with a secondary amine to give a tertiary amine, but undergo an internal loss of halogen acid to form, presumably, an unsaturated compound.

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A PRELIMINARY EXAMINATION OF ROTENONE AND SOME OF ITS DEGRADATION PRODUCTS

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For many years considerable interest has been taken in a number of tropical plants, extracts of which have been used as fish and arrow poisons. More recently there has been a commercial development of these plants as sources of insecticides, and in this connection the root of *Derris elliptica Benth.* or *Tuba* has been widely used. On the agricultural side considerable attention has been given to the properties of *Derris* and it seemed of interest to obtain some light on the chemical structure and properties of the toxic principle.

A toxic principle from the root was first isolated as an amorphous substance by Greshoff,[1] who assigned it the name "Derrid." This worker showed that derrid did not contain nitrogen and was not a glucoside. Wray[2] also appears to have isolated the same substance, though he assigned to it the name "Tubain."

Derrid was also obtained by van Sillevoldt[3] as an amorphous powder, m. p. 73°, with an empirical formula $C_{33}H_{30}O_{10}$. It was suggested that it contained three mexthoxyl groups and two hydroxyl groups, although benzoylation or acetylation failed. Further, "anhydroderrid," yellow needles, m. p. 210–214°, of formula $C_{33}H_{28}O_9$, was obtained. A crystalline substance, m. p. 158°, from the roots of *Derris elliptica Benth.* was obtained by Lenz,[4] who gave it the name "Derrin." Takeo Ishikawa[5] isolated a toxic substance, m. p. 163.5°, from the same source and assigned to it the name "tubotoxin" and the empirical formula $C_{18}H_{18}O_5$. The work of the previous author was extended by Kariyone and Atsumi,[6] who confirmed the above formula. This compound, which in alcoholic solution reduced Fehling's solution, formed a phenylhydrazone, $C_{18}H_{18}O_5{=}N{\cdot}NHC_6H_5$, m. p. 255°, and a monoxime, $C_{18}H_{18}O_5{=}NOH$, m. p. 245°. It was suggested that a diacetyl derivative, m. p. 125–155°, was obtained but

[1] Greshoff, *Ber.*, **23**, 3538 (1890).
[2] Wray, *Pharm. J.*, [3] **23**, (1892).
[3] van Sillevoldt, *Arch. Pharm.*, **237**, 595 (1899).
[4] Lenz, *ibid.*, **249**, 298 (1911).
[5] Takeo Ishikawa, *Japan Med. Lit.*, **1**, 7 (1916).
[6] Kariyone and Atsumi, *J. Pharm. Soc. Japan*, **491**, 6 (1923).

the analyses did not bear this out. A methoxyl estimation showed the presence of 1.5 methoxyl groups in the molecule. Further work by Kariyone, Kimura and Kondo[7] showed that tubotoxin in hot alcoholic solution added on a molecule of hydrogen chloride (needles, m. p. 194°) and a molecule of hydrogen bromide (needles, m. p. 185°). Tubotoxin, whose formula in conformity with more recent work had been altered to $C_{19}H_{18}O_5$, on reduction in presence of palladium and barium sulfate, yielded dihydrotubotoxin, $C_{19}H_{20}O_5$, m. p. 216°, which was confirmed by the preparation of an oxime. When warmed with alcoholic potash at water-bath temperature, tubotoxin yielded "tubaic acid," $C_9H_{10}O_3$, m. p. 129°, which decolorized bromine and potassium permanganate solutions, and formed a methyl ester, m. p. 54°, and a mono-acetyl derivative, m. p. 133°. This acid may be reduced under the same conditions as tubotoxin to give dihydrotubaic acid, m. p. 166°, which was also obtained by the fission of dihydrotubotoxin with alcoholic potash. The methyl ester of this acid melts at 79° and the acetyl derivative at 169°.

In a later communication Kariyone and Kondo[8] revised the formula of tubaic acid to $C_{12}H_{12}O_4$ and stated that this acid is isomeric with "rotenic acid," m. p. 182°, obtained by the fusion of tubotoxin with potash, and which yielded an acetyl derivative, m. p. 154°.

Contemporaneous with Kariyone and his collaborators, Takei[9] was also examining the same toxic substance from *Derris elliptica Benth.* He found that this principle was identical with rotenone isolated by Kazuo Nagai[10] from *Derris chinensis Benth.* Takei[9] had shown that rotenone had the formula $C_{19}H_{18}O_5$, which was supported by an examination of the phenylhydrazone, m. p. 254°. He found that rotenone contained 1.5 OCH_3 groups; was converted into isorotenone by concentrated sulfuric acid; was degraded into an acid, $C_{10}H_{10}O_3$, m. p. 129°, which reacted with bromine; and on fusion with potash was transformed into rotenic acid, $C_9H_{10}O_3$ (or $C_6H_6O_2$). Further, the acid $C_9H_{10}O_3$ (m. p. 182°) yielded a mononitro derivative (m. p. 168°) and from its reaction with ferric chloride he suggested that it was a dimethyl-*o*-hydroxybenzoic acid.

