[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

ROTENONE. XX. THE STRUCTURE OF TUBAIC ACID

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In a brief note recently published in THIS JOURNAL[1] it was reported that on catalytic hydrogenation tubaic acid ($C_{12}H_{12}O_4$) yields a mixture of dihydrotubaic ($C_{12}H_{14}O_4$) and tetrahydrotubaic ($C_{12}H_{16}O_4$) acids. The latter was stated to be 2,4-dihydroxy-3-isoamylbenzoic acid, and its relation to tubaic acid was indicated. The present paper presents the experimental evidence for these statements.

Tetrahydrotubaic acid is practically insoluble in cold chloroform, in alcoholic solution it gives a violet color with ferric chloride, and it is optically inactive. It readily forms a diacetyl derivative ($C_{16}H_{20}O_6$) and a monomethoxy acid ($C_{13}H_{18}O_4$). The monomethoxy acid resists further methylation. When heated to its melting point the tetrahydro acid loses carbon dioxide, forming an alkyl resorcinol.

The fact that tetrahydrotubaic acid is practically insoluble in cold chloroform, in contrast to tubaic and dihydrotubaic acids, which are soluble in this solvent, suggests that tetrahydrotubaic acid contains an hydroxyl group para to the carboxyl group, as para hydroxy acids are insoluble in cold chloroform.[2]

Tubaic acid is optically active, but isotubaic acid ($C_{12}H_{12}O_4$), which differs from tubaic acid in the position of a double bond, is optically inactive. The resolution of dihydroisotubaic acid,[3] the levo form of which is identical with dihydrotubaic acid, proves that the isomerization of tubaic acid to isotubaic acid is due to the migration of a hydrogen atom originally attached to an asymmetric carbon atom. It follows, therefore, that the asymmetric carbon atom in tubaic acid possesses one hydrogen atom.

Tetrahydrotubaic acid also is optically inactive, indicating that one of its hydroxyl groups is formed by the opening of a ring containing an indifferent oxygen atom which is attached on one side to an asymmetric carbon atom. The disappearance of the optical activity of tubaic acid on drastic hydrogenation, as well as in the isomerization of tubaic to isotubaic acid, is proof that tubaic acid has but one asymmetric center.

Diacetyltetrahydrotubaic acid, which is easily obtained from the tetrahydro acid by the action of acetic anhydride and sodium acetate, is converted into a monoacetyltetrahydrotubaic acid when it is refluxed in a

[1] Haller and LaForge, THIS JOURNAL, **53**, 4460 (1931).

[2] Meyer, "Analyse und Konstitutionsermittlung organischen Verbindungen," 5th ed., 1931, p. 418.

[3] Butenandt and Hildebrandt, *Ann.*, **477**, 245 (1930); Takei, Koide and Miyajima, *Ber.*, **63**, 1369 (1930).

solution of potassium acetate in absolute alcohol. In alcoholic solution this monoacetyl derivative gives a violet color with ferric chloride.

On methylation of tetrahydrotubaic acid with dimethyl sulfate and dilute alkali, its monomethoxymethyl ester is obtained, which is readily saponified, to the monomethoxy acid ($C_{13}H_{18}O_4$).

Attempts to methylate the second hydroxyl group were unsuccessful.

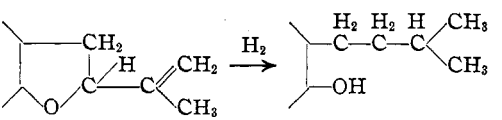
The fact that one of the hydroxyl groups in tetrahydrotubaic acid resists methylation, and the corresponding acetyl derivative is easily saponified, indicates that the hydroxyl group involved is di-ortho substituted.

When heated to its melting point the tetrahydro acid loses carbon dioxide and is converted into a crystalline substance ($C_{11}H_{16}O_2$) which gives the characteristic reactions of resorcinol.

That only one alkyl group is present in the diphenol and that this group is an isoamyl group is shown by the following facts: on oxidation with potassium permanganate tubaic acid yields only acetic acid, whereas under the same conditions isotubaic and dihydrotubaic acids give isobutyric acid.

Takei[4] isolated isovaleric acid by alkali fusion of isotubanol ($C_{11}H_{12}O_2$), (decarboxylated isotubaic acid), and he also demonstrated the presence of the isoallyl group in tubaic acid. On ozonization of tubanol methyl ether, he obtained a methyl ether methyl ketone, which on oxidation with hypoiodide yielded an iodine-containing carboxylic acid that had one carbon atom less than the methyl ketone. We have ozonized the acetyl derivative of tubaic acid ($C_{14}H_{14}O_5$) and obtained from the ozonide a product which analyzes for a compound of formula $C_{13}H_{12}O_6$ and gives the reactions of a methyl ketone.

