of the amylase in the presence of 0.01 *M* acetate is negligible and is more than offset by the increase in reproducibility of results over those obtained in unbuffered systems.

Changes in the concentration of phosphate from 0.01 to 0.10 *M* do not influence the activity of the enzyme if the hydrogen-ion activities of the systems are suitably adjusted.

If the hydrogen-ion activities of the systems are suitably adjusted, the activity of this amylase is the same in the presence of 0.01 *M* acetate or of 0.01 *M* phosphate.

In measurements of the activity of the amylase of *Aspergillus oryzae*, acetate is preferable to phosphate because of its more efficient buffer effect in the region of hydrogen-ion activities which are optimal for the action of this amylase.

In measurements of thirty minutes at 40° with 2% starch containing 0.01 *M* acetate, the amylase of *Aspergillus oryzae* is most active saccharogenically at hydrogen-ion activities of *P*H 5.3 to *P*H 5.5.

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

THE "YELLOW COMPOUNDS" RESULTING FROM THE DECOMPOSITION OF ROTENONE IN SOLUTION

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RECEIVED APRIL 1, 1931 PUBLISHED JUNE 8, 1931

The literature on *Deguelia* (Derris) contains numerous references to "yellow compounds" which are obtained in the process of isolating the active insecticidal principles, or which result from the treatment of rotenone ($C_{23}H_{22}O_6$, m. p. 163°), the most important constituent, with various reagents.[1]

Greshoff's[2] derrid, described as an amorphous material melting at 61°, and decomposing at 160°, was probably a mixture of rotenone and certain yellow materials. The same investigator later reported the melting point of pure derrid as 204°.[3]

Van Sillevoldt[4] obtained from the roots of *Deguelia elliptica* a pale yellow substance melting at 73° which he also called derrid. This, on treatment with an alcoholic solution of hydrogen chloride, gave yellow needles

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[1] Clark, *Science*, **71**, 396 (1930), has isolated a yellow crystalline material, toxicarol, from the root of *Deguelia sp.* and it is possible that other yellow compounds are associated with rotenone in this root. This paper, however, deals primarily with such yellow compounds as result from the decomposition of rotenone in solution.

[2] Greshoff, *Ber.*, **23**, 3537 (1890).

[3] Greshoff, *Meded. 'Slands Plant.*, **25**, 47 (1898).

[4] Van Sillevoldt, *Arch. Pharm.*, **237**, 595 (1899).

melting at 214°, which he called anhydroderrid. He stated that this material was also present in crude derrid.

Power,[5] on treatment of the resin from the chloroform extraction of stems of *Deguelia trifoliata* (*Derris uliginosa*), obtained a material identical with the anhydroderrid of Van Sillevoldt.

In the course of a chemical investigation of the constituents of *Deguelia elliptica* root, Tattersfield and Roach[6] obtained, in addition to rotenone, three yellow crystalline compounds. These materials were formed on fractionation of the alcoholic extracts of the root. One of these compounds corresponded to the anhydroderrid of Van Sillevoldt, another melted at 222–224°, and the third decomposed at 280°. They crystallized in the form of long, yellow needles and were only slightly soluble in organic solvents. These investigators were unable to obtain anhydroderrid from derris extract which had been freed of rotenone. They concluded that all three yellow compounds were secondary products of the extraction process.

Butenandt,[7] by treatment of rotenone with mild oxidizing agents, obtained a yellow crystalline compound melting at 218°, which he called dehydrorotenone and which he showed has the empirical formula $C_{23}H_{20}O_6$. LaForge and Smith[8] obtained a melting point of 225° for this compound.

Takei[9] obtained, on oxidation of rotenone with chromium trioxide in glacial acetic acid, a yellow compound melting at 298°. This he named rotenonone. Butenandt[7] also prepared this compound and assigned to it the formula $C_{23}H_{20}O_7$; whereas LaForge and Smith[8] assigned it the formula $C_{23}H_{18}O_7$. The latter investigators have shown that rotenonone can be obtained from dehydrorotenone and that dehydrorotenone precedes the formation of rotenonone from rotenone. Butenandt has suggested that dehydrorotenone and rotenonone are possibly the "yellow compounds" which are so frequently mentioned in the literature as accompanying rotenone. These two compounds are practically non-toxic to fish and insects.

