dyes for cotton, whereas the sulfanilic acid derivatives were not fast to cotton and were completely washed out.

The colors of the resulting skeins indicate that the antimony group (the stibinic acid group) is more strongly auxochromic in character than is the sulfonic acid group. Table III lists the colors of the various dyes.

Our thanks are extended to Dr. R. E. Rose, of the Technical Laboratory of E. I. du Pont de Nemours and Co., who kindly furnished the skeins for this work and the color designations of the finished products, and also to Mr. C. Slagle of this Laboratory who assisted in preparing the dyed skeins.

Summary

1. The method of preparing stibanilic acid has been improved.
2. Three series of azo dyes containing antimony have been made and tested against trypanosomiasis in the white rat.
3. The effect of antimony on color has been ascertained.
4. Several other antimonials have been described.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE]

VARIOUS EFFECTS PRODUCED BY THE ACTION OF LIGHT UPON THE ISOMERIC MODIFICATIONS OF CERTAIN POLYPEPTIDE-HYDANTOINS

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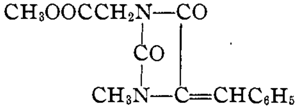
As a result of the investigation of a fairly large number of pairs of geometrical isomers corresponding to different derivatives of benzal[1] and anisalhydantoin,[2] the interesting observation has frequently been made that during the process of preparation and purification of these substances, minute quantities of very high-melting and very insoluble compounds were formed. These substances were markedly different in their properties from any of the major products taking part in the particular transformations but were never formed in quantities sufficient to admit of a study of their properties or even of their analysis. Recent work on the isomeric modifications of methyl-N-3-methylbenzalhydantoin-N-1-acetate and of the corresponding acids,[1] has at last made it possible to arrive

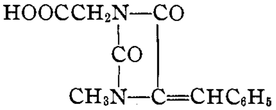
I



$$\begin{array}{l} CH_3OOCCH_2N{-}CO \\ \quad\quad\quad\quad | \quad\quad\ | \\ \quad\quad\quad\ CO \quad\ | \\ \quad\quad\quad\quad | \quad\quad\ | \\ \quad\quad CH_3N{-}C{=}CHC_6H_5 \end{array}$$

(a) m. p., 65.5–66.5°
(b) m. p., 98.5–99.5°

II



$$\begin{array}{l} HOOCCH_2N{-}CO \\ \quad\quad\quad | \quad\quad\ | \\ \quad\quad\ CO \quad\ | \\ \quad\quad\quad | \quad\quad\ | \\ CH_3N{-}C{=}CHC_6H_5 \end{array}$$

(a) m. p., 186.5–187.5°
(b) m. p., 198.5–199.5°

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[1] Hahn and Evans: The preparation and properties of these substances have been described in a paper which was received by THIS JOURNAL, July 23, 1927.

[2] Hahn and Gilman, THIS JOURNAL, 47, 2953 (1925).

at a definite and more or less detailed knowledge of some of the conditions which favor the formation of these very insoluble and high-melting compounds. The phenomenon has, for example, now been shown definitely to involve either a polymerization or an intramolecular rearrangement of a single pure substance and to be accelerated if not induced by the action of light. The changes which take place occur slowly in the presence of ordinary daylight, are hastened by bright sunlight and are very markedly accelerated under the influence of ultraviolet light from a mercury arc lamp. Certain of these compounds have been observed to suffer these changes when exposed to light in the *solid* state but the transformation is distinctly favored by the solution of the substance either in alcohol or ether. In particular, ether solutions which contain minute traces of iodine as a catalyst have been observed to give very satisfactory results under the action of ultraviolet light. The rate of transformation varies to a marked degree under what appear to be the same conditions in the case of different substances. The changes which are produced by the action of light are not limited to the formation of the above-mentioned high-melting and insoluble compounds but are fairly complex, as will be apparent from a consideration of the following examples.

I (a) The lower-melting ester, m. p., 65.5–66.5°, whether present in solid crystalline form or when partially dissolved and partially suspended in alcohol or ether, is transformed under the action of light in the following three ways: (i). It isomerizes to give the corresponding higher-melting modification. This was identified by a mixed melting-point determination with an analyzed specimen, m. p., 98.5–99.5°, which had been obtained by synthesis.[3] (ii) It decomposes with the splitting off of benzaldehyde. This product was identified by its odor, its reaction with Tollens' reagent and finally by its conversion into benzoic acid. The benzoic acid which was produced in this way was identified by its properties and by a mixed melting-point determination with a pure laboratory specimen (Kahlbaum preparation). This decomposition takes place spontaneously even when pure, dry crystals are allowed to stand in stoppered glass containers, so that the substance cannot be kept unchanged under any conditions prevailing in the laboratory for periods of more than a few months. Incompletely purified specimens, m. p., 63–66°, begin to show signs of decomposition in the course of one week. The other product or products resulting from this decomposition have not been isolated. (iii) It polymerizes or isomerizes to form a substance which is very insoluble in all ordinary solvents and which melts at 278°.