From the foregoing facts it follows that grouping A is present in tubaic acid and that on drastic hydrogenation it is changed to grouping B.



A B

Tubaic acid and all rotenone derivatives which are converted by hydrogenation into phenolic compounds therefore contain the grouping $-\overset{1}{O}-\overset{2}{CH}-\overset{3}{C}=\overset{4}{CH_2}$. This grouping is comparable with that of a conjugated system, and hydrogenation takes place with the opening of the ether ring.[5] This reaction is explained by the assumption that a 1,4-addition of hydrogen (at —O— and C_4) first takes place, followed by the formation of the double linkage at 2,3. This double linkage is further reduced in the formation of

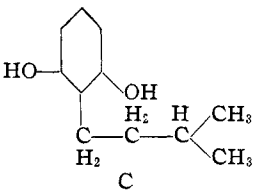
[4] Takei, Koide and Miyajima, *Ber.*, **63**, 1369 (1930).

[5] Schöpf, *Ann.*, **452**, 237 (1927); **483**, 157 (1930).

tetrahydrotubaic and dihydrorotenonic acids, but remains unsaturated in rotenonic acid, which therefore contains the grouping $-\overset{H_2}{C}-\overset{H}{C}=C(CH_3)_2$. This reaction is analogous to the formation of β-tetrahydrodesoxycodeine from desoxycodeine-C.[6]

The position of the alkyl group in the resorcinol derivative is either 2, 4 or 5 (formula C). Position 4 is excluded because this alkyl resorcinol is known. Its melting point is 62–62.5°, while the compound obtained from tetrahydrotubaic acid melts at 85°. Position 5 is excluded because alkyl groups meta to the hydroxyl groups in resorcinol do not give a fluorescein reaction. Also it is more probable that the two linkages on the benzene nucleus of the original oxide ring of tubaic acid are ortho to each other rather than meta, and therefore the alkyl group and the hydroxyl group in tetrahydrotubaic acid are also ortho to each other rather than meta. It follows, therefore, that the alkyl group occupies position 2.

The behavior of the tetrahydro acid indicates that the carboxyl group is ortho to one of the hydroxyl groups and para to the other. Tetrahydrotubaic acid is best represented by D.



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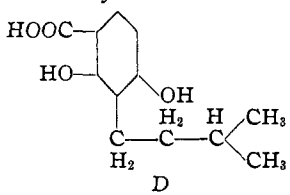

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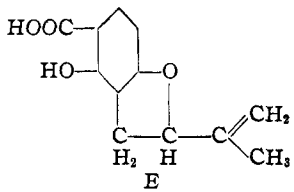


E

Structure E, which satisfactorily accounts for all the known facts, is now proposed for tubaic acid.

The relation of tubaic acid to rotenone has been discussed in a previous paper.[7]

Tubaic acid was considered to be a secondary decomposition product because at that time[8] the rotenone molecule was supposed to contain a lactone group and also because it was difficult to account otherwise for the failure to obtain tubaic acid when rotenone derivatives were oxidized with hydrogen peroxide, as tubaic acid is stable to this oxidizing agent. The fact that no tubaic acid is obtained is satisfactorily accounted for, however, with consideration of the observation of Dakin[9] on the oxidation of hydroxy aromatic aldehydes and ketones with hydrogen peroxide. Dakin has shown that hydroxy aromatic aldehydes and ketones in which the free hydroxyl group is in the ortho or para position to the aldehyde or ketone group, are oxidized by hydrogen peroxide with the formation of polyphenols. *No substituted benzoic acid is formed in the reaction.*

[6] Small and Cohen, THIS JOURNAL, **53**, 2221 (1931).

[7] LaForge and Haller, *ibid.*, **54**, 810 (1932).

[8] LaForge, Haller and Smith, *ibid.*, **53**, 4400 (1931).

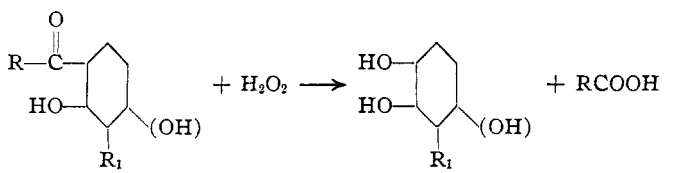
[9] Dakin, *Am. Chem. J.*, **42**, 477 (1909).

