somewhat less saturated under the sunlight lamp than under the mazda, with iodine numbers of 106.6 and 103.9, respectively. Sterols were recovered as digitonides to the extent of 37 and 34 mg. per 100 mg. of dry tissue in these respective samples. These data supply limited evidence of increased dehydrogenation under the higher proportion of short-wave radiation.

Summary

Elimination of wave lengths shorter than about 3900 or 5200 Å. from radiation resembling sunlight decreased the assimilation of nitrate and conserved carbohydrates in young wheat plants. The primary factors to which increased reducing power under shorter radiation could be attributed were increased tissue contents of chlorophylls and sulfhydryl compounds. Paths are indicated by way of which these factors could function as trigger mechanisms in the reduction of nitrate. Depression of pentosan formation was associated with increased assimilation of nitrate. Most of the compositional effects in the tissue could be attributed to variations in the proportion of blue-violet light, but the reduction of nitrate to nitrite was more distinctly associated with long ultra-violet radiation. Apparently the high proportion in sunlight of radiation from 3900 to 4920 Å. and the low proportion above 8000 Å., in comparison with the light sources here tested, favor the assimilation of nitrate by plants.

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[CONTRIBUTION FROM THE DIVISION OF INSECTICIDE INVESTIGATIONS, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, U. S. DEPARTMENT OF AGRICULTURE]

The Dimorphism of Rotenone

BY ERNEST L. GOODEN AND CHARLES M. SMITH

The fact that rotenone occurs in two forms characterized by melting points of 163° and about 180° was briefly referred to by Butenandt and Hildebrandt[1] and later considered more fully by Cahn.[2] This phenomenon has also been studied in the Insecticide Division and, since certain characteristics of the two materials not previously recorded have been determined, the results of this study are given here.

No attempt has been made to confirm the statement of Butenandt and Hildebrandt that the higher melting form of rotenone can be prepared from the ordinary form by grinding, but the transition during melting-point determinations and the melting and resolidification of rotenone that was rapidly heated to a temperature between the two melting points have been observed. Neither of these two methods of preparation gives a product suitable for microscopical study, but the higher melting material can be prepared easily in well-crystallized form by the process given in the following section.

Preparation of Materials

The sample of low-melting rotenone was obtained from cubé root, being crystallized once from carbon tetrachloride as the solvate, and then twice from a 1:1 mixture of acetone and water as rotenone itself.

The high-melting rotenone was prepared from this sample by immersing it in a petroleum oil less volatile than kerosene and heating to 140–150°. The mixture was maintained in this temperature range, with frequent stirring, at least until examination with a polarizing microscope showed apparently complete transformation of the original thin six-sided plates to chunky, granular crystals.[3] The petroleum oil was decanted, and the crystals were washed by decantation with petroleum ether and dried at room temperature. Four batches were prepared in this manner, with different periods of heating, lasting from one to five hours. None of the batches was pure white, as it is presumed they should have been, but the one heated least was the best looking preparation and was considered most nearly pure.

That this material was not a solvate was proved by microanalysis[4] and a determination of the opti-

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

[2] Cahn, *J. Chem. Soc.*, 1129 (1934).

[3] No method of proving the completeness of the transformation was devised. The writers feel certain that it was nearly enough complete to demonstrate a difference in density if any considerable difference existed. The optical data are, of course, not affected by this uncertainty, for they were determined on individual crystals known to be of the high-melting form.

[4] The writers wish to thank Dr. J. R. Spies for determinations of carbon and hydrogen.

cal rotatory power in acetone at 20° (*i. e.*, below the transition temperature), both of which agreed within experimental limit with the values for the low-melting form.

