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THE STEREOCHEMISTRY OF ROTENONE

by

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B. S., University of Illinois
(1956)

Submitted in partial fulfillment
of the requirements for the
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at the

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May, 1960

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Department of Chemistry, May, 1960

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THE STEREOCHEMISTRY OF ROTENONE

by

James Stanley Kaltenbronn

Submitted to the Department of Chemistry in May, 1960 in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy.

ABSTRACT

Exhaustive ozonization of dihydrotubaic acid (X), followed by oxidation of the ozonization mixture with hydrogen peroxide led to (+)-3-hydroxy-4-methylpentanoic acid (XII). Synthesis of this acid and resolution with quinine gave (-)-3-hydroxy-4-methylpentanoic acid. This acid was reduced to the diol (XIV) with lithium aluminum hydride, converted to the primary tosylate (XV), and this reduced again with lithium aluminum hydride to L-(-)-ethylisopropylcarbinol (XVI). This establishes the D configuration for the (+) acid obtained from ozonization and demands the D (R) configuration at C20 in rotenone.

Ozonization of acetyldihydrorotenone (XIX) led to D-(-)-glyceric acid and establishes an S configuration at C8 in rotenone. Spectral and optical rotatory dispersion evidence bearing on the ring juncture at C7-C8 is discussed. Although preliminary evidence points to a cis ring juncture in rotenone, definitive assignment is not possible until infrared studies on some model compounds have been completed by Prof. L. Crombie.

APPENDIX

The irradiation of rotenone, dihydrorotenone, and isorotenone in aqueous tetrahydrofuran led to very low yields of the respective cleavage products, tubaic acid, dihydrotubaic acid, and isotubaic acid. The neutral fragment expected could not be detected. Irradiation of rotenone in methanolic tetrahydrofuran and dihydrorotenone in tetrahydrofuran-cyclohexylamine gave no detectable cleavage products.

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THE STEREOCHEMISTRY OF ROTENONE

INTRODUCTION

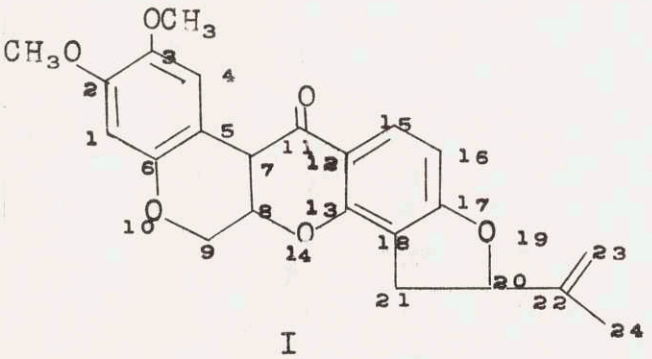
About 100 years ago, travelers in the East Indies, South America and Africa reported the use of certain plants as an aid in catching fish. Botanists have shown that some of the most potent of these fish-poisoning plants, commonly called tuba, timbo, cubé, and haiari, belong to the family Fabaceae, or Papilionaceae, and the genera Derris, Milletia, Tephrosia, Lonchocarpus, and Mundulia. These genera owe their toxic properties to one or more chemically closely related substances, the most important of which is rotenone[1].

The roots of these plants have been in use for many years and are still being used in large quantities for the preparation of dusts and sprays for combatting many injurious insects. More than six and one-half million pounds of Derris and Lonchocarpus, the most important genera commercially, were imported into the United States in 1940. Derris is obtained from British Malaya and the Dutch East Indies, while Lonchocarpus comes from South America[2].

In 1933, Clark isolated rotenone from the roots of Tephrosia virginiana L.[3], the most abundant species of Tephrosia indigenous to the United States. Although early analytical studies showed considerable variation in the amount of insecticidal constituents, selective breeding experiments now make it possible to produce a root of uniformly high quality[4].

The rotenone-bearing plants have been shown to be toxic to many widely different species of insects, acting as both a contact and a stomach poison. Rotenone and allied materials in large doses have some toxic effect on man and the higher animals, but they are far less toxic than the arsenicals used for insecticidal purposes.

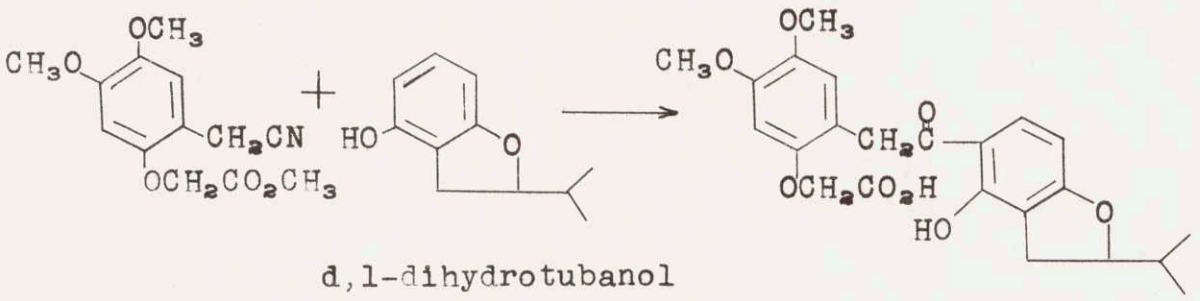
The structure of rotenone was determined in 1932 by three groups of workers who arrived at the correct structure at about the same time[5,39,40] Haller and LaForge in the United States, Butenandt in Germany, and Takei in Japan were the chief investigators. The proof of structure was made an even more difficult task because many of the compounds exist in dimorphic crystalline modifications with variable melting points and occasionally compounds fail to give depressions on mixed melting point determination. A discussion of the reactions which led to the elucidation of structure I can be found in several places[1,5]



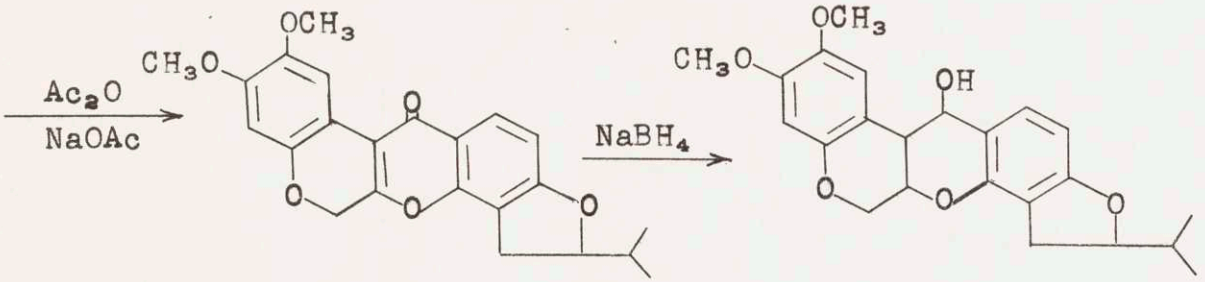
I

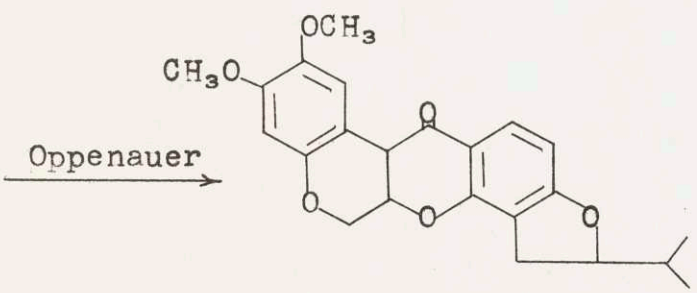
Although rotenone has not been completely synthesized, Robertson and coworkers in England have published extensively during the last twenty-five years on the attempted synthesis of rotenone and related compounds.[6,7,8] Shamshurin in Russia has also done work in this area, but he has so far been concerned mainly with the synthesis of degradation products of rotenone.[9]

Recently, Miyano and Matsui have succeeded in the total synthesis of d,l-dihydrorotenone using the following scheme.[10]



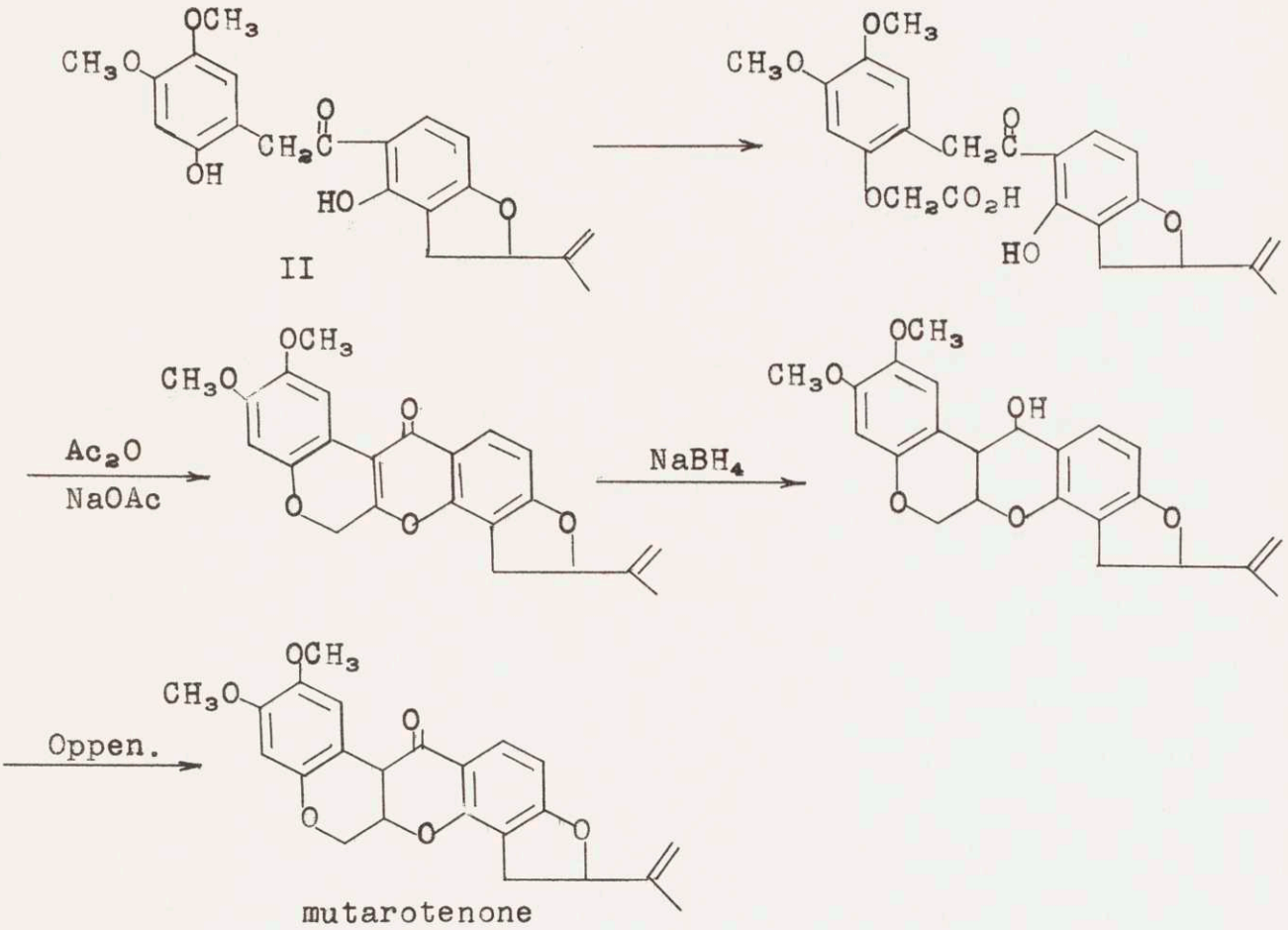
d,l-dihydrotubanol





d,l-dihydrorotenone

Rotenone itself has been obtained by the same authors[11] in a partial synthesis using a similar method but starting with derritol (II), a degradation product of rotenone.



mutarotenone

The key step in these syntheses was the unexpected reduction of the C7-C8 double bond by sodium borohydride. Until now it had been impossible to reduce this double bond by any known method.

