.93 g. (56 mmoles) of freshly distilled boron trifluoride etherate in 20 ml. of diglyme. The reaction mixture was stirred for an additional 2.75 hr., 20 ml. of water was added cautiously (gas evolution), and then a solution of 3.0 g. (75 mmoles) of sodium hydroxide in 25 ml. of water was added. The mixture was stirred for 15 min., 15 ml. of 30% hydrogen peroxide was added slowly, and the mixture was stirred for 18 hr. and diluted with 50 g. of ice. The mixture was extracted with four portions of ether, the combined ethereal extracts were washed with ice–water and dried, and the solvent was evaporated. The residual oil (3.32 g.) was dissolved in ether and chromatographed on alumina (activity III). Elution with ether gave 2.51 g. of an oil and elution with benzene yielded 0.62 g. of a viscous liquid. Rechromatography of the ether eluate with benzene yielded an additional 0.32 g. of viscous liquid. The total yield of benzene eluate was 0.94 g., corresponding to a 27% yield of triol.

Anal. Calcd. for $C_{20}H_{40}O_3$ (328.52): C, 73.12; H, 12.27. Found: C, 72.88; H, 12.10.

The n.m.r. spectrum of triol in deuteriochloroform showed a broad high-field envelope at τ 7.7–9.0 and two small peaks at 6.28 (three protons) and 6.75 (three protons). Successive dilutions of the sample shifted the last peak to τ 7.35, 7.63, and finally 8.00, while the other peaks remained stationary.

A mixture of 0.50 g. (1.53 mmoles) of dihydrocembrenetriol, 5.0 ml. (53 mmoles) of acetic anhydride, and 0.75 g. of anhydrous sodium acetate was heated at 145° for 5 hr. The reaction mixture was processed in the usual fashion and there was obtained 0.68 g. of an oil. This oil was dissolved in ether, the ethereal solution was filtered through alumina (activity III), and the ether was evaporated to yield 0.557 g. (83%) of triacetoxydihydrocembrene **3**. The viscous oil was evaporatively distilled at 180–200° (0.05 mm.), ϵ_{200} 2400.

Anal. Calcd. for $C_{26}H_{46}O_6$ (454.63): C, 68.68; H, 10.20. Found: C, 68.60; H, 10.06.

The n.m.r. spectrum of the triacetate in carbon tetrachloried showed a broad high field band at τ 7.7–9.6 and a smaller broad band centered at 5.20 (3 protons).

2-Isopropyl-5-oxohexanoic Acid from Piperitone.—A solution of 7.80 g. (51 mmoles) of piperitone, $[\alpha]^{24}\text{D}$ −20.5° (*c* 2.30, $CHCl_3$), in 88 ml. of methanol was ozonized at 0° until ozone was no longer absorbed. The reaction mixture was added to 80 ml. of water and 10 ml. of 30% hydrogen peroxide, allowed to stand at room temperature for 5 hr., heated under reflux for 11 hr., and cooled to room temperature. Excess 5% potassium bicarbonate solution was added, and the aqueous solution was extracted with three portions of ether. The aqueous layer was acidified with concentrated sulfuric acid and extracted continuously with ether for 39 hr. The ethereal extract was dried, and the solvent was evaporated to yield 5.84 g. (66.5%) of crude 2-isopropyl-5-oxohexanoic acid, $[\alpha]^{26}_{D}$ +5.45° (c 2.58, $CHCl_3$), semicarbazone m.p. 152–155°. The enantiomer is reported[21] to have $[\alpha]_{D}$ −5.0°.

The methyl ester was prepared by allowing the crude acid to react with 50 ml. of methanol and 1 ml. of concentrated sulfuric acid on a steam bath for 6 hr. The reaction mixture was processed in the usual manner and the pure ester was obtained by v.p.c. using a 5 ft. × 0.25 in. Carbowax column at 210°. The ester had n^{21}_{D} 1.4335, $[\alpha]^{25}_{D}$ +10.1° (c 2.67 $CHCl_3$), and the semicarbazone melted at 124.0–125.5°.

Anal. Calcd. for $C_{11}H_{21}N_3O_3$ (243.30): C, 54.30; H, 8.70; N, 17.27. Found: C, 54.26; H, 8.54; N, 17.01.

The enantiomer is reported[21] to have $[\alpha]_{D}$ −9° and the semicarbazone m.p. 128.0–129.5°.