During an investigation of the solubility of rotenone it was noted that solutions of rotenone in certain organic solvents changed, on standing, from colorless to various depths of yellow.[10] This change does not occur with equal rapidity in all solvents. Thus at room temperature pyridine solutions become deep amber in color within a few days, chloroform and ethylene dichloride solutions exhibit this change more slowly, in acetone and diacetone alcohol the change takes place only very slowly and in benzene and alcohol the change is almost imperceptible. This alteration in

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[5] Power, *Pharm. Arch.*, **5**, 145 (1902); **6**, 1 (1903).

[6] Tattersfield and Roach, *Ann. Appl. Biol.*, **10**, 1 (1923).

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

[8] LaForge and Smith, THIS JOURNAL, **52**, 1091 (1930).

[9] Takei, *Biochem. Z.*, **157**, 1 (1925).

[10] Jones, *Ind. Eng. Chem.*, **23**, 387 (1931).

color is accompanied by a change in the optical rotation of the solution, the rates of the two changes being roughly proportional. The solutions also lose their toxicity to fish and insects.[11]

Pyridine solutions of rotenone on standing for several days deposited long, yellow, needle-like crystals. Solutions in acetone, amylene dichloride, chloroform, diacetone alcohol and other solvents gave similar yellow crystals. However, the crystals did not separate spontaneously from these solvents, as was the case with the pyridine solutions, but only after long standing and final boiling and evaporation of the solutions to a small volume.

The present investigation has proved conclusively that the changes occurring in solution are a result of the oxidation of the rotenone by air. Thus the change is accelerated by greater exposure to air, but when rotenone is dissolved in pyridine through which nitrogen has been passed and the solution kept in an atmosphere of nitrogen, no color change takes place and no deposition of crystals is to be observed even on long standing. The change is not influenced by differences in light intensity.

A chemical examination of the yellow crystalline product of the decomposition has shown it to be a mixture of dehydrorotenone and rotenonone, both oxidation products of rotenone. The proportion of the two materials in the product deposited seems to depend upon several factors, chief of which are temperature, access of air and the length of time the solution is allowed to stand. The first product deposited seems to be dehydrorotenone, and as the decomposition progresses rotenonone is formed. These crystalline materials are only the initial products of the decomposition. The reaction continues beyond the formation of these two compounds, resulting in a complex mixture of other oxidation products of rotenone.

A sample of the yellow crystalline material removed from a pyridine solution of rotenone soon after the deposition of crystals began was found to melt at about 225°. This same material in chloroform solution had an optical rotation of $[\alpha]_D^{20}$ $-43°$. Butenandt and Hildebrandt[12] obtained $[\alpha]_D^{18}$ $-40.5°$ for dehydrorotenone in chloroform solution. Samples of yellow crystalline material separating at early stages from other pyridine solutions had melting points ranging from 220 to 240°. Probably rotenonone was present in some of these samples.

That rotenonone is present in increasing amounts in the mixture separating from pyridine solutions after longer standing or on more severe treatment (increased temperature or bubbling of air through the solution) was also established. Thus a sample of crystalline material, separated from a pyridine solution which had been allowed to stand over a long period of time, melted at about 280°. Several recrystallizations of this material

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[11] Davidson and Jones, *J. Econ. Entomol.*, **24**, 257 (1931).

[12] Butenandt and Hildebrandt, *Ann.*, **477**, 245 (1930).

from acetic acid (no doubt causing further decomposition) raised the melting point to 298°. When mixed with an equal quantity of pure rotenonone it caused no depression of the melting point. Another sample of yellow material separating from a pyridine solution was recrystallized twice from ethyl acetate. Its melting point was found to be about 290°. The minimum and the intermediate indices of refraction[13] of both these samples were identical with those of pure rotenone. The maximum index was higher than that of the readily available reference liquids.

The yellow crystalline material separating from a pyridine solution of rotenone was found to be non-toxic to goldfish.[14] Both dehydrorotenone and rotenonone are practically non-toxic to fish.

The needle-like, yellow crystals obtained from amylene dichloride and diacetone alcohol solutions of rotenone also appeared to be mixtures of dehydrorotenone and rotenonone.

