

Structural Studies on Terebinthone from *Schinus terebinthefolius*

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A new triterpene, terebinthone, has been isolated from the berries of *Schinus terebinthefolius* (Radd.). Analyses indicate the formula $C_{30}H_{46}O_3$. Spectral evidence indicates that the compound is a diketone with one group in the C-3 position and the second in a highly hindered position. Reduction and hydrogenation studies supported these findings. Ozonolysis produced acetaldehyde showing that the single double bond is in the side chain as an ethylidene group. The third oxygen of terebinthone is believed to be in a cyclic ether ring.

THE TREE *Schinus terebinthefolius* (Radd.), also known as the Brazilian pepper tree, belongs to the family *Anacardiaceae*. It is a small evergreen tree found in south and central Florida. During the winter months numerous clusters of small, bright red berries appear (1). These berries are said to exert a paralyzing effect on birds upon ingestion.

This study was initiated in an effort to evaluate the principle producing this action, to identify it as well as other major isolable products chemically, and to determine if there may be anything of therapeutic value. In the course of the study, the berries were initially defatted with hexane. From this solvent there was obtained a crystalline substance, which, though not possessing the aforementioned pharmacology, still was of considerable interest chemically. This compound is the subject of this first communication.

Analyses of this compound, provisionally named terebinthone, and several derivatives indicated that the molecular formula $C_{30}H_{46}O_3$ best fit the data. Further confirmation of this formula was found in the closeness of the Rast molecular weight to the theoretical value.

It was concluded that terebinthone was a triterpene from the finding of four C-methyl groups, gem dimethyl and terminal methyl group absorption in the infrared (1385 and 1365 cm^{-1}) (2, 3), the close similarity in the infrared spectra of terebinthone and its derivatives with other triterpenes, color tests, and the molecular formula.

Perbenzoic acid titration (4) indicated the presence of one double bond. Infrared analysis showed two ketonic carbonyl group absorptions at 1708 and 1690 cm^{-1} . The remaining oxygen function did not appear as a carbonyl or hydroxyl

group in the infrared spectrum, but an absorption at 1095 cm^{-1} was observed in terebinthone and several other derivatives which has been assigned to the C—O—C group of a cyclic ether (5, 6).

The presence of two carbonyl groups and one double bond indicated that terebinthone is basically tetracyclic, and with the cyclic ether would be pentacyclic. This conclusion is in agreement with the molecular formula.

A closer examination of the carbonyl groups indicated that the peak at 1708 cm^{-1} could be ascribed to an isolated aliphatic or isolated six-membered ring ketone (3, 7). Terebinthone showed no absorption in the conjugated carbonyl region of the ultraviolet, confirming the fact that both keto groups are isolated. It was concluded that the 1708 cm^{-1} absorption peak was due to a 3-keto group from the fact that terebinthone gave a positive Zimmermann reaction (8) and an absorption maximum at 367 $m\mu$ for the mono-2,4-dinitrophenylhydrazone derivative. Reich (9) has shown that absorption in the 367–369 $m\mu$ region is due to the presence of this derivative of a 3-keto group.

The 1703–1690 cm^{-1} region is reported (2, 10) to be due to a saturated keto group in a hindered position. This is in agreement with the absorption of the second carbonyl group of terebinthone (1690 cm^{-1}). The fact that this group was hindered was shown by the fact that only a mono-2,4-dinitrophenylhydrazone could be formed even under forcing conditions. This derivative still showed the 1690 cm^{-1} absorption.

Reduction of terebinthone with lithium aluminum hydride (11) gave a diol, terebinthol, $C_{30}H_{50}O_3$. This compound had only one acylable function, forming a mono-3,5-dinitrobenzoate, confirming the presence of a highly hindered keto group in terebinthone. The 1095 cm^{-1} absorption (cyclic ether) indicated that no change had occurred in the ether ring. Terebinthol gave a positive Liebermann-Burchard reaction (12) indicative of a 3-hydroxyl group.