Oxidative Rearrangements of Pentacyclic Triterpenes. A Method for the Synthesis of Certain Naturally Occurring Triterpenes from α- and β-Amyrin[1]

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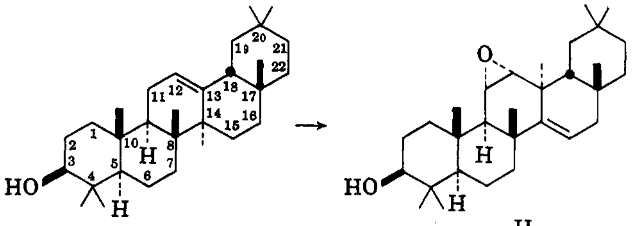
Several new rearrangement reactions of pentacyclic triterpenes are described, a number of which involve rearrangement to a carbon skeleton that is thermodynamically less stable than the initial skeleton. These and related rearrangements provide the basis for a general approach to the partial synthesis of a number of naturally occurring triterpenes, *e.g.*, friedelin. In essence the method involves the coupling of a rearrangement step with an exothermic reaction such as electrophilic addition to a carbon–carbon double bond. Specific illustrations which are presented include the syntheses of 11α,12α-oxidotaraxerol (II), taraxerol, and multiflorenol (XXVI) from β-amyrin.



I + O_2 → II + H_2O

During a program of studies in the field of pentacyclic triterpenes at the University of Illinois some 10 years ago,[2] it was discovered that ethanolic solutions of a mixture of α- and β-amyrins (obtained from *Manila elemi* resin) upon prolonged exposure to air and sunlight at room temperature deposit a colorless crystalline solid of composition $C_{30}H_{48}O_2$. It was ascertained from subsequent studies that this new substance was *not* present in the original mixture, that it is an oxidation product derived from β-amyrin, and that its formation involves some novel and interesting chemistry. At the outset it was apparent that this product was the result either of carbon rearrangement or functionalization at a saturated carbon, or both, and, since these topics were under active study in our laboratory,[2b,3,4] the incidental discovery assumed a surprising relevance to the pre-existing research program. The new photooxidation product obtained from β-amyrin (I) was eventually shown to be a taraxerene derivative (II) by the chemical studies which are detailed in this paper, and a new kind of oxidative rearrangement was uncovered. The taraxarene skeleton is unstable relative to the olean-12-ene (β-amyrin) system as shown by the rearrangement of taraxerol (II minus the epoxide function) to β-amyrin (I) under acid catalysis.[5,6] In the conversion I → II, therefore, there is an intrinsic driving force which overcomes the energetically unfavorable change in the arrangement of carbon and hydrogen and the skeletal rearrangement can be considered as "powered" by the oxidation. Clearly, rearrangements can be differentiated generally with reference to whether the carbon-skeletal change is energetically favorable or unfavorable. The former can be effected merely by the application of a suitable catalytic agent (*e.g.*, an acid), but the latter require coupling to an exergonic (free-energy releasing) reaction which provides the thermodynamic driving force. This distinction serves a useful purpose in the analysis of certain synthetic and biosynthetic problems; it is made here to underscore the broader aspects of the rearrangement I → II, which are also considered in subsequent sections of this paper. The generalization of the idea of oxidatively driven rearrangements leading to less stable carbon skeletons provides a clue to the interesting problem of reversing exergonic rearrangements such as the friedelanol → olean-13,18-ene multigroup rearrangement.[2b] A part of this paper deals with this particular synthetic problem

(1) (a) Submitted in honor of Professor Louis F. Fieser. (b) Presented in part at the Gordon Conference on Natural Products, New Hampton, N. H., Aug. 1960.

(2) Several of the main objectives of these investigations have already been reached and described in publications dealing with structure, synthesis, and stereochemistry in the pentacyclic triterpene series. These include (a) the stereochemistry of the α-amyrin group, E. J. Corey and J. J. Ursprung, *Chem. Ind.* (London), 1387 (1954), and *J. Am. Chem. Soc.*, **78**, 183 (1956); (b) the structure and stereochemistry of friedelin and the multigroup rearrangement from the friedelan to the olean series, E. J. Corey and J. J. Ursprung, *ibid.*, **77**, 3667, 3668 (1955); **78**, 5041 (1956); (c) the total synthesis of pentacyclosqualene, E. J. Corey and R. R. Sauers, *ibid.*, **79**, 3925 (1957); **81**, 1739 (1959); (d) the partial synthesis of α-amyrin from a β-amyrin derivative, E. J. Corey and E. W. Cantrall, *ibid.*, **80**, 499 (1958); **81**, 1745 (1959); and (e) the total synthesis of a β-amyrin derivative, oleana-11,13(18)-diene, E. J. Corey, H.-J. Hess, and S. Proskow, *ibid.*, **81**, 5258 (1959); **85**, 3979 (1963). The last reference in each group is a detailed paper which includes a bibliography of important papers on related topics. (f) For a recent review on pentacyclic triterpenes, see J. Simonsen and W. C. J. Ross, "The Terpenes," Vol. V, Cambridge University Press, London, 1957.

(3) E. J. Corey and W. R. Hertler, *ibid.*, **80**, 2903 (1958); **81**, 5209 (1959).

(4) E. J. Corey and R. W. White, *ibid.*, **80**, 6686 (1959).

(5) J. M. Beaton, F. S. Spring, R. Stevenson, and J. L. Stewart, *J. Chem. Soc.*, 2131 (1955).

(6) C. J. W. Brooks, *ibid.*, 1675 (1955).