I (b). The corresponding higher-melting ester, m. p., 98.5–99.5°, behaves in much the same way as the lower-melting ester under the action

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[3] The same change is produced very rapidly and quantitatively in alcohol solution under the action of hydrogen chloride gas. Compare Ref. 1.

of light but is distinguished by the fact that it is relatively much more stable and that its rate of transformation is relatively slower.

(i) It isomerizes to give the corresponding lower-melting modification, m. p., 65.5–66.5°, which was identified by comparison with the analyzed specimen. (ii) It decomposes with the formation of benzaldehyde, which latter was identified as described above. (iii) It polymerizes or isomerizes to give the same insoluble product, m. p., 278°, that was obtained in the case of the lower-melting isomer. The identity of the product which was obtained in the two cases was established by analyses and by means of a mixed melting-point determination.

II (a). The lower-melting corresponding acid, m. p., 186.5–187.5°, appears to be extremely stable under the action of light and suffered no appreciable change in its melting point on standing and no precipitation of an insoluble compound from its solutions even after these had been exposed to radiation from a mercury arc lamp over a period of many weeks.[4]

II (b). The higher-melting isomer suffers the following transformations under the action of light:

(i) It isomerizes to give the lower-melting modification, which was identified by means of a mixed melting-point determination with a pure analyzed specimen. (ii) It has not been observed to decompose with the splitting off of benzaldehyde. (iii) It polymerizes or isomerizes to give an acid which melts with decomposition at 290–294°. This substance is extremely insoluble in all ordinary solvents, but may be recrystallized from boiling acetic acid. It is not identical with the insoluble product which was obtained from the corresponding esters, since a mixture of the two products melted at 265–288°. The product from the esters is neutral to litmus and phenolphthalein, while the product from the above acid shows distinctly acid properties.

A comparison of these results shows that two different insoluble and high-melting rearrangement products are formed by the action of light upon the isomeric modifications of methyl-N-3-methylbenzalhydantoin-N-1-acetate (I, a and b) and upon the corresponding higher-melting acid (II, b), respectively. This is significant in that it suggests at once that the —$COOCH_3$ and —COOH groups are still intact in the final product of the reaction. A comparison of the results which were obtained from the analysis of the two products, moreover, approximates very closely to the results obtained from the analysis of the original ester and acid, respectively. Further, the insoluble acid product, m. p., 290–294°, exactly neutralizes one equivalent of base, the reaction being accompanied by the

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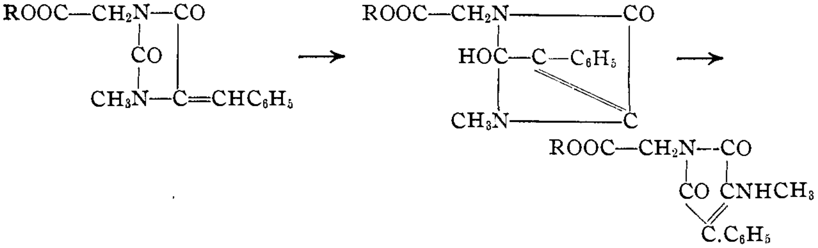
[4] It is, however, rapidly and quantitatively transformed into the corresponding higher melting isomer when its acetic acid solution is boiled with hydrochloric acid. Compare Ref. 1.

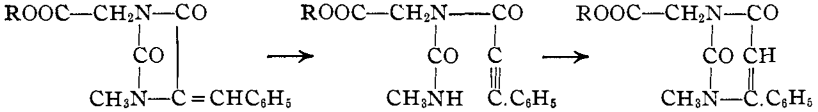
complete solution of the substance. These facts admit of the assumption that the transformation has been of the nature either of a polymerization (which could be accounted for by the presence of unsaturated ethylene linkages in the original esters and acid) or of an isomerization reaction. The possibility that the reaction is one of polymerization cannot, unfortunately, be settled by means of molecular weight determinations, since the products under consideration are too insoluble to admit of laboratory manipulation by either boiling- or freezing-point methods.

In the event of isomerization two possible rearrangements might be conceived to take place:[5]

A.



B.



The corresponding acid might in either case be assumed to behave similarly. If a compound of the first type were formed, it should show basic properties as it contains a CH_3NH group. Since, however, neither of the high-melting products is soluble to any extent in concentrated hydrochloric acid, the possibility of a rearrangement of this kind would seem to be discounted. On the other hand, the rearrangement of the hydantoin to a pyrimidine would, according to advices from Dr. Johnson, be in general agreement with the observed solubilities of the new products, and if, by any chance, such a transformation can actually be demonstrated it would represent a new generic relationship between hydantoins and pyrimidines and would be of immediate biochemical interest. Typical illustrations of the procedures which have been employed will now be given.