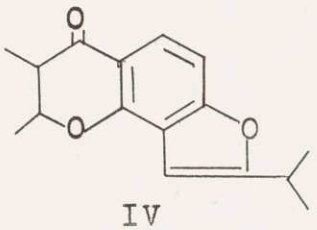
The only work done on the stereochemistry of rotenone has been that of Cahn, Phipers, and Boam.[12] They noted that

when 1-10 % ethanolic potassium hydroxide solution was added to rotenone in acetone, the rotation fell rapidly reaching a value which then remained constant for many hours. On working up the solution, they found, in addition to small amounts of oxidation products, a new substance, m.p.146°, $[\alpha]_D$ -83° (benzene) which they called mutarotenone. Mutarotenone is, however, not a single substance. When dissolved in carbon tetrachloride, rotenone separates as a crystalline solvate containing one molecule of carbon tetrachloride of crystallization. The mother liquor yielded a gum which has never been obtained crystalline, but which seems to be the C7-C8 epimer of rotenone.

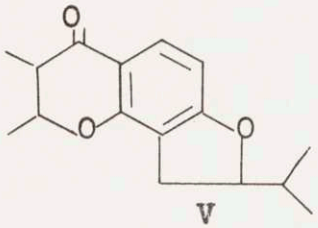
With iodine, mutarotenone gave an almost quantitative yield of (-)-dehydrorotenone (III), showing that all components of the compound have a levorotatory C20. On isomerization with sulfuric acid, d,l-isorotenone (IV) was formed showing that the second component of mutarotenone is epimeric with rotenone at both C7 and C8. Thus, mutarotenone is a 1:1 mixture of rotenone and its C7-C8 epimer, (+)-epirotenone.

Hydrogenation studies on (+)-epirotenone are in agreement with the structure proposed. When (+)-epirotenone was hydrogenated in ethyl acetate over platinum oxide, (+)-dihydroepirotenone (V) was formed which, as expected, was not enantiomeric with (-)-dihydrorotenone. On hydrogenation in

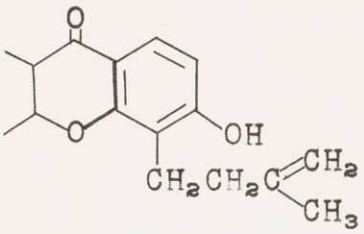
dioxane solution in the presence of potassium acetate over a palladium-barium sulfate catalyst, a phenolic gum was obtained which was believed to be impure (+)-rotenonic acid (VI), since cyclization yielded (+)-dihydrodeguelin (VII), [(+)-dihydro-β-rotenone.]



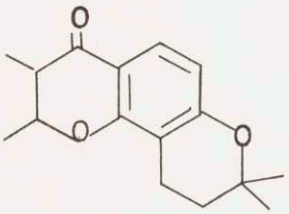
IV



V

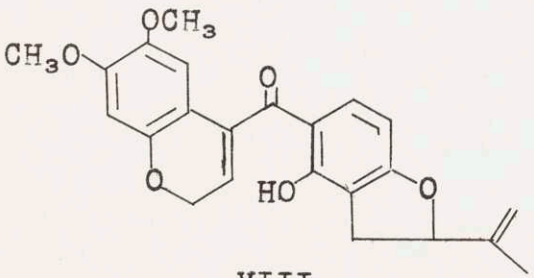


VI



VII

The epimerization of both C7-C8 can be accounted for by assuming a retro-Michael reaction giving the open form VIII in which asymmetry at C7 and C8 is destroyed. Ring closure then gives rotenone and (+)-epirotenone.

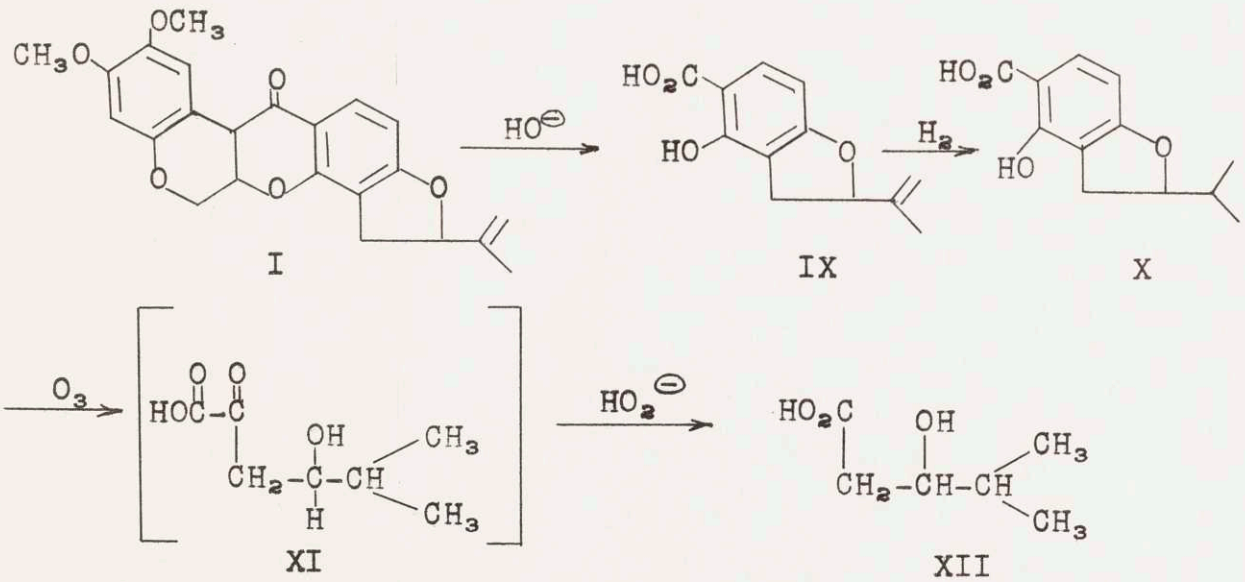


VIII

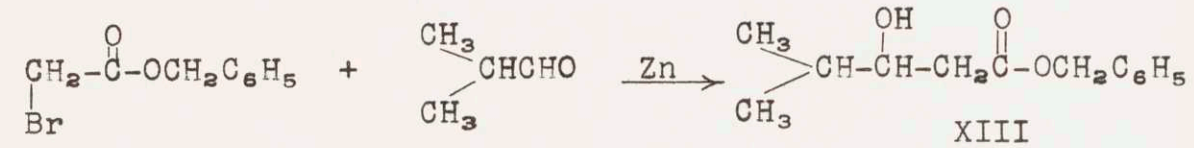
RESULTS AND DISCUSSION

Considering the easy racemization[12] of C7 and C8 and knowing of no way to relate either of these centers to C20, we decided to determine the absolute configuration at C20 and C8 separately. The absolute configuration at C20 was determined in the following manner. Dihydrotubaic acid (X), which can be obtained from rotenone by the action of strong base and reduction of the resulting tubaic acid (IX);[13] was used as the starting material.

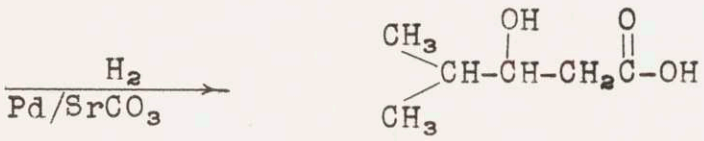
Ozonization[14,15] of dihydrotubaic acid in chloroform at 0° and chromatography of the reaction product on silica gel led to an oil which seemed to be a mixture of XI and XII as judged from the infrared spectra. (Carbonyl adsorption at 1742 $cm.^{-1}$ and 1712 $cm.^{-1}$) Oxidation of the mixture with basic peroxide gave pure XII, $[\alpha]_D$ +26° (chloroform).



Since the amount of acid isolated was too small to enable us to carry out the necessary reactions to relate it to glyceraldehyde, synthetic d,l-3-hydroxy-4-methylpentanoic acid (XII) was prepared and resolved *via* the quinine salt.



XIII



XII

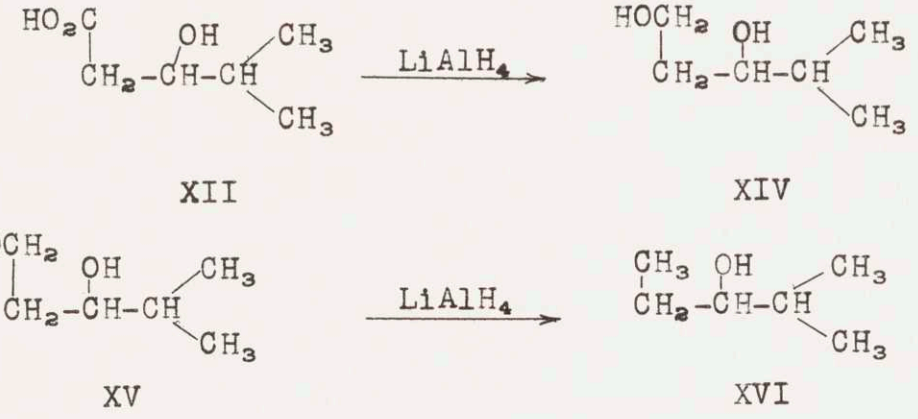
Benzylbromoacetate was prepared by transesterification of ethyl bromoacetate, and condensed with isobutyraldehyde under Reformatsky conditions.[16] Since the resulting hydroxy-ester XIII, on distillation, gave closely boiling fractions with widely different indices of refraction (apparently due to partial dehydration), the crude hydroxyester was used for the hydrogenolysis.

The hydrogenolysis was carried out in ethyl acetate solution over a $Pd/SrCO_3$ catalyst[17] at room temperature and atmospheric pressure. Since β-hydroxyacids are somewhat unstable, distillation was not attempted, and the acid was purified by chromatography on silica gel. The infrared spectrum of the acid XII and its *p*-bromophenacyl ester were identical with those of the acid isolated from the ozonolysis

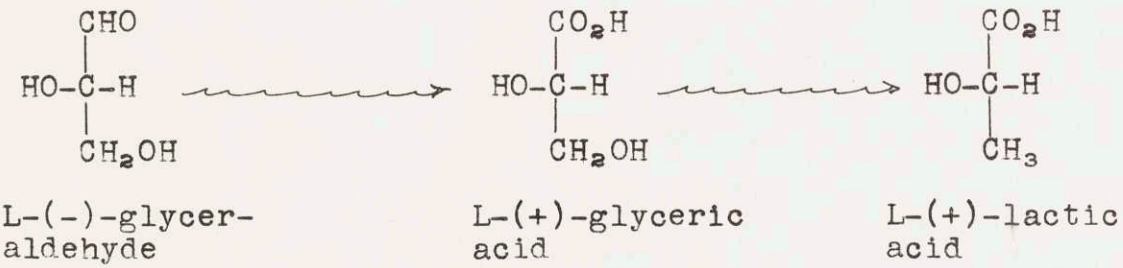
of dihydrotubaic acid.