The failure of Cahn to get crystalline high-melting rotenone by working in tetralin and in xylene at 100° was very likely due to the fact, considered later, that the transition temperature is apparently above 100°. Even if it is below this, his method would probably fail because of the very rapid retransformation which occurs in such good solvents as tetralin and xylene. For example, if a mount of high-melting rotenone is made in amyl acetate, and examined under the microscope, the transformation is seen to begin almost immediately, small crystals of the low-melting form appearing in the liquid surrounding each crystal of the high-melting form. Further, in one experiment ethyl ether was used to wash, by decantation, a lot of high-melting rotenone prepared by the method described in this paper, and the entire mass transformed before the washing could be completed. It is only the very low solubility of rotenone in petroleum fractions that accounts for the success of our method.

General Appearance

Figure 1 shows the most characteristic habit of the ordinary low-melting form, the small faces beveling two opposite edges being commonly present. Thicker crystals have at times been obtained, and these might be difficult to differentiate from those of the high-melting form by casual inspection. The crystals of the high-melting form, shown in Fig. 2, contain the same faces as those of the low-melting form, and others in addition. A comparison of the two compounds with respect to the plane angles of the faces that are parallel to the optic axial planes shows large differences, indicating a distinct difference in crystallographic axial ratios.

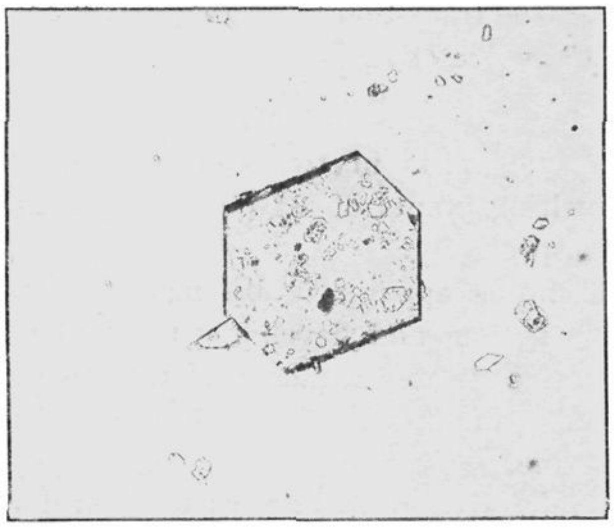

Fig. 1.—Low-melting rotenone (the common form) × 60.

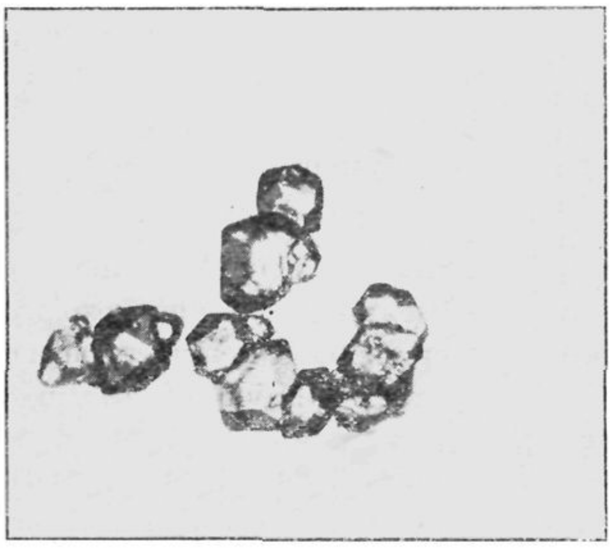

Fig. 2.—High-melting rotenone (the unstable form) × 60.

Optical Characteristics

The crystals of low-melting rotenone have symmetrical extinction and are biaxial with the axial plane parallel to the large face upon which the crystals normally lie.

The crystals thus permit the direct determination of the α- and γ-indices, which were found to be 1.616 and 1.662, respectively, in good agreement with the values given by Clark.[5] The α-index is shown when the beveled edges are parallel to the vibration direction of the polarizer. Pieces suitable for the measurement of β are difficult to obtain, but by working with thick crystals grown especially for the purpose it was possible to determine it as being 1.635. The accuracy of these figures (±0.003) is not great enough for determining the optical character, nor could this character be determined from the interference figures. From the parallelism of the optical directions and the principal crystallographic directions it is inferred that the crystals belong to the orthorhombic system.

