pected, if one hydrogen were fixed for each dye molecule reduced and if the chlorophyll molecule contained one transferrable hydrogen. The small amounts of deuterium found in the chlorophyll samples could easily be accounted for as due to traces of deuterated impurities.

To test whether deuterium gained in the reaction might have been lost during the process of separation, an experiment was performed with deuterated *o*-nitrophenol. Most of its deuterium was lost when the compound was passed through the paper column under conditions similar to those prevailing in the chlorophyll separations. However, subsequent to the completion of these experiments, it was demonstrated[4] that chlorophyll does not exchange with D_2O in either homogeneous or heterogeneous liquid systems. Therefore, if deuterium had been introduced photochemically into the chlorophyll molecule, it would have replaced a non-labile hydrogen and presumably would not have been lost during the separation.

These results indicate that, under the experimental conditions used, the mechanism of the sensitized reaction does not involve a primary removal of a hydrogen atom from the chlorophyll. However, it is not justifiable to conclude that this primary step is excluded from the mechanisms of other chlorophyll-sensitized reactions or of this same reaction occurring in other solvents (*e.g.*, methanol). The slowness of dark exchange between chlorophyll and deuterium oxide suggests that it would be feasible to repeat the present experiments using deuterated methanol as a solvent.

These results are similar to those of Ruben and co-workers[5] and of Calvin and Aronoff,[6] who were unable to demonstrate significant uptake of tracer hydrogen in the chlorophyll of *Chlorella* photosynthesizing in heavy water. It may also be remarked that Krasnovsky and his co-workers[7] have presented evidence for the reversible reduction of chlorophyll by ascorbic acid in pyridine. They have also demonstrated that, in the same solvent, chlorophyll sensitizes the oxidation of ascorbic acid by riboflavin and by certain dyes.

Experimental

Ascorbic acid containing about 40% D/(D + H) was prepared by exchange of normal U.S.P. acid with excess heavy water and was analyzed with a mass spectrometer. Butter yellow was purified by repeated chromatography and recrystallization (m.p. 116.5°). Other reagents and solvents have been described elsewhere.[4]

Since oxygen inhibits the reaction under study, the mixture was boiled vigorously and sealed under a vacuum prior to illumination. The light source was a 1000-watt water-jacketed tungsten lamp, covered by a Corning H.R. 3480 filter.

After illumination, the reaction mixture was transferred to petroleum ether by methods already described[4] and adsorbed at the top of a 2 × 30 cm. chromatography column. (The adsorbent, Whatman Ashless Filter Paper Pulp, had been packed under suction from aqueous suspension, then washed with over 500 ml. each of solvent grade acetone and petroleum ether.) Residual butter yellow, occasional traces of pheophytin and the product amines were completely removed by petroleum ether and benzene, whereupon the chlorophyll was eluted with ether. Only the leading 50-75% of the band was collected; it exhibited the normal absorption spectrum and fluorescence of chlorophyll-a.

Partial infrared spectra of chlorophyll from four of the runs showed no bands due to deuterium or to decomposition products. All samples were burned and analyzed for deuterium by methods previously described.[4]

Acknowledgments.—We are happy to acknowledge the generous aid and useful comments of the following persons: Dr. A. H. Brown, Dr. A. W. Frenkel and Mr. J. M. Daly of the Department of Botany and Dr. B. L. Crawford of the School of Chemistry.

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Some Substituted 5-Benzalhydantoins and 5-Benzylhydantoins[1]

BY HENRY P. WARD

RECEIVED MARCH 31, 1952

A significant feature of the new hydantoins reported in this paper is their physiologically active[2] phenethylamine skeleton structure. They were made as precursors to phenylalanines which yield on decarboxylation phenethylamines related to mescaline.