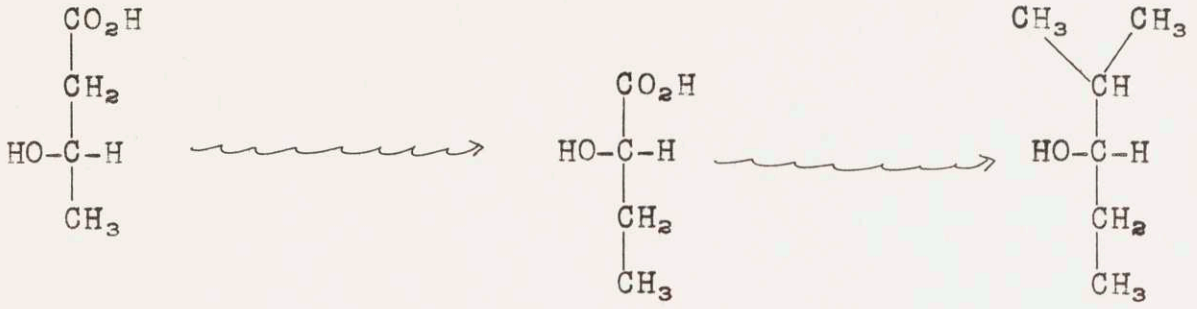
The β-hydroxyacid XII was resolved via its quinine salt. Two recrystallizations from ethyl acetate gave a salt which could be converted to optically pure 3-hydroxy-4-methylpentanoic acid, $[\alpha]_D$ -25° (chloroform).

The resolved acid was reduced with lithium aluminum hydride to give the (-)-diol XIV, $[\alpha]_D$ -7° (chloroform), which was converted to the monotosylate XV, which in turn was reduced to (-)-2-methyl-3-pentanol XVI, $[\alpha]_D$ -8.5° (ethanol).



(-)-2-methyl-3-pentanol (XVI) has been related to L-(-)-glyceraldehyde in the following manner.

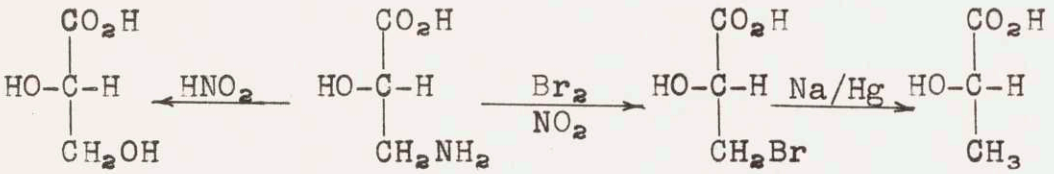




L-(+)-β-hydroxy-butyric acid | L-(+)-α-hydroxy-butyric acid | L-(-)-2-methyl-3-pentanol

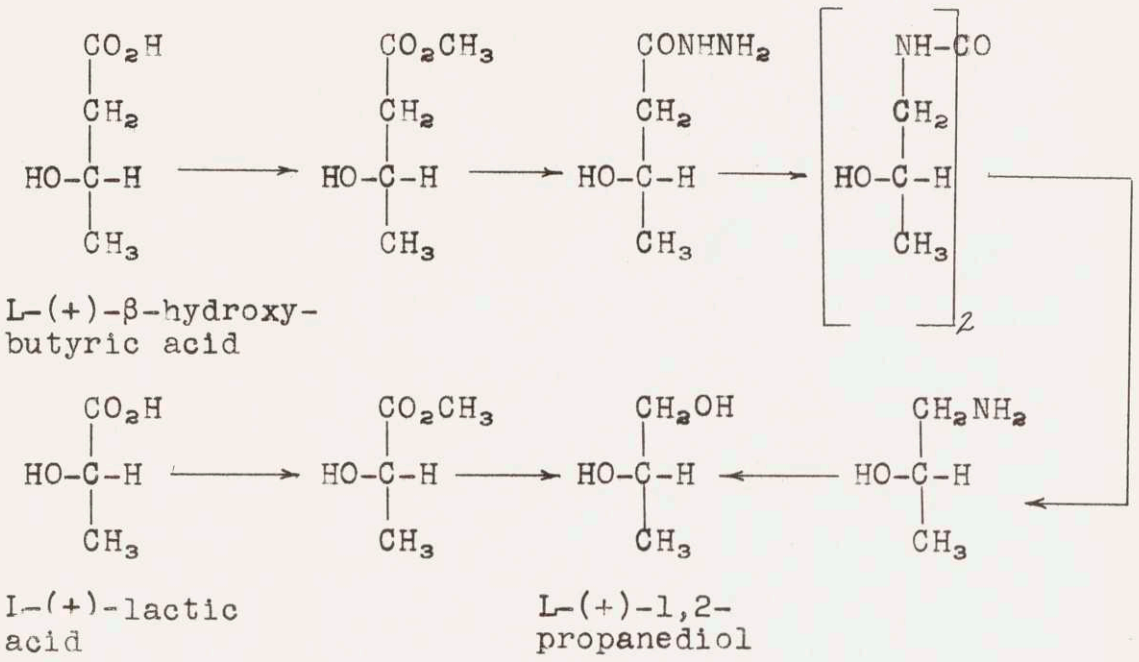
(The wavey line indicates a relationship, not necessarily a direct transformation).

L-(-)-glyceraldehyde has been oxidized by Wohl and Schellenberg[18] to L-(+)-glyceric acid using mercuric oxide. Freudenberg[19] has obtained both L-(+)-glyceric acid and L-(+)-lactic acid from L-(-)-isoserine by the following reaction scheme, thereby relating L-(+)-glyceric acid to L-(+)-lactic acid.

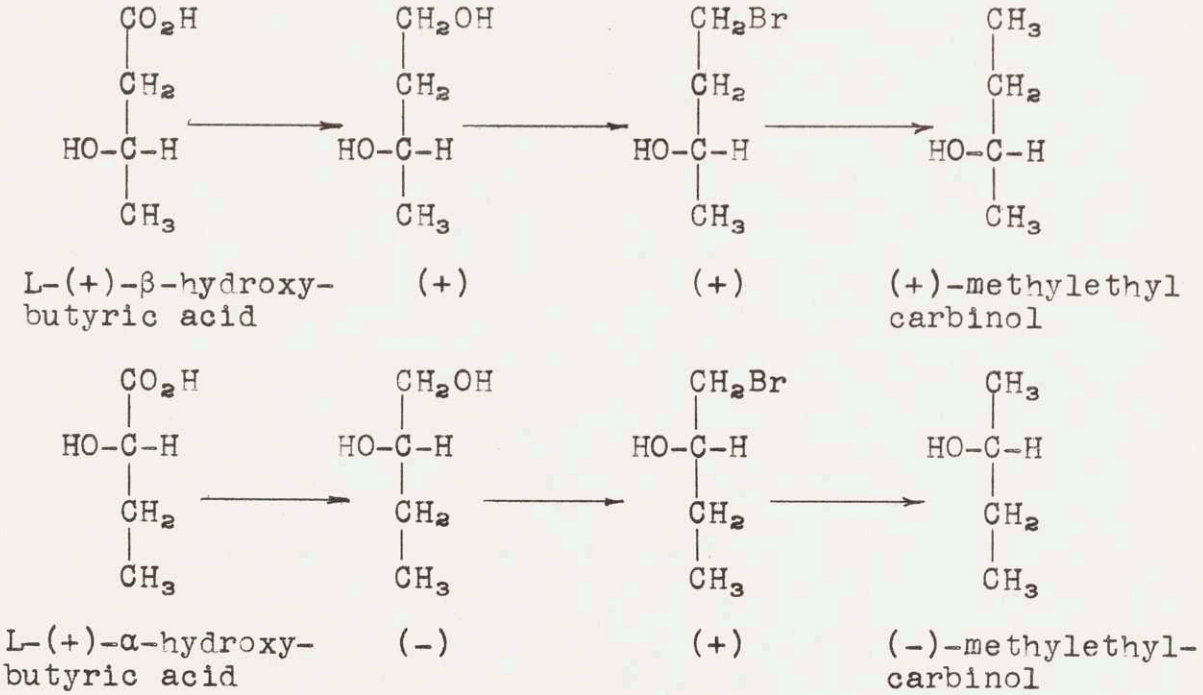


L-(+)-glyceric acid | L-(-)-iso-serine | (+) | L-(+)-lactic acid

L-(+)-lactic acid and L-(+)-β-hydroxybutyric acid have been related by converting both to the same diol.[20,21]

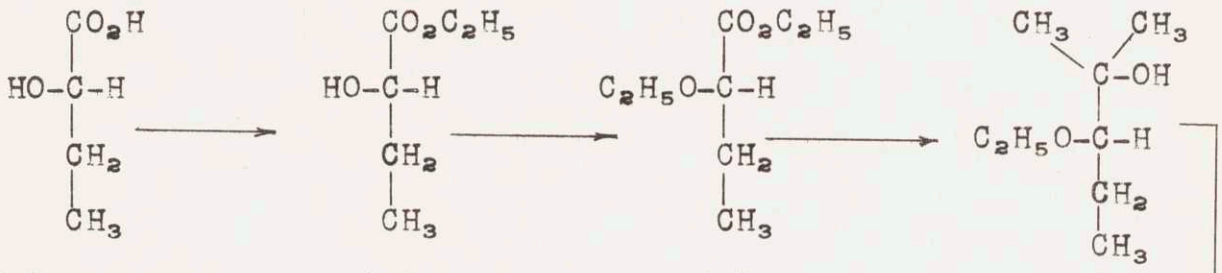


L-(+)-β-hydroxybutyric acid was related to L-(+)-α-hydroxybutyric acid by converting the two acids to enantiomeric alcohols.[22]

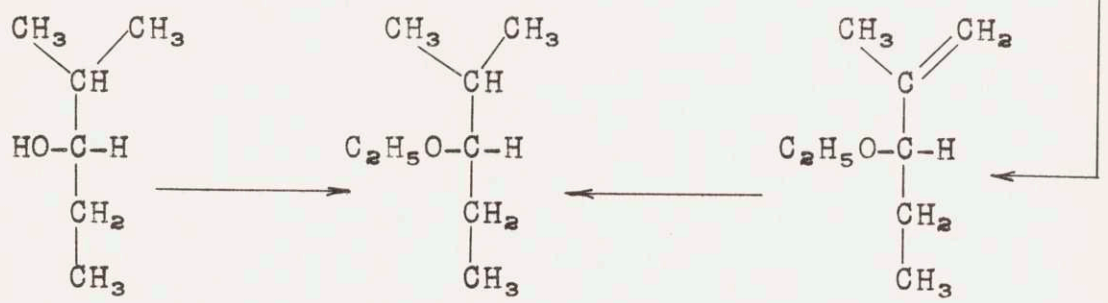


Since in one case the carboxyl group ends up as a methyl group and in the other as an ethyl substituent, the fact that enantiomeric alcohols were formed establishes that the two starting hydroxy acids are members of the same (L) series.