On treating rotenone at 150° with acetic anhydride in acetic acid, or with chromic anhydride in acetic acid at 50°, a compound of empirical formula $C_{18}H_{16}O_5$ (m. p. 233°) was obtained. On oxidation of rotenone with chromic anhydride in acetic acid at a higher temperature, a compound of empirical formula $C_{17}H_{14}O_5$ (m. p. 298°) was produced to which he assigned the name "rotenonone." The heating of rotenonone with fused potash yielded rotenic acid.

[7] Kariyone, Kimura and Kondo, *J. Pharm. Soc. Japan*, **514**, 1049 (1924).

[8] Kariyone and Kondo, *ibid.*, **518**, 376 (1925).

[9] (a) Takei, *Bull. Inst. Phys. Chem. Research Japan*, **2**, 485 (1923); (b) *Biochem. Z.*, **157**, 1 (1925).

[10] Kazuo Nagai, *J. Chem. Soc. Tokyo*, **23**, 740 (1902).

In a later communication Takei[11] revised almost completely his original paper. Rotenone was now assigned the formula $C_{23}H_{22}O_6$ and this formula was confirmed by analysis of the phenylhydrazone, the oxime, the addition product with hydrogen chloride and a re-examination of isorotenone and its oxime. At the same time rotenic acid was given the formula $C_{12}H_{12}O_4$ and was further confirmed by acetylation and preparation of a dimethyl derivative and its nitration product.

It was found on gradually raising the temperature of rotenic acid to 210° and then to 300° two products were obtained: a phenol, $C_{11}H_{12}O_2$ (m. p. 42°), and an alkali-insoluble substance (m. p. 136°) of probable formula $C_{22}H_{22}O_4$. The phenol was characterized by the preparation of a *p*-tolylsulfonyl derivative and of a methyl ether.

In 1925, and therefore previous to the publication by Takei,[11] the discrepancies between the results of the various investigators had been noticed, and it was decided to reopen the problem in an attempt to establish a formula and a partial structure for the same principle of *Derris elliptica Benth.* In view of the drastic revision to which Takei[11] has subjected his previous work,[9b] it seems necessary to obtain independent confirmatory evidence. It has now been shown that the composition of rotenone is best expressed by the molecular formula $C_{23}H_{22}O_6$ and that it forms an oxime, $C_{23}H_{22}O_5{=}NOH$, a phenylhydrazone, $C_{23}H_{22}O_5{=}N{\cdot}NHC_6H_5$, and an addition product with hydrogen chloride, $C_{23}H_{22}O_6{\cdot}HCl$. Further proof is afforded by the preparation of the hydrobromide of rotenone and the oxime of the hydrochloride. The formation of isorotenone, its oxime and its phenylhydrazone has been established, again confirming the work of Takei.[11]

On oxidation of rotenone with chromic anhydride in acetic acid under conditions described by Takei,[9b] a substance having the same analysis and melting point was obtained, agreeing with a revised formula $C_{21}H_{18}O_6$.

The action of alcoholic potash yielded an acid $C_{12}H_{12}O_4$ (m. p. 129°)[8] which is presumably identical with $C_{10}H_{10}O_3$ described by Takei.[9b] Rotenic acid, which was obtained by the fusion of rotenone with potash, has been shown to have the formula $C_{12}H_{12}O_4$.[8,11] The formula of this acid has been further confirmed by the preparation of a mononitro derivative and a methyl ester. Rotenic acid on heating at about 210° until evolution of carbon dioxide had ceased yielded a compound $C_{23}H_{22}O_5$ (m. p. 103°).

Experimental

Rotenone (m. p. 163°) was prepared by extracting the powdered root with ether and crystallization of the product, which separated on cooling from carbon tetrachloride and then alcohol.

Anal. Calcd. for $C_{23}H_{22}O_6$: C, 70.05; H, 5.6; OCH_3, 15.7; mol. wt., 394. Found:

[11] Takei, *Ber.*, **61**, 1003 (1928).

C, 69.6, 69.5, 69.7; H, 5.85, 5.6, 5.9; OCH_3, 15.0, 15.1; mol. wt. in $C_2H_4Br_2$: 403, 376, 381; in $CHBr_3$, 386.

Rotenone-oxime.—A mixture of 5 g. of rotenone, 3.7 g. of hydroxylamine hydrochloride and 4.2 g. of fused sodium acetate dissolved in 80 cc. of absolute ethyl alcohol was refluxed for three to four hours. The hot solution was filtered and the crystals which separated from the filtrate on cooling were filtered, washed with water and on repeated recrystallization from methyl alcohol formed colorless needles, m. p. 252°.

