5.   The interpretation of the maximum point in the curve of lactic acid production from gentiobiose and melibiose is based on the assumption that a 6-hexosido-glucose-4,5-enediol must have formed in each case in the regions of higher alkalinity.   A mechanism is given to explain the decreasing yield of this acid after the point of maximum production has been passed.

COLUMBUS, OHIO

ROTENONE. XVI.
INTERPRETATION OF SOME CHARACTERISTIC REACTIONS OF ROTENONE

BY F. B. LAFORGE, H. L. HALLER AND L. E. SMITH

During the past four or five years, research by several groups of investigators in different parts of the world has produced a large number of important facts bearing on the structure of the rotenone molecule.

Many of the observed reactions are apparently without analogy, and it has not been possible to coördinate any group of facts with a satisfactory structural formula.

From the observations of Butenandt[1] and others it has been established that the rotenone molecule ($C_{23}H_{22}O_6$) consists of two components each containing eleven carbon atoms joined through a carbonyl group.   One of these components contains a benzene ring to which two methoxyl groups are attached; the other is related to tubaic acid.   Cleavage takes place at the carbonyl group when rotenone and its derivatives are subjected to oxidation or hydrolysis.

$$(C_{11}H_{11}O_3)CO(C_{11}H_{11}O_2)$$
Derric acid component   Tubaic acid component

Rotenone and most of its derivatives contain a lactone group which is peculiar in that it is opened by hydrogenation, with the formation of desoxy acids.[2]   The desoxy acids are formed with loss of an asymmetric center.[3] As will be shown later, the lactone ring is located in that part of the molecule which yields tubaic acid.   The conversion of the lactones into acids in order to render them soluble in alkali, is taken advantage of in the peroxide oxidation process described in previous articles.[4]

[1] Butenandt, *Ann.*, **464**, 253 (1928).

[2] LaForge and Smith, THIS JOURNAL, 51, 2574 (1929); **52**, 1088 (1930); **52**, 1091 (1930); **52**, 3603 (1930).

[3] Haller and LaForge, *ibid.*, **53**, 3426 (1931).

[4] LaForge and Smith, *ibid.*, (a) **52**, 1091 (1930); (b) **52**, 3603 (1930); (c) Smith and LaForge, *ibid.*, **52**, 4595 (1930).

Among the observations recorded by Butenandt in his first article, three have proved to be of unusual interest: (1) the formation of the phenolic compound derritol ($C_{21}H_{22}O_6$) from rotenone, with the loss of two carbon atoms and the simultaneous formation of rotenol ($C_{23}H_{24}O_6$), by reduction with zinc in alkaline solution; (2) the formation of dehydrorotenone ($C_{23}H_{20}O_6$) by mild oxidation, a reaction which involves the loss of two hydrogen atoms and the disappearance of one of the two original asymmetric centers of rotenone; (3) the formation of derrisic acid ($C_{23}H_{24}O_8$) from dehydrorotenone by the addition of two molecules of water in the presence of alkali.

Derritol, rotenol, dehydrorotenone and derrisic acid are formed by changes in the methoxyl-containing part of the rotenone molecule. Oxidation of these derivatives or their corresponding desoxy acids in this Laboratory has resulted in the isolation of the methoxyl-containing part of all four, in the form of acids, the structures of which have been definitely established with the exception of the one obtained from rotenol.

From derritol, after methylation of the phenol group, 2,4,5-trimethoxy-phenylacetic acid was obtained,[4c] which was further degraded to 2,4,5-trimethoxybenzoic acid.[5] This acid was also obtained by direct oxidation of derritol methyl ether.[5] Rotenol yielded netoric acid,[4c] a monobasic dimethoxy acid ($C_{12}H_{14}O_5$), the structure of which is not yet fully established. 2-Hydroxy-4,5-dimethoxybenzoic acid was obtained by permanganate oxidation of dehydrorotenone in acetone solution.[6] Derritol also yielded the same acid by this method.[5] Rissic acid[4b] was obtained by peroxide oxidation of dehydrodihydrorotenonic acid, the desoxy acid corresponding to dehydrodihydrorotenone. Rissic acid was shown to be 3,4-dimethoxy-6-carboxy-phenoxyacetic acid (A).[7] Derrisic acid, on peroxide oxidation, yielded derric acid[4a] which has been shown to have the structure (B).[7]