The final relationship was ascertained by a synthesis of L-(-)-ethylisopropylcarbinol from L-(+)-α-hydroxybutyric acid:[23]



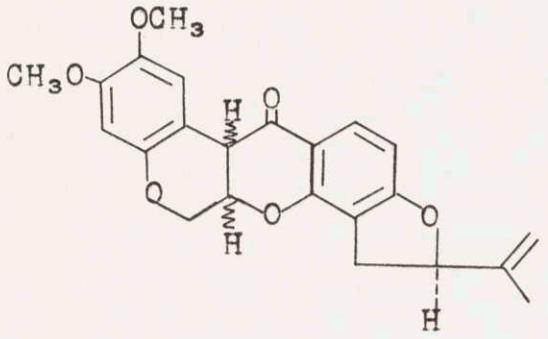
L-(+)-α-hydroxy-butyric acid (-) (-) (-)



L-(-)-ethylisopropyl-carbinol (-) (-)

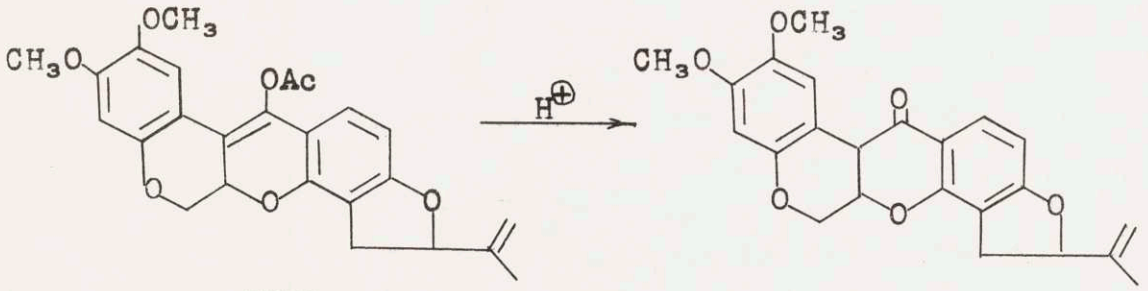
This relates (-)-2-methyl-3-pentanol to L-(-)-glyceraldehyde, and because (-)-2-methyl-3-pentanol has been related to (-)-3-hydroxy-4-methylpentanoic acid, the (+)-3-hydroxy-4-methylpentanoic acid isolated from the ozonization of dihydrotubaic acid must be related to

D-(+)-glyceraldehyde (the R series according to Prelog's convention).[24] Thus rotenone must have the D (R) configuration at C20, and the partial stereostructure of rotenone is as follows.



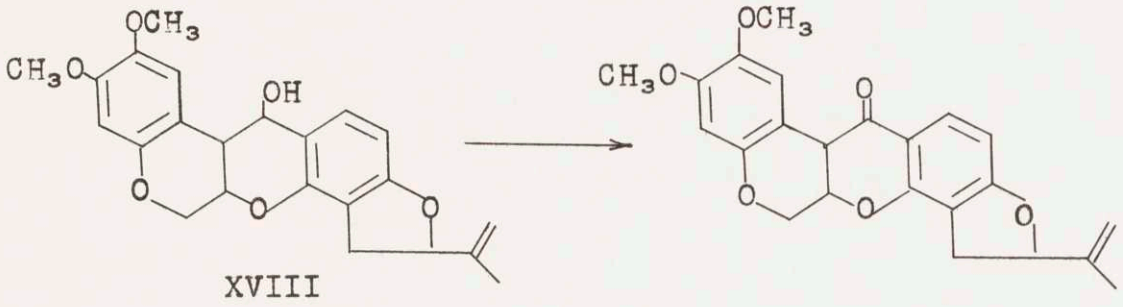
Early in the work on the configuration at C7 and C8 the assumption was made that the trans ring juncture is more stable than the cis. Recent work, however, (vide infra) has questioned the correctness of this assumption. That rotenone is indeed in the more stable form is shown by the following reactions:

a) Hydrolysis of the enol acetate XVII derived from rotenone regenerates rotenone:[12]



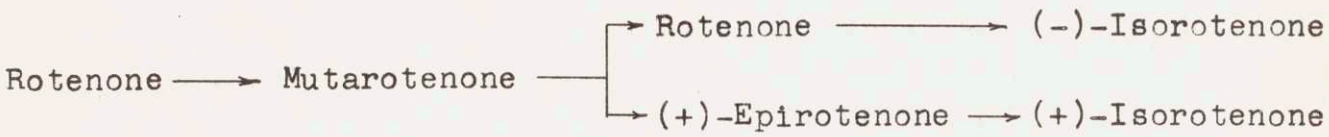
XVII

b) Oppenauer oxidation of the alcohol XVIII derived from rotenone gives mutarotenone, a 1:1 mixture of the two possible trans (or cis) forms.[11]



XVIII

c) Acid catalyzed isomerization of rotenone gives (-)-isorotenone while (+)-epirotenone is converted to (+)-isorotenone.



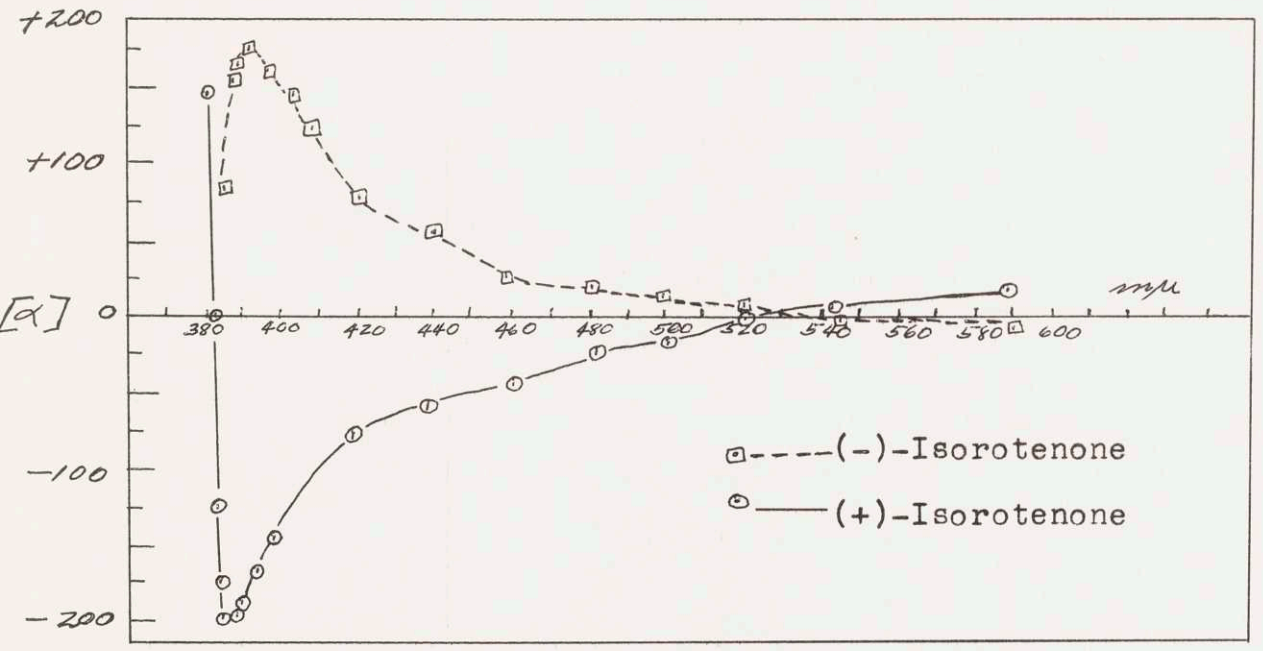
In this case the two isorotenones are enantiomeric, showing that the epimerization at C7-C8 has resulted in only one kind of ring juncture, and this is the type of ring juncture found in rotenone.

We hoped to arrive at a tentative answer concerning the absolute configurations at C7 and C8 by measuring the optical rotatory dispersion curves of a suitable derivative of rotenone.

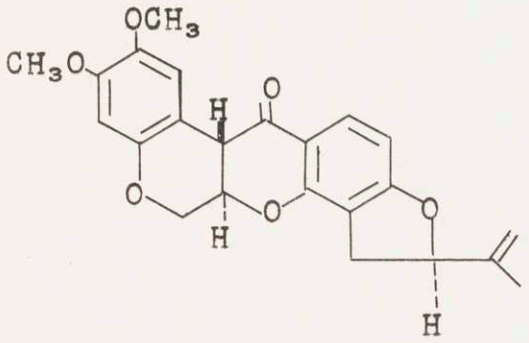
Rotenone when treated with mild base (sodium acetate in acetone) undergoes racemization at C7 and C8 and forms mutarotenone, which, as already mentioned, is a 1:1 mixture

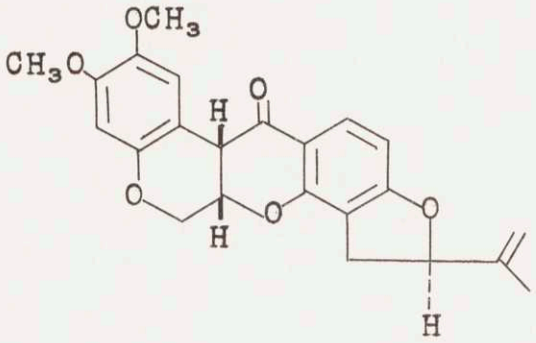
of rotenone and (+)-epirotenone.[12] It has not been possible to obtain (+)-epirotenone in crystalline form, but it has been obtained in 95 % purity as judged from optical rotation. The two epimers, rotenone and (+)-epirotenone when treated with concentrated sulfuric acid in glacial acetic acid are converted to (-)-isorotenone, $[\alpha]_D$ -73° and (+)-isorotenone, $[\alpha]_D$ +75°, respectively. The infrared spectra in solution of the two isorotenones are identical.

The optical rotatory dispersion curves of (+) and (-)-isorotenone have been measured;[25] the curves being mirror images, the curve of (-)-isorotenone having a positive Cotton effect, and that of (+)-isorotenone a negative Cotton effect.



Applying the Moffitt-Woodward rules[26] for both a trans and a cis ring juncture, one can arrive at the following tentative structures for rotenone.

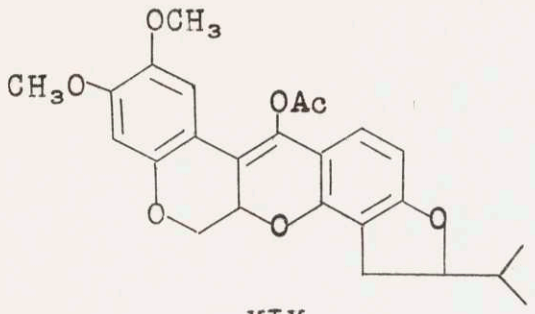




In addition chemical evidence for assignment of absolute configuration at C8 has been sought.

When rotenone is treated with acetic anhydride in the presence of sodium acetate, an enol acetate XVII is formed in which C8 has been racemized. Fortunately, the epimer with C8 corresponding to rotenone is less soluble and can be separated.[12] That this product has the same configuration as rotenone at C8 is shown by the fact that acid hydrolysis of the enol acetate gives back rotenone in 90 % yield, a reaction not expected to affect C8.