Anal. Calcd. for $C_{23}H_{22}O_6$=NOH: C, 67.5; H, 5.6; N, 3.4. Found: C, 67.9, 67.4, 67.75; H, 5.3, 5.65, 5.85; N, 3.4, 3.5, 3.45.

Rotenone phenylhydrazone was prepared by refluxing for five hours a mixture of 5 g. of rotenone, 7.5 g. of phenylhydrazine hydrochloride and 4.2 g. of fused sodium acetate dissolved in 50 cc. of absolute alcohol. The solid product which separated was filtered, washed with water and on crystallization from alcohol yielded fine yellow needles, m. p. 245°.

Anal. Calcd. for $C_{23}H_{22}O_5 = NNHC_6H_5$: C, 71.9; H, 5.8; N, 5.8. Found: C, 71.6; H, 5.7; N, 6.0.

Rotenone hydrochloride was best prepared in the cold by dissolving with shaking 2 g. of rotenone in 100 cc. of glacial acetic acid saturated with hydrogen chloride and leaving for two to three days. The greenish-yellow solution, on addition of water, precipitated a solid which after filtering, drying and recrystallizing from alcohol had a m. p. of 197°. On concentration of the alcoholic mother liquors, in addition to a further quantity of rotenone hydrochloride, there was obtained a very small quantity of a solid forming yellow crystals from alcohol (m. p. 255°) containing chlorine.

Anal. Calcd. for $C_{23}H_{22}O_6$·HCl: C, 64.1; H, 5.35; Cl, 8.25; OCH_3, 14.4; mol. wt., 430.5. Found: C, 64.2, 63.95, 64.5; H, 5.35, 5.3, 5.5; Cl, 8.1, 7.95; OCH_3, 14.1; mol. wt., 442.

The oxime of rotenone hydrochloride was obtained by refluxing a mixture of 5 g. of rotenone hydrochloride, 3.7 g. of hydroxylamine hydrochloride and 4.2 g. of fused sodium acetate, dissolved in 100 cc. of absolute alcohol, for six hours. The hot solution after filtering deposited crystals which gave on repeated crystallization from alcohol colorless needles, m. p. 239° (with decomposition).

Anal. Calcd. for $C_{23}H_{22}O_6$=NOH·HCl: C, 61.95; H, 5.4; Cl, 7.95; OCH_3, 13.9. Found: C, 62.65, 62.60; H, 5.45, 5.60; Cl, 7.95; OCH_3, 14.0.

Rotenone hydrobromide was prepared in a manner similar to rotenone hydrochloride using glacial acetic acid saturated with hydrogen bromide. In this case the product separated from solution and after twenty-four hours was filtered off; it crystallized from methyl alcohol in long white needles, m. p. 190°.

Anal. Calcd. for $C_{23}H_{22}O_6$·HBr: C, 58.1; H, 4.85; Br, 16.85. Found: C, 57.7, 58.1; H, 4.85, 4.95; Br, 16.75.

Isorotenone was obtained by the gradual addition of 30 cc. of concentrated sulfuric acid to 5 g. of rotenone in 40 cc. of glacial acetic acid. After addition was complete the red solution was poured into ice water; the solid which was precipitated was crystallized from alcohol in small needles, m. p. 184°.

Anal. Calcd. for $C_{23}H_{22}O_6$: C, 70.05; H, 5.6. Found: C, 69.5; H, 5.65.

Isorotenone phenylhydrazone, which was prepared in the same manner as rotenone phenylhydrazone, on crystallization from alcohol formed fine yellow needles, m. p. 230°.

Anal. Calcd. for $C_{23}H_{22}O_5$=$NNHC_6H_5$: C, 71.9; H, 5.8; N, 5.8. Found: C, 71.95, 71.43; H, 5.73, 5.5; N, 5.85, 6.05.

No appreciable action takes place on passing hydrogen chloride into a hot alcoholic

solution of isorotenone for fourteen hours nor had concentrated sulfuric acid any action on rotenone hydrochloride.

Rotenonone was obtained by the slow addition of a solution of 10 g. of chromic anhydride in glacial acetic acid to a hot acetic acid solution of 5 g. of rotenone. On crystallization of the product from acetic acid, yellow needles were obtained, m. p. 300°.

Anal. Calcd. for $C_{21}H_{18}O_6$: C, 68.85; H, 4.9. Found: C, 68.7; H, 5.3.

Rotenic acid, obtained by the fusion of rotenone with potash, crystallized from benzene in white needles, m. p. 183°.

Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.45; equiv., 220. Found: C, 65.4; H, 5.35; equiv., 220, 226.

A Zeisel determination for the methoxyl group gave a negative result.