(A)   (B)
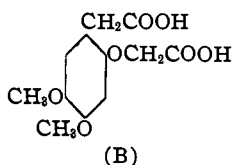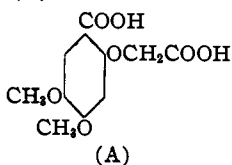
Derric acid is readily oxidized to rissic acid,[8] which in turn is converted by elimination of one carboxyl group, into decarboxyrissic[7,9] acid, which was shown by synthesis to be 3,4-dimethoxyphenoxyacetic acid.[7]

With the structure of these acids established it is now possible to picture

[5] Smith and LaForge, THIS JOURNAL, 53, 3072 (1931).

[6] Unpublished results obtained in this Laboratory.

[7] LaForge, THIS JOURNAL, 53, 3896 (1931).

[8] LaForge and Smith, ibid., 52, 2878 (1930).

[9] Takei, Miyajima and Ono, Ber., 64, 248 (1931).

the probable mechanism of the formation of derritol, rotenol, dehydro-rotenone and derrisic acid.

Derritol and Rotenol.—Butenandt,[1] has suggested that derritol contains the grouping (C) and that it is formed by hydrolysis and reduction from the grouping (D) contained in rotenone. This mechanism appears to

(C)   (D)
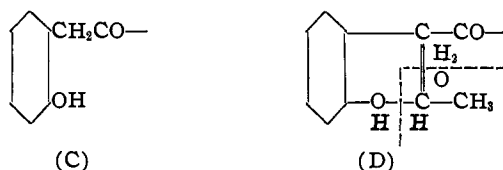
be substantially correct, as we have obtained 2,4,5-trimethoxyphenyl-acetic acid (E)[10] from derritol methyl ether.[4c] It has been definitely established, however, that the part of the rotenone molecule in question contains an asymmetric carbon atom[11] and for this reason as well as for others which will be referred to later rotenone must be represented by formula (F). This grouping undergoes rearrangement under the influence

(E)   (F)
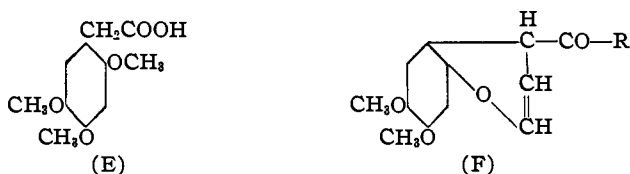
of alkali whereby the double bond shifts to the α,β-position, where it is subject to hydrolysis and reduction substantially as suggested by Butenandt.
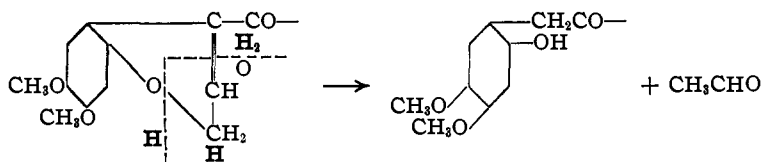
Rotenol is formed simultaneously with the derritol. In this case it seems probable that after the double bond has shifted to the α,β-position it

(G)

is reduced instead of hydrolyzed and that rotenol is represented by formula (G). This assumption is supported by the fact that rotenol yields netoric acid ($C_{12}H_{14}O_5$), which would correspond to the acid containing the grouping in the formula given above. This theory is also in agreement with the fact that isorotenol and rotenolic acid are optically inactive,[3] for

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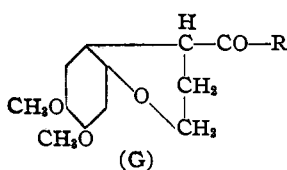
[10] The correct melting point for this acid is 83° instead of 93°.[4c]

