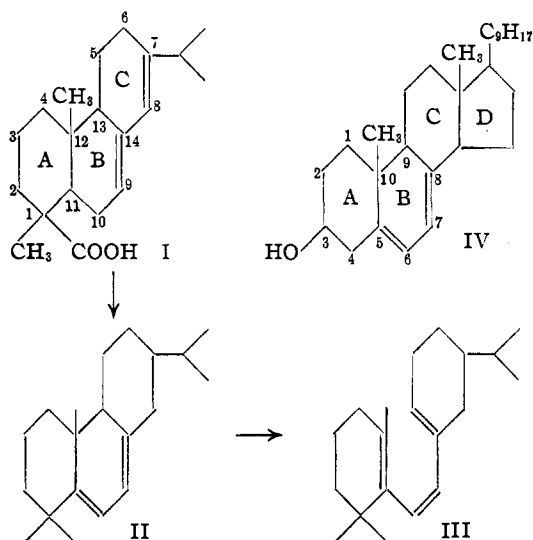


[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY]

The Irradiation of Abietic Acid with Ultraviolet Rays^{1,2}BY RONALD F. BROWN,³ G. BRYANT BACHMAN AND STANLEY J. MILLER

If the 12, 13 bond of abietic acid (I), or of any of the isomers of abietic acid, could be broken, as can be done with the 9, 10 bond of ergosterol (IV), 7-dehydrocholesterol, and certain other sterols, by irradiation with ultraviolet rays, a convenient path to the preparation of substances of physiological interest would be available.



It seems agreed that the system of conjugated double bonds between positions 5 and 8 in the sterol system (IV) is requisite for the development of antirachitic properties upon irradiation.⁴ None of the oleoresinous acids of established structure bear a similarly situated system of bonds between positions 11 and 14 (II). Nevertheless, this group of acids is characterized by lability of structure and ease of oxidation. Abietic acid is recognized only as a relatively stable isomer into which some of the other forms change upon exposure to acids and heat. Ultraviolet light is a means of converting energy poor molecules into energy rich forms and it is possible to visualize a transformation such as I \rightarrow II as one of the results of the absorption of energy by a molecule

of abietic acid, though not necessarily by a single step.

Little attention has been given to the irradiation of the oleoresinous acids with ultraviolet light other than as an aid in the elucidation of structure.⁵ Wienhaus, Ritter and Sandermann⁶ observed that the exposure of an alcoholic solution of levopimaric acid⁷ to the light of the mercury quartz lamp yielded a non-crystalline product, $[\alpha]_D^{30}$, which could be converted into retene upon treatment with palladium charcoal. Since the conditions under which this experiment was carried out were not clearly stated, and since the authors speak of a similar irradiation as an oxidation,⁸ it may be assumed that air was not excluded from the solutions while they were being exposed to the rays.

Abietic acid possesses a strong absorption maximum in the ultraviolet at $237.5 \text{ m}\mu$ ⁹ This corresponds closely to the mercury line at $237.8 \text{ m}\mu$,¹⁰ which is the strongest of the weak peaks in the emission of the mercury quartz lamp below the much more intense peak at $253.7 \text{ m}\mu$. Consequently, in the present work, no attempt was made to filter the light, and the irradiations were carried out by exposing solutions of abietic acid to the lamp for varying periods of time. Three solvents were used: hexane, benzene and ethanol. Benzene absorbs ultraviolet light in the region desired, but was used in an attempt to employ an energy carrier. The alcohols are transparent down to $200 \text{ m}\mu$ ¹¹ but have been reported to dehydrogenate in the vapor phase to aldehydes and hydrogen.¹² The extent of this reaction in the liquid phase was assumed to be

(5) Kraft, *Ann.*, **524**, 1 (1936); **520**, 133 (1935); Fieser and Campbell, *THIS JOURNAL*, **60**, 159 (1938).

(6) Wienhaus, Ritter and Sandermann, *Ber.*, **69B**, 2198 (1936).