Methyl Ester of Rotenic Acid.—A solution of 1 g. of rotenic acid in ether was treated with an ethereal solution of diazomethane prepared from 3 cc. of nitrosomethylurethan and allowed to stand at ordinary temperature for three days. On evaporating the ether, an oil was obtained which went solid after washing with dilute sodium carbonate and water. On crystallization from methyl alcohol, colorless prisms (m. p. 39–40°) were obtained. This ester may also be prepared by the action of methyl iodide on the silver salt of rotenic acid.

Anal. Calcd. for $C_{12}H_{11}O_4CH_3$: C, 66.65; H, 6.0. Found: C, 66.15, 66.7; H, 5.6, 5.8.

Nitrorotenic acid was obtained by the careful addition of 2 cc. of a 10% solution of nitric acid (sp. gr. 1.42) in glacial acetic acid to 0.5 g. of $C_{12}H_{12}O_4$ dissolved in 15 cc. of glacial acetic acid. The solution was warmed gently for a few minutes and left overnight. On addition of water a yellow solid was precipitated which crystallized from alcohol and water in yellow needles, m. p. 187°.

Anal. Calcd. for $C_{12}H_{11}O_4NO_2$: N, 5.3. Found: N, 5.2, 5.1.

Action of Heat on Rotenic Acid.—Two g. of rotenic acid was heated between 216 and 225° until evolution of carbon dioxide had ceased. The oily residue was washed with dilute caustic soda and the residual white solid crystallized from methyl alcohol; it formed small, white crystals (m. p. 104°). In alcoholic solution it gave a green color with ferric chloride.

Anal. Calcd. for $C_{23}H_{22}O_5$: C, 73.0; H, 5.8; mol. wt., 378. Found: C, 73.0, 73.4; H, 5.9, 6.15; mol. wt., 396, 405.

The alkaline filtrate on acidification gave a substance of phenolic character which could not be obtained in a pure state.

Tubaic acid, which was prepared by the action of alcoholic potash on rotenone, yielded colorless needles on crystallization from water, m. p. 128–129°.

Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.45; equiv., 220. Found: C, 65.55 65.5; H, 5.45, 5.65; equiv., 221.

No methoxyl grouping was found to be present.

The author wishes to thank Messrs. Guthrie and Co., Ltd., East India merchants, London, and the Agricultural Department F. M. S. for the gift of a considerable amount of the Derris used in the above work.

Summary

1. Rotenone has been shown to have the formula $C_{23}H_{22}O_6$ and to form a monoxime and a phenylhydrazone. It adds on one molecule of hydrochloric acid or hydrobromic acid.

2. Isorotenone is formed by the action of sulfuric acid on rotenone, and yields a phenylhydrazone.

3. Rotenic acid, which is obtained by the fusion of rotenone with potash, has the formula $C_{12}H_{12}O_4$. It yields a monomethyl ester and a mononitro derivative.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF NEBRASKA]

THE ACTION OF ALKYL CHLOROCARBONATES ON STIBANILIC ACID[1]

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Studies in the field of organic antimonials have been carried out in connection with investigations of protozoal diseases because in some of these diseases the antimonials appear to be of greater value than the arsenicals.

It was the object of this investigation to prepare some of the carbalkoxy derivatives of *p*-aminostibinic acid and to study some of their properties. Methyl, ethyl, propyl, *iso*propyl, butyl, *iso*butyl, β-chloro-ethyl and γ-chloropropyl chlorocarbonates were condensed with *p*-stibanilic acid. Only one of the products, *p*-carbethoxyaminophenylstibinic acid was found reported in the literature.[2]

Two general methods, with various modifications, have been used for the preparation of *p*-aminophenylstibinic acid or *p*-stibanilic acid from *p*-aminoacetanilide; the first, by diazotization of *p*-aminoacetanilide and subsequent addition to alkaline antimony trioxide;[3] the second, by formation of the double salt in acid solution[4] and its addition to a solution of sodium hydroxide.[5] In both cases the acetyl group was removed by hydrolysis with a 5% solution of sodium hydroxide.

In this study both of the above methods were tried in the preparation of *p*-stibanilic acid with about equal results. With either method several difficulties were encountered. Not only was the material hard to handle mechanically but the yields were low. In the method finally adopted *p*-amino-acetanilide was diazotized and slowly added to an alkaline solution of antimony trioxide containing a little glycerol. The foam which forms was kept down by vigorous stirring and by regulating the

[1] Some of the compounds described in this paper are being studied pharmacologically under the direction of Dr. A. S. Loevenhart, Department of Pharmacology, University of Wisconsin.

[2] Brachmachari, *Indian J. Med. Research*, **10**, 508 (1922).

[3] German patent 254,421 (1911).

[4] May and Gray, *J. Chem. Soc.*, 3174 (1926).

[5] Dunning and Reid, THIS JOURNAL, **48**, 2959 (1926); **49**, 2869 (1927).