[11] Butenandt and Hildebrandt, *Ann.*, **477**, 249 (1930).

the original optically active center would be lost with the shifting of the double bond and the reduction product would be racemic. Rotenol is converted by oxidation into dehydrorotenol,[12] which might be represented by formula (H). Dehydrorotenol is easily reconverted into rotenol by reduction with zinc and alkali,[12] a property which is common to α,β-unsaturated ketones. The above-mentioned scheme as applied to rotenol, however, is suggested with caution, because as yet the structure of netoric acid is not fully established and also for the reason that the presence of the ketone group in rotenol has not been demonstrated with satisfaction. We have an indication of its presence in isorotenol, which is reduced by Clemmenson's reagent to isodesoxyrotenol ($C_{23}H_{26}O_5$).[12] Butenandt considered rotenol[1] to be the carbinol corresponding to rotenone, but there is no evidence to support this assumption.
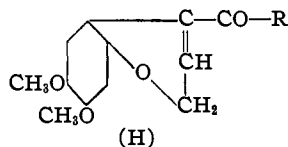
Rotenol is much more easily cleaved by alkali than is rotenone and gives an unexpectedly large yield of isotubaic acid.[13] It is possible that in addition to the reaction stated above some change may have taken place in that part of the molecule from which tubaic acid is derived and concerning which our knowledge is as yet very incomplete.

Dehydrorotenone.—Dehydrorotenone may be prepared from rotenone by a number of mild oxidative agents, the most convenient being iodine in alcoholic potassium acetate solution.[1] In this process the acetyl derivative of an hydroxy rotenone is obtained as an intermediary product.[4a] The hydroxyrotenone, named rotenolone, is easily dehydrated to dehydrorotenone.[4a] Thus, by this method, the first step of the reaction is probably one of iodine substitution. The iodine is then replaced by the acetic acid radical, forming acetylrotenolone, which is easily hydrolyzed to rotenolone.

If rotenone is expressed by formula (I) in which R is the part of the molecule containing the lactone group and the easily reducible double bond and from which tubaic acid is derived, then rotenolone would be the corresponding hydroxyl derivative (J). Dehydrorotenone would result from the loss of the elements of water from rotenolone and would be expressed by formula (K).[14]
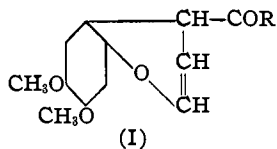
[12] Haller and LaForge, THIS JOURNAL, **53**, 2271 (1931).

[13] Haller and LaForge, *ibid.*, **52**, 4505 (1930).

[14] This cyclic allenic formula is admittedly without supporting analogy, but it is the only one we have been able to propose which is in agreement with the facts so far observed. It is therefore offered as a tentative expression which may later have to be modified or withdrawn. Attempts are now being made to prepare simple derivatives containing the grouping in question in order to study their properties.

$$\text{(J)} \qquad \text{CH}_3\text{O}\diagdown\text{C(OH)—CO—R} \qquad\qquad \text{(K)} \qquad \text{CH}_3\text{O}\diagdown\text{C—CO—R}$$
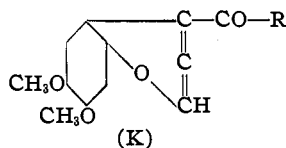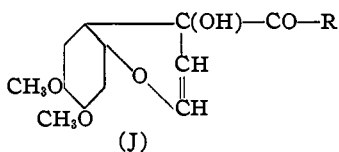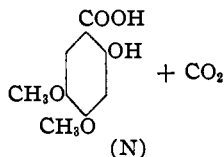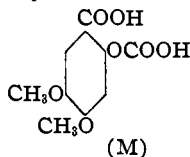
This reaction would explain the disappearance of the original asymmetric center of rotenone in the formation of the dehydro compounds.