The benzalhydantoins reported here were made by condensing the following aldehydes with hydantoin: 2,4-dichlorobenzaldehyde, 2-ethoxybenzaldehyde, 3,4-diethoxybenzaldehyde, 4-hydroxy-3-ethoxybenzaldehyde and 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde). The condensations were usually effected in glacial acetic acid and acetic anhydride in the presence of fused sodium acetate after the method first used by Wheeler and Hoffman.[3] It was found more satisfactory to convert the phenolic aldehydes to hydantoins by using diethylamine as the condensing agent in pyridine solution.[4]

The benzylhydantoins were made from the benzalhydantoins by several reduction methods in addition to those cited. The 2,4-dichlorobenzalhydantoin was most readily reduced to the corresponding hydantoin with hydriodic acid in acetic acid. The alkoxy substituted benzalhydantoins were reduced in 1 *N* NaOH solution with Raney nickel and hydrogen.

Table I summarizes data for the hydantoins.

Experimental

The following procedures are representative examples by which the benzalhydantoins, $ArCH{=}C(CO{-}NH)(CO{-}NH)$, and the benzylhydantoins, $ArCH_2{-}CH(CO{-}NH)(CO{-}NH)$, in Table I were prepared.

5-(2,4-Dichlorobenzal)-hydantoin.—A mixture of 10 g. of hydantoin, 15 g. of 2,4-dichlorobenzaldehyde, 9 g. of fused sodium acetate, 58 ml. of glacial acetic acid and 2 ml. of

(1) The author is grateful to the Research Corporation for a grant toward the support of this work.
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TABLE I

C-5 SUBSTITUTED BENZALHYDANTOINS AND C-5 SUBSTITUTED BENZYLHYDANTOINS

Hydantoin	Formula	Yield, %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
2,4-Dichlorobenzal	$C_{10}H_6O_2N_2Cl_2$	60	310–312	46.71	46.91	2.35	2.51
2,4-Dichlorobenzyl	$C_{10}H_8O_2N_2Cl_2$	56	226–227	46.35	46.51	3.11	3.15
2-Ethoxybenzal	$C_{12}H_{12}O_3N_2$[a]	68	222–223	62.08	62.18	5.17	5.21
2-Ethoxybenzyl	$C_{12}H_{14}O_3N_2$	80	171–172	61.50	61.91	5.98	5.98
3,4-Diethoxybenzal	$C_{14}H_{16}O_4N_2$[a]	65	245–247	60.83	61.06	5.83	5.84
3,4-Diethoxybenzyl	$C_{14}H_{18}O_2N_2$[b]	75	186–187	60.40	60.09	6.50	6.48
4-Hydroxy-3-ethoxybenzal	$C_{12}H_{12}O_4N_2$	76	263–265	58.07	58.03	4.88	4.87
4-Hydroxy-3-ethoxybenzyl	$C_{12}H_{14}O_4N_2$[b]	60	194–195	57.57	57.43	5.63	5.60
4-Hydroxy-3,5-dimethoxybenzal	$C_{12}H_{12}O_5N_2$[c]	45	304–306	54.50	54.39	4.57	4.49
4-Hydroxy-3,5-dimethoxybenzyl	$C_{12}H_{14}O_5N_2$[b]	60	225–226	54.11	53.67	5.29	5.30

[a] Yellow needles made by procedure used for dichlorobenzalhydantoin. [b] White crystals made by procedure used for ethoxybenzylhydantoin. [c] Yellow crystals made by procedure used for 4-hydroxy-3-ethoxybenzalhydantoin.

acetic anhydride was gently refluxed on an oil-bath for 3 hours. After 2 hours a yellow solid separated from the liquid and soon accumulated. The heating was continued another hour. The mixture was cooled, filtered, and the solid product washed with 50 ml. of ethanol and 50 ml. of water. The dried product weighed 11.1 g. An additional 2.1 g. separated from the filtrate; total yield 60%. Crystallization from acetic acid gave pale yellow crystals.