Hydrogenation of terebinthone to dihydro-

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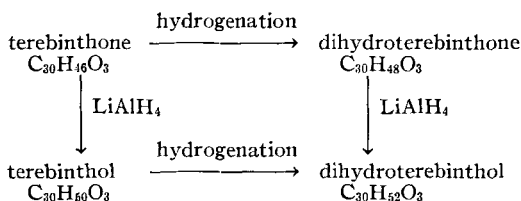
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terebinthone, $C_{30}H_{48}O_3$, with the measured consumption of hydrogen equivalent to one double bond, confirmed the presence of one reactive double bond in the molecule. Dihydroterebinthone gave a positive Zimmermann reaction and formed only a mono-oxime even under vigorous conditions (13). The 1095 cm.^{-1} absorption was present indicating no cleavage of this ring.

Hydrogenation of terebinthol and lithium aluminum hydride reduction of dihydroterebinthone gave the same compound, dihydroterebinthol, $C_{30}H_{52}O_3$.



The compound retained the 1095 cm.^{-1} absorption peak (cyclic ether) in the infrared. A mono-tosylate was prepared which confirmed the fact that all four of these compounds contain a highly hindered oxygen function.

Zaffaroni (14) described a technique useful for partial characterization of steroids which consists of measurement of absorptions of concentrated sulfuric acid solutions from 200 to 500 $m\mu$. The principal maxima for the four compounds above was at 304 $m\mu$. Absorption between 280 and 296 $m\mu$ is associated with an α,β -unsaturated ketone (15). The fact that all four compounds exhibited the same maxima clearly indicates that no change has occurred in the positions of the oxygen functions and that the double bond, when present, does not exhibit any effect on any of these.

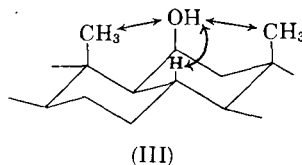
The Wolff-Kishner reduction (16) of dihydroterebinthone gave a major product with a molecular formula of $C_{30}H_{50}O_2$. The infrared analysis showed absorption at 1095 cm.^{-1} (cyclic ether) and 1703 cm.^{-1} which must be due to the same hindered carbonyl group previously discussed, since a successful Wolff-Kishner reduction depends upon the formation of a phenylhydrazone intermediate and no derivatives could be made of one carbonyl group. Furthermore, this compound, 3-deoxy-dihydroterebinthone gave no Zimmermann reaction, indicating that the 3-keto group had been reduced.

There can be no question that the hindrance of one oxygen function in these compounds is of the magnitude of the C-11 position in steroids or the C-7 and C-11 positions of tetracyclic triterpenes. In the latter case, the C-7 carbonyl group of

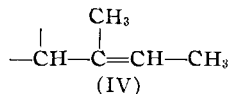
lanostane-7,11-dione-3-yl acetate (I) has been reduced by the Wolff-Kishner method (17) to the 11-monoketone (II). Though this does not



exclude the possibility of a C-7 keto group in terebinthone, or other possibilities as well, the evidence strongly suggests that the hindered keto group might well be in the C-11 position. If reduction of this keto group occurs to give an 11β -hydroxyl group, then this would account for the failure to form a derivative of this function. The 11β -hydroxyl group is practically unacylable (18) due to steric strain from two angular methyl groups and a hydrogen (III).



Ozonolysis (19) of terebinthone gave a volatile compound identified through its 2,4-dinitrophenylhydrazone as acetaldehyde. It appeared likely from this evidence that the double bond was located in the side chain as an ethylidene group. In an attempt to learn more of the environment of this unsaturation, terebinthone was treated with dry hydrogen chloride (20) to induce rearrangement if possible. The product, which could not be purified, was treated with ozone as before. The volatile product, identified through a derivative, was methyl ethyl ketone. From these reactions it is possible to conclude that the side chain must possess the arrangement (IV).



In regard to the location of the cyclic ether function, no evidence is yet available though studies are continuing in this laboratory.