Isorotenone and dihydrorotenone yield corresponding dehydro derivatives.[4a] Isodehydrorotenone may be prepared either by oxidation of isorotenone or by isomerization of dehydrorotenone.[10] Dehydrodihydrorotenone is obtained by oxidation of dihydrorotenone or by hydrogenation of dehydrorotenone.[4a] In the last-mentioned process, the larger part of the dehydrorotenone is converted, by the opening of the lactone ring, into dehydrodihydrorotenonic acid.
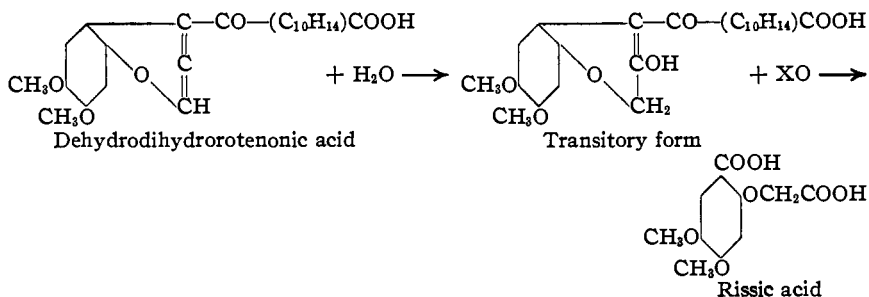
$$\text{(L)} \qquad \text{CH}_3\text{O} \diagdown \text{C*—CO—} \quad \text{C*H}$$
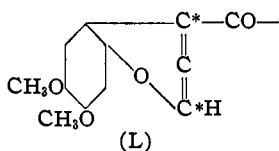
Direct oxidation of dehydrorotenone or any of its derivatives in acetone with permanganate yields 2-hydroxy-4,5-dimethoxybenzoic acid,[6] a process which may be illustrated as follows.

Dehydrorotenone (L) is oxidized at the carbon atoms indicated by * to the acid (M) which is unstable and yields the acid (N).

$$\text{(M)} \qquad \text{CH}_3\text{O}\diagdown\overset{\text{COOH}}{\underset{}{\bigcirc}}\text{OCOOH} \qquad\qquad \text{(N)} \qquad \text{CH}_3\text{O}\diagdown\overset{\text{COOH}}{\underset{}{\bigcirc}}\text{OH} + \text{CO}_2$$

Dehydrodihydrorotenonic acid is obtained as mentioned above by hydrogenation of dehydrorotenone and would still contain the cumulated double bonds, characteristic of the dehydro series, which are not affected by catalytic hydrogenation.

Dehydrodihydrorotenonic acid yields rissic acid on peroxide oxidation in alkaline solution.[4b] The reactions involved are related to the mechanism of the formation of derrisic acid as will be shown below. They may be illustrated as follows

$$\text{CH}_3\text{O}\diagdown\text{C—CO—(C}_{10}\text{H}_{14})\text{COOH} \quad + \text{H}_2\text{O} \longrightarrow \text{CH}_3\text{O}\diagdown\text{C—CO—(C}_{10}\text{H}_{14})\text{COOH} \quad + \text{XO} \longrightarrow$$

Dehydrodihydrorotenonic acid   Transitory form

$$\text{CH}_3\text{O}\diagdown\overset{\text{COOH}}{\underset{}{\bigcirc}}\text{OCH}_2\text{COOH}$$
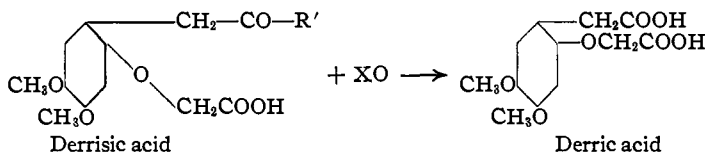
Rissic acid