The yellow material melting at 222–224° obtained by Tattersfield and Roach as a result of boiling alcohol solutions of rotenone was no doubt principally dehydrorotenone and the material decomposing at 280° was probably largely rotenonone.

It was also found that dihydrorotenone (in which the double bond in the side chain has been reduced) behaves similarly to rotenone in pyridine solution, as does isorotenone (in which the double bond has migrated). The pyridine solutions of these materials become deep yellow in color and needle-like, yellow crystals separate, which in the case of dihydrorotenone are no doubt a mixture of dehydrodihydrorotenone and dihydrorotenonone, and in the case of isorotenone probably consist of dehydroisorotenone and isorotenonone

Gimlette,[15] describing work done by Durham, states that crystals of rotenone are altered and reddened by exposure to light. The material in this case was no doubt impure or perhaps contained solvent of crystallization. A sample of pure, crystalline rotenone exposed to ordinary light conditions in this Laboratory showed no change in color after standing for more than a year. No change was observed in melting point, specific optical rotation or toxicity against fish and insects.

Conclusions

1. Rotenone in solution in various organic solvents decomposes, the rate of this decomposition depending on the solvent, temperature, access of air and other factors.
2. This change is an air oxidation of the rotenone, the initial products

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[13] Crystallographic examination was made by George L. Keenan of the Food and Drug Administration.

[14] Piscicidal tests were made by W. A. Gersdorff of the Insecticide Division.

[15] Gimlette, "Malay Poisons and Charm Cures," London, 1923, 2d ed., 260 pp.

of the decomposition being the yellow, crystalline compounds dehydrorotenone and rotenonone.

3. Similar changes are undergone by dihydrorotenone and isorotenone in pyridine solution, analogous compounds no doubt being formed.

4. Dry, crystalline rotenone undergoes no change on long standing.

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STUDIES ON LEVULINIC ACID. I. ITS PREPARATION FROM CARBOHYDRATES BY DIGESTION WITH HYDROCHLORIC ACID UNDER PRESSURE[1]

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RECEIVED APRIL 2, 1931 PUBLISHED JUNE 8, 1931

Priority of discovery of the fact that dilute mineral acids acting upon hexoses or any carbohydrate yielding them on hydrolysis cause the formation, among other substances, of levulinic acid must be accorded to Mulder[2] in that the compound which he isolated from such a reaction mixture and described as glucinic acid was later found by Grote and Tollens[3,4] to be identical with the substance which they obtained from inulin under similar conditions.[4] It is to the latter investigators that the common name for this keto acid is due.

The traditional equation for the formation of this acid

$$C_{12}H_{22}O_{11} = 2CH_3COCH_2CH_2COOH + 2HCOOH + H_2O$$

by no means represents a complete picture. Subsidiary reactions take place to such an extent that the best yields[5] of levulinic acid which have been reported as a direct result of studied attempts so to guide the course of this reaction that a greater conversion of carbohydrate might be rapidly

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[1] This paper, and others already published by R. W. Thomas on the general theme of the sugars as chemical raw materials [THIS JOURNAL, **52**, 2028, 3010 (1930)], forms part of a thesis presented to the Graduate Faculty of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1931.

[2] Mulder, *J. prakt. Chem.*, **21**, 219 (1840).

[3] Grote and Tollens, *Ber.*, **7**, 1375 (1874).

[4] Mulder's discovery has been many times verified. Since his day the following carbohydrates have been made to yield this acid: starch [Grote and Tollens, *Ber.*, **7**, 1379 (1874)]; dextrose, levulose [Grote, Kehrer and Tollens, *Ann.*, **206**, 228 (1881)]; lactose [Rodewald and Tollens, *ibid.*, **206**, 231 (1881)]; galactose [Kent and Tollens, *ibid.*, **227**, 228 (1885)]; raffinose [Rischbiet and Tollens, *ibid.*, **232**, 193 (1886)]; sorbose [Wehmer and Tollens, *ibid.*, **243**, 320 (1888)]; Smith and Tollens, *Ber.*, **33**, 1286 (1900); and mannose [Fischer and Hirschberger, *ibid.*, **22**, 370 (1889)].

[5] (a) McKenzie, "Organic Syntheses," John Wiley and Sons, Inc., New York, 1929, Vol. IX, p. 50; (b) Sah and Ma, THIS JOURNAL, **52**, 4880 (1930).