High-melting rotenone is also biaxial with symmetrical extinction, and again many crystals lie upon a face which is parallel to the axial plane, so that, except for the greater thickness, the general aspect is similar to that of ordinary rotenone. The indices of refraction are, however, significantly different, having the following values:

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[5] Clark, *Science*, **70**, 478 (1929).

$\alpha = 1.600$, $\beta = 1.650$, $\gamma = 1.685$. The various indices are shown in the same comparative positions as with low-melting rotenone. The interference figures definitely prove the substance to be optically negative, which fact is in accord with the relative values of the indices. The optic axial angle is greater than 65°, the limit of the microscope used. From these data it appears probable that high-melting rotenone also belongs to the orthorhombic system.

Density

Density determinations on both forms of rotenone were first made by the displacement method in a pycnometer, the immersion medium being a 0.3% water solution of gelatin which improved the wetting power of the water and prevented the retention of air bubbles. The two forms were found to be indistinguishable in density within the limits of accuracy of the procedure. the value being 1.33 g. per cc. at 20°.[6]

This result was checked by suspension experiments with solutions of potassium iodide which had densities the same as and slightly different from that of the solids. No difference in behavior could be demonstrated.

Melting Point

Practically all observers agree in assigning a melting point of 163° to ordinary rotenone, and this figure was obtained on the material used in this investigation. Butenandt and Hildebrandt[1] found a melting point of 176° for the high-melting rotenone prepared by them by grinding the ordinary form, and Cahn[2] observed melting points of from 176 to 183° for material prepared by dry heating. At various times in this Laboratory values of 179 to 181° have been observed with samples of ordinary rotenone that evidently were converted during the course of the melting-point determination. Attempts to establish the melting point more definitely on the material used in this investigation failed. A series of observations conducted in a manner similar to that usually employed in this Division gave a range of 5°, with a mean of 175°. Then a series of tests was made in which the melting-point tube was plunged into a bath previously adjusted to some temperature between 170 and 180°, and in one instance prompt melting occurred at 174°. When the tubes were put into a bath previously brought to 170° (approximately) and then slowly heated, melting points of 174, 174 and 175° were obtained on three separate batches. The behavior was such, however, that we feel these values are too low, and are inclined to suspect that the samples may have contained small amounts of impurities either decomposition products formed during the heating of the rotenone or some petroleum oil dissolved or entrained in the crystals at the time of their formation.

Transition Temperature

There seems to be no doubt that the rotenone melting at 163° is the stable form at room temperature; for, as stated before, the transformation of the high-melting form into it can be watched under the microscope in a mount prepared with a good solvent such as amyl acetate. No estimate of the transition temperature has been published except the figure 125° given by Roark,[6] and that figure was based on some work by one of the writers in which portions of low-melting rotenone were immersed in white oil (a petroleum fraction) and maintained at various fixed temperatures, the occurrence of transformation being watched for by periodic microscopic examination. Transformation was plainly evident at 131°, and apparently did not take place at 120°. In the absence of definite knowledge that the white oil does not affect the transition point, it cannot be said that the true transition temperature will have the estimated value of 125°.

Summary

Rotenone exists in two enantiotropic forms, with melting points of 163° and approximately 180°, respectively.

Both forms apparently belong to the orthorhombic system and develop with similar habit except for a greater tendency of the low-melting form to appear in crystals tabular parallel to the optic plane.

The refractive indices are as follows: for low-melting rotenone, $\alpha = 1.616$, $\beta = 1.635$, $\gamma = 1.662$; for high-melting rotenone, $\alpha = 1.600$, $\beta = 1.650$, $\gamma = 1.685$. The high melting form is optically negative, the other practically without sign.

Both forms have a density of 1.33 g. per cc. at 20°.

The transition temperature has not been determined.

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(6) Note that this is appreciably higher than the 1.27 given for low-melting rotenone by R. C. Roark [*Ind. Eng. Chem.*, **25**, 639–642 (1933)]. That figure was merely a rough approximation.