Derrisic Acid.—When dehydrorotenone is boiled with alcoholic alkali it adds two molecules of water and is converted into derrisic acid, $C_{23}H_{24}O_8$.[11] This reaction is illustrated graphically as follows
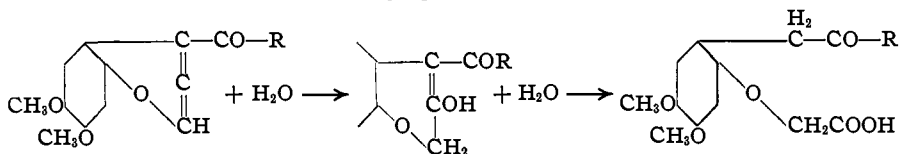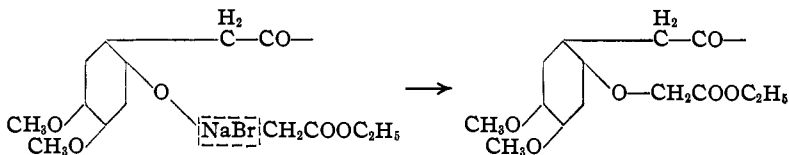

Derrisic, dihydro and isoderrisic acids are oxidized by hydrogen peroxide in alkaline solution to derric acid.

Derrisic acid   Derric acid

The above formula has also been confirmed by synthesis of derrisic, di-hydro- and isoderrisic acids from derritol, dihydro- and isoderritol. When dihydro- or isoderritol is treated with bromoacetic ester in the presence of sodium ethylate in about molecular proportions, condensation takes place probably as indicated below.

The esters are easily converted into the corresponding dihydro- and iso-derrisic acids.

Derritol by the same treatment yields derrisic acid ethyl ester but at the same time a small quantity of dehydrorotenone is formed which must be removed by crystallization or by saponifying the ester and reësterifying the recrystallized derrisic acid.

The α-hydroxyrotenonic acids[15] described by us, which are easily con-verted into the dehydro acids, probably contain a tertiary hydroxyl group.

The fact that rotenolone gives derritol and rotenol[4a] is explained by the assumption that the group $-\overset{|}{C}(OH)COR$ would be first reduced to $-\overset{|}{C}HCOR$, the characteristic arrangement of rotenone, after which the re-action would proceed as indicated above in the discussion of derritol and rotenol.

The Part of the Rotenone Molecule from which Tubaic Acid is De-rived.—Tubaic acid ($C_{12}H_{12}O_4$) was first prepared by Kariyone[16] by the
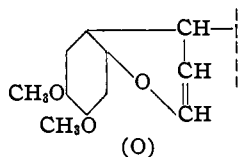
[15] LaForge and Smith, THIS JOURNAL, **52**, 3603 (1930).

[16] Kariyone, Kimura and Kondo, J. Pharm. Soc. Japan, No. 514, 1049 (1924).

action of strong alkali on rotenone. It is easily converted into an isomeric acid. Tubaic or isotubaic (rotenic) acid has since been obtained from a number of rotenone derivatives including all of those discussed in detail in this article.[13] These acids have been studied by Takei and others.

In addition to being represented by the formula given above, it seems likely that both tubaic and isotubaic acids contain an indifferent oxygen atom and that tubaic acid contains the easily reducible double bond present in rotenone. This double bond changes its position when tubaic acid is converted into isotubaic acid, and the change is the same as that involved in the formation of the compounds of the iso series. In its more stable position the double bond is reduced only with difficulty, and in the iso series the lactone ring is no longer opened by hydrogenation to form desoxy acids.

As a result of our investigations described above it is apparent that the indifferent oxygen atom of rotenone is contained in that part of the molecule containing the methoxyl groups whose structure is indicated by formula (O). The group represented by R would have the formula $C_{11}H_{11}O_2$ and the corresponding acid, assuming that cleavage takes place as indicated, by $C_{12}H_{12}O_4$, which is the formula for tubaic acid. As many of the derivatives of rotenone react as lactones, the lactone group must be contained in the part of the molecule from which tubaic acid is derived. There is, however, no indication of a lactone group in tubaic acid, which seems to contain an indifferent oxygen atom in addition to a carboxyl and an hydroxyl group. None of the desoxy acids derived from any of the rotenone derivatives by hydrogenation yield either tubaic or isotubaic acid, but they yield compounds whose structure is as yet unknown.[13]

In fact, except in the case of the shifting of the double bond, tubaic acid shows none of the characteristic reactions of rotenone, and we are obliged to consider it as a secondary reaction product, the process of the formation of which is as yet very obscure.