Reduction of acetylrotenone with hydrogen over a 5 % palladium on barium sulfate catalyst gives acetyldihydrorotenone XIX, m.p. 208.5-210.5°, $[\alpha]_D$ -161°

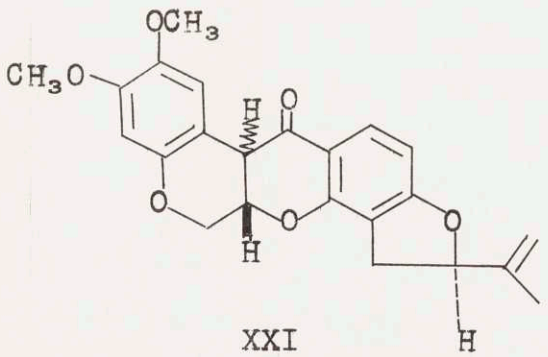


XIX

It seems clear that exhaustive ozonization of acetyldihydrorotenone should yield glyceric acid which contains the asymmetric carbon atom originally present at C8. In addition, C20 will be converted, as before, to (+)-3-hydroxy-4-methylpentanoic acid. This work has been carried out in the dihydro series, not for the purpose of isolating D-(+)-3-hydroxy-4-methylpentanoic acid, but to have a compound as different from glyceric acid in polarity as possible, in order to facilitate separation. Attempts to prepare acetylisorotenone by isomerization of acetylrotenone led to hydrolysis and isolation of (-)-isorotenone. Attempts to prepare it from (-)-isorotenone by various methods (isopropenyl acetate, acetic anhydride with acid catalyst) could not be effected without racemization at C8.

Acetyldihydrorotenone was subjected to exhaustive ozonization at 0° (50 % excess of theoretical amount of ozone), the solvent removed under reduced pressure, and the residue refluxed with water for one-half hour. The water was removed and the residue reozonized for one hour, and worked up as

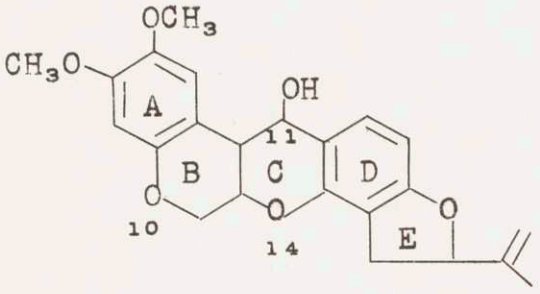
before. The thick, brown oil obtained was chromatographed on Dowex 1 X10 (acetate form), and the various fractions checked by paper chromatography to see which ones should be combined. The glyceric acid had an R_f of 0.49, while the D-(+)-3-hydroxy-4-methylpentanoic acid had an R_f of 0.67. Since there also appeared to be a contaminant of high R_f (0.99), the combined fractions were resubjected to ion-exchange chromatography, giving 160 mg. of a clear oil consisting of glyceric acid and D-(+)-3-hydroxy-4-methylpentanoic acid. The mixture was converted to the p-bromophenacyl esters and separated into the pure components. The derivative of 3-hydroxy-4-methylpentanoic acid had $[\alpha]_D$ +12.2°, m.p. 73-73.5°, thus confirming our earlier results. The p-bromophenacyl ester of glyceric acid had $[\alpha]_D$ -1.49±0.2°, m.p. 109-111°, undepressed when mixed in several varying proportions with authentic samples of D-(-)-glyceric acid p-bromophenacyl ester. Thus rotenone has the S (Prelog's convention) configuration at C8.



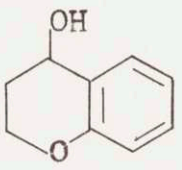
XXI

The configuration at C8 is opposite to that predicted by the Moffitt-Woodward rule for a trans ring juncture, but is in accord with a cis formulation.

Recently we have had communication with Prof. Crombie in England who has investigated the high resolution infrared spectrum of the C11 alcohol XVIII, and has noticed a shift of $\Delta\nu$=47 $cm.^{-1}$ between bonded and non-bonded hydroxyl.[27] This



XVIII



XX

he interprets as hydrogen bonding between the C11 hydroxyl and the ether oxygen at position 10. Oxygen at position 14 has been eliminated as a possible site for hydrogen bonding since model compound XX shows only a free hydroxyl. If this is correct, rotenone must have cis fused rings. However, Prof. Crombie has not excluded the possibility of hydrogen bonding to the benzene ring A,[28,29] and is at present measuring the infrared spectra of model compounds in an effort to resolve this question. It might be mentioned that the value of $\Delta\nu$=47 $cm.^{-1}$ is higher than those usually observed for hydrogen bonding with the π-electrons of a phenyl ring. In the

β-phenylethanols;[28] the values of the shifts were in the range of 31-38 $cm.^{-1}$

Thus, though the preliminary evidence may speak for a cis ring juncture, final assignment must await the results of the model study. Until this question is resolved, one must write for rotenone the above partial stereostructure XXI.

EXPERIMENTAL

All boiling points are uncorrected. Melting points were taken on a Kofler Micro Hot Stage and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord, and are in chloroform unless otherwise specified. Rotations were determined on an O. C. Rudolf and Sons polarimeter, No. 344. Refractive indices were taken on a Bausch and Lomb refractometer. The activity of adsorbants used for chromatograms was determined by the adsorption of dyes according to the procedure of Brockmann.[30] Microanalyses were done by the Microanalytical Laboratory of M.I.T.

Rotenone (I).---The rotenone used in this work was obtained from S. B. Penick and Company, N. Y., and was purified by recrystallization from ethanol, m.p. 164-165°, $[\alpha]_D^{25}$ -225° (c, 1.07 benzene). [Lit.[12] m.p. 163°, $[\alpha]_D$ -226° (benzene)].

Tubaic Acid (IX).---This compound was prepared from rotenone by the action of 5 % ethanolic potassium hydroxide according to the procedure of Takei and Koide;[13] m.p. 128.5-130°. [Lit. m.p. 129°].

Dihydrotubaic Acid (X).---Reduction of tubaic acid in ethyl acetate over 5 % palladium on barium sulfate gave dihydrotubaic acid, m.p. 167-168°, $[\alpha]_D^{25}$ -91° (chloroform). [Lit.[13] m.p. 166°, $[\alpha]_D^{20}$ -82° (chloroform)].

Ozonolysis of Dihydrotubaic Acid.---A solution of 1.05 g. (4.73 mmoles) of dihydrotubaic acid in 50 ml. of chloroform was ozonized at 0° for 3.5 hours. (8 mg. O_3/min.) The chloroform solution was decanted from the reaction vessel which contained a small amount of a white crystalline compound adhering to the walls of the vessel. Removal of the chloroform at room temperature under reduced pressure left a yellow oil which was emulsified with 25 ml. of water and the mixture allowed to reflux for one-half hour. The aqueous solution was extracted continuously with ether for 17 hours, the ether dried over sodium sulfate, and the solvent removed under reduced pressure giving a brown oil which solidified slowly. The crude solid was treated with benzene and the benzene soluble portion chromatographed on silica gel. (Davison Mesh Size 28-200). With benzene-ether (1:1) ten fractions (350 mg.) were eluted which appeared to be a mixture of XI and XII as judged from the infrared spectrum. (Carbonyl adsorption at 1742 $cm.^{-1}$ and 1712 $cm.^{-1}$).

Peroxide Oxidation.---The above brown oil (270 mg.) was dissolved in 10 ml. of 4 % aqueous sodium hydroxide to which 4 ml. of 30 % aqueous hydrogen peroxide was added all at once with cooling. After standing at room temperature for one hour the solution was warmed to 50° for 15 minutes. Water (20 ml.) was then added and sulfur dioxide bubbled into the solution

until it was acidic to Congo Red. The acidic solution was then extracted with three 50-ml. portions of ether, and the ether dried over sodium sulfate. Removal of the ether gave 208 mg. of a clear oil which was chromatographed on silica gel. There was eluted with chloroform-ether (20:1) 154 mg. of a clear oil, having a carbonyl frequency at 1712 $cm.^{-1}$ and showing infrared bands characteristic of carboxylic acids. The acid had $[\alpha]_D^{25}$ +26.4±0.6° (c, 2.08 chloroform).

The p-bromophenacyl ester melted at 73.5-74°, $[\alpha]_D^{26}$ +13.6±1° (c, 0.74 chloroform).

Calcd. for $C_{14}H_{17}O_4Br$ (M.W. 329.20) C, 51.08; H, 5.21

Found C, 51.38; H, 5.33

Benzyl Bromoacetate.---A solution of 54 g. (0.32 mole) of ethyl bromoacetate, 40 g. (0.37 mole) of benzyl alcohol, and 1 g. of p-toluenesulfonic acid-mono-hydrate were heated at 90° for 6 hours. On raising the bath temperature, 12.3 ml. of ethanol was collected. The reaction mixture was poured into water and the organic layer separated. The aqueous layer was extracted with ether and the combined organic phases washed with saturated sodium bicarbonate, water, and then dried over magnesium sulfate. Removal of the ether under reduced pressure and distillation of the residue through a 6-inch Vigreux column gave the product, b.p.137-138°/8 mm., $n^{25}D$ 1.5460. [Lit.[31] b.p. 143°/10 mm.].

Benzyl 3-hydroxy-4-methylpentanoate (XIII).---In a 500 ml., 3-necked flask equipped with stirrer and reflux condenser there was placed 16.8 g. (0.257 mole) of purified granulated zinc. About 20 ml. of a solution consisting of 58.8 g. (0.257 mole) of benzyl bromoacetate and 19.4 g. (0.27 mole) of isobutyraldehyde in 100 ml. of benzene and 40 ml. of ether was added and the solution warmed until the reaction started. The remainder of the solution was added dropwise over a period of one hour, and heating continued to maintain gentle reflux. After the addition was complete, the solution was heated for an additional hour, cooled, and extracted with two 150-ml. portions of cold 10 % sulfuric acid. The organic layers were washed with 150 ml. of saturated sodium bicarbonate and then with 150 ml. of water, dried over magnesium sulfate, and the solvent removed under reduced pressure. Since attempted distillation gave closely boiling fractions of widely differing refractive index and infrared spectra, the crude product was used in the following step.

3-Hydroxy-4-methylpentanoic Acid (XII).---A solution of 20 g. of benzyl 3-hydroxy-4-methylpentanoate in 125 ml. of ethyl acetate was hydrogenolyzed at room temperature and atmospheric pressure using a 7 % palladium on strontium carbonate catalyst. After the theoretical amount of hydrogen had been taken up (7.5 hours), the solution was filtered through Celite and the solvent removed under reduced pressure.

Chromatography on silica gel gave the purified product. The infrared spectrum was identical with that of the (+)-acid isolated from the ozonolysis of dihydrotubaic acid.

The p-bromophenacyl ester had m.p. 83.5-84° and an infrared spectrum in solution identical with that of the (+) ester from the ozonolysis.