Most of the rotenone derivatives which are cleaved by hydrogen peroxide possess an hydroxyl group ortho to the carbonyl group, and in some cases an hydroxyl group is present in both the ortho and para positions.

Derrisic acid has an hydroxyl group ortho to the carbonyl group. In this case, on oxidation with hydrogen peroxide, cleavage takes place between the carbonyl group and the benzene ring with the formation of derric acid and probably a catechol derivative, which is for the most part oxidized further by the excess of peroxide. No tubaic acid is formed in the process.

Dehydrodihydrorotenonic acid possesses an hydroxyl group para to the carbonyl group. In the oxidation of this compound hydrolysis of the ortho (ether) oxygen linkage probably takes place first, and oxidation then proceeds with the formation of risic acid.

Methylderritolic and dihydrorotenolic acids have a hydroxyl group in the ortho position as well as in the para position, and oxidation takes place without the formation of tubaic acid. On the other hand such compounds as rotenone itself and rotenonic acid are not cleaved by hydrogen peroxide. These reactions are illustrated as follows



Experimental

Tetrahydrotubaic Acid (2,4-Dihydroxy-3-isoamylbenzoic Acid).—One gram of tubaic acid dissolved in 25 cc. of ethyl acetate was reduced with hydrogen at 48 lb. pressure, with 0.5 g. of freshly prepared reduced platinum oxide as catalyst. The reduction was allowed to proceed for two hours. The solution was filtered and concentrated to dryness on the steam-bath. The mixture of dihydro and tetrahydrotubaic acid was dissolved in 95% alcohol. The solution, heated to boiling, was diluted with an equal volume of hot water and filtered through charcoal.

The crystals which deposited on cooling were found to be pure dihydrotubaic acid. On concentration of the filtrate a second crop of crystals was obtained. This was filtered off, dried and then washed several times with cold chloroform. The remaining substance was crystallized from 20% alcohol. It can also be recrystallized from benzene, chloroform or toluene. It melted at 206° with decomposition, and was optically inactive. The yield was about 0.1 g.

Anal. Subs., 0.0822, 0.0806: CO_2, 0.1931, 0.1903; H_2O, 0.0529, 0.0526. Calcd. for $C_{12}H_{16}O_4$: C, 64.25; H, 7.20. Found: C, 64.07, 64.40; H, 7.20, 7.30. *Titration.* Subs., 0.0297: 1.34 cc. of *N*/10 KOH. Calcd. mol. wt., 224.13. Found: acid equivalent, 222.

Attempts were made to increase the yield of tetrahydrotubaic acid by the use of different solvents as well as by the addition of pinene to the ethyl acetate solution. Ethyl alcohol (95%) and glacial acetic acid were employed as solvents, but in all cases there was no appreciable increase in the amount of tetrahydrotubaic acid formed.

Diacetyltetrahydrotubaic Acid.—Two-tenths gram of tetrahydrotubaic acid was

refluxed in 5 cc. of acetic anhydride and 0.2 g. of anhydrous sodium acetate for one hour. After most of the solvent had been removed by distillation, water was added. The precipitated oil soon crystallized. The substance was filtered off and dissolved in 10 cc. of 95% alcohol. After the solution had been refluxed for one hour, it was diluted with an equal volume of hot water, filtered through charcoal and allowed to crystallize. The diacetate which separated melted at 143°. The yield was 0.18 g.

Anal.[10] Subs. (mg.), 3.052, 3.997: CO_2, 7.020, 9.151; H_2O, 1.750, 2.335. Calcd. for $C_{16}H_{26}O_6$: C, 62.31; H, 6.52. Found: C, 62.73, 62.44; H, 6.42, 6.54. *Titration.* Subs., 0.0338: 2.15 cc. of *N*/20 KOH. Calcd. mol. wt., 308.16. Found: acid equivalent, 314.

To 0.0338 g. of the diacetyltetrahydrotubaic acid was added 8.75 cc. of *N*/20 KOH. The solution was heated on the steam-bath for one-half hour, cooled and the excess of alkali was titrated with *N*/10 hydrochloric acid. The reaction required 3.28 cc. of *N*/20 potassium hydroxide; calcd. for 2 acetyl: 3.28 cc. of *N*/20 KOH.