Experimental

Dihydroderrisic Acid Ethyl Ester from Dihydroderritol.—One and one-half grams of dihydroderritol[17] was dissolved in 30 cc. of absolute alcohol to which were added 3.7 cc. of alcohol containing 2.5 g. of sodium in 100 cc. (about the calculated quantity of sodium), and 0.7 g. of bromoacetic ester. The solution was heated in a sealed tube for one and one-half hours at 100°. A mass of crystals separated on cooling which was recrystallized from 95% alcohol. The substance crystallizes as long needles melting at 128°. The yield was 0.8 g. When mixed with dihydroderrisic acid ethyl ester no depression of the melting point was observed. Owing to the very characteristic crystal form the synthetic material was easily identified by crystallographic methods with dihydroderrisic acid ethyl ester.

[17] LaForge and Smith, THIS JOURNAL, **51**, 2580 (1929).

Anal. Subs., 0.0790: CO_2, 0.1884; H_2O, 0.0457. Calcd. for $C_{25}H_{30}O_8$: C, 65.50; H, 6.55. Found: C, 65.04; H, 6.43.

Dihydroderrisic Acid from the Synthetic Dihydroderrisic Acid Ethyl Ester.—Two-tenths of a gram of the synthetic ester was boiled for five minutes with 10 cc. of alcohol containing an excess of sodium ethylate. The sodium salt of the acid separated during the process. About 30 cc. of water was added and the solution acidified. The separated acid was recrystallized from dilute alcohol and melted at 171°, which is the melting point of pure dihydroderrisic acid. No depression was noted when the acid was melted with dihydroderrisic acid and a crystallographic examination also showed their identity.

Anal. Subs., 0.0754: CO_2, 0.1761; H_2O, 0.0440. Calcd. for $C_{23}H_{26}O_8$: C, 64.16; H, 6.09. Found: C, 63.69; H, 6.05.

Isoderrisic Acid Ethyl Ester from Isoderritol.—One gram of isoderritol was treated with sodium ethylate and bromoacetic ester in the manner described for the preparation of dihydroderrisic acid ethyl ester. The crystals which separated on cooling were recrystallized from 95% alcohol and melted at 128°. The yield was 0.3 g. In a subsequent experiment it was found that the same yield was obtained by refluxing at ordinary pressures. When mixed with isoderrisic acid ethyl ester no depression of the melting point was noted.

Anal. Subs., 0.0774: CO_2, 0.1860; H_2O, 0.0424. Calcd. for $C_{25}H_{28}O_8$: C, 65.78; H, 6.14. Found: C, 65.66; H, 6.08.

Esterification of Isoderrisic Acid.—As the ethyl ester of isoderrisic acid has not yet been described, it was prepared by boiling 0.9 g. of isoderric acid in 10 cc. of absolute alcohol containing 1 g. of sulfuric acid for three hours. The ester crystallized out on cooling and was recrystallized from 95% alcohol. The melting point was 128°.

Isoderrisic Acid from the Synthetic Ester.—Two-tenths of a gram of ester was heated with 10 cc. of alcohol containing an excess of sodium ethylate for five minutes, water was then added and the solution acidified. The separated acid was recrystallized from dilute alcohol and melted at 156°. Butenandt records the melting point of isoderrisic acid as 149°, but we have found 156° to be the melting point of the pure substance. When mixed with pure isoderrisic acid the synthetic acid showed no depression of the melting point and a crystallographic examination showed their complete identity.

Anal. Subs., 0.0733: CO_2, 0.1738; H_2O, 0.0390. Calcd. for $C_{23}H_{24}O_8$: C, 64.45; H, 5.65. Found: C, 64.66; H, 5.91.