EXPERIMENTAL

All melting points were taken on the Kofler apparatus unless otherwise stated. Analyses were performed by the Weiler and Strauss Microanalytical Laboratory, Oxford, England, and Galbraith Laboratories, Knoxville, Tenn. Spectral work was done by the Department of Chemistry, University of Florida.

Plant Material.—The ripe berries of *Schinus terebinthifolius* (Radd.) were gathered during the winter months of 1959–1960 and 1960–1961 near St. Petersburg, Fla.¹ A voucher specimen of the plant is deposited in the herbarium of the University of Florida. The berries were air-dried for 3 days then minced in a meat grinder.

Isolation of Terebinthone.—Nine hundred grams of the minced berries was extracted with hexane in a Soxhlet extractor for 24 hours. The hexane was gently expelled under an air stream leaving a brown, viscous oil weighing 117 Gm. A crystalline deposit gradually formed in the oil and several crops were filtered from it. An average of 4 Gm. of crystalline material was obtained from several extractions or about 0.45% of the crude berries. The crude material was recrystallized several times from acetone and finally from alcohol to give fine needles, m.p. 178–179°, $[\alpha]_D^{25} - 72.6^\circ$ (chloroform). Chromatography of the material on a Celite column did not improve these values. The name terebinthone was provisionally assigned to this compound.

Anal.—Calcd. for $C_{30}H_{48}O_3$: C, 79.24; H, 10.20; mol. wt., 454; C-methyl, 13.3 (four). Found: C, 79.22; H, 10.22; mol. wt. (Rast), 445, 456; C-methyl, 12.7.

Infrared analysis indicated absorption at 1708 and 1690 cm^{-1} (two carbonyls), 1640 cm^{-1} (double bond), 1385 and 1365 cm^{-1} (gem dimethyl and angular methyl) and 1095 cm^{-1} (cyclic ether). Ultraviolet analysis indicated absorption at 213 $m\mu$ ($E = 7272$). The compound gave a positive Zimmermann test and a negative test with tetranitromethane. The material was insoluble in hot sodium hydroxide solution.

Terebinthone Mono-2,4-dinitrophenylhydrazone.—A solution of 60 mg. of terebinthone in 10 ml. of aldehyde-free alcohol was treated with 25 ml. of U. S. P. 2,4-dinitrophenylhydrazine solution and refluxed for 4 hours. The product was isolated in the usual way and recrystallized from ethanol-ethyl acetate to give orange needles, m.p. 221–223°.

Anal.—Calcd. for $C_{38}H_{50}N_4O_6$: C, 68.11; H, 7.94; N, 8.83. Found: C, 68.75; H, 7.57; N, 9.19.

Ultraviolet analysis indicated maximum absorption at 367 $m\mu$ ($E = 1409$). Infrared analysis showed absorption at 1690 cm^{-1} (carbonyl).

Perbenzoic Acid Titration of Terebinthone.—A 40-mg. sample of terebinthone was dissolved in purified chloroform at 5° and made up to exactly 10 ml. with the same solvent. Exactly 9 ml. of this solution was withdrawn and added to exactly 5 ml. of a solution of perbenzoic acid in benzene at 5°. The mixture was swirled and kept at 5° for 24 hours. A blank was prepared using 9 ml. of purified chloroform. At the end of 24 hours, a 2-ml. aliquot was added to a solution of 1 Gm. potassium iodide, 1 ml. acetic acid, and 20 ml. of water. The liberated iodine was titrated with 0.01 *N* sodium thiosulfate. The perbenzoic acid consumed was equivalent to 0.98 double bond. At the end of 48 hours, the acid consumption was equivalent to 1.1 double bonds.