Calcd. for $C_{14}H_{17}O_4Br$ (M.W. 329.20) C, 51.08; H, 5.21

Found C, 51.52; H, 5.49

Resolution of 3-Hydroxy-4-methylpentanoic Acid.---A solution of 5.44 g. (0.041 mole) of d,l-acid in 50 ml. of ethyl acetate was added all at once to a warm solution of quinine in 200 ml. of ethyl acetate. The solution was concentrated under reduced pressure and the salt allowed to crystallize. There was obtained 8.0 g. of the quinine salt, m.p. 147.5-151.5°, $[\alpha]_D$ -143.5±0.5° (ethanol). Recrystallization from ethyl acetate gave 5.7 g. of quinine salt, m.p. 151.5-153.5°, $[\alpha]_D^{25}$-145±2° (c, 1.03 ethanol). The free acid was obtained by dissolving the quinine salt in water, adding 10 % sulfuric acid until acidic to Congo Red, and extracting with ether. Drying over sodium sulfate and removal of the ether under reduced pressure gave 2.9 g. of resolved acid having $[\alpha]_D^{28}$-24.7±0.6° (c, 0.98 chloroform). The infrared spectrum was identical with that of the (+) acid isolated from ozonolysis of dihydrotubaic acid.

The p-bromophenacyl ester had m.p. 74-75°, $[\alpha]_D^{25}$ -14.4±0.8° (c 2.09 chloroform) and an infrared spectrum identical with that of (+) ester from the ozonolysis.

Calcd. for $C_{14}H_{17}O_4Br$ (M.W. 329.20) C, 51.08; H, 5.21

Found C, 51.42; H, 5.39

(±)-4-Methylpentane-1,3-diol (XIV).---To a suspension of 0.76 g. (0.02 mole) of lithium aluminum hydride in 25 ml. of ether was added dropwise a solution of 1.92 g. (0.0145 mole) of (±)-3-hydroxy-4-methylpentanoic acid over a period of one-half hour. After the addition was complete, the solution was refluxed for two hours, 5 ml. of water added, and the ether decanted. The residue was washed with several portions of ether, and the combined ether phases dried over sodium sulfate. Removal of the ether under reduced pressure and distillation of the crude diol in a micro Hickman column gave 1.26 g. (73.5 %) of the product which distilled at a bath temperature of 130°/10 mm.

The di-α-naphthylurethane had m.p. 136-137°.

Calcd. for $C_{28}H_{28}O_4N_2$ (M.W. 456.52) C, 73.66; H, 6.18

Found C, 73.60; H, 6.13

(-)-4-Methylpentane-1,3-diol (XIV).---In a similar manner, reduction of 2.9 g. (0.022 mole) of (-) acid ($[\alpha]_D$ -24.7°) with 1.14 g. (0.03 mole) of lithium aluminum hydride gave 2.1 g. of crude diol which was distilled through a micro

Hickman column at a bath temperature of 130-135°/13 mm. The diol had n_D^{25} 1.4482, $[\alpha]_D^{27}$ -6.9±0.2° (c 2.84 chloroform) and an infrared spectrum identical with that of (±)-diol.

The di-α-naphthylurethane had m.p. 134.5-136.5°.

Calcd. for $C_{28}H_{28}O_4N_2$ (M.W. 456.52) C, 73.66; H, 6.18

Found C, 73.96; H, 6.41

(±)-1-Tosyl-4-methyl-3-pentanol (XV).---To a solution of 1.26 g. (10.66 mmoles) of 4-methylpentane-1,3-diol in 15 ml. of anhydrous pyridine was added with cooling 2.03 g. (10.66 mmoles) of p-toluenesulfonyl chloride, and the solution left standing at room temperature for eleven hours. The solution was then poured into 100 ml. of ether, extracted with 50 ml. of 5 % hydrochloric acid, 25 ml. of 2 % sodium carbonate, and then with 25 ml. of water. Drying of the ether phase over sodium sulfate and removal of the ether under reduced pressure gave 1.47 g. (50.3 %) of the crude tosylate as an oil. The infrared spectrum had the characteristic tosylate bands at 1176 $cm.^{-1}$ and 915 $cm.^{-1}$ Attempted distillation and attempted chromatography on Woelm Neutral Alumina, Act. I, led to decomposition of the tosylate.

(-)-1-Tosyl-4-methyl-3-pentanol (XV).---In a similar manner, 1.39 g. (11.78 mmoles) of diol ($[\alpha]_D$ -6.9°) treated with 2.24 g. (11.78 mmoles) of p-toluenesulfonyl chloride in 10 ml. of anhydrous pyridine gave 1.8 g. of crude tosylate

having characteristic tosylate bands at 1176 $cm.^{-1}$ and 915 $cm.^{-1}$ in the infrared spectrum which was identical with that of (±)-1-tosyl-4-methyl-3-pentanol.

(±)-2-Methyl-3-pentanol (XVI).---To a suspension of 228 mg. (6 mmoles) of lithium aluminum hydride in 10 ml. of anhydrous ether was added dropwise 1.47 g. (5.4 mmoles) of crude tosylate over a period of 15 minutes. After the addition was complete, the solution was refluxed for an additional four hours, 5 ml. of water added, and the ether decanted. The residue was washed with several portions of ether, the ether dried over sodium sulfate, and removed by distillation through a 12-inch Vigreux column. Distillation of the residue in a micro Hickman flask at atmospheric pressure gave 258 mg. (47 %) of the product distilling at a bath temperature of 135-140°, $n^{25}D$ 1.4173 [Lit.[32] $n^{20}D$ 1.4175].

The 3,5-dinitrobenzoate had m.p. 81-82°.

Calcd. for $C_{13}H_{16}O_6N_2$ (M.W. 296.28) C, 52.70; H, 5.44

Found C, 52.97; H, 5.60

(-)-2-Methyl-3-pentanol (XVI).---In like manner 1.8 g. (6.6 mmoles) of tosylate from the (-)-diol was reduced with 250 mg. (6.6 mmoles) of lithium aluminum hydride. The product was distilled at atmospheric pressure in a micro Hickman column, the product distilling at a bath temperature of 135-145°, 411 mg. (61 %), $n^{25}D$ 1.4184, $[\alpha]_D^{27}$ -8.5±0.25°

(c, 3.31 ethanol). [Lit.[28] $[\alpha]_D$ -9.8° (neat), and for the other enantiomorph $[\alpha]_D$ +16.4° (c, 1.06 ethanol)[33]].

The 3,5-dinitrobenzoate had m.p.89-92°, $[\alpha]_D^{26}$+4.9±0.6° (c, 1.1 chloroform).

Calcd. for $C_{13}H_{16}O_6N_2$ (M.W. 296.28) C, 52.70; H, 5.44

Found C, 52.28; H, 5.45

The infrared spectra of both the alcohol and its 3,5-dinitrobenzoate were identical with those of the (±)-alcohol.

Mutarotenone.---The action of anhydrous sodium acetate in ethanol on rotenone according to the procedure of Cahn, Phipers, and Boam gave mutarotenone as a colorless solid, m.p. 146.5-148.5°, $[\alpha]_D$ -83.3° (benzene). [Lit.[12] m.p. 146°, $[\alpha]_D$ -83° (benzene)].

(+)-Epirotenone.---A solution of 2.9 g. of mutarotenone in carbon tetrachloride was left standing for two days. There was collected 1.7 g. (84.3 %) of the rotenone-carbon tetrachloride complex. The carbon tetrachloride in the filtrate was removed leaving an oil which was dissolved in ether and left standing for three days. Three hundred milligrams of mutarotenone separated and was removed by filtration. Removal of the ether gave 1.38 g. of a white foam $[\alpha]_D$ +34°, corresponding to 91 % (+)-epirotenone.

Four hundred eighty milligrams of the above foam was chromatographed on 40 g. of Merck acid washed alumina, Act. I.

On elution with benzene, (+)-epirotenone with $[\alpha]_D$ +44° (benzene) corresponding to 94.4 % (+)-epirotenone was isolated.

(-)-Isorotenone (IV).---To a solution of 500 mg. (1.27 mmoles) of rotenone in 5 ml. of glacial acetic acid at 60° was added dropwise 3 ml. of conc. sulfuric acid over a period of 15 minutes. After the addition was complete, the reaction mixture was poured into 25 ml. of water and the crude (-)-isorotenone collected on a filter. Recrystallization from carbon tetrachloride gave 330 mg. (66 %) of pure (-)-isorotenone, m.p. 180-181°, $[\alpha]_D^{26}$ -76° (c, 1.02 benzene), [Lit.[12] m.p. 184°, $[\alpha]_D$ -73° (benzene)].

(+)-Isorotenone (IV).---In like manner, 1.23 g. of (+)-epirotenone gave 600 mg. of (+)-isorotenone, m.p. 179.5-181.5°, $[\alpha]_D^{25}$ +73° (benzene), [Lit.[12] m.p. 182°, $[\alpha]_D$ +75° (benzene)].

Acetylrotenone (XVII).---Rotenone (60 g.), anhydrous sodium acetate (30 g.), and acetic anhydride (950 ml.) were heated at reflux for one hour, cooled, poured into 1.6 l. of water, and the acetic anhydride hydrolyzed by stirring for one hour. The solvent was then decanted from the sticky solid, the solid washed with several portions of water, and then crystallized from one liter of ethanol, giving 23 g. of colorless crystals, m.p. 160-163°. Recrystallization from ethanol yielded 20.8 g. , m.p. 162-164°. [Lit.[12] m.p. 159-160°].

In another preparation, the other dimorphic crystalline

modification was obtained, m.p. 139.5-141.5°, $[\alpha]_D^{25}$ -161° (c, 1.0 benzene). [Lit.[34] m.p. 137°, $[\alpha]_D$ -172° (benzene)].

Hydrolysis of Acetylrotenone to Rotenone.---A solution of 100 mg. of acetylrotenone in 4.3 ml. of ethanol and 0.8 ml. of conc. hydrochloric acid was heated at reflux for one hour, poured into 10 ml. of water, and the gummy residue collected by filtration. Recrystallization from ethanol gave rotenone, m.p. 160-163°, $[\alpha]_D^{25}$-225° (c, 1.07 benzene). [Lit.[12] m.p. 163°, $[\alpha]_D$ -226° (benzene)].

Acetyldihydrorotenone (XIX).---A solution of 20.6 g. (0.0437 mole) of acetylrotenone in 300 ml. of acetone with 2 g. of 5 % palladium on barium sulfate was reduced at room temperature and atmospheric pressure over a period of three hours. The solution was filtered to remove catalyst, and the residue recrystallized from ethanol giving 16.8 g. of faintly yellow crystals, m.p. 208.5-210.5°, $[\alpha]_D^{26}$ -161° (c, 1.4 benzene). [Lit.[35] m.p. 209-211°].

Ozonization of Acetyldihydrorotenone.---Through a solution of 5.0 g. (11.4 mmoles) of acetyldihydrorotenone in 100 ml. of methanol-chloroform (1:1) at 0° was passed ozone (8 mg./min.) for 12 hours (50 % excess of theory). The solution turned brown and then became a bright yellow. After the ozonization was complete, the solvent was removed under reduced pressure at room temperature and the resulting oil

mixed with 50 ml. of water and refluxed for one-half hour. Removal of the water under reduced pressure at 50° left a brown residue. This residue was dissolved in 100 ml. of methanol-chloroform (1:1), ozonized for one hour, and worked up as before leaving 4.6 g. of a brown solid. This material was chromatographed on ion exchange resin (Dowex 1 X10, acetate form, 100-200 mesh) in a column 17 mm. X 14.5 cm., using 1 N acetic acid as elutant. Fractions of 25 ml. were collected and were checked by paper chromatography to determine in which fractions the glyceric acid was present. Since there appeared to be a contaminant with high R_f (0.96-1.00) present, the ion exchange chromatography was repeated. Removal of the solvent under reduced pressure at 50° gave 160 mg. of an oil, a mixture of glyceric and 3-hydroxy-4-methylpentanoic acid.