Derrisic Acid Ethyl Ester and Dehydrorotenone from Derritol.—The procedure employed was the same as that described for the preparation of isoderrisic acid ethyl ester. The reaction mixture was refluxed at ordinary pressure. The yield was 0.3 g. from 1.5 g. of derritol. The material contained a small quantity of a yellow substance which is very difficultly soluble in warm alcohol. It was separated and recrystallized from glacial acetic acid. It melted at 224°. A mixed melting point with dehydrorotenone showed no depression and the substance was definitely identified as dehydrorotenone by crystallographic methods.

After removal of the dehydrorotenone the ester melted at 98° and the melting point was not depressed when mixed with derrisic acid ethyl ester. It is most convenient to saponify the ester and to re-esterify the purified derrisic acid.

Anal. Subs., 0.0800, 0.0817, 0.0815: CO_2, 0.1937, 0.1957, 0.1976; H_2O, 0.0451, 0.0450, 0.0453. Calcd. for $C_{25}H_{28}O_8$: C, 65.78; H, 6.14. Found: C, 66.03, 65.33, 66.12; H, 6.25, 6.12, 6.17.

Derrisic Acid from Synthetic Ester.—The ester was saponified with a slight excess of sodium ethylate and the acid isolated by acidification and dilution with water. It

was recrystallized from 50% alcohol and melted at 152°. A mixture of the synthetic material and derrisic acid showed no depression of the melting point.

Summary

The reactions involving the formation of derritol, rotenol, dehydro-rotenone and derrisic acid from rotenone are explained on the basis of their oxidation products. All of these reactions are concerned with the methoxyl containing part of the rotenone molecule which is best expressed by the formula.(P).
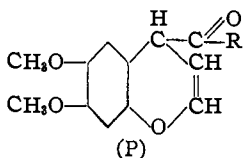
(P)

WASHINGTON, D. C.

THE EXHAUSTIVE O-METHYLATION OF QUERCETIN

BY A. S. GOMM AND M. NIERENSTEIN

Pentamethylquercetin (III) was first prepared by Waliaschko[1] by converting quercetin (I) into trimethylquercetin by means of dimethyl sulfate and alkali and by the action of dimethyl sulfate on the potassium salt of trimethylquercetin. Later, Wunderlich[2] showed that Waliaschko's trimethylquercetin was the 7,3',4'-trimethyl ether, as monomethylphloroglucinol and veratric acid are obtained on hydrolysis with alkali and since it is known that an hydroxyl group in the *peri*-position (position 5 in quercetin) to a carbonyl is refractory to methylation.[3]

Doubt was, however, thrown by Herzig[4] on Waliaschko's trimethylquercetin, and Herzig expressed the opinion that it was probably 3,7,3',4'-tetramethylquercetin (II). We have repeated Waliaschko's methylation, which we found to proceed in every respect as described by him, and we have at the same time confirmed Herzig's contention. Waliaschko's trimethylquercetin is thus shown to be 3,7,3',4'-tetramethylquercetin (m. p. 159–160°; Waliaschko and Wunderlich give m. p. 154°), which would also obviously yield monomethylphloroglucinol and veratric acid on hydrolysis as recorded by Wunderlich.[5]

The methylation of quercetin in the presence of alkali is, however, open to the danger of nuclear methylation, as shown by Perkin,[6] and this can be

1 Waliaschko, *Archiv. Pharm.*, **242**, 225 (1904); *Ber.*, **42**, 727 (1909).

2 Wunderlich, *Archiv. Pharm.*, **246**, 241 (1908).

3 See, for example, Kostanecki and Dreher, *Ber.*, **26**, 76 (1893); Schunck and Marschlewski, *J. Chem. Soc.*, **65**, 185 (1894); Perkin, *ibid.*, **103**, 1632 (1913).

4 Herzig, *Monatsh.*, **33**, 685 (1912).

5 See Watson, Sen and Medhi, *J. Chem. Soc.*, **107**, 1482 (1915), who have thus used tetramethylquercetin and not the supposed trimethyl derivative in their work.

6 Perkin, *J. Chem. Soc.*, **103**, 1632 (1913).