Ozonolysis of Terebinthone.—A solution of 200 mg. of terebinthone in 25 ml. of carbon tetrachloride at 0° was treated with a stream of 3% ozonized oxygen for 30 minutes. Water was then added to the reaction mixture and the volatile constituents were then steam distilled into a solution containing 200 mg. of 2,4-dinitrophenylhydrazine, 14 ml. of 40% sulfuric acid, and 6 ml. of water. About half of the carbon tetrachloride was steam distilled to insure completeness of the distillation. The distillate mixture was shaken and the organic layer separated. The aqueous phase was then extracted with ether which was added to the carbon tetrachloride solution. The residue from this solution was recrystallized from ethanol to give needles, m.p. 143–145°. A mixed melting point with the corresponding acetaldehyde derivative gave no depression and the infrared spectra were identical.

Acid Treatment of Terebinthone.—A solution of 400 mg. of terebinthone in 30 ml. of distilled chloroform was treated with a slow stream of dry hydrogen chloride for 7 hours. The hydrogen chloride and the chloroform were then removed under vacuum leaving an oily residue which could not be crystallized. This residue was then treated with ozone as previously described. The 2,4-dinitrophenylhydrazone obtained, m.p. 104–106°, gave no depression in melting point when mixed with the corresponding derivative of methyl ethyl ketone.

Dihydroterebinthone.—A solution of 1 Gm. of terebinthone in 30 ml. of ethyl acetate was hydrogenated at 1 atmosphere using prerduced platinum oxide as a catalyst. The consumption of hydrogen was equivalent to 92% of the theoretical amount for one double bond. The product from evaporation of the ethyl acetate was recrystallized from methanol to give 940 mg. of needles, m.p. 152–153.5°, $[\alpha]_D^{25} - 80.8^\circ$ (chloroform).

Anal.—Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 78.83; H, 10.78.

Infrared analysis indicated strong absorption at 1700 cm^{-1} (carbonyl). The compound gave a positive Zimmermann test.

Dihydroterebinthone Mono-oxime.—A solution of 225 mg. of dihydroterebinthone and 650 mg. of hydroxylamine hydrochloride in 25 ml. of dry pyridine was refluxed for 20 hours. The pyridine was removed under vacuum and the residue recrystallized from methanol-water to give microcrystals, m.p. 164–166°.

Anal.—Calcd. for $C_{30}H_{49}NO_3$: C, 76.38; H, 10.47; N, 2.97. Found: C, 76.20; H, 10.62; N, 3.26.

Infrared analysis indicated absorption at 1700 cm^{-1} (carbonyl).

Terebinthol.—A suspension of 400 mg. of lithium aluminum hydride in 30 ml. of dry, peroxide-free ether was stirred for 1 hour. To this was added dropwise a solution of 500 mg. of terebinthone in dry, peroxide-free ether. Stirring was continued for 24 hours. At the end of this time, 1 ml. of 10% sodium hydroxide solution was added dropwise, followed by 1.2 ml. of water, then stirring continued for another 18 hours. The reaction mixture was then treated with water and the ether phase separated. The residue from the ether was recrystallized from acetone-hexane to give prisms, m.p. 189.5–191.0°, $[\alpha]_D^{25} - 49.1^\circ$ (chloroform).

Anal.—Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99;

¹ The authors wish to thank Mr. Lloyd Remington and his students at Boca Ciega High School for gathering the material. We are also indebted to them for introducing to us the local folklore concerning this plant's action.

active hydrogen (two), 0.41. Found: C, 79.08; H, 10.59; active hydrogen, 0.39.

Infrared analysis indicated a strong absorption at 3400 cm^{-1} (hydroxyl) and 1035 cm^{-1} (C—O). There was no absorption in the carbonyl region. The compound gave positive Liebermann-Burchard and Salkowski tests but negative Tortelli-Jaffe and tetranitromethane tests.

Terebinthol Mono-3,5-dinitrobenzoate.—A solution of 100 mg. of terebinthol and 500 mg. of 3,5-dinitrobenzoyl chloride in 3 ml. of dry pyridine was heated for 1 hour on a steam bath then allowed to stand at room temperature for 5 hours. This solution was then added to 10 ml. of water and the precipitate filtered off and washed with 2% sodium carbonate and water. The crude material was chromatographed on an alumina-Celite (1:2) column. The lower of two bands, developed with benzene, was cut from the column and extracted with chloroform. The residue from the chloroform was recrystallized from methanol, m.p. 119–121°.