Monoacetyltetrahydrotubaic Acid.—Two-tenths gram of diacetyltetrahydrotubaic acid was dissolved in 2 cc. of absolute alcohol containing 0.2 g. of potassium acetate. The solution was refluxed for one hour and then diluted with an equal volume of water. After the solution had cooled, 0.5 cc. of 20% sulfuric acid was added carefully. The substance, which separated immediately, was crystalline. It was filtered off, washed with water and dried. The yield was 0.08 g. It melted at 156°. In alcoholic solution it gave a purple color with ferric chloride.

Anal. Subs. (mg.), 3.223, 3.406: CO_2, 7.452, 7.869; H_2O, 1.991, 2.068. Calcd. for $C_{14}H_{18}O_5$: C, 63.13; H, 6.82. Found: C, 63.07, 63.01; H, 6.91, 6.79. *Titration.* Subs., 0.0270: 2.05 cc. of *N*/20 KOH. Calcd. mol. wt., 266.14. Found: acid equivalent, 264.

To 0.0270 g. of monoacetyl tetrahydrotubaic acid was added 5.20 cc. of *N*/20 potassium hydroxide. The solution was heated on the steam-bath for one-half hour. It was then cooled, and the excess alkali was titrated with *N*/10 hydrochloric acid. The reaction required 4.07 cc. of *N*/20 potassium hydroxide. Calcd. for 1 acetyl, 4.07 cc. of *N*/20 KOH. On addition of excess acid to the solution obtained above, tetrahydrotubaic acid separated. It was identified by its melting point.

Methyltetrahydrotubaic Acid (2-Hydroxy-4-methoxy-3-isoamylbenzoic Acid).—To 0.2 g. of tetrahydrotubaic acid dissolved in 5 cc. of 5% potassium hydroxide solution was added 0.5 cc. of dimethyl sulfate. The solution was frequently shaken and was kept alkaline by further addition of alkali. After two hours, it was acidified with dilute sulfuric acid. The precipitate was filtered off, washed with water and then refluxed in 95% alcohol to which had been added 1 cc. of 5% potassium hydroxide in order to saponify any ester that had formed. The solution was concentrated, diluted with an equal volume of water, filtered through charcoal and acidified with dilute sulfuric acid. On cooling, the methoxy acid separated. The yield was 0.18 g. The acid was recrystallized from dilute alcohol. It can also be crystallized from benzene. It melted at 156°. In alcoholic solution it gave a purple color with ferric chloride.

Anal. Subs. (mg.), 3.387, 4.300: CO_2, 8.110, 10.357; H_2O, 2.300, 2.939. Calcd. for $C_{13}H_{18}O_4$: C, 65.51; H, 7.62. Found: C, 65.30, 65.69; H, 7.60, 7.65.

An attempt was made to methylate the second hydroxyl group by using a large excess of methyl iodide and silver oxide and also with methyl iodide and potassium carbonate, but in each case unchanged starting material was recovered.

[10] We are indebted to Mr. J. R. Spies of this Division for the microcombustions reported in this paper.

Tetrahydrotubanol (2-Isoamylresorcinol).—Five-tenths gram of tetrahydrotubaic acid was heated in a Wood's metal bath at 215–225° for about five minutes. When the evolution of carbon dioxide, which took place vigorously at first, had ceased, the remaining reddish liquid was distilled under reduced pressure. On cooling it readily crystallized. The substance was recrystallized from chloroform–petroleum ether. It can also be recrystallized from benzene–petroleum ether (b. p. 37–75°). It melted at 85°.

Anal. Subs. (mg.), 3.560, 3.936: CO_2, 9.504, 10.521; H_2O, 2.807, 3.115. Calcd. for $C_{11}H_{16}O_2$: C, 73.28; H, 8.95. Found: C, 72.81, 72.90; H, 8.82, 8.86.

The compound gave a precipitate with bromine water, and a cherry red color with Millon's reagent. With Lieberman's reagent, an intense blue color was obtained. This soon turned quite dark and on dilution with water the color practically disappeared. There was no change on addition of alkali. When the substance was dissolved in chloroform, and a small piece of potassium hydroxide was added a rose-red color was slowly produced around the edges of the potassium hydroxide (Guareschi–Lustgarten reagent).[11] In alcoholic solution no color test was obtained with ferric chloride, but in aqueous solution a purplish-blue color was formed with this reagent. The color disappeared on addition of sodium bicarbonate.

With formaldehyde and sulfuric acid a red ring slightly tinged with violet was obtained.[12] After fusion with phthalic anhydride and a drop of concentrated sulfuric acid, the diluted solution became red with a green fluorescence when excess alkali was added.