(7) The levopimaric acid used, m. p. $138-139^\circ$, $[\alpha]_D^{30} = -113.3^\circ$, acid number 186.4, was distinguished from abietic acid by the speed with which the addition of maleic anhydride took place at room temperature.

(8) Wienhaus and Sandermann, *Ber.*, **69B**, 2202 (1936), in reference to the irradiation of the benzoquinone adduct of levopimaric acid.⁷

(9) Kraft, *Ann.*, **520**, 133 (1935).

(10) Ellis, Wells and Heyroth, "The Chemical Action of Ultraviolet Rays," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1941, pp. 67, 133, 134.

(11) Bielecki and Henri, *Ber.*, **45**, 2819 (1912); Harris, *THIS JOURNAL*, **55**, 1940 (1933); see ref. 10 also.

(12) Ref. 10, pp. 401-402.

(1) Based upon a thesis submitted by S. J. Miller to the faculty of Purdue University in partial fulfillment of requirements for the degree of Master of Science, August, 1942.

(2) This problem, proposed by R. F. B. and begun in 1941 by R. F. B. and S. J. M., was completed in 1942 by G. B. B. and S. J. M.

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(4) Fieser, "Natural Products Related to Phenanthrene," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1937, pp. 380-381.

small enough to allow the use of ethanol as a solvent for the irradiations.

If hexane or benzene was used as the solvent, there was no apparent change in the abietic acid even though the exposure to the ultraviolet was allowed to continue for nine days. The material irradiated in ethanol, however, could be divided into three to four crystalline fractions. Extensive recrystallization resulted in the isolation of two crystalline fractions, other than abietic acid, of melting points 90–100°, and 120–130°. No systematic differences in the yields of the various fractions with length of time of irradiation was noted other than an increase in the total material converted.

Since the recrystallized fractions from the irradiations were still impure, the largest fraction of the first crystallization was used in the subsequent work on purification and identification. The maleic anhydride addition product was identified as the diene adduct of abietic acid.¹³ Dehydrogenation of the original fraction yielded retene. Chromatographic adsorption of a hexane solution of the material (not completely soluble) on activated carbon (Norite) gave only abietic acid. The hexane insoluble residue could only be an oxidation product of abietic acid (or of some isomer), since a reduction or the rupture of a ring would yield substances as soluble or more soluble than abietic acid. Of the two possibilities, it seemed more likely that oxidation had occurred.

In order to identify the hexane insoluble material, the work of Ruzicka and collaborators¹⁴ on the oxidation of abietic acid with alkaline potassium permanganate was repeated. In order to separate the products, these workers treated the cold solution of the barium salts for prolonged periods with carbon dioxide. In this manner, the unchanged abietic acid was freed and separated first, followed by the dihydroxyabietic acid. Treatment of the remaining solution with dilute sulfuric acid liberated impure tetrahydroxyabietic acid. The separation was not sharp, we found, and necessitated extensive recrystallization. Since it was known that hexane would dissolve the abietic acid, it seemed desirable to locate a solvent which would separate the hydroxy-acids by extraction. Trials with the

ether series of solvents showed that dibutyl ether extraction of the residue from the hexane extraction of the crude oxidation product served this purpose and removed the dihydroxyabietic acid. Addition of hexane to the dibutyl ether solution caused the dihydroxyabietic acid to separate in pure form. The residue from the dibutyl ether extraction after further washing with hexane, proved to be identical with the tetrahydroxyabietic acid already prepared.

This convenient procedure for the separation of the oxidation product, when employed on the hexane insoluble residues from the material obtained from the irradiations in alcohol, enabled us to identify dihydroxyabietic and tetrahydroxyabietic acids. Other materials may have been formed in small amount during the irradiations, but careful reworking of all mother liquors at every stage failed to produce any other crystalline material.