Experimental

The tendency of methyl-N-3-methyl-4-benzalhydantoin to undergo decomposition under the action of light was first noticed when a pure, dry, crystalline specimen, m. p., 65.5–66.5°, which had been analyzed and left in a stoppered glass specimen bottle was found after the lapse of three

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[5] These two possible configurations have been suggested by Professor Treat B. Johnson and it now seems probable that the synthesis of a pyrimidine corresponding to the second formula (B) will be attempted next year by Miss Evans at Yale University under the direction of Dr. Johnson.

months to have passed into an oil. When the stopper of the container was removed a strong odor of benzaldehyde was detected. Other specimens were later observed to behave in the same way, the period of time required for liquefaction varying from two weeks to six months under different conditions. Specimens which were less pure, m. p., 63–66°, liquefied very rapidly. The process was also found to be accelerated by radiations from a quartz mercury arc lamp.[6] The oily products which were obtained in a series of experiments were combined and distilled with steam. The steam distillate yielded a small amount of solid material which was removed by filtration. It melted at 115–118° and after recrystallization from water was identified as benzoic acid by means of a mixed melting point with a pure specimen of benzoic acid of known constitution. Samples of the aqueous filtrate from this substance when treated with Tollens' reagent gave a silver mirror. The main portion of the distillate was, therefore, oxidized with permanganate, neutralized with sodium hydroxide and evaporated to small volume. The clear solution which was obtained in this way, when acidified with hydrochloric acid, gave a precipitate of benzoic acid which was identified by comparison with a pure specimen of this substance. An oily residue which remained in the distilling flask after the steam distillation was separated from the supernatant aqueous layer and treated with alcohol. This procedure resulted in its partial solution and the simultaneous separation of an insoluble substance which melted at 276–278°. The alcohol filtrate from the latter was found to contain a mixture of the two isomeric esters II and III. These were partially separated by a process of fractional crystallization.

When the ester, m. p., 65.5–66.5°, was dissolved in alcohol or ether and the clear solution allowed to stand in diffused daylight, it gradually became cloudy and a fine, white, crystalline precipitate was observed to settle on the bottom and walls of the containing vessel. The formation of this precipitate was greatly accelerated by subjecting the solution to the radiations from a mercury lamp. Under this treatment, continued over a period of two weeks in the presence of a trace of iodine as a catalyst, 1.0 g. of ester, m. p., 65.5–66.5°, yielded approximately 0.1 g. of insoluble precipitate, m. p., 276–278°. The alcohol filtrate from this substance smelled strongly of benzaldehyde and on evaporation yielded 0.4 g. of a substance melting at 94–98°. This after recrystallization from alcohol was identified as the higher-melting, isomeric modification of the original ester by comparison with a pure specimen of known constitution. The alcohol filtrates from this substance when combined and evaporated yielded an oil which did not crystallize.

The ester (III), m. p., 98.5–99.5°, was observed to behave similarly. Thus, pure crystalline analyzed specimens which had stood in glass-stoppered containers for a period of one year were found to have become opaque and to have suffered a change in melting point to 97–245°. When recrystallized from boiling alcohol, this mixture was readily

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[6] 220-volt, direct current type, manufactured by the Hanovia Chemical and Manufacturing Company, Newark, N. J.

resolved into an insoluble substance, m. p., 276–278°, which was removed by filtration, and the original ester, m. p., 94–98°. The same effect was produced in ten weeks by exposing a sample of the ester to the action of radiations from a mercury lamp. In this case the product melted at 90–235°, while a sample of the same specimen which was kept in the dark showed no appreciable change in its melting point. The formation of the insoluble substance, m. p., 276–278°, was still further accelerated by partly dissolving and partly suspending the ester, m. p., 98.5–99.5°, in alcohol or ether and subjecting the mixture to the action of radiations from a mercury lamp. Thus 5 g. of ester suspended in 80 cc. of alcohol-free ether and exposed in this way for three weeks yielded 0.2 g. of an insoluble substance melting at 274–278°. In this case 3.6 g. of the original ester, m. p., 94–98°, was recovered and in addition to this 0.2 g. of the lower-melting isomer, m. p., 65.5–66.5°, was isolated as the result of fractional recrystallization of the resulting mixture from alcohol. Traces of benzoic acid were also separated and identified. The formation of the insoluble substances, m. p., 274–278°, appears to represent a time reaction. Thus, in general a clear ether solution becomes cloudy in the course of about a week and in two weeks an appreciable precipitate has formed. If this is removed by filtration and the clear filtrate exposed to the further action of light an *immediate* cloudiness becomes apparent. It should be noted that invariably at the beginning of any experiment there appears to be an appreciable period of time (usually about one week) which is required for the initial reaction. In one case 1.7 g. of ester, m. p., 98.5–99.5°, dissolved in 80 cc. of cold methyl alcohol bcame slightly turbid after a lapse of 15 hours and yielded 0.2 g. of a precipitate, m. p., 274–277°, after an exposure of three weeks.