Anal.—Calcd. for $\text{C}_{37}\text{H}_{52}\text{N}_2\text{O}_8$: C, 68.07; H, 8.03; N, 4.29. Found: C, 68.59; H, 7.74; N, 4.58.

Infrared analysis indicated absorption at 3400 cm^{-1} (hydroxyl).

Dihydroterebinthol.—(a) *Hydrogenation of Terebinthol.*—A solution of 17 Gm. of terebinthol in ethyl acetate was hydrogenated as previously described. The residue from the ethyl acetate was chromatographed on alumina. One gram of product was eluted with ether and recrystallized from methanol to give rods, m.p. 159–161°, $[\alpha]_D^{25}$ –38.7° (chloroform).

Anal.—Calcd. for $\text{C}_{30}\text{H}_{50}\text{O}_3$: C, 78.20; H, 11.38; Found: C, 78.49; H, 11.27.

(b) *Reduction of Dihydroterebinthone.*—An ethereal solution of 350 mg. of dihydroterebinthone was reduced with lithium aluminum hydride as previously described. This product, m.p. 159–161°, gave no depression in melting point with the compound obtained by the hydrogenation of terebinthol. The infrared spectra were also identical.

Dihydroterebinthol Mono-tosylate.—A solution of 100 mg. of dihydroterebinthol and 600 mg. of *p*-toluenesulfonyl chloride in 5 ml. of pyridine was heated on a water bath for 4 hours. The solution was then cooled, diluted with aqueous acid, and extracted with ether. The ether solution was washed with 5% sodium carbonate, dried, and the residue recrystallized from methanol to give needles, m.p. 144–146°.

Anal.—Calcd. for $\text{C}_{37}\text{H}_{56}\text{O}_6\text{S}$: C, 72.54; H, 9.15; S, 5.23. Found: C, 72.24; H, 9.40; S, 4.99.

Infrared analysis indicated absorption at 3400 cm^{-1} (hydroxyl).

Wolf-Kishner Reduction of Dihydroterebinthone (3-Desoxy-dihydroterebinthone).—Two hundred milligrams of sodium in 50 ml. of distilled, dry diethylene glycol was heated to 180°, 2.4 ml. of

anhydrous hydrazine was added, and refluxing continued for 5 minutes. The solution was then cooled to 140° and 1 Gm. of dihydroterebinthone added, followed by 5 ml. of diethylene glycol. Refluxing was resumed with intermittent shaking to dissolve the compound completely. Refluxing was continued for 22 hours at 180°, then some hydrazine was allowed to escape until the refluxing temperature rose to 210°. This temperature was maintained for another 22 hours. The reaction mixture was cooled, diluted with water, and extracted with benzene. The residue from the benzene weighed 50 mg. and could not be further purified.

The aqueous phase was acidified with 10% acetic acid and extracted with benzene. The residue from the benzene, weighing 200 mg., was recrystallized from ethanol-water to give crystals, m.p. 137–139°, $[\alpha]_D^{25}$ –37.8° (chloroform).

Anal.—Calcd. for $\text{C}_{30}\text{H}_{50}\text{O}_2$: C, 81.39; H, 11.38; mol. wt., 442. Found: C, 81.10; H, 11.33; mol. wt. (Rast), 444.

Infrared analysis showed a single carbonyl absorption (1703 cm^{-1}) and cyclic ether (1095 cm^{-1}). The compound gave no Zimmermann reaction.

Absorption Spectra in Sulfuric Acid.—The absorption spectra between 220 and 700 μ of concentrated sulfuric acid solutions of terebinthone, terebinthol, dihydroterebinthone, and dihydroterebinthol were obtained after time intervals of 5, and 15 minutes, 2 and 24 hours. The principal maxima in each case was observed at 304 μ . A secondary peak in the terebinthone solution at 258 μ was observed to disappear with the passage of time.

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