Under the conditions employed here, since efforts were made to exclude air during the irradiations, the oxidation might have occurred during the subsequent operations on the isolated products. This was not considered probable since the crude product from the irradiations in hexane or benzene was quite pure abietic acid and no oxidized material could be isolated if the residues were subjected to the same treatment as the material from the ethanol irradiations. Thus, the oxidation could only have occurred during the irradiation, and the role of the alcohol was suspected. Tests with aldehyde reagents showed that the ethanol used was aldehyde free before irradiation, but contained more than traces of aldehyde after irradiation. In the presence of water, acetaldehyde might be able to act as an oxidizing agent on abietic acid. Even though the solvent used was dried over magnesium, time did not allow us to prepare some even more carefully dried ethanol, and to use anhydrous conditions throughout, because, under these conditions, the ethoxy-acids rather than the hydroxy-acids would be expected to form. Even better, perhaps, a test of the action of a moist alcoholic solution of acetaldehyde on abietic acid could have been made. However, an irradiation was carried out with an alcoholic solution of abietic acid in a fused quartz test-tube immersed in a fused quartz flask which contained pure ethanol to act as a filter. The test-tube was evacuated by the usual technique, and exposed

(13) Whether maleic anhydride is added to levopimaric or to abietic acid, the adduct is the same; see refs. 6 and 8, also Ruzicka and Bacon, *J. Soc. Chem. Ind.*, **55**, 546 (1936).

(14) Ruzicka and Meyer, *Helv. chim. acta*, **5**, 315 (1922); **6**, 1097 (1923); Ruzicka and Sternbach, *Helv. chim. acta*, **21**, 565 (1938); **23**, 333 (1940); **24**, 492 (1941).

to the mercury lamp for one week. The abietic acid solution was unchanged, the acid recovered in pure form, and the solvent gave a negative aldehyde test. The alcohol used in the filter, however, gave a positive test.

Thus it has been shown that abietic acid was unaffected by exposure to ultraviolet light. If ethanol was used as a solvent for the irradiation, the abietic acid was oxidized even though air and oxygen were excluded from the system.

Experimental Part¹⁵

Abietic Acid.—Sodium tetra-abietate, m. p. 180–181°, was prepared according to the method of Palkin and Harris.¹⁶ Samples of abietic acid were prepared from the salt as needed, dried in a vacuum desiccator over phosphoric anhydride, and used immediately. From 1.0 g. of the salt, about 0.9 g. of abietic acid could be obtained of average melting point 160 to 170°, and $[\alpha]_D -80^\circ$.

Hexane.—The hexane used as a solvent for the irradiations was purified by shaking with portions of concentrated sulfuric acid until no coloration was produced. After washing with water until neutral, the hexane was dried and distilled over phosphoric anhydride. The fraction boiling between 60 and 70° was collected and used as needed.

Ethanol.—Absolute ethyl alcohol was treated with magnesium and mercuric chloride in the usual manner to remove all but traces of water. The alcohol was distilled from the mixture as needed, using ordinary precautions to prevent the absorption of water vapor from the air by the distillate.

Benzene.—Thiophene-free benzene was dried and stored over sodium.

Irradiation of Abietic Acid.—The light source used was a commercial mercury quartz lamp (Hanovia Alpine Sun Lamp). Two types of fused quartz containers were used: test-tubes of approximately 20-ml. capacity, and 250-ml. Erlenmeyer flasks. While no attempt was made to control the concentrations of the solutions within close limits, the solutions in hexane used were approximately 1 g. in 10 ml., in benzene and in alcohol, 2 g. in 10 ml. No attempt was made to control the temperature during the irradiations. Since the containers were placed close to the tubes of the lamp, the contents became warm in a few minutes, probably reaching a temperature of 50 to 70°.

The solvent chosen for a given run was distilled and collected in flasks, and boiled for a few minutes to expel dissolved air. The solvent was then added to the weighed quantity of abietic acid which had been placed in the quartz container. The containers were clamped in position about 4 to 6 cm. from the lamp tubes and evacuated through the stopcock inserted into the rubber stopper. The evacuation caused the solvent to boil, and the boiling was continued for several minutes in order to remove dis-

solved air. The vacuum was shut off by closing the stopcock and the lamp was turned on.

During the first runs with alcohol as the solvent, it was found that no change occurred until the material had been exposed to the rays for at least six hours. During the later trials, some of the irradiations were allowed to continue for as long as nine days. The usual time of exposure was seven days.