Ozonization of Acetyltubaic Acid.—A stream of ozonized oxygen was passed into a solution of 1 g. of acetyltubaic acid in 15 cc. of chloroform for four hours Most of the chloroform was then removed by distillation. Water was added to the remaining sirup, and the solution was boiled for fifteen minutes. It was then cooled, and extracted with ether. The ether extract was dried over sodium sulfate. On removal of the ether, the substance readily crystallized. It was washed with isopropyl ether and recrystallized from a solution of 20% acetic acid—80% butyl ether. The yield was 0.2 g. It melted at 145°.

Anal. Subs., 0.0832, 0.0845: CO_2, 0.1784, 0.1834; H_2O, 0.0356, 0.0358. Calcd. for $C_{13}H_{12}O_6$: C, 59.07; H, 4.58. Found: C, 58.48, 59.19; H, 4.79, 4.74. Subs., 0.0475: cc. of $N/10$ KOH, 1.87. Calcd. mol. wt., 264.1. Found: acid equivalent, 254.

The substance reduced Fehling's solution, and with sodium hydroxide and iodine it gave iodoform. The same compound was obtained when acetyltubaic acid was ozonized in glacial acetic acid.

Conversion of Tetrahydrotubanol to Tetrahydrotubaic Acid.—Two-tenths gram of tetrahydrotubanol was refluxed in 50 cc. of a saturated solution of sodium bicarbonate for half an hour. The solution was cooled, filtered and acidified with dilute sulfuric acid. The precipitate was filtered off, washed with water and dried. It was then twice recrystallized from benzene. It melted at 206° and was identified as tetrahydrotubaic acid.

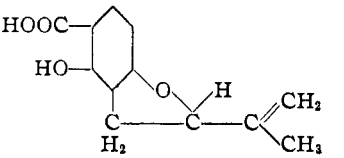
Summary

Tubaic acid is reduced by hydrogenation with platinum oxide catalyst to a mixture of dihydro and tetrahydrotubaic acid.

Tetrahydrotubaic acid is 2,4-dihydroxy-3-isoamylbenzoic acid, and tubaic acid is represented by the formula

[11] Rosenthaler, "Der Nachweis organischen Verbindungen," 1923, p. 240.

[12] Mulliken, "Identification of Pure Organic Compounds," 1908, Vol. I, p. 24.



An explanation of the mechanism of the oxidation of rotenone derivatives by hydrogen peroxide is given.

The behavior of tubaic acid on hydrogenation is analogous to that of desoxycodeine-C. Both contain the same groupings and both give tetrahydrophenols under the same conditions.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, CASE SCHOOL OF APPLIED SCIENCE]

QUINAZOLINES. III. THE INTERACTION OF ANILINE WITH 2-CHLORO-4-ALKOXYQUINAZOLINES AND 2-CHLORO-4-KETODIHYDROQUINAZOLINE

BY N. A. LANGE AND F. E. SHEIBLEY

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When 2,4-dichloroquinazoline in alcohol is boiled with sodium acetate, a compound with properties and a composition corresponding to a chloroketodihydroquinazoline is always found among the reaction products. In an earlier communication it was stated that by analogy with the similarly formed 2-chloro-4-alkoxyquinazolines this substance was most likely 2-chloro-4-ketodihydroquinazoline but that attempts to confirm this view by converting it into the known 4-ketodihydroquinazoline and 2-ethoxy-4-ketodihydroquinazoline were unsuccessful.[1]

The ease with which aniline replaces both of the chlorine atoms of 2,4-dichloroquinazoline with phenylamino groups suggested the possibility of transforming 2-chloro-4-alkoxyquinazolines into 2-anilino derivatives. By treating 2-chloro-4-methoxyquinazoline (I) and 2-chloro-4-ethoxyquinazoline (III) with aniline in alcohol, hydrochlorides of 2-anilino-4-methoxyquinazoline (IV) and 2-anilino-4-ethoxyquinazoline (VI), respectively, are obtained. These last, on hydrolysis with dilute hydrochloric acid in the presence of aniline, or simply on heating, when the elements of an alkyl chloride are evolved, revert to the known 2-anilino-4-ketodihydroquinazoline (V), a compound which results directly when the previously mentioned chloroketo compound is treated with an alcoholic solution of aniline, and thus identifies the latter as 2-chloro-4-ketodihydroquinazoline (II).

A confirmation of the orientation of the halogen and alkoxy groups in the chloro-alkoxy derivatives arrived at in a previous paper[2] is afforded

[1] Lange and Sheibley, THIS JOURNAL, **53**, 3871 (1931).

[2] Lange, Roush and Asbeck, *ibid.*, **52**, 3696 (1930).