Paper Chromatography.---The fractions were checked using the system devised by Palmer[36,37] Spots, 1 cm. or less in diameter, containing 80 μl. of the solution from the ion exchange column were applied to W. and R. Balston, Ltd. 3 mm. paper along a line drawn 2.1 cm. from the bottom, the spots being placed 3 cm. apart. The paper was bent into the form of a cylinder, stapled so the ends did not quite touch, and placed upright on the bottom of the chromatography tank which had been filled to a depth of 1.5 cm. with developing solvent. A solvent system consisting of

a mixture of ethyl ether, 88 % formic acid, and water in the proportions of 5:2:1 was used. At the end of 2.5 hours, during which time the solvent front traveled approximately 18 cm., the paper was dried with an air blower and sprayed with 0.04 % bromophenol blue in alcohol. The glyceric acid showed an R_f of 0.49 and was accompanied by 3-hydroxy-4-methylpentanoic acid with an R_f of 0.67.

p-Bromophenacyl Esters of the Mixture of Acids.--- The mixture of acids (160 mg.) was neutralized with 1 N sodium hydroxide, and 401 mg. of p-bromophenacyl bromide in 6 ml. of ethanol added and the solution refluxed for 5 hours. The solution was cooled, diluted with 25 ml. of water and the esters allowed to crystallize. After filtering and drying, the precipitate was extracted three times with boiling hexane, and the insoluble portion crystallized from benzene-hexane. Recrystallization from the same solvents gave 35.2 mg. of the pure p-bromophenacyl ester of glyceric acid, m.p. 109-111°, $[\alpha]_D^{25}$ -1.49±0.2°, (c, 4.36 acetone). The sample showed no depression when mixed with varying proportions of authentic p-bromophenacyl ester of D-(-)-glyceric acid, $[\alpha]_D^{25}$ -1.9±0.2°, and the infrared spectra of both esters were identical.

Calcd. for $C_{11}H_{11}O_5Br$ (M.W. 303.12) C, 43.58; H, 3.66

Found C, 43.19; H, 3.51

Chromatography of the hexane soluble portion on Woelm Neutral Alumina (Act. IV) gave 43 mg. of the p-bromophenacyl ester of (-)-3-hydroxy-4-methylpentanoic acid, m.p. 73-73.5°, $[\alpha]_D^{25}$ +12.2±0.2° (c, 2.31 chloroform), having an infrared spectrum identical with that of the same acid isolated earlier from the ozonization of dihydrotubaic acid.

Resolution of d,l-Glyceric Acid.---Glyceric acid was resolved using quinine according to the procedure of Anderson;[38] and gave a calcium salt having $[\alpha]_D^{24}$+14.4° (c, 2.12 water). [Lit. $[\alpha]_D^{20}$ +14.0° (water)].

The p-bromophenacyl ester had m.p. 109-111°, $[\alpha]_D^{25}$ -1.9±0.2° (c, 5.62 acetone).

The p-bromophenacyl ester of d,l-glyceric acid had m.p. 120-122°.

Calcd. for $C_{11}H_{11}O_5Br$ (M.W. 303.12) C, 43.58; H, 3.66

Found C, 43.18; H, 3.53

BIBLIOGRAPHY

1. F. B. LaForge, H. L. Haller, and L. E. Smith, Chem. Revs., 12, 181 (1933).

2. H. L. Haller, L. D. Goodhue, and H. A. Jones, Chem. Revs., 30, 33 (1942).

3. E. P. Clark, Science, 77, 311 (1933).

4. L. Zechmeister, Fortschritte der Chemie Organischer Naturstoffe, Springer, Vienna, 1953, Vol. 10, p. 436.

5. A. Butenandt and W. McCartney, Ann., 494, 17 (1932).

6. W. Hilton, R. W. H. O'Donnel, F. P. Reed, A. Robertson, and G. L. Rusby, J. Chem. Soc., 423 (1936).

7. H. I. King, R. H. Holland, F. P. Reed, and A. Robertson, J. Chem. Soc., 1672 (1948).

8. G. Parker and A. Robertson, J. Chem. Soc., 1121 (1950).

9. A. A. Shamshurin, J. Gen. Chem. (USSR), 16, 1877 (1946).

10. M. Miyano and M. Matsui, Ber., 92, 2487 (1959).

11. M. Miyano and M. Matsui, Ber., 91, 2044 (1958).

12. R. S. Cahn, R. F. Phipers, and J. J. Boam, J. Chem. Soc., 513 (1938).

13. S. Takei and M. Koide, Ber., 62, 3030 (1929).

14. E. Hardegger, H. Gempeler, and A. Züst, Helv. Chim. Acta, 40, 1819 (1957)., and earlier papers cited.

15. H. Schmid and A. Ebnöther, Helv. Chim. Acta, 34, 1041 (1951).

16. R. L. Shriner in Org. Reactions, 1, 1 (1942).

17. R. E. Bowman, J. Chem. Soc., 325 (1950).

18. A. Wohl and R. Schellenberg, Ber., 55, 1404 (1922).

19. K. Freudenberg, Ber., 47, 2027 (1914).

20. P. A. Levene and H. L. Haller, J. Biol. Chem., 65, 49 (1925).

21. P. A. Levene and H. L. Haller, J. Biol. Chem., 67, 329 (1926).

22. P. A. Levene and H. L. Haller, J. Biol. Chem., 74, 343 (1927).

23. P. A. Levene and R. E. Marker, J. Biol. Chem., 101, 413 (1933).

24. R. S. Cahn, C. K. Ingold, and V. Prelog, Experientia, 12, 81 (1956).

25. We wish to thank Prof. K. Wiesner and Dr. F. Bickelhaupt, Univ. of New Brunswick, for the measurements.

26. W. Moffitt, R. B. Woodward, C. Djerassi, and W. Klyne, to be published; C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Company, Inc., New York, N. Y., 1960.

27. L. P. Kuhn, J. Am. Chem. Soc., 74, 2492 (1952); 76, 4323 (1954)

28. P. R. Schleyer, C. Wintner, D. S. Trifan, and R. Bacskai, Tetrahedron Letters, No. 14, 1 (1959).

29. I. M. Goldman and R. O. Crisler, J. Org. Chem., 23, 751 (1958).

30. H. Brockmann and H. Schodder, Ber., 74, 73 (1945).

31. H. T. Clarke, J. Chem. Soc., 416 (1910).

32. A. Pukirev, Trans. Inst. Pure Chem. Reagents (USSR), No. 16, 73 (1939). C. A. 37, 4686 (1943).

33. R. H. Pickard and J. Kenyon, J. Chem. Soc., 103, 1923 (1913).

34. R. S. Cahn and J. J. Boam, J. Soc. Chem. Ind., 54, 42T (1935).

35. L. E. Smith and F. B. LaForge, J. Am. Chem. Soc., 54, 2996 (1932).

36. J. K. Palmer, Science, 123, 415 (1956).

37. J. K. Palmer, Connecticut Agr. Expt. Sta. Bull., No. 589 (1955).

38. E. Anderson, Am. Chem. J. 42, 401 (1909).

39. F. B. LaForge and H. L. Haller, J. Am. Chem. Soc., 54, 810 (1932).

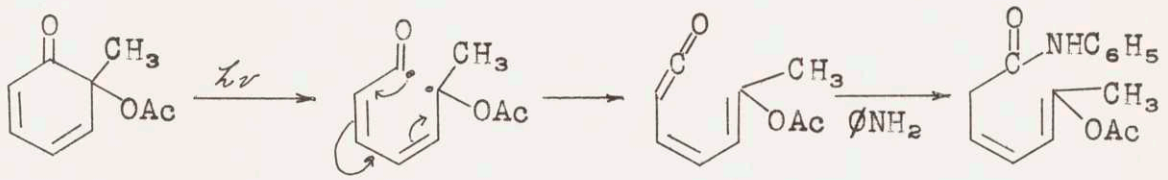
40. S. Takei, S. Miyajima, and M. Ono, Ber., 65, 1041 (1932).

APPENDIX

THE IRRADIATION OF ROTENONE
AND
RELATED COMPOUNDS

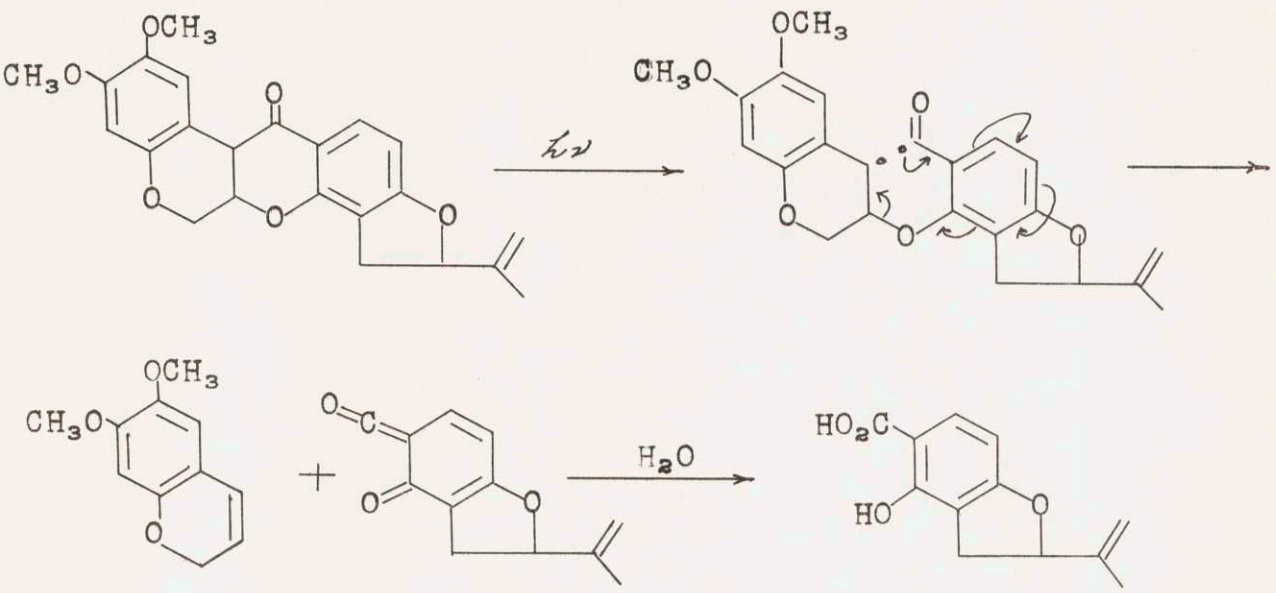
RESULTS AND DISCUSSION

Barton and Quinkert[1] have investigated the irradiation of 6,6-disubstituted cyclohexadienones of type I and have found that in ether solution saturated with water, diene acids of type III are obtained in high yield. In addition, when these cyclohexadienones are irradiated in dry ether in the presence of aniline, the anilides are formed in high yield. Barton has interpreted these results by suggesting that the cleavage reaction proceeds through ketenes of type II which, formally speaking, are simply derived from I by redistribution of electrons.