At the conclusion of a run, the solution was washed into a flask and the solvent removed by distillation, the last traces of solvent being removed under vacuum. The residue was chipped out, dried in a vacuum desiccator, and the melting point determined. The melting point was used as a rough measure of the amount of decomposition of the abietic acid by comparing it with the melting point of the acid before exposure.¹⁷ In this way it was noted that if decomposition occurred, the solutions darkened before any indication of change could be obtained by means of the melting point test.

Isolation and Reactions of the Irradiation Products.—The crude product isolated from the irradiations in hexane for periods up to seven days melted completely at 155°. Similarly, the crude product from benzene solutions exposed for five hours to the rays had a melting point of 157°. The material from the alcoholic solutions had much lower melting points, 125–135°. This crude material was dissolved in ethyl alcohol and water added at the boiling point until a cloudiness persisted. The solution was cooled slowly and the precipitated material collected by suction filtration. The process of adding water was repeated on each successive filtrate until no further material could be obtained. The last fraction obtained was usually oily and this was combined with those from each succeeding run and stored in ethanol solution under an atmosphere of natural gas. When the amount collected had become great enough, the solution was extracted with benzene, the benzene removed, and the residue fractionally precipitated from alcohol with water as described.

Each solid fraction was refractionated by the same technique and those fractions of similar melting point combined. In this manner, the irradiated material was separated eventually into three fractions: A, m. p. 150–160°, melting point of a mixture with abietic acid of m. p. 160°, 160°; B, m. p. 120–130°; and C, m. p. 90–100°. From a typical run in which 3.70 g. of abietic acid was irradiated for seven days in 15 ml. of ethanol, the first crystal fractions obtained were I, 0.15 g., m. p. 135–145°; II, 2.75 g., m. p. 130–140°; and III, 0.75 g., m. p. 125–135° for a total of 3.65 g. of recovered material. From the combined residues of several runs, a small amount of IV could be isolated, m. p. 80–100°.

Several attempts were made to purify III by means of the quarter-sodium salt. The first portion obtained on recrystallization from alcohol, m. p. 175–180°, proved to be abietic acid salt, mixed melting point 176°. The second portion, m. p. 150–155°, after acidification, m. p. 120–125°, was evidently still a mixture.

The maleic anhydride addition product was prepared

(15) All melting points are uncorrected. The rotations were determined in 95% alcohol, 8.65 g. of solution containing about 1 g. of solute.

(16) Palkin and Harris, *THIS JOURNAL*, **66**, 1935 (1934).

(17) Although it is known that the melting point of abietic acid is a poor constant for use in identification, the method served satisfactorily here. When necessary, rotations were determined. When no range is reported, the figure given is that for complete solution.

from 1.45 g. of III according to Wienhaus and Sander-mann.⁸ The only pure product isolated was the adduct of abietic acid, 0.5 g., m. p. 227–228°.

Fraction III, 1.0 g., was treated with 5 g. of selenium for twelve hours at 330°. After removal of the colloidal selenium by means of Norite in hexane solution, 0.1 g. of retene was recovered, m. p. 96–97°, melting point with an authentic sample of retene prepared in similar manner from abietic acid, 96°.

The crude irradiation product, 5 g., was extracted with 200 ml. of hexane (very small insoluble residue) and the solution passed through a chromatographic column of Norite. All of the dissolved material was found to be adsorbed since the solvent left no residue upon distillation. Elution of the column with 50% hexane in benzene, 150 ml., then with 150 ml. of benzene and distillation of the solvents produced no residues. A final elution with 150 ml. of a 50% solution of ethanol in benzene produced a small amount of abietic acid, m. p. 150°, after removal of the solvent. Extrusion of the column, extraction of each of eight sections with hot alcohol, filtration, and distillation produced only abietic acid as a residue. This remaining abietic acid was found to have been located in the lower three sections of the column.

Since this result indicated that hexane would dissolve only abietic acid from the crude irradiation product, 2 g. of III was washed with 100 ml. of hexane. The residue remaining softened and foamed at 90°, sintered at 100–110°, and melted completely at 129–130°. The crystalline material isolated from the hexane had a melting point of 160°, $[\alpha]_D -80^\circ$, and was evidently abietic acid.