5-(2,4-Dichlorobenzyl)-hydantoin.—A mixture of 10 g. of 5-(2,4-dichlorobenzal)-hydantoin, 15 ml. of glacial acetic acid, 5 ml. of acetic anhydride and 70 ml. of hydriodic acid (sp. gr. 1.5) was refluxed for 2 hours on an oil-bath at 118°. The mixture was distilled with steam to remove acid, cooled, and filtered; yield 5.7 g. (56%). The product crystallized from acetic acid as white needles.

5-(2-Ethoxybenzyl)-hydantoin.—Five grams of 5-(2-ethoxybenzal)-hydantoin in 50 ml. of 1 *N* NaOH was hydrogenated at room temperature in the presence of 5 g. of Raney nickel in a Parr hydrogenator at 40 p.s.i. for 5 hours. The catalyst was filtered off and filtrate was made acid with dilute hydrochloric acid; 4.1 g. (80%) of white precipitate was obtained. Recrystallization from hot water yielded white platelets.

5-(4-Hydroxy-3-ethoxybenzal)-hydantoin.—Twenty-five grams of 4-hydroxy-3-ethoxybenzaldehyde, 15 g. of hydantoin, 25 ml. of pyridine and 12 ml. of diethylamine were refluxed gently for 8 hours; the mixture was allowed to stand overnight and the tan colored precipitate was filtered and washed with cold ethanol; yield 28.2 g. (76%). The product was recrystallized from dilute acetic acid.

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Paper Chromatography of Azo-dyes from Arylamines and Sulfanilamide[1]

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RECEIVED DECEMBER 7, 1951

A diazotized arylamine, coupled with different phenolic compounds, gives a typical and very sensitive color reaction. This reaction is currently used in the determination of *p*-aminobenzoic acid and sulfanilamide in biological material.[2] The fact that it cannot discriminate among different isomers of arylamines leads to difficulties when the major problem is that of determining *p*-aminobenzoic acid in the presence of either anthranilic acid or sulfanilamide.

Azo-dyes resulting from the coupling of α-naphthol with the diazotized arylamine were separated on paper chromatograms by two different methods. In the first method, the chromatograms were developed with tenth molar sodium hydroxide. The spots could be identified by their position and difference in color: the faster *o*-aminobenzoic acid derivative was orange in color, fluorescent in ultraviolet light, and turned yellow in strong alkali; the slower *m*-aminobenzoic acid spot was orange; the *p*-aminobenzoic acid derivative was pink and stayed so in strong alkali. The sulfanilamide spot could not be separated from the *p*-aminobenzoic acid spot.

Spraying these chromatograms with indicator solutions showed that the ascending solvent separated into different *p*H zones. A neutral zone ran first, an alkaline zone, having a *p*H of about 11, followed, and last was a zone having the alkalinity of the hydroxide used (*p*H 13). The formation of a neutral water zone running ahead of the alkaline solution was observed first by Schoenbein.[3] The second zone was due to sodium carbonate, which separated from the solvent, and which was produced by a reaction of the ascending solvent with atmospheric carbon dioxide. These *p*H zones suggested that the separation of the chromatographed spots might depend upon different adsorption rates of the dyes at a given *p*H and upon their displacement at various *p*H values. This is supported by the fact that in weaker alkali (0.05 *N*) the movement of the spots was slower, while in stronger alkali (0.2 *N*) they were shifted near the upper margin of the liquid. In a carbon dioxide atmosphere the ascending hydroxide was neutralized, giving carbonate in which the spots remained stationary.

The other method of separating azo-dyes of arylamines and sulfanilamide was by partition chromatography, as described by Lederer for the separation of indicators.[4] The following table gives *RF* values for different compounds which were prepared as above. Amyl alcohol, shaken with a 10% solution of concentrated ammonia, was used as a solvent.

TABLE I

Azo-dye of	*RF*
o-Aminobenzoic acid	0.63
m-Aminobenzoic acid	.09
p-Aminobenzoic acid	.05
Sulfanilamide	.50

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