The insoluble products, m. p., 274–278°, which were obtained as the result of these experiments were found to be identical whether formed from the lower- or higher-melting esters. This material as obtained from all sources was combined and purified by recrystallization from boiling benzene. The process was an extremely tedious one because of the very slight solubility of the substance (0.3 g. in 600 cc.) but was satisfactory in so far that a pure product melting sharply at 278° was finally obtained.

Anal. Calcd. for $C_{14}H_{14}O_4N_2$: N, 10.21. Found: 10.26.

This composition is the same as that found in the case of the original esters[7] m. p., 65.5–66.5° and 98.5–99.5°, respectively.

The corresponding isomeric acids, m. p., 186.5–187.5° and 198.5–199.5°, respectively, were observed to differ from each other very considerably in their behavior under the action of light. The former appears to be remarkably stable both in the solid state and in solution. Some action takes place as shown by a change in melting point to 150–180° after an exposure of five months. The change was, however, not sufficient to allow of the separation and identification of any of the transformation products and no trace of an insoluble residue was detected in recrystallizing the above mixture.

The higher-melting isomeric acid, m. p., 198.5–199.5°, was readily transformed into a mixture having a melting point of 190–285° under the action of light. This very definite change was observed in the case of pure, dry, crystalline specimens which had been allowed to stand in stoppered glass containers in ordinary daylight for about a year, but a sample of the same specimen showed an appreciable though smaller change in melting point (to 197–200°) during the course of a month. Both products when treated with alcohol were only partially dissolved and when the resulting solution was filtered an insoluble residue melting with decomposition at 292–294° was obtained.

When the acid, m. p., 198.5–199.5°, partially dissolved and partially suspended in either methyl alcohol or ether, was placed in a quartz bottle and subjected to the action

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[7] Compare Ref. 1.

of radiations from a mercury lamp, the same transformation took place much more rapidly. Thus 5.0 g. of acid gave a precipitate of 0.05 g. of insoluble material, m. p. 292–294°, during the course of two days and the amount of this product increased to 0.3 g. at the end of six weeks. The filtrate from this, on evaporation, yielded 2 g. of a substance which melted at 186–188.5° and which was identified as the corresponding lower-melting isomer after recrystallization and comparison with an analyzed specimen of this compound which had been obtained by synthetic methods. No benzaldehyde was detected as a by-product in any of these transformations. The initial reaction required approximately the same time as was observed in the case of the corresponding esters.

The product melting at 292–294° was not identical with the high-melting and insoluble product which was obtained from the corresponding esters since a mixture of the two substances was found to melt at 265–288°. This new compound is, moreover, an acid which is slightly soluble in water and quickly decolorizes aqueous alkaline solutions containing phenolphthalein. It was, moreover, obtained by boiling 0.1 g. of the product, m. p., 278°, with a mixture of 5 cc. of glacial acetic acid and 10 cc. of concentrated hydrochloric acid for twenty-four hours. The substance is almost insoluble in alcohol, ether and chloroform and so very slightly soluble in benzene that it was not practicable to recrystallize it from this solvent. In this respect it differs markedly from the product, m. p., 278°, which was obtained by the action of light on the corresponding isomeric esters. It is somewhat more soluble in boiling glacial acetic acid (0.5 g. in 1000 cc.), from which it separates in the form of a white, crystalline powder, but unfortunately its melting point was not noticeably improved as a result of this treatment.

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: N, 10.77. Found: 9.97, 10.17.

These results correspond closely with those which were obtained in the case of the original acid, m. p., 198.5–199.5°, namely, 10.65 and 10.63. The fact that the values found are so much lower than the calculated might be accounted for by assuming that traces of an impurity are present which could not be removed by recrystallization from acetic acid.

This new acid exactly neutralizes one equivalent of potassium hydroxide as calculated for its simplest molecular formula. Thus, when 0.0346 g. is suspended in 40 cc. of water, to which a drop of phenolphthalein has been added, and titrated against standard potassium hydroxide, it passes into solution and an end-point is reached when 0.0086 g. of base or an equivalent of 0.0059 g. of potassium has been added. The calculated amount of potassium required to neutralize an acid having the formula $C_{13}H_{12}O_4N_2$ is equal to 0.0052 g.

Summary

Certain types of hydantoin derivatives which contain unsaturated ethylene linkages and which exist in geometrically isomeric modifications undergo three distinctly different types of rearrangement and decomposition when subjected to the action of light. While the exact nature of these changes is not known in all cases, the phenomena would seem to be of definite importance from the standpoint of biochemistry.

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