I II III

In analogy to this reaction, the irradiation of rotenone and its derivatives has been carried out, both for the investigation of the reaction and as a possibility of obtaining cleavage products which could be used in a projected synthesis of rotenone. With rotenone the reaction should proceed as follows.



The reaction did indeed occur; however, the yields were poor. On irradiation of 5.0 g. of rotenone in tetrahydrofuran and water for 46 hours, there was obtained 230 mg. of acidic material, which, on chromatography gave 50 mg. (1.8 %) of tubaic acid. No neutral material corresponding to the other fragment could be isolated. Irradiation of rotenone for a longer period of time (9.5 days) gave no improvement in yield. A control experiment was run which gave 43 mg. of acidic material, but no tubaic acid was present.

Since polymerization was extensive, the irradiation lamp becoming coated with brown polymer, it was felt that the reaction might be improved by irradiating dihydrorotenone.

Irradiation of 4.2 g. of dihydrorotenone in tetrahydrofuran and water for 46 hours gave 440 mg. of acidic material which on chromatography gave 102 mg. (4.3 %) of

dihydrotubaic acid. Irradiation for a longer period of time gave no improvement in yield. In both irradiations no polymer was formed on the irradiation lamp. The other fragment expected could not be detected in the neutral material.

Irradiation of 4.0 g. of (-)-isorotenone in tetrahydrofuran and water for 47 hours gave 21 mg. (0.9 %) of isotubaic acid.

In all these irradiations, the Hanau Lamp was used. The spiral coil irradiator was also used, but in all cases only very small amounts of the respective acids were isolated.

Both lamps have also been used to irradiate rotenone in the presence of methanol and dihydrorotenone in the presence of cyclohexylamine. In all cases no cleavage products could be isolated from either the neutral or acidic portions of the irradiation mixture.

EXPERIMENTAL

Melting points were taken on a Kofler Micro Hot Stage and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord, and are in chloroform unless otherwise specified. Rotations were determined on an O. C. Rudolf and Sons polarimeter, No. 344. The activity of adsorbants used for chromatograms was determined by the adsorption of dyes according to the procedure of Brockmann[2]. Microanalyses were done by the Microanalytical Laboratory of M. I. T.

Irradiation of Rotenone.---A solution of 5.0 g. (0.0127 mole) of rotenone in 250 ml. of tetrahydrofuran and 50 ml. of water was irradiated with a Hanau lamp for 46 hours while passing nitrogen (purified with Fieser's solution[3]) through the solution. After the specified irradiation time, the solvent was removed under reduced pressure, and the residue taken up in 250 ml. of ether. The ether solution was extracted with three 100-ml. portions of 5 % sodium carbonate solution, the carbonate phase acidified with 10 % sulfuric acid until acidic to Congo Red, and the acidic solution extracted with three 150-ml. portions of ether and dried over sodium sulfate. Removal of the ether gave 230 mg. of a brown oil which was chromatographed on silica gel with chloroform. After an initial, unidentified 113 mg. fraction of a brown oil, there

was eluted with chloroform-ethyl acetate (9:1), 50 mg. (1.8 %) of crude tubaic acid. Recrystallization from hexane gave pure tubaic acid, m.p. 128.5-129.5°, [Lit.[4] m.p. 129°], undepressed on admixture with authentic tubaic acid. The infrared spectrum was identical with that of authentic tubaic acid.

The neutral portion was chromatographed on 150 g. of Merck Acid Washed Alumina (Act. I), but no characterizable material other than rotenone could be isolated.

Control Reaction.---A solution of 5.0 g. of rotenone in 225 ml. of tetrahydrofuran and 75 ml. of water was left standing in the dark for two days. On working up the solution as before, 43 mg. of acidic material was obtained. Chromatography on silica gel with chloroform gave no separation. The infrared spectrum had a band at 1748 $cm.^{-1}$, but had no absorption characteristic of carboxylic acids, nor any bands corresponding to those of tubaic acid.

Dihydrorotenone.---Catalytic reduction of rotenone (16 g.) in 200 ml. of acetone with 1.2 g. of 5 % palladium on barium sulfate catalyst gave dihydrorotenone, m.p. 209-211°, $[\alpha]_D$ -227° (benzene). [Lit.[5] $[\alpha]_D$ -225.2° (benzene)].

Irradiation of Dihydrorotenone.---A solution of 4.2 g. (0.0106 mole) of dihydrorotenone in 150 ml. of tetrahydrofuran and 50 ml. of water was irradiated for 46 hours with a Hanau lamp while passing nitrogen (purified by Fieser's

solution) through the system. Work-up as before gave 440 mg. of a brown oil which was chromatographed on silica gel. After an initial, unidentified fraction of 136 mg. of a brown oil, there was eluted with chloroform-ethyl acetate (9:1), 102 mg. (4.3 %) of crude dihydrotubaic acid. Sublimation at 130° (0.06 mm.) and recrystallization from methanol-water gave 49 mg. (2.1 %) of pure dihydrotubaic acid, m.p. 165-167°, [Lit.[4] m.p. 166°], undepressed on admixture with authentic sample. The infrared spectrum was identical with that of authentic dihydrotubaic acid.

Chromatography of the neutral portion on 150 g. of Woelm Neutral Alumina (Act. I) gave no characterizable material other than dihydrorotenone.

Methyl Ester of Dihydrotubaic Acid.---Dihydrotubaic acid (240 mg.) was esterified with diazomethane in ether. Recrystallization from methanol-water gave 224 mg. of crystalline product, m.p. 76.5-78.5°. [Lit.[6] m.p. 75°].

Irradiation of Rotenone in the Presence of Methanol.---Five grams (0.0127 mole) of rotenone in 100 ml. of tetrahydrofuran and 100 ml. of methanol was irradiated with the Hanau lamp for 108 hours using the same set-up as before. After the specified time, the solvent was removed under reduced pressure and taken up with 250 ml. of ether. Rotenone (2.48 g.) separated and was removed by filtration. The ether

solution was extracted with three 100-ml. portions of 5 % sodium hydroxide, the basic solution acidified with 10 % sulfuric acid until acidic to Congo Red, and the acidic solution extracted with three 150-ml. portions of ether. Drying over sodium sulfate and removal of the ether under reduced pressure gave 425 mg. of a brown oil. Chromatography on silica gel gave no fractions corresponding to the methyl ester of tubaic acid.

Chromatography of the neutral portion on Woelm Neutral Alumina (Act. III) also gave no fractions whose infrared corresponded to the methyl ester of tubaic acid.

N-Cyclohexylamide of Dihydrotubaic Acid.---To 96 mg. (4 mmoles) of magnesium turnings covered with ether was added dropwise 0.26 ml. (4 mmoles) of methyl iodide in 5 ml. of ether. After stirring for one-half hour, all the magnesium had disappeared. The solution was refluxed for 15 minutes, cooled in an ice bath, and 400 mg. (4 mmoles) of cyclohexylamine in 5 ml. of ether added dropwise. After the addition was complete, the ice bath was removed and 218 mg. (0.925 mmole) of the methyl ester of dihydrotubaic acid in 10 ml. of ether was added slowly. After the addition was complete, the solution was refluxed for one-half hour, then stirred at room temperature for 4 hours. The solution was then extracted with 10 ml. of 10 % hydrochloric acid, 10 ml. of sat. sodium

bicarbonate, and 20 ml. of water. Drying over magnesium sulfate and removal of the ether gave 261 mg. of a yellow oil, which was chromatographed on silica gel. There was eluted with benzene 62 mg. of a crystalline product. Several recrystallizations from methanol-water gave 40 mg. of the pure N-cyclohexylamide of dihydrotubaic acid, m.p. 138-139°. The infrared spectrum showed the characteristic amide bands at 1648 $cm.^{-1}$ and 1525 $cm.^{-1}$

Calcd. for $C_{18}H_{25}O_3N$ (M.W. 303.38) C, 71.26; H, 8.31

Found C, 70.92; H, 8.36

Irradiation of Dihydrorotenone in the Presence of Cyclohexylamine.---Dihydrorotenone (4.0 g.) in 125 ml. of tetrahydrofuran and 50 ml. of cyclohexylamine was irradiated for 60 hours with a Hanau lamp using the same set-up as before. After the irradiation was complete, the solvent was removed under reduced pressure and the residue taken up in chloroform. The chloroform solution was extracted with 100 ml. of 10 % hydrochloric acid, 100 ml. of 1 N sodium hydroxide, and three 100-ml. portions of water (until neutral to litmus).

The sodium hydroxide extract was acidified with 10 % sulfuric acid (Congo Red) and extracted with two 100-ml. portions of chloroform. Drying over sodium sulfate and removal of the chloroform gave 107 mg. of a brown oil. Chromatography on silica gel gave no fractions whose infrared

spectrum corresponded to N-cyclohexylamide of dihydrotubaic acid or to dihydrotubaic acid itself.

Removal of the solvent from the neutral portion gave 9 g. of a brown oil. Chromatography on silica gel and on Merck Acid Washed Alumina (Act. I) and rechromatography of various fractions on silica gel and alumina gave no fractions whose infrared corresponded to that of the N-cyclohexylamide of dihydrotubaic acid or which showed the amide II band at 1525 $cm.^{-1}$

Isotubaic Acid.---A solution of 100 mg. of tubaic acid in 1 ml. of glacial acetic acid was warmed to 50°, and 0.5 ml. of conc. sulfuric acid added dropwise. After standing for 5 minutes, the solution was poured into 5 ml. of water and the precipitate collected. The crude acid was purified by sublimation at 130°/0.05 mm., m.p.183-184.5°, [Lit.[7] m.p. 186°].

Irradiation of (-)-Isorotenone.---A solution of (-)-isorotenone in 150 ml. of tetrahydrofuran and 50 ml. of water was irradiated with a Hanau lamp for 47 hours, using the same set-up as before. Work-up in a manner similar to that used in the irradiation of rotenone gave 122 mg. of a brown oil. Chromatography on silica gel gave 21 mg. (0.9 %) of crude isotubaic acid, whose infrared spectrum was identical with that of authentic sample.

BIBLIOGRAPHY

1. D. H. R. Barton and G. Quinkert, Proc. Chem. Soc., 197 (1958). D. H. R. Barton and G. Quinkert, J. Chem. Soc., 1 (1960).

2. H. Brockmann and H. Schodder, Ber., 74, 73 (1945).

3. L. Fieser, J. Am. Chem. Soc., 46, 2639 (1924).

4. S. Takei and M. Koide, Ber., 62, 3030 (1929).

5. F. B. LaForge and L. E. Smith, J. Am. Chem. Soc., 51, 2574 (1929).

6. S. Takei, M. Koide, and S. Miyajima, Ber., 63, 1369 (1930).

7. H. L. Haller and F. B. LaForge, J. Am. Chem. Soc., 52, 2480 (1930).

BIOGRAPHICAL SKETCH

The author was born on November 21, 1934 in New Baden, Illinois. He received his primary and secondary education in that city, attending St. George's Parochial School and the New Baden Community High School, graduating in June 1952. In September, 1952 he entered the University of Illinois, obtaining a B. S. in Chemistry in June, 1956. In September, 1956 he entered the Massachusetts Institute of Technology to undertake graduate study leading to the doctorate in organic chemistry.