Oxidation of Abietic Acid.—From the oxidation of 5 g. of abietic acid with alkaline potassium permanganate, 15 g., and separation of the products by treatment of the barium salts with carbon dioxide according to Ruzicka and co-workers¹⁴ there was obtained 0.5 g. of dihydroxyabietic acid, m. p. 150–153°, and 0.4 g. of tetrahydroxyabietic acid, m. p. 200–220°. A small portion of the latter acid was heated with dilute sulfuric acid for three hours, and the melting point of the product, α -tetrahydroxyabietic acid, 248–250°, was identical with that reported.¹⁴

Tests with small portions of the crude product from the potassium permanganate oxidation of abietic acid were made with various solvents. Di-isopropyl ether dissolved practically all of a test portion, while di-isoamyl ether caused the material to become oily. Di-*n*-butyl ether seemed to dissolve part of the material but left a solid residue. A repetition of the oxidation with 5 g. of abietic acid, and treatment of the crude product with 100 ml. of hexane at room temperature, resulted in 3.5 g. of residue, m. p. about 130°. The hexane solution was distilled to isolate the residue, 1.0 g., m. p. 160°. The hexane insoluble residue was washed with 40 ml. of di-*n*-butyl ether, and the residue again washed with hexane to remove occluded ether. The residue, 2.0 g., had a melting point of 200–220°, and produced no lowering of the melting point when mixed with material of the same melting point isolated by the carbon dioxide method. Addition of hexane

to the di-*n*-butyl ether solution caused the dissolved material to precipitate, 1.5 g., which, after collection and thorough washing with hexane, had a melting point of 150–153°. This material likewise did not lower the melting point of the corresponding substance isolated by the carbon dioxide method.

Identification of Irradiation Products.—Fraction III, 2.0 g., was treated with hexane and di-*n*-butyl ether as described, and resulted in 0.5 g. of hexane soluble material, m. p. 160°, 1.0 g. of hexane insoluble, di-*n*-butyl ether soluble material, m. p. 150–153°, and 0.5 g. of hexane insoluble di-*n*-butyl ether insoluble material, m. p. 200–220°. These three substances were identified, respectively, as abietic acid, dihydroxyabietic acid and tetrahydroxyabietic acid since mixtures with authentic samples showed no lowering of the melting points.¹⁸

One irradiation of seven days was carried out during which the solution of abietic acid in alcohol was contained in a fused quartz test-tube which was fitted through a one-hole rubber stopper into a quartz flask which was filled with alcohol. This served to filter the ultraviolet rays through alcohol. Otherwise the procedure used was the same as has been described. The crude product, m. p. 155°, $[\alpha]_D -75^\circ$, was unchanged abietic acid. The alcohol used as the solvent in the test-tube gave a negative test with Fuchsin aldehyde reagent, both before and after exposure. The alcohol in the flask, which had served as a filter, gave a negative test before and a positive test after exposure with the same reagent. In addition, 100 ml. of the latter alcohol was treated after exposure with an excess of 2,4-dinitrophenylhydrazine hydrochloride. The 2,4-dinitrophenylhydrazone of acetaldehyde, 0.5 g., m. p. 164–165° after one recrystallization from alcohol, was obtained.

Summary

1. Unfiltered ultraviolet light has been shown to have no effect on abietic acid when in solution in hexane or benzene.

2. Abietic acid has been shown to be oxidized to dihydroxyabietic and tetrahydroxyabietic acids when an alcoholic solution was irradiated with unfiltered ultraviolet light. If the light was filtered through alcohol, the abietic acid remained unchanged.

3. An improved procedure for the separation of abietic acid, dihydroxyabietic acid and tetrahydroxyabietic acid by solvent extraction has been devised.

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(18) Since potassium permanganate is known to give diols of the *cis*- or *meso*-configuration when used to oxidize ethylenic bonds, it is interesting to note that the oxidation here during irradiation